Bioreactor Engineering for Bacterial Production of Biomolecules from CO_2

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CO_2 : the context

- **Global emissions of greenhouse gases as CO_2 are a major cause of global warming**

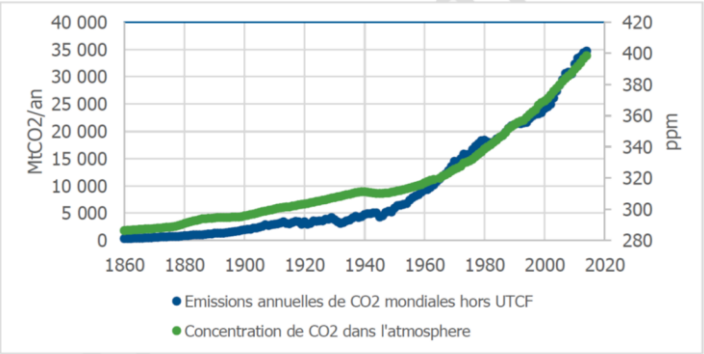


Figure 3 : Évolution du CO_2 atmosphérique hors UTCF (Utilisation des terres, leurs changements et la forêt) depuis 1850 jusqu'à aujourd'hui
Source : WRI-CAIT et NASA

- **CO2 a by-product of :**

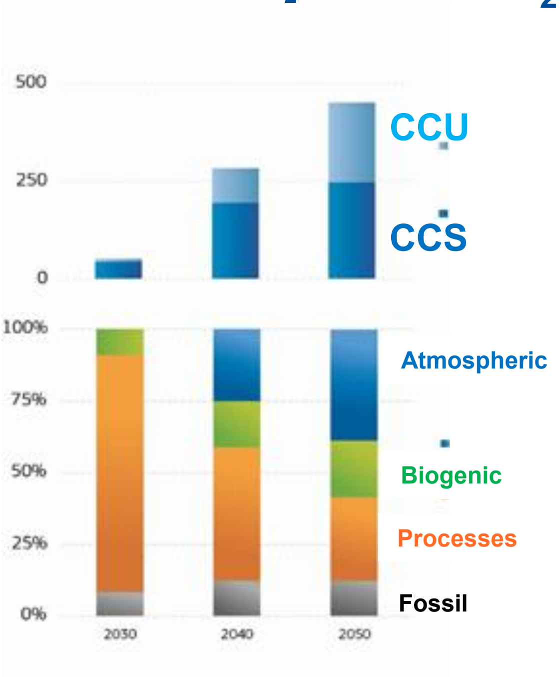
EU CO2-emissionsin 2014 (Mt) by sector		
Energy industries	1065,5	60%
Chemical industries	245,1	14%
Metal industries	166	9%
Construction industries	144	8%
Paper industries	77	4%
Waste management	55	3%
Others (mining, agriculture, food...)	26,1	1%

Kircher & Schwarz (2020) CO_2 and CO as feedstocks Sustainable Carbon Sources for the Circular. Springer Eds

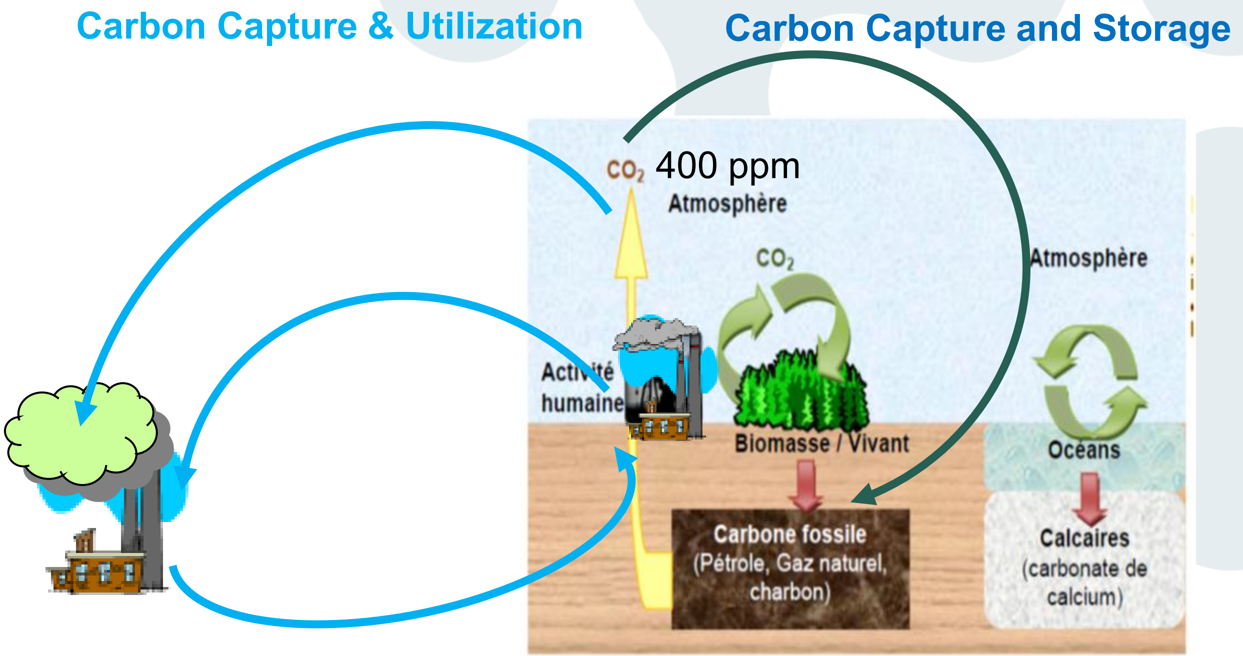
- **European Union is committed to achieving economy-wide climate neutrality by 2050 to limit global warming to 1.5 °C**
  - **EU directives target 450 Mt CO_2 captured per year *(50 Mt by 2030, 240 Mt by 2040)***

CO_2 : the context

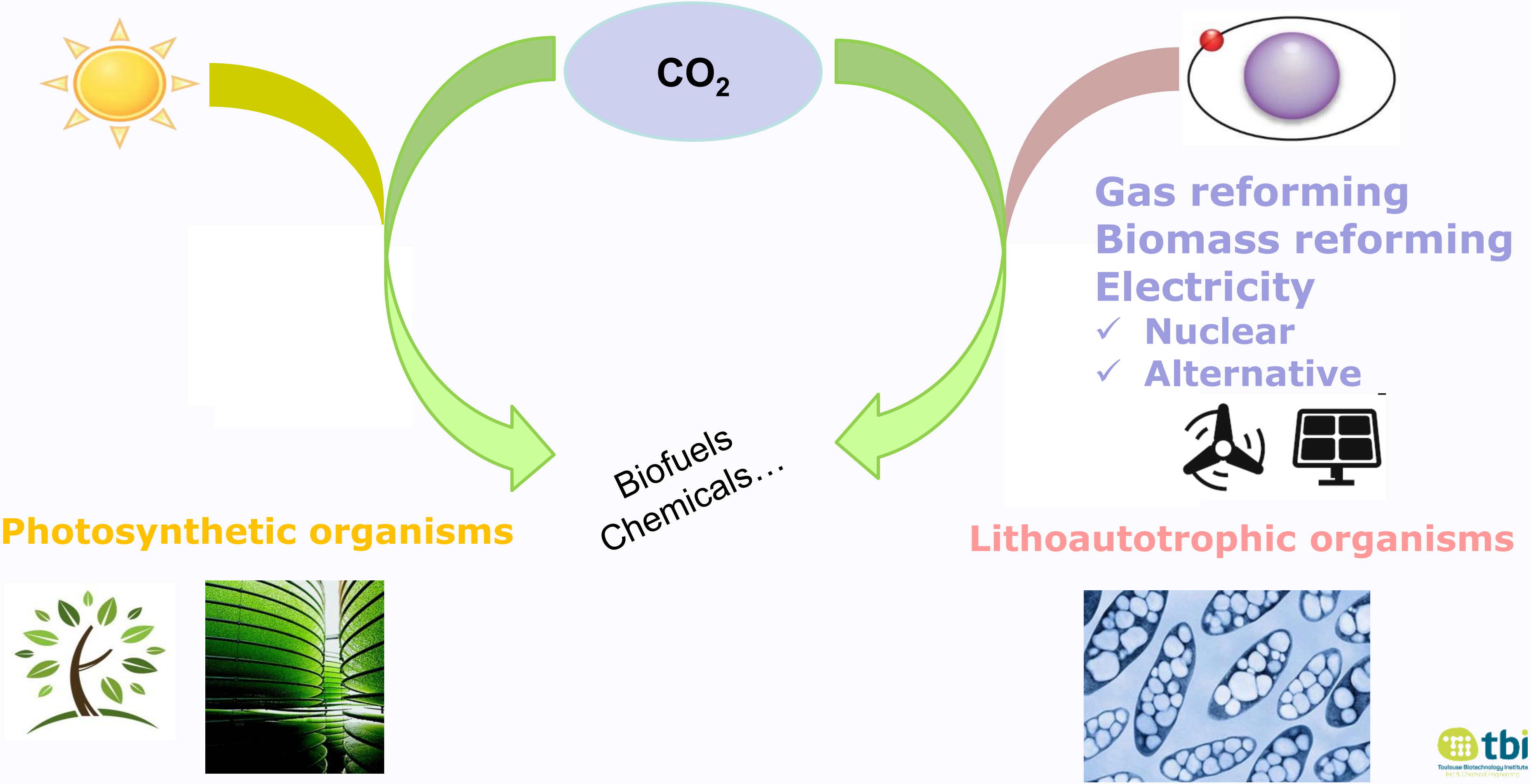
How to reinject the CO_2 emissions into a circular economy ?



COMMUNICATION FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT
Towards an ambitious Industrial Carbon Management for the EU. Document 52024DC0062. 06/02/2024



CO_2 : the biotechnological way



CO2 : the biotechnological way

Best achievement so far : LanzaTech process of ethanol production from Steel industry gases (syngases)

- Carbon Monoxide (CO) is the C-source
- Anaerobic process by acetogenic bacteria
- Industrial facilities in Belgium, India, China, USA

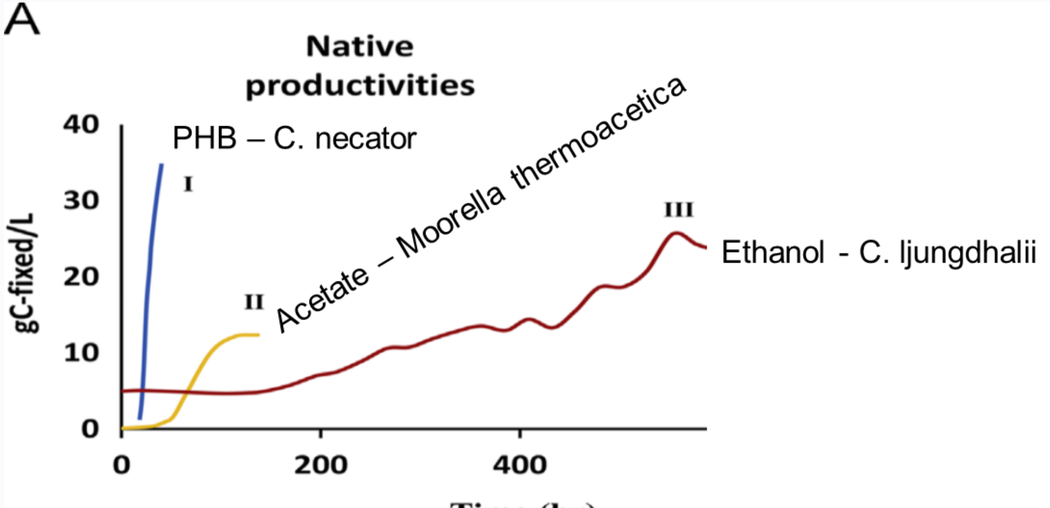
https://lanzatech.com/

however 90% of industrial emissions contain

- **CO_2 and not CO**
- **O_2**

and need to be completed with H_2

Other promising routes are <u>aerobic</u> autotrophic organisms

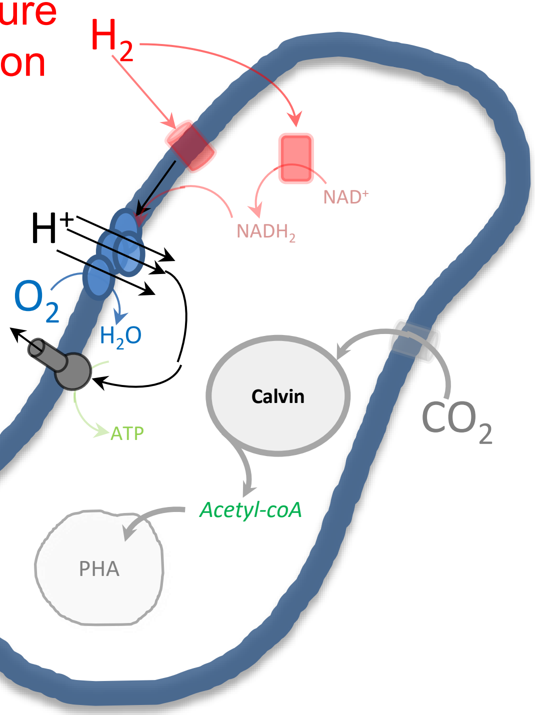


Nybo et al. Metabolic Engineering 30 (2015) 105–120

Autotrophic growth : Gas requirements ($H_2/O_2/CO_2$)

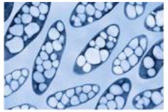
Soluble and membrane hydrogenases
Among the most O_2 tolerant in the nature
but still inhibited by low O_2 concentration

Aerobic respiration

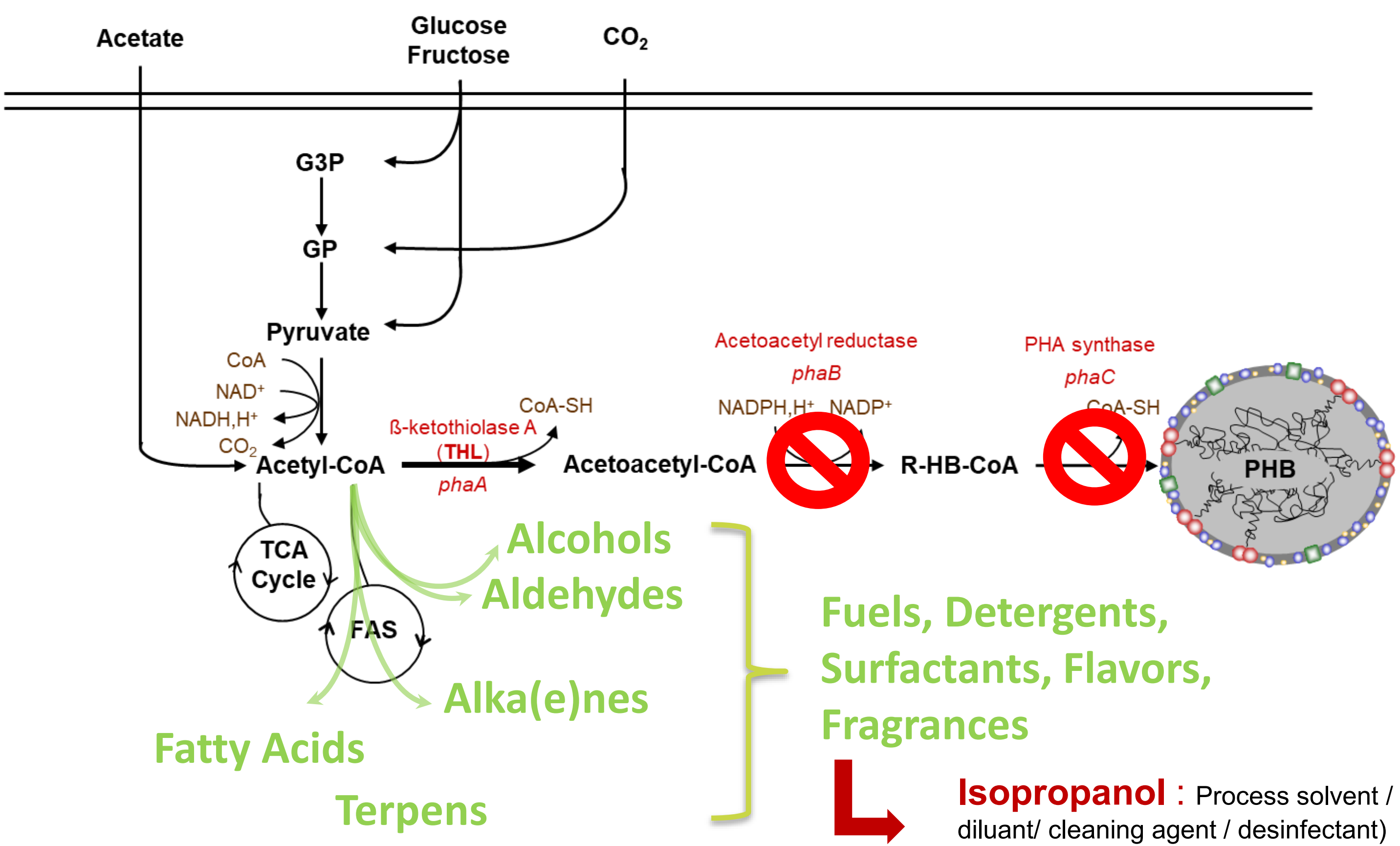


Carbon fixation via the Calvin-Benson-Bassham cycle

Cupriavidus necator :
a CO_2-based platform for
- Bioplastics
- SC Proteins
- Chemicals


Grousseau *et al.*, 2013 Bioresource technology

Cupriavidus necator as a chemical platform from CO_2



Isopropanol : Process solvent / Chemical intermediates / diluant/ cleaning agent / desinfectant)

Market size : 2,94 billion (2021) / 4,44 billion (2030)

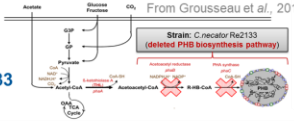
Iterative Approach : Strain Engineering ⟺ Biochemical Engineering

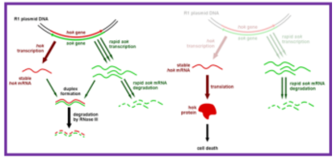
Strain Engineering for expression stability

Strain

C.necator **Re2133**

From Grousseau et *al.*, 2014



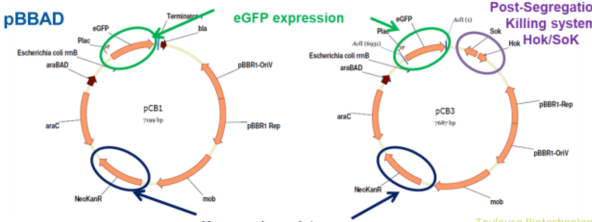


Plasmid

Backbone **pBBAD**

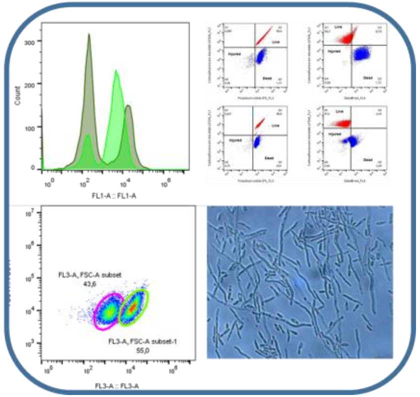
eGFP expression

Post-Segregational Killing system Hok/SoK



Kanamycin resistance

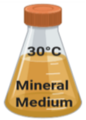
Toulouse Biotechnology



Sub-Population distribution

Fermentation Engineering

In flasks



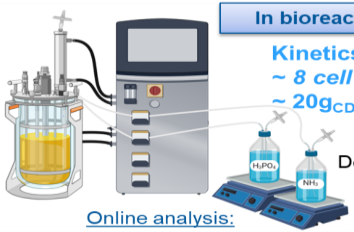
Kinetics during 10-12h
~ 2 cell generations
~ 1g_{CDW}/L

Mineral Medium A
Described in *Lu*, 2013

Offline analysis

- Optical density
- Flow Cytometry
- Multiplate reader
- HPLC

In bioreactors



Kinetics during 30-50h
~ 8 cell generations
~ 20g_{CDW}/L

Mineral Medium B
Described in *Crepin*, 2016

Online analysis:

- pH = 7
- pO2 >20% (aerobic conditions)
- Stirring
- Airflow
- Temperature = 30°C

Offline analysis

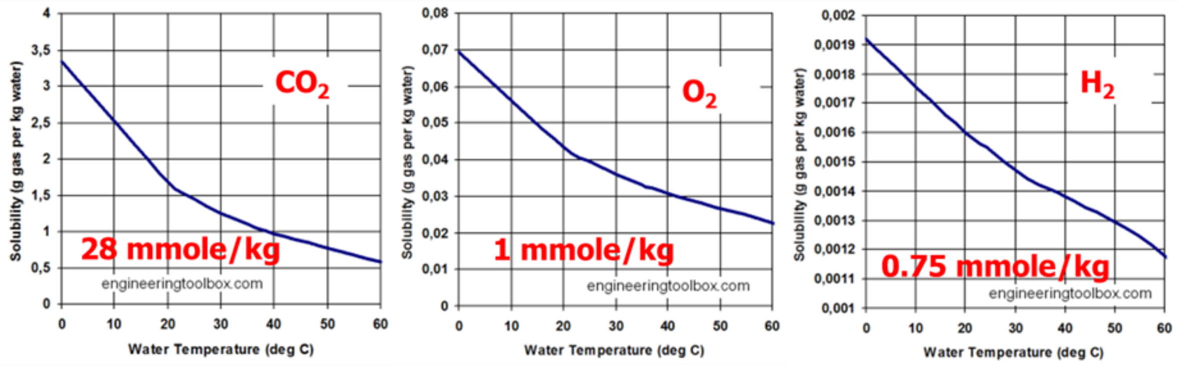
- Optical density
- Flow Cytometry
- Multiplate reader
- HPLC
- Plate count

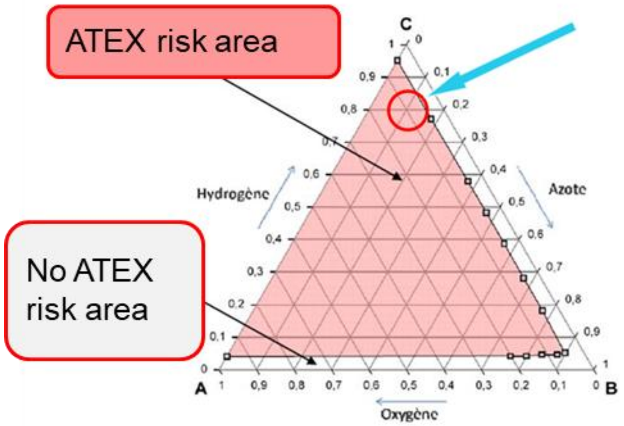
Gas supply management ($H_2/O_2/CO_2$)

The optimal gas stoichiometry (8:1:1 $H_2:CO_2:O_2$) is within the explosive range for the molar fractions of H_2 and O_2

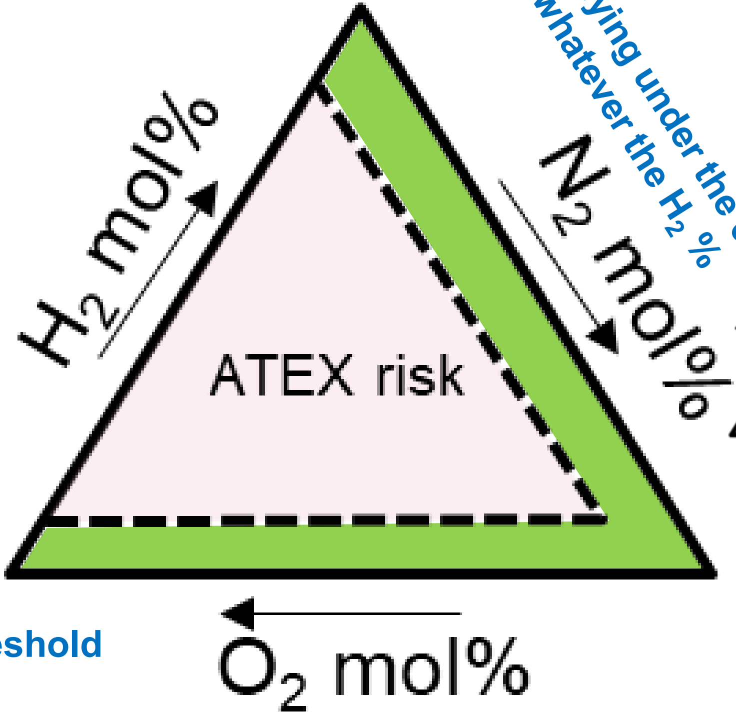
Optimal gas transfer to the microbial cell suspension
Low solubility of H_2 and O_2

Solubility of pure gases (at 1 atm)

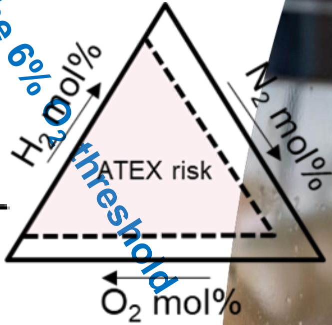




Gas supply management ($H_2/O_2/CO_2$)
Flying around the « Bermuda triangle »



Flying under the 6% O_2 threshold
whatever the H_2 %

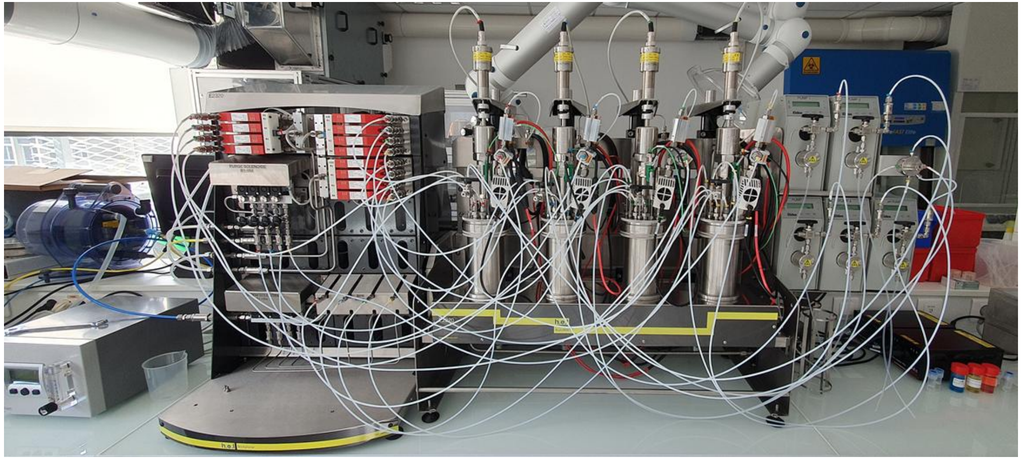


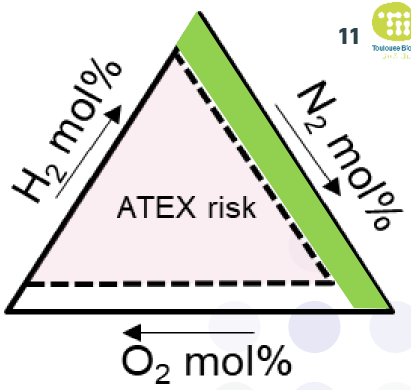
Flying under the 4% H_2 threshold
whatever the O_2 %

Gas supply management (H_2/Air/CO_2)

Confidentiel

Bioreactor design





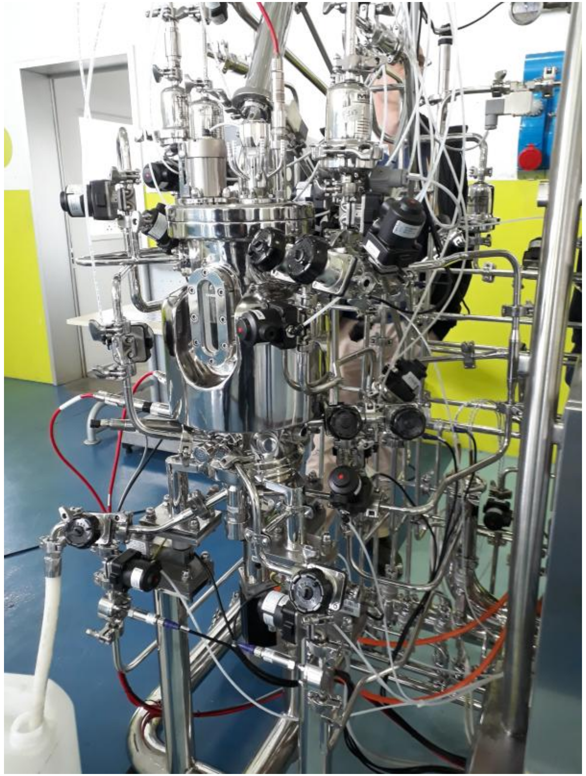
Gases supply by 3 independent spargers

- Air
- H_2
- CO_2

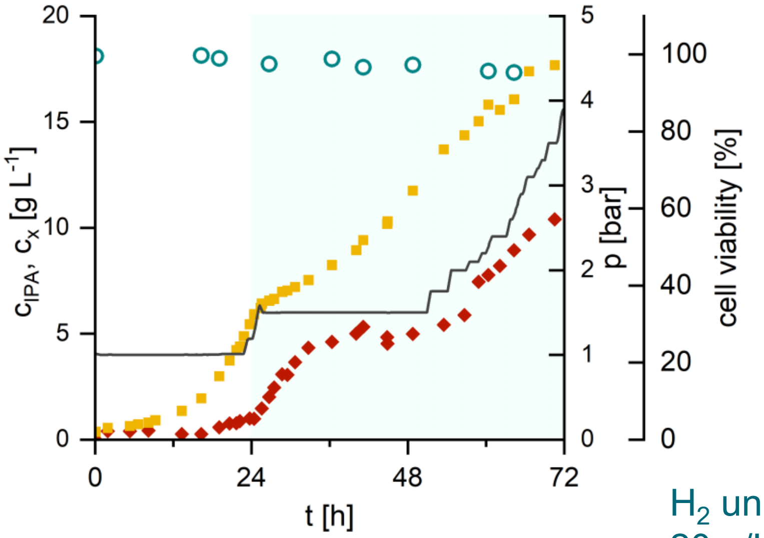
High pressure control system

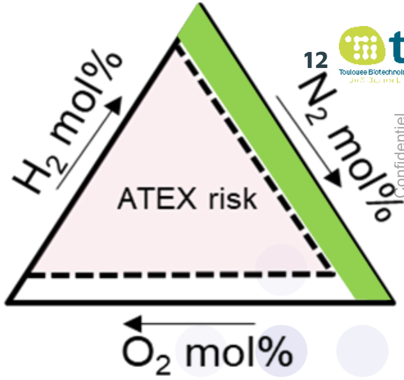
Gas feed control system to avoid explosive environment by controlling O_2% below 6%

Gas supply management ($H_2/O_2/CO_2$)



C. necator Re2133/pEG7c



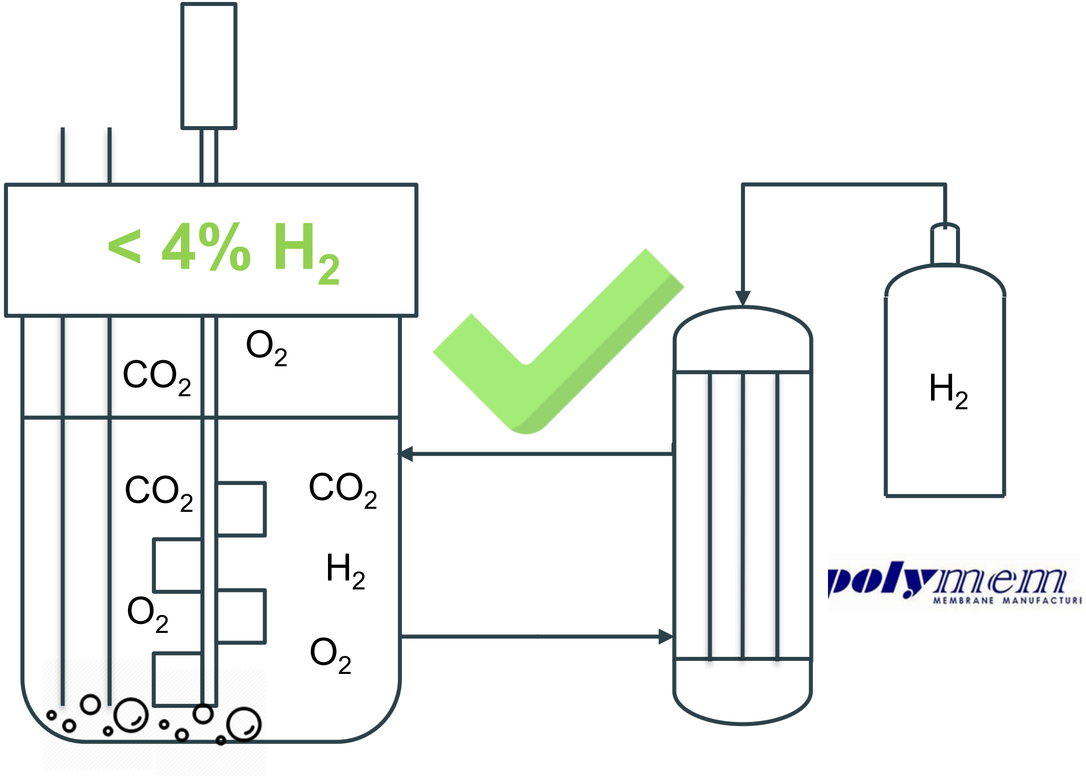


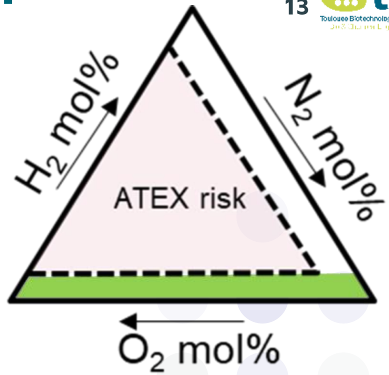
H_2 unlimited growth
20 g/L Biomass, μ_{max} = 0,13 h^{-1}
%H_2 Headspace high

11 g/L IPA – 1.5 g/L Acetone

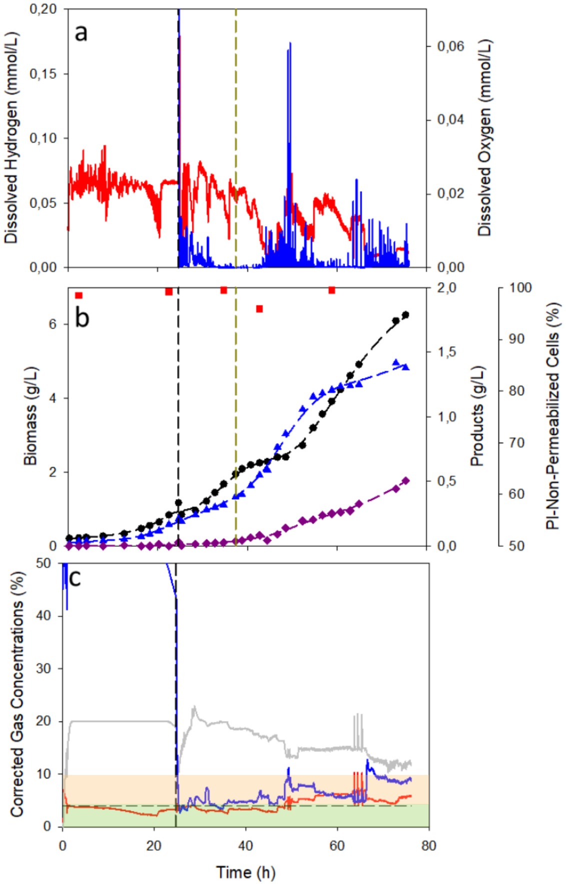
Weickardt et al., 2023 unpublished data

Gas supply management through Membrane Contactor





Gas supply management through Membrane Contactor

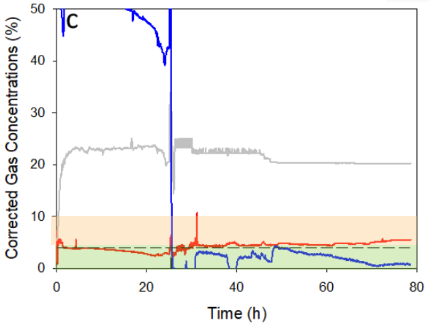


H_2 unlimited growth

2,5 g/L Biomass

1,4 g/L IPA – 0.05 g/L Acetone

Low H_2 atmosphere : 1-10%



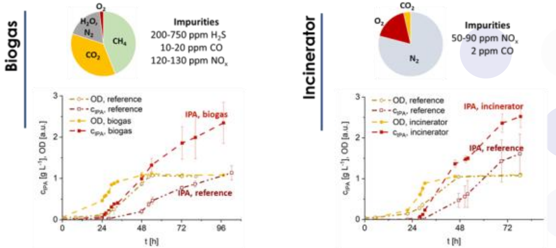
DiBisceglie et al., 2026 Biores. Technol.

Take home messages

- ✓ **Bioreactor design and fermentation strategy allow to reach cell densities of 20-30 gL^{-1} from CO_2 under non-explosive atmosphere AND no-gas limitation**

- ✓ **Metabolic engineering development in *C. necator* allows to produce various molecules (alcohols, alkanes, terpenes, …) from CO_2 (> 11 $g\ L^{-1}$ IPA)**

- ✓ **Remaining challenges in terms of**
  - ✓ **Operating with real CO_2-rich industrial effluents**
  - ✓ **Strain robustness for robust process**



Industrial biogas: 40 % supplemented with 50 % H_2, 10 % O_2
Synthetic biogas: 16 % CH_4, 12 % CO_2, 42 % H_2, 10 % O_2

Incinerator offgas: 70 % supplemented with 30 % H_2
Synthetic gas: 56 % N_2, 4 % CO_2, 30 % H_2, 10 % O_2

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Collaborators

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Dr R. Kratzer (TUG)
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Dr S. Schmidt (UG)

Pr D. Holtmann (KIT)
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