Biotechnological processes for the high efficiency conversion of CO_2 from industrial waste streams into high value products

Annabel Serpico[1], Alba Suárez[1], Laura Pérez[1], Adrià Marí[1], Deborah Cornadó[1], Paula Félix[1], Simone Savino [2], Guiomar Sánchez[1]

aserpico@leitat.org

[1] Biotechnology and microbiology R&D department, LEITAT technological centre, Terrassa

[2] Faculty of Science and Engineering, University of Groningen, Groningen

1. BIOCON-CO2 vision

The main objective of BIOCON-CO2 is to develop and validate a flexible platform to biologically transform CO_2 into added-value chemicals and plastics. The versatility and flexibility of the platform is based on 3 main stages (CO_2 solubilization, bioprocess and downstream) which will be combined together as puzzle pieces. BIOCON-CO2 will develop 4 MCFs based **using CO_2 from iron&steel industry** as a direct feedstock to produce 4 commodities with application in chemicals and plastics sectors using 3 different biological systems: anaerobic microorganisms (C3-C6 alcohols by Clostridia), aerobic microorganisms (3-hydroxypropionic acid by Acetobacter) and enzymes (formic acid by recombinant resting *E. coli* cells and lactic acid by multi-enzymatic system) (Fig. 1).

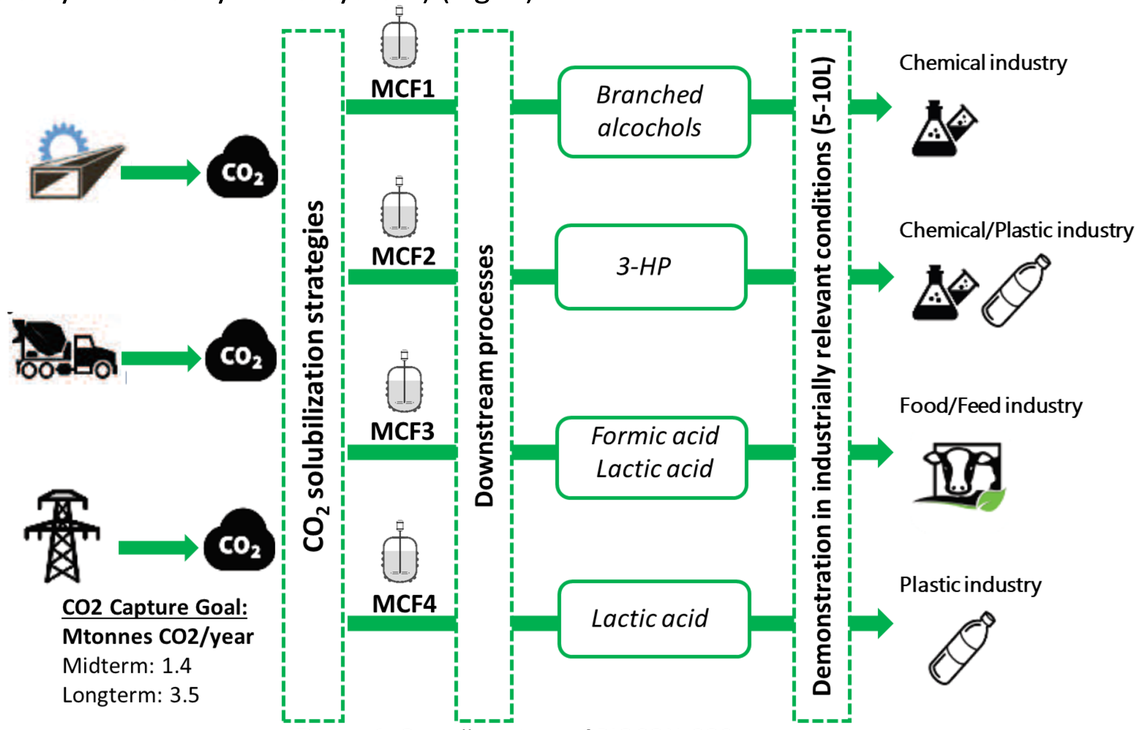

Figure 1. Overall strategy of BIOCON-CO2.

2. *C. necator* as MCF

Cupriavidus necator H16 grows to high-cell densities in lithoautotrophic, heterotrophic or mixotrophic conditions. It is able to fix CO_2 via the CBB cycle[1]. *C. necator* H16 naturally accumulates polyhydroxy alkanoates (PHAs), mostly polyhydroxy butyrate (PHB), under nutrient restriction[2] (Fig.2).

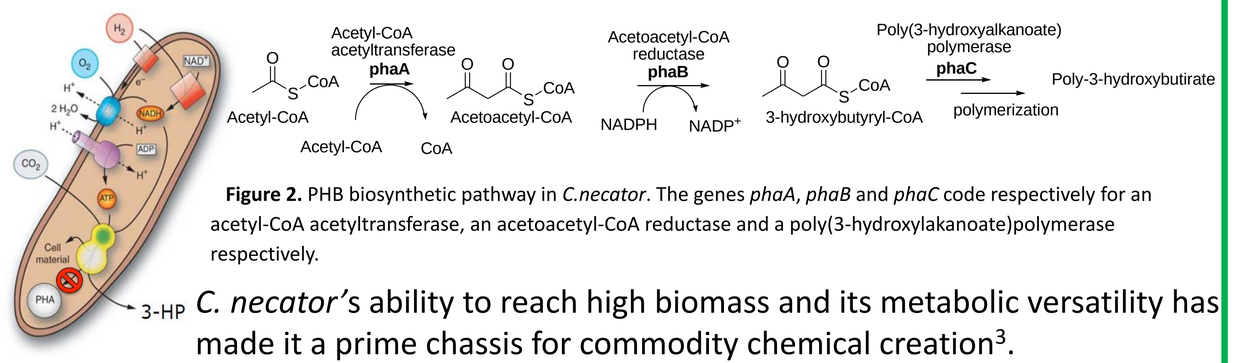

Figure 2. PHB biosynthetic pathway in *C.necator*. The genes *phaA*, *phaB* and *phaC* code respectively for an acetyl-CoA acetyltransferase, an acetoacetyl-CoA reductase and a poly(3-hydroxylakanoate)polymerase respectively.

C. necator's ability to reach high biomass and its metabolic versatility has made it a prime chassis for commodity chemical creation[3].

This versatile, non-pathogenic microorganism is of great biotechnological interest as it has been already industrially applied for the production of the biodegradable thermoplastic Biopol[4]. For the aforementioned reasons, *C. necator* has been elected as the microorganism of choice for 3-HP production and metabolic engineering will be performed for its production.

3. Biofilm formation

Microbial biofilms exhibit several advantages compared to planktonic cells. It protects against shear forces, nutrition deficiency, free radicals, pH changes and antimicrobial agents. As BIOCON-CO2 is performed in harsh conditions and in presence of several contaminants, biofilm formation was tested for *C. necator.*

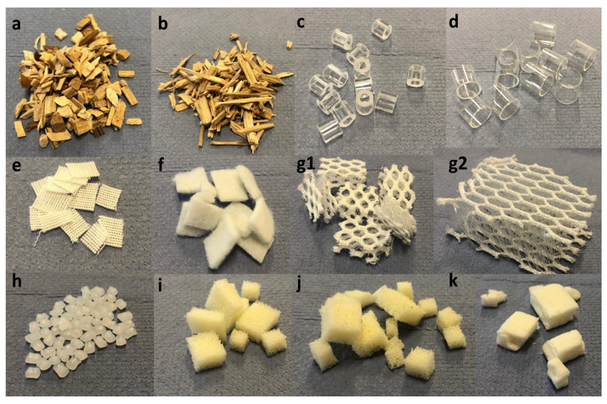

Figure 3. Packing materials selected for biofilm test. In order a) beechwood, b) eucalyptus,c) Raschig rings 6mm, d) Raschig rings 10mm, e) PES woven, f) PES non-woven, g1) PES 3D material , g2) zoom in of the PES 3D material, h) PP pellets,i) Foam PU60, j) Foam PU65, k) Foam PIR.

An extensive bibliographic research has been carried out to select 11 different materials with properties suitable for biofilm formation and CO_2 adsorption (Fig.3).

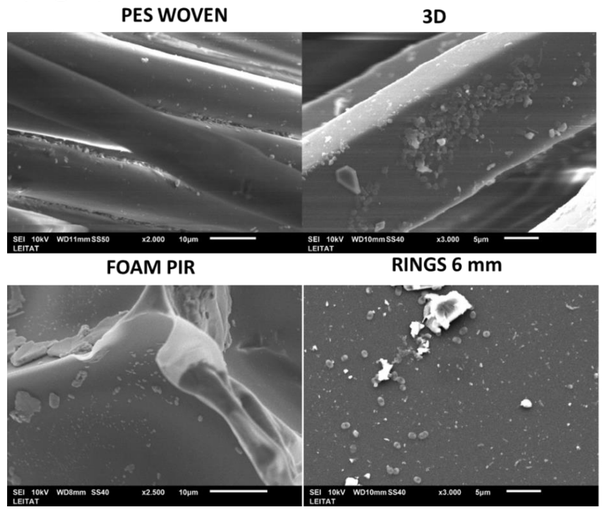

Figure 4. SEM images of biofilm observed on PES woven, PES 3D, foam pir and rings 6 mm respectively.

According to the cell count, the best materials are PES non-woven, PES woven, PES 3D, eucalyptus and beechwood respectively (Fig. 5). These materials were selected for further analysis.

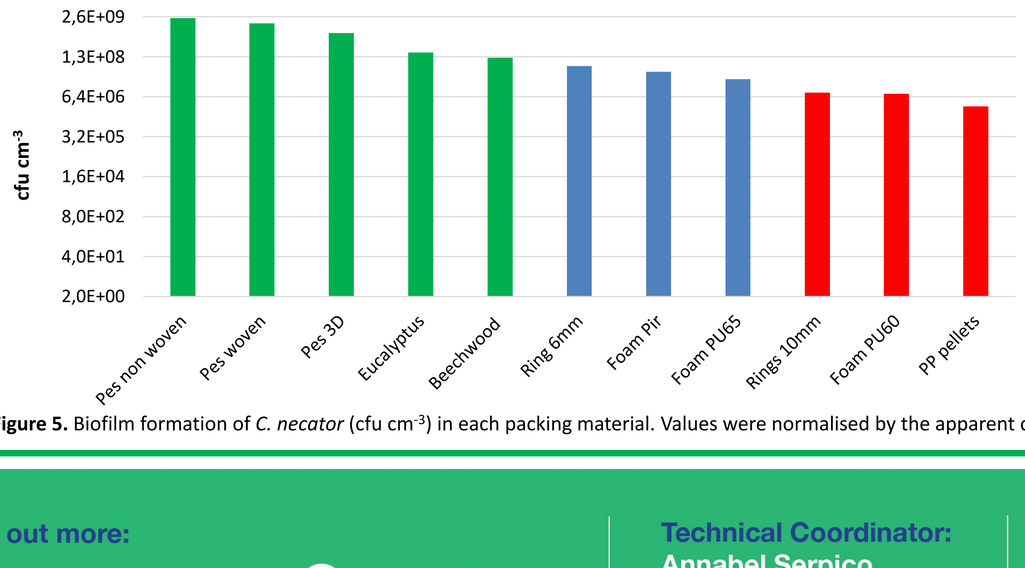

Figure 5. Biofilm formation of *C. necator* (cfu cm^{-3}) in each packing material. Values were normalised by the apparent density.

4. hCAII as biocatalyst

The carbonic anhydrase enzyme (CA) catalyses the conversion of CO_2 into bicarbonate (HCO_3^-) at extremely high turnover rates[5]. The chosen CA is the most active CA isoform described, the human one (hCAII) which displays a k_{cat} of 10^6 s^{-1}.

$$CO_2 + H_2O \overset{CA}{\rightleftharpoons} HCO_3^{\ominus} + H^{\oplus}$$

A recombinant construct for hCAII was obtained and expression conditions optimized. The hCAII stability of samples stored at a RT and at 4ºC was evaluated overtime during 15 days. A dramatic loss of activity of ~ 35% was observed after just one day in both conditions. As the final substrate of the reaction will be CO_2 from iron&steel industry, gaseous contaminants present in these effluents have been tested for inhibition. Four bottles, A, B, C and D were tested for 6 hours (gas flow 50 mL/min at 30ºC) and the activity of hCAII evaluated. Only one of the bottle, with sulphur containing species, strongly inhibits hCAII.

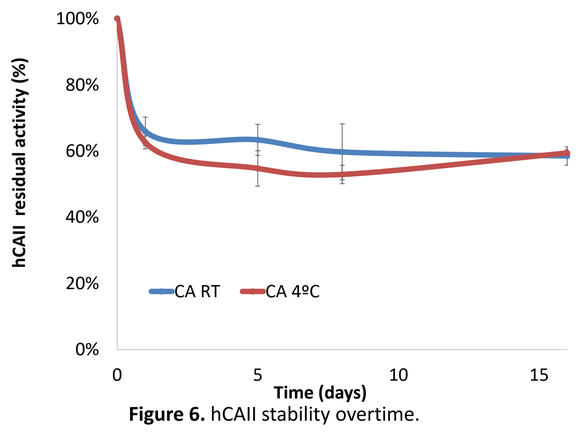

Figure 6. hCAII stability overtime.

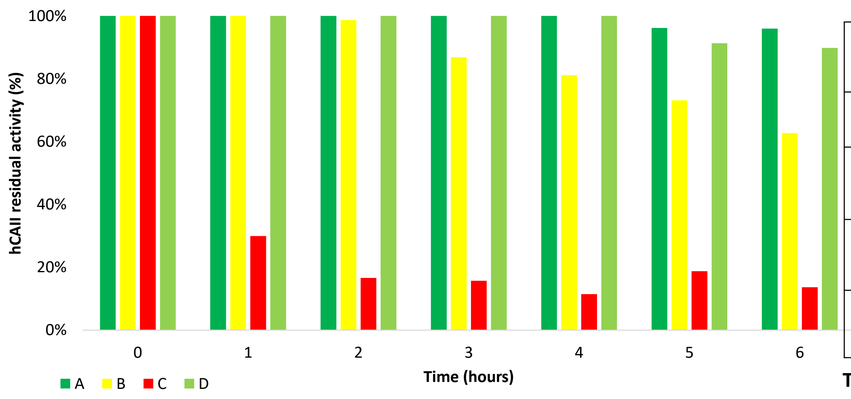

Figure 7. hCAII activity overtime with A, B, C and D.

	Gases	Final concentration in the bioreactor (ppm)
A	Oxygen (O_2)	40480
	Carbon dioxide (CO_2)	46640
B	Oxygen (O_2)	46000
	Carbon dioxide (CO_2)	53000
	Carbon monoxide (CO)	53000
C	Hydrogen Sulfide (H_2S)	120
	Carbonyl sulphide(COS)	28
	Carbon disulphide (CS_2)	8.5
D	Methane (CH_4)	125000
	Ethylene (C_2H_4)	9000
	Acetylene (C_2H_2)	500

Table 1. Composition of the contaminant gas bottle tested in the study.

The hCAII lack of stability and the inhibition by sulphur containing compound have been analyses by RUG to find mutants with improved features. Two different approaches have been adopted for the two limitations and 8 preliminary mutations have been selected for further analysis.

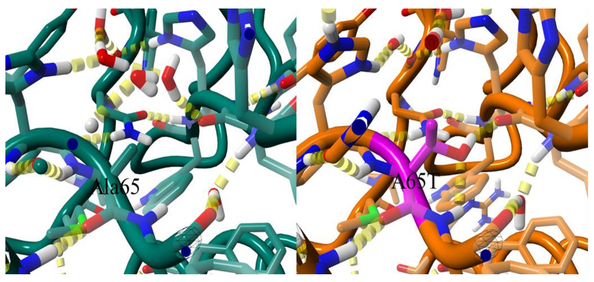

Figure 8. Example of one of the selected mutants. A65T mutation introduced in silico via FRESCO and selected for further investigation.

References

[1] Heinrich D. *et al.* (2018) *Microbiol Biotechnol.* 11(4):647-656
[2] Kohlmann Y *et al.* (2011) *J Proteome Res* 10(6):2767–2776
[3] Reinecke & Steinbüchel, (2008), *J Mol Microbiol Biotechnol.* 16(1-2):91-108
[4] Shimizu R. *et al.* (2015) *Scientific Reports*. 5, 11617
[5] Alvizo *et al.* (2014) *PNAS* 111(46):16436–41.

Find out more:
www.biocon-co2.eu
@BIOCON_CO2

Technical Coordinator:
Annabel Serpico
aserpico@leitat.org

Administrative Coordinator:
Xavier Ponte Font
xponte@leitat.org

Communication & Press:
Marieke Reuver
BIOCON-CO2@aquatt.ie

This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement no. 761042 (BIOCON-CO_2). This output reflects the views only of the author(s), and the European Union cannot be held responsible for any use which may be made of the information contained therein.