ConCO$_2$rde: Using CO$_2$ as a resource

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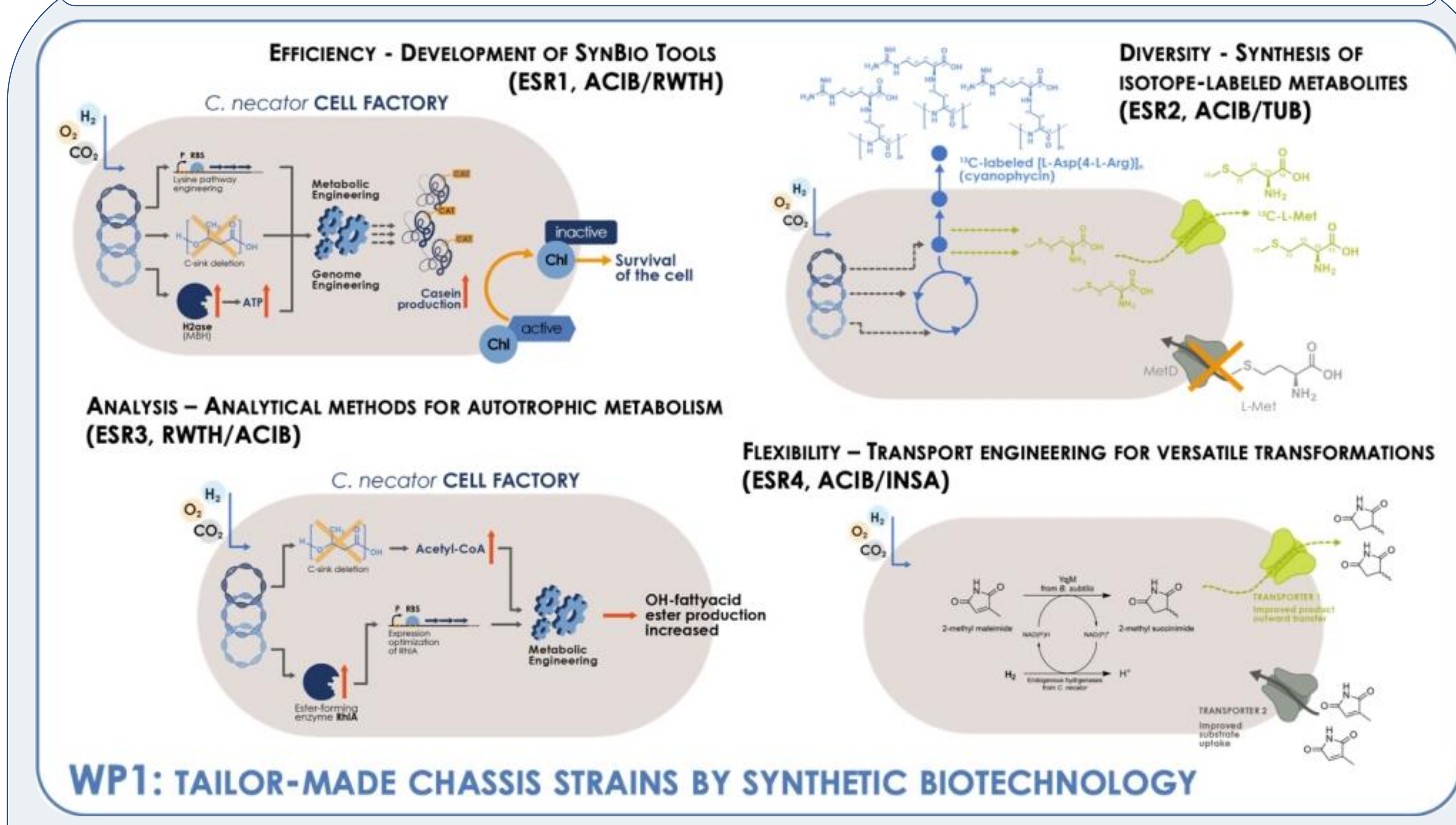
Funded by the European Union

Background

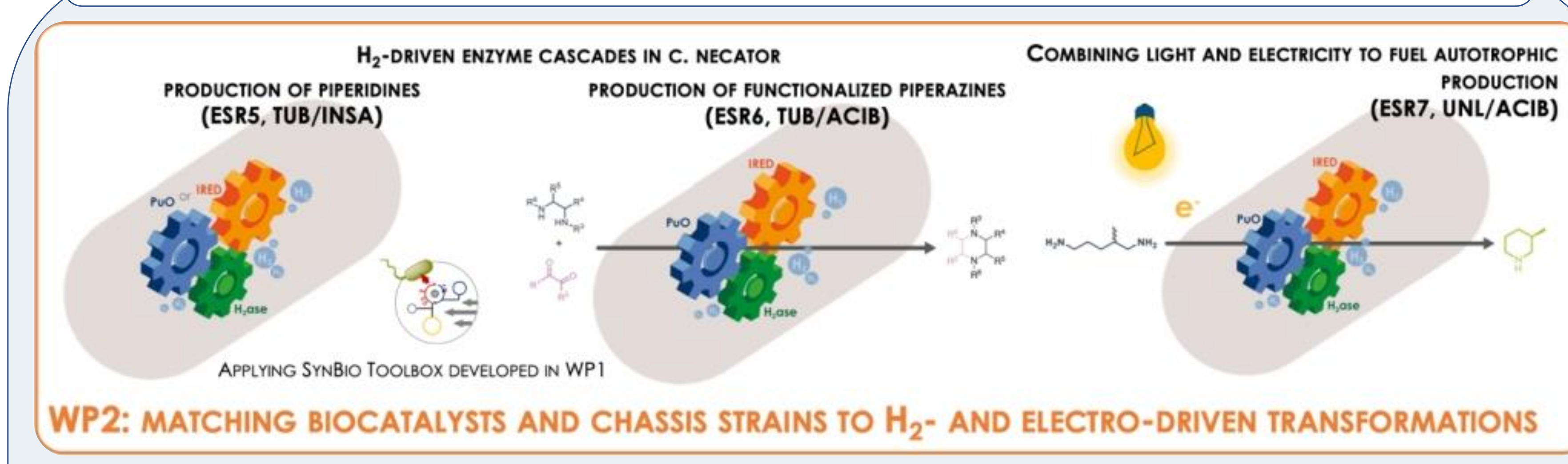
As a result of human activity, the atmospheric concentration of carbon dioxide (CO_2) has increased by 50 % since the start of the industrial revolution, significantly contributing to global warming [1]. In contrast to the classical petrochemical industry that uses fossil fuels, with biotechnology renewable resources can be utilized as a carbon source, reducing the impact on the environment. The ConCO$_2$rde project goes one step further and strives to valorize CO_2 waste streams as primary carbon source, meeting market demands and societal concerns. Chemolithoautotrophic microorganisms such as *Cupriavidus necator* and *Rhodopseudomonas palustris* naturally fix CO_2. By expanding the engineering toolbox for these microorganisms, the project aims to develop robust microbial cell factories to produce pharmaceutical precursors, amino acids for the food industry and more. Together with 17 leading academic and industrial organizations, and 11 young scientists, the ConCO$_2$rde Interactive Training Network explores new concepts to exploit the strengths of autotrophy and strives to establish efficient gas-driven processes.

WP1: Strain development by synthetic biology and metabolic engineering



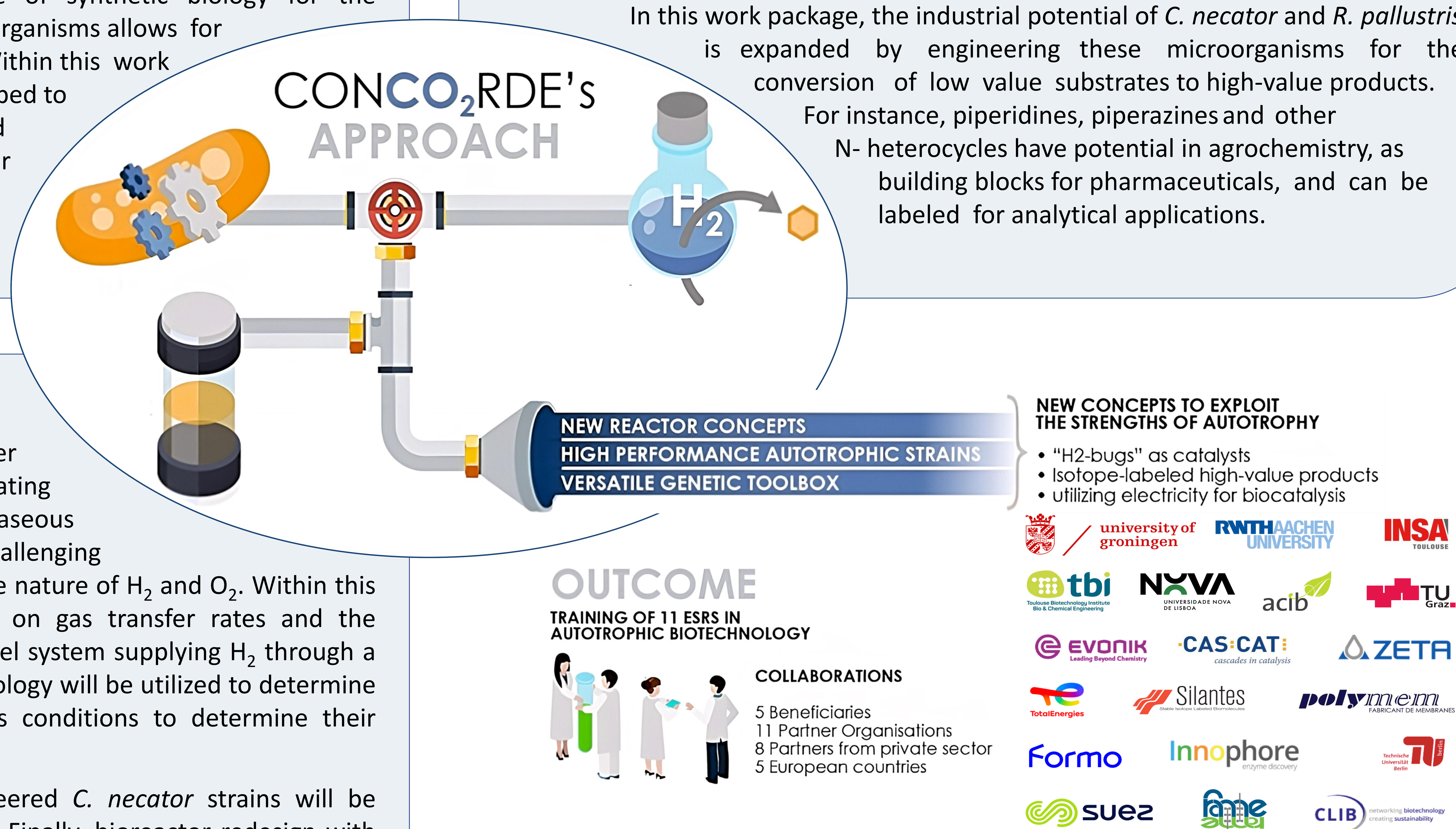
The development of efficient strains is a crucial step towards fully exploiting the industrial potential of a microorganism. The use of synthetic biology for the modification of the metabolism of autotrophic microorganisms allows for more controlled expression of products of interest. Within this work package, various genetic engineering tools are developed to Easily modify *C. necator*. Additionally, flux analysis and metabolic engineering will be employed to redirect or delete endogenous pathways. Finally, this knowledge will be applied to develop new recombinant strains, expanding the production potential of *C. necator.*

WP2: H_2- and electro-driven synthesis of high value products



The chemical production of complex value-added products is expensive, generates substantial waste, and often uses toxic compounds. For this reason, enzyme cascades are gaining attention in the manufacturing of fine chemicals. They offer an excellent sustainable alternative since enzymes are enantio- and stereoselective and work under mild reaction conditions. However, many enzymes require expensive cofactors. Exploiting microorganisms' native cofactor regeneration systems offers an affordable solution to this problem. Together with engineered enzyme cascades, novel compounds can be produced through whole-cell bioconversion.

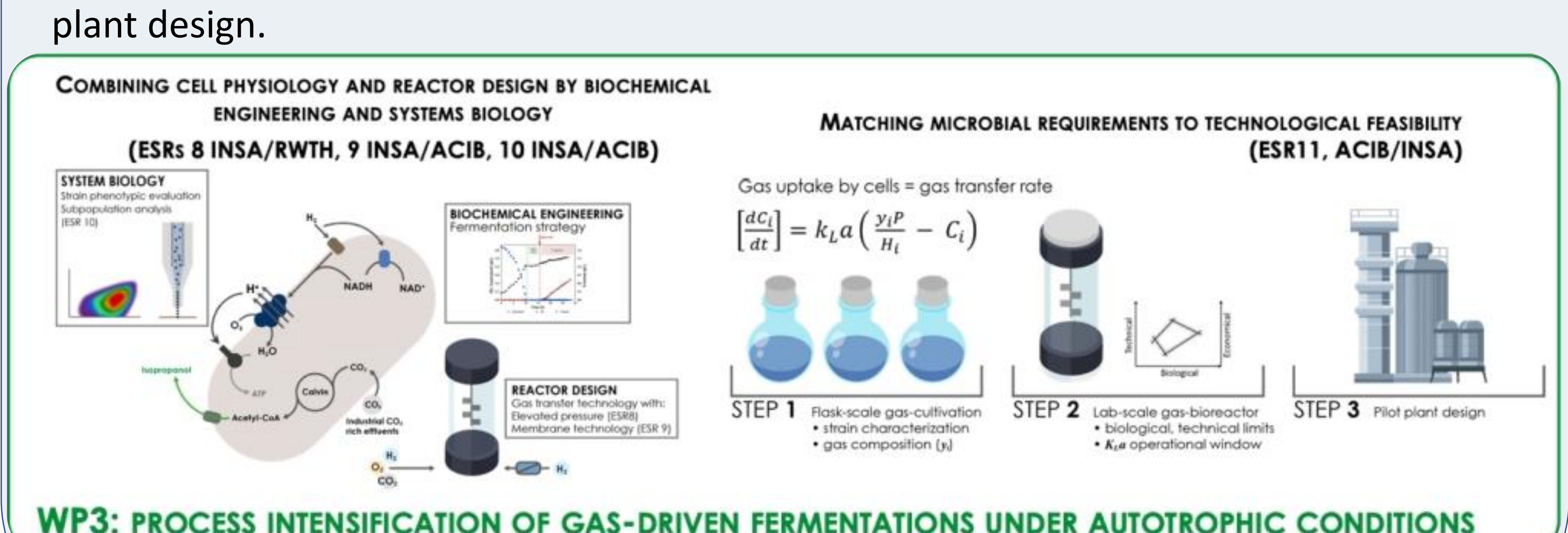
In this work package, the industrial potential of *C. necator* and *R. pallustris* is expanded by engineering these microorganisms for the conversion of low value substrates to high-value products. For instance, piperidines, piperazines and other N- heterocycles have potential in agrochemistry, as building blocks for pharmaceuticals, and can be labeled for analytical applications.



WP3: Reactor design and up-scaling of gas-driven processes

The use of autotrophic microorganisms in gas fermentation has recently gained momentum, however several problems remain to be addressed. When cultivating *C. necator* in bioreactors, providing the required gaseous substrates (CO_2, H_2, and O_2) in excess becomes challenging because of the limited gas solubility and the explosive nature of H_2 and O_2. Within this work package, the influence of elevated pressure on gas transfer rates and the fermentation process are studied. Additionally, a novel system supplying H_2 through a membrane will be assessed. Furthermore, systems biology will be utilized to determine the homogeneity of the cell culture under various conditions to determine their robustness against the harsh industrial settings.

Various fermentation strategies on different engineered *C. necator* strains will be explored to obtain high biomass and product yields. Finally, bioreactor redesign with novel safety concepts will be investigated with subsequent scale-up including a pilot plant design.



Cupriavidus necator and *Rhodopseudomonas palustris*

Cupriavidus necator is a gram negative soil bacterium. It can fix CO_2 through the Calvin-Benson-Bassham (CBB) cycle using H_2 as an energy source and O_2 as a terminal electron acceptor. *C. necator* is best known for its natural polyhydroxyalkanoate (PHA) production which can amount up to 80 % of its biomass [2]. This bacterium is attractive for the industrial applications because of its versatile metabolism and capacity to produce high-value products such as alcohols, fuel additives and bioplastics.

Rhodopseudomonas palustris is a photosynthetic gram negative bacterium. In contrast to *C. necator, R. palustris* assimilates CO_2 in strictly anaerobic conditions and uses light as an energy source. Its reducing power can be obtained from an electrical circuit in a bioelectrochemical cell. Just like *C. necator*, this bacterium can also produce PHAs[3].

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 955740. This poster reflects only the author's view, the Agency is not responsible for any use that may be made of the information it contains.

References

[1] Fox, A. (Smithsonian Magazine, 2021)

[2] Fiorese, M. L., Freitas, F., Pais, J., Ramos, A. M., De Aragão, G. M. F., & Reis, M. A. M. *Engineering in Life Sciences*, *9*(6), 454–461. (2009)

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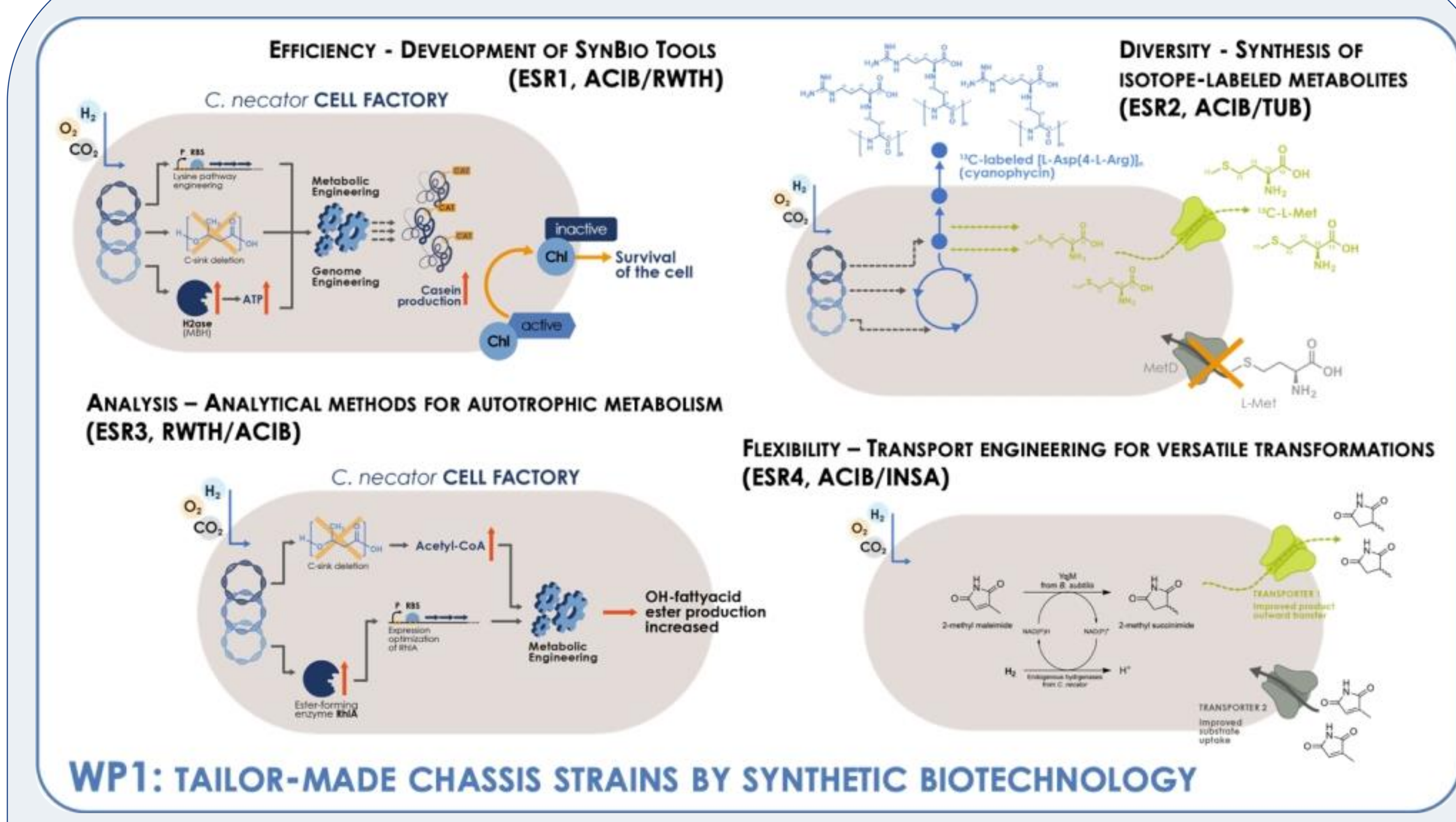

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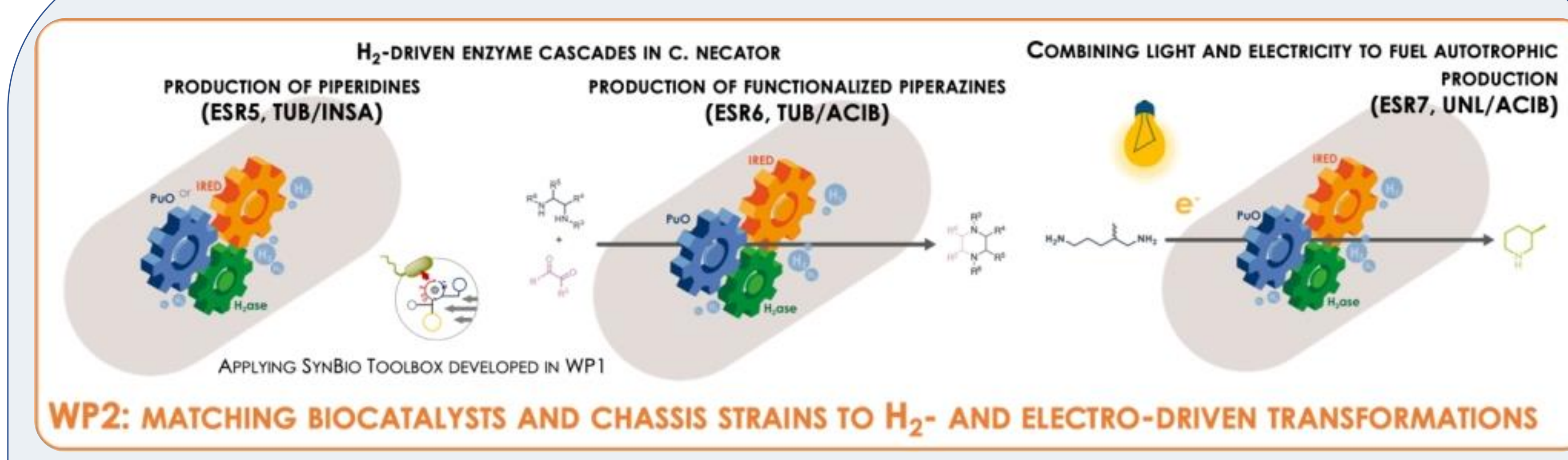

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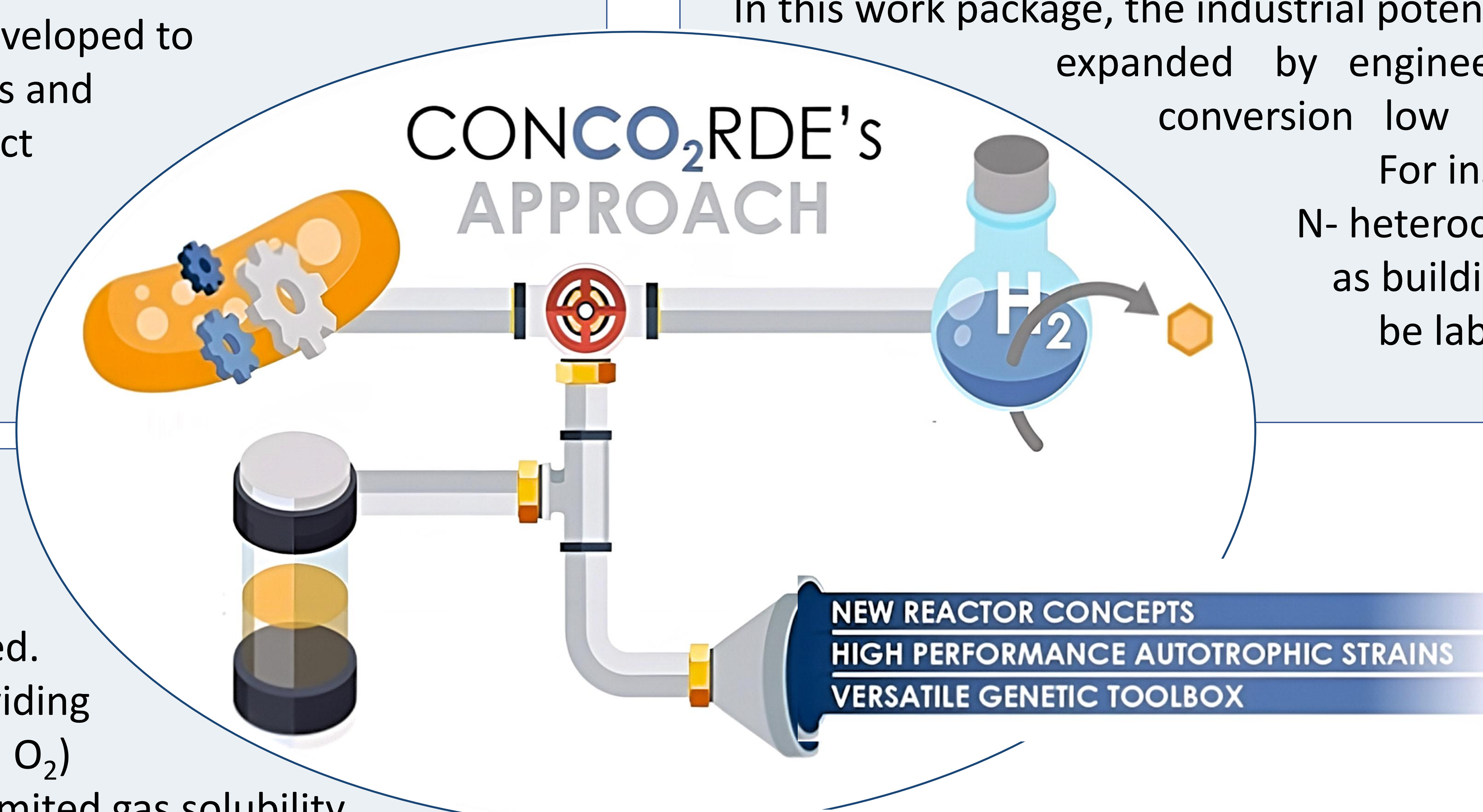

NEW CONCEPTS TO EXPLOIT THE STRENGTHS OF AUTOTROPHY
- "H2-bugs" as catalysts
- Isotope-labeled high-value products
- utilizing electricity for biocatalysis

OUTCOME

TRAINING OF 11 ESRS IN AUTOTROPHIC BIOTECHNOLOGY

COLLABORATIONS

5 Beneficiaries
11 Partner Organisations
8 Partners from private sector
5 European countries

WP3: Reactor design and up-scaling of gas-driven processes

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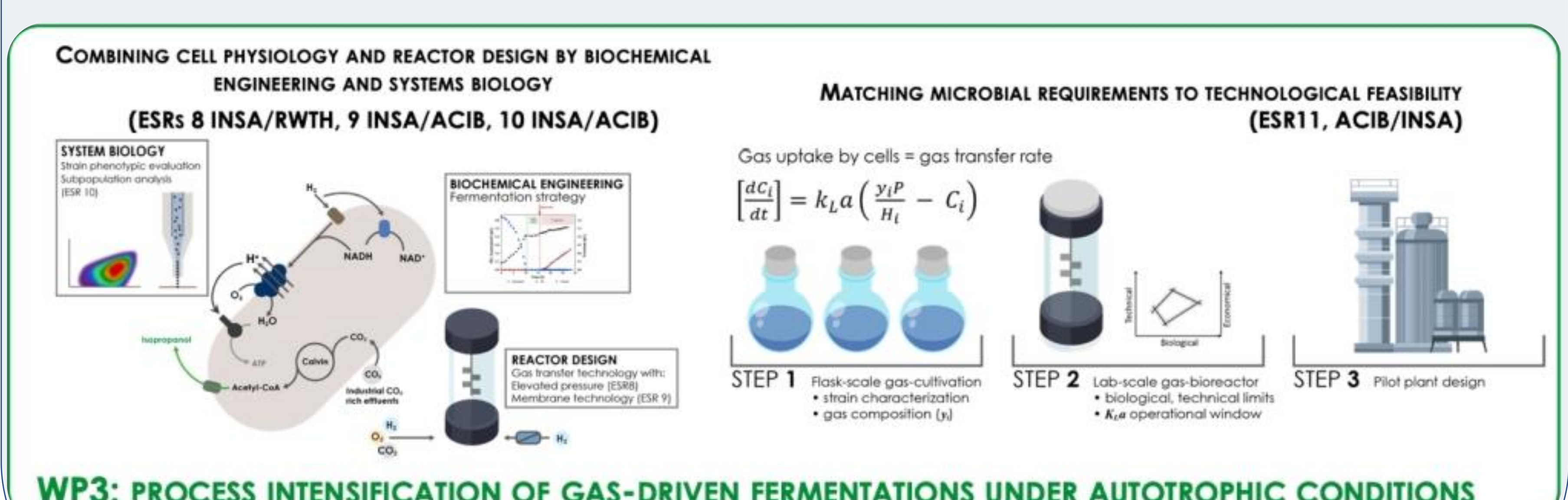

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