BioRECO₂VER

8th Conference on CO_2 as Feedstock for Fuels, Chemistry and Polymers

Heleen De Wever and project partners, 24 March 2020

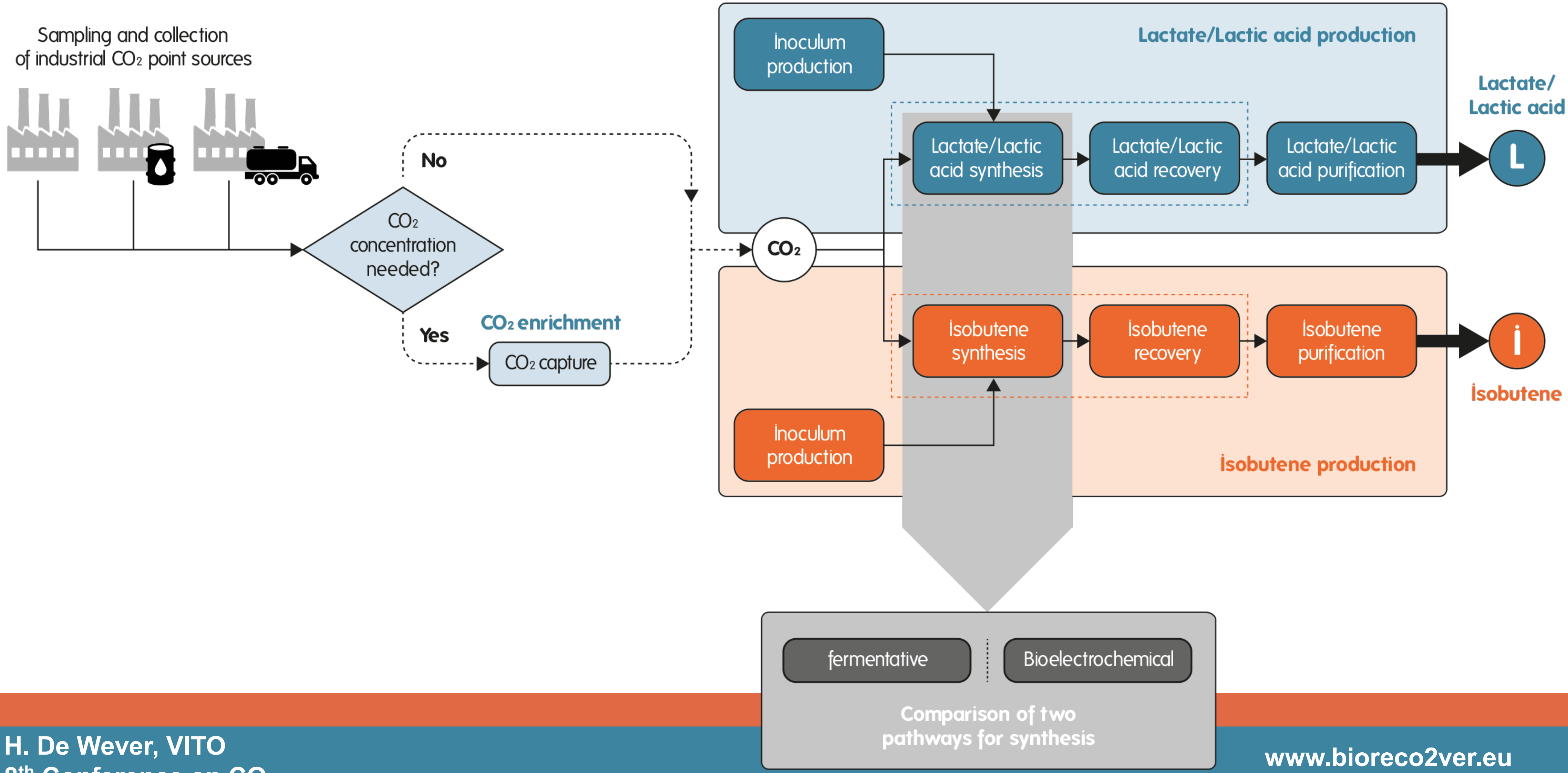
Horizon 2020
European Union Funding
for Research & Innovation

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 760431.

General information

Title	Biological routes for CO_2 conversion into chemical building blocks
Acronym	BioRECO$_2$VER
Work program topic	BIOTEC-5-2017: Microbial platforms for CO_2-reuse processes
Type of action	Research and Innovation Action
Start date	1st January 2018
End date	31th December 2021
EU budget	€ 6,812,187.50
Coordinator	VITO

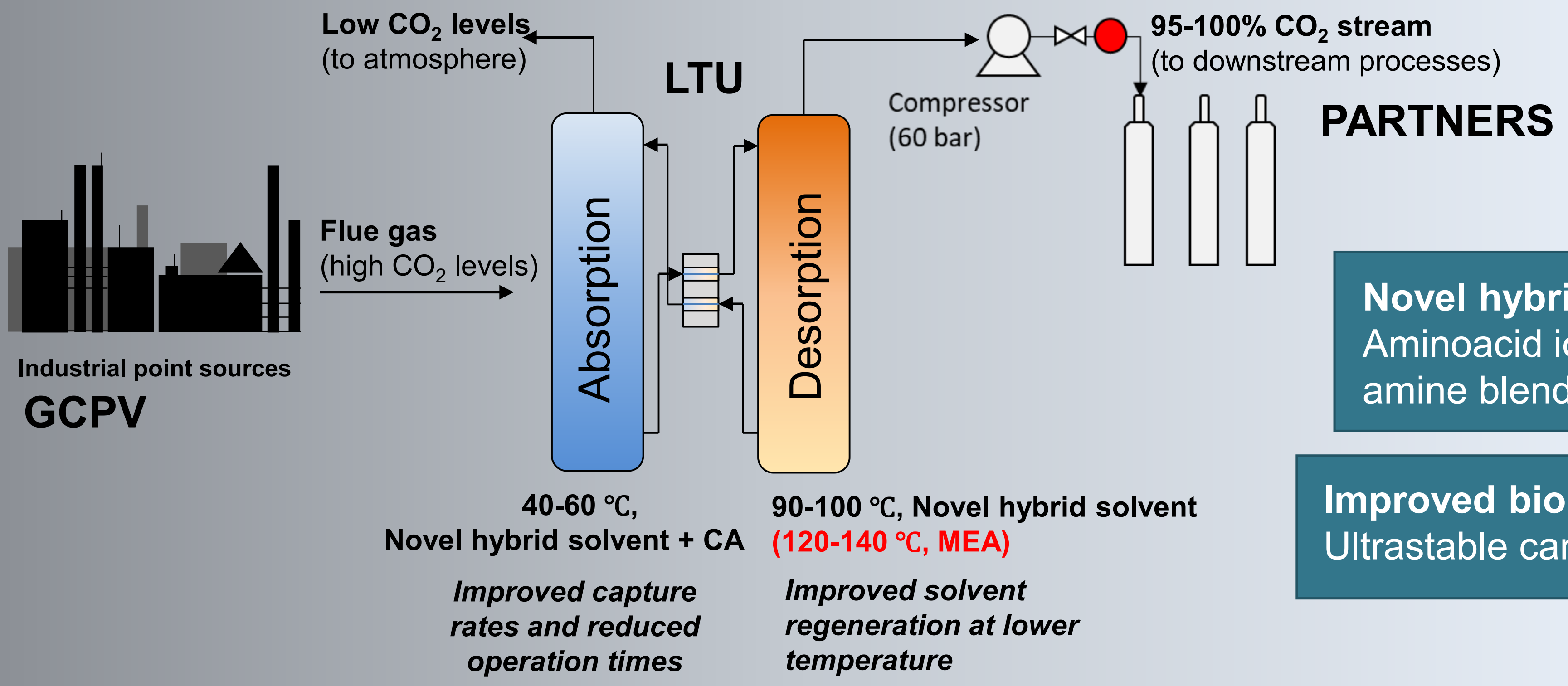
Overall project concept



Project Consortium

- 4 Industries
- 4 SMEs
- 2 universities
- 2 RTOs

CO_2 capture and pretreatment - An overview



Low CO_2 levels
(to atmosphere)

LTU

Compressor
(60 bar)

95-100% CO_2 stream
(to downstream processes)

PARTNERS

Flue gas
(high CO_2 levels)

Absorption

Desorption

Industrial point sources

GCPV

40-60 ℃,
Novel hybrid solvent + CA

Improved capture rates and reduced operation times

90-100 ℃, Novel hybrid solvent
(120-140 ℃, MEA)

Improved solvent regeneration at lower temperature

Novel hybrid solvent:
Aminoacid ionic liquid (AAIL): amine blend

Improved biocatalyst:
Ultrastable carbonic anhydrase (CA)

$$(R_1R_2R_3)N + AAIL + CO_2 \overset{CA}{\rightleftarrows} (R_1R_2R_3)NH^+ + AAIL\text{-carbamate} + HCO_3^-$$

Enzyme improvement by directed evolution

Library construction:

1000 epCA variants generated with error-prone PCR

Library screening:

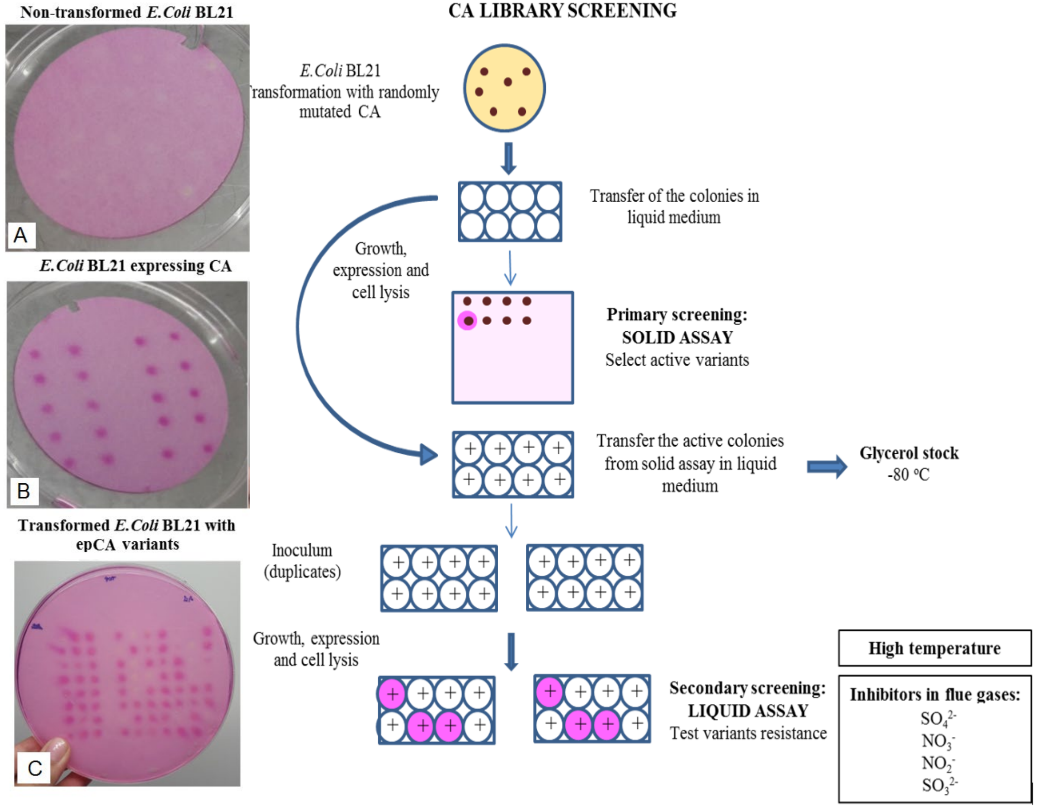
- Primary screening to select active variants

- Secondary screening to select variants with resistance to inhibitors

Scaled-up production of most promising variants

Sequencing for identification of mutations

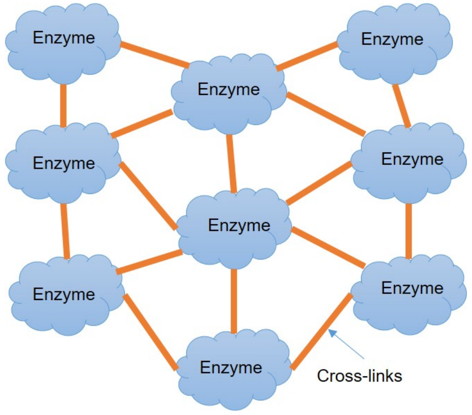
- **3 mutants showed 50% increased resistance to flue gas inhibitors**



Enzyme improvement by immobilization

Applied techniques for CA immobilization:

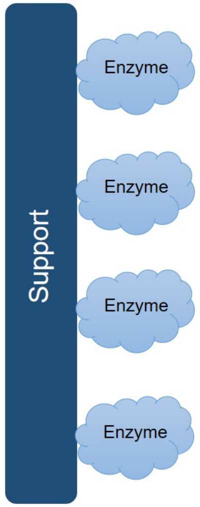
Cross-linking



>95% residual activity employing a novel crosslinker

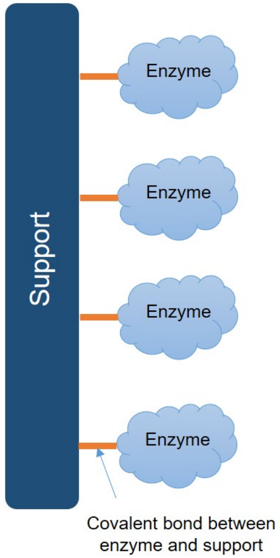
High thermostability employing stabilizing agents

Adsorption



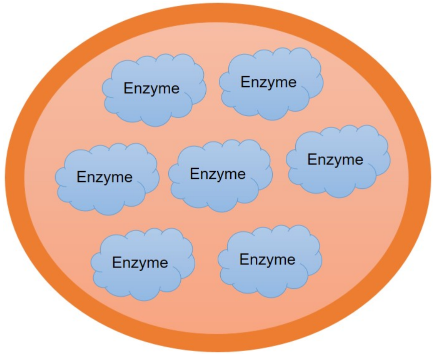
>80%
residual activity

Covalent bonding



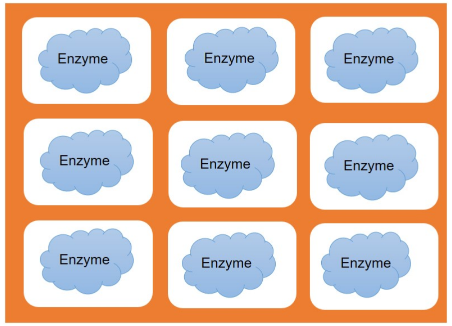
>30%
residual activity

Encapsulation



>45%
residual activity

Entrapment



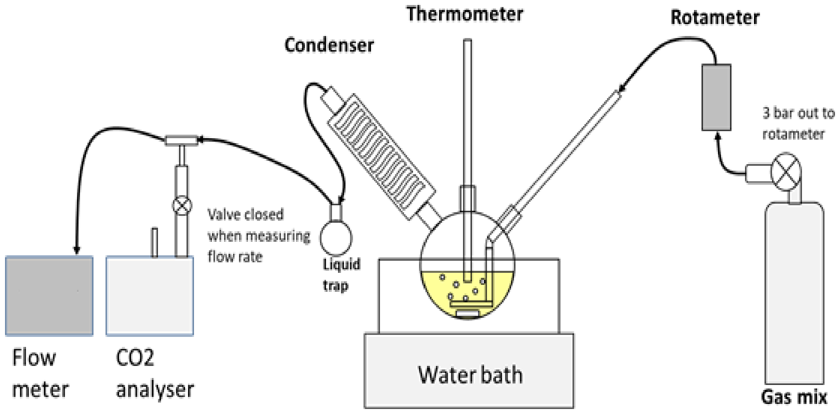
>50%
residual activity

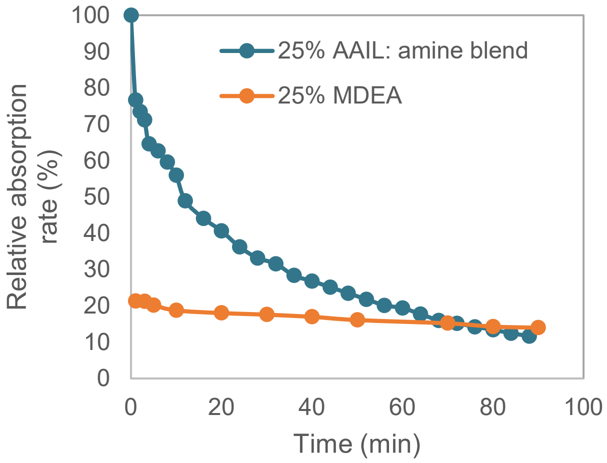
High thermostability due to protection by the support

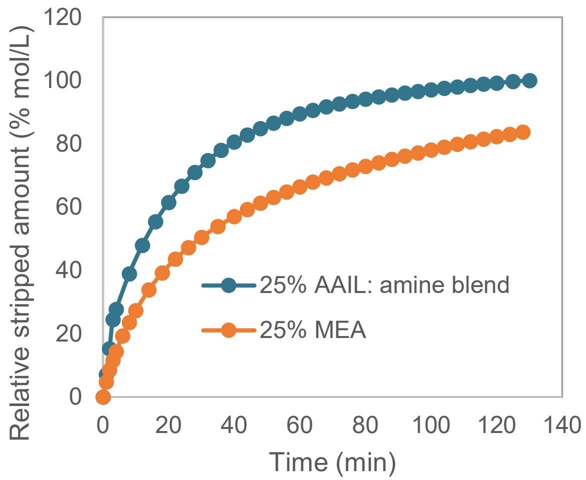
Development of a solvent with competitive absorption and desorption properties

Screening of different AAIL:tertiary amine blends in small scale resulted in selection of solvent with:

- **5-fold higher initial rate**
- **2-fold higher CO_2 load compared to MDEA**
- **2-fold higher regeneration at 80°C**
- **>15% reduction in desorption T compared to MEA**







MDEA: Methyl diethanolamine; MEA: Monoethanolamine

CO_2 capture in large-scale packed bed absorption equipment

Scaled-up trials revealed even higher benefit with use of the developed solvent:

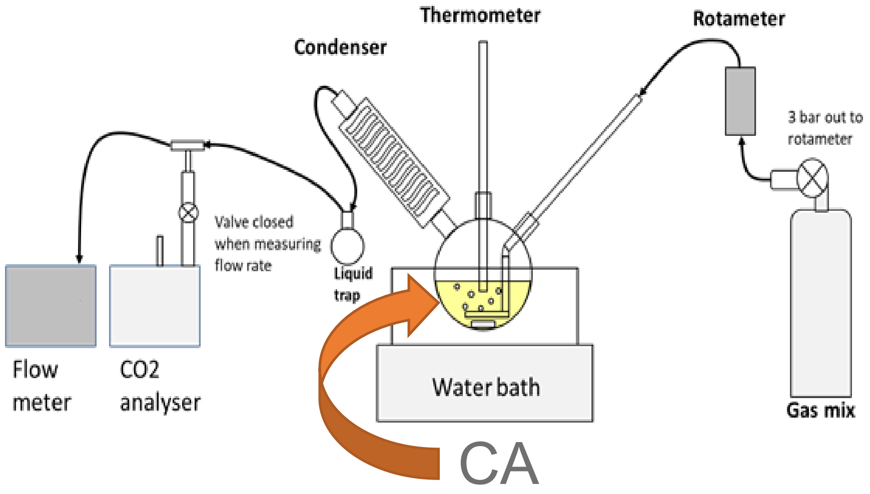
Solvent	Relative K_Ga (%)
25% MEA	100
25% MDEA	3.07
25% AAIL: amine blend	30.86

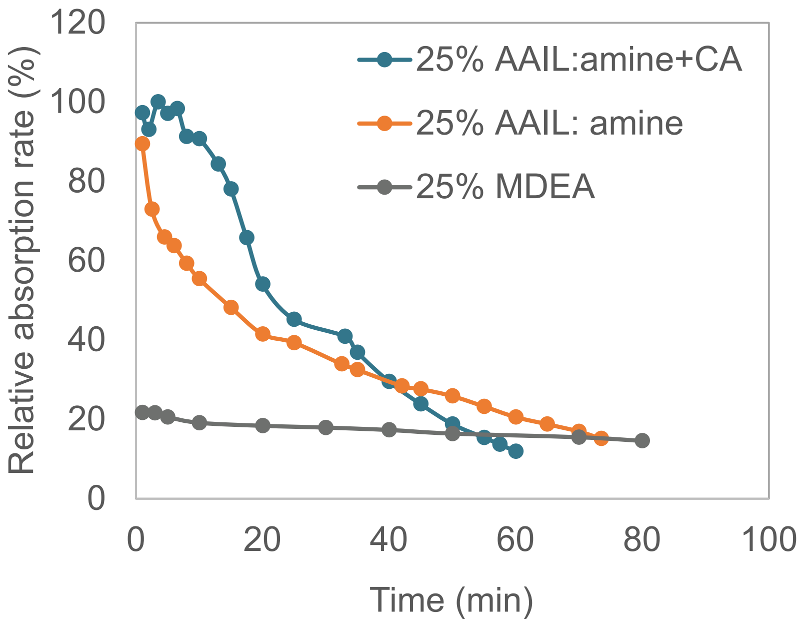
- **10-fold increase in mass transfer coefficient (K_GA) compared to MDEA**

1m (80mm ID) packed column (Raschig rings)

MDEA: Methyl diethanolamine; MEA: Monoethanolamine

Integration of CA with novel hybrid solvent for efficient CO_2 capture





- **Reduced operation times by 25%**
- **32% increase in captured CO_2 compared to the non-enzymatic reaction**

MDEA: Methyl diethanolamine

CO_2 capture and pretreatment

Conclusions:

- An ultrastable CA was improved by protein engineering and immobilization for increasing stability towards harsh and high temperature environment
- An enzyme compatible novel hybrid solvent was developed with competitive absorption and desorption properties

Next steps:

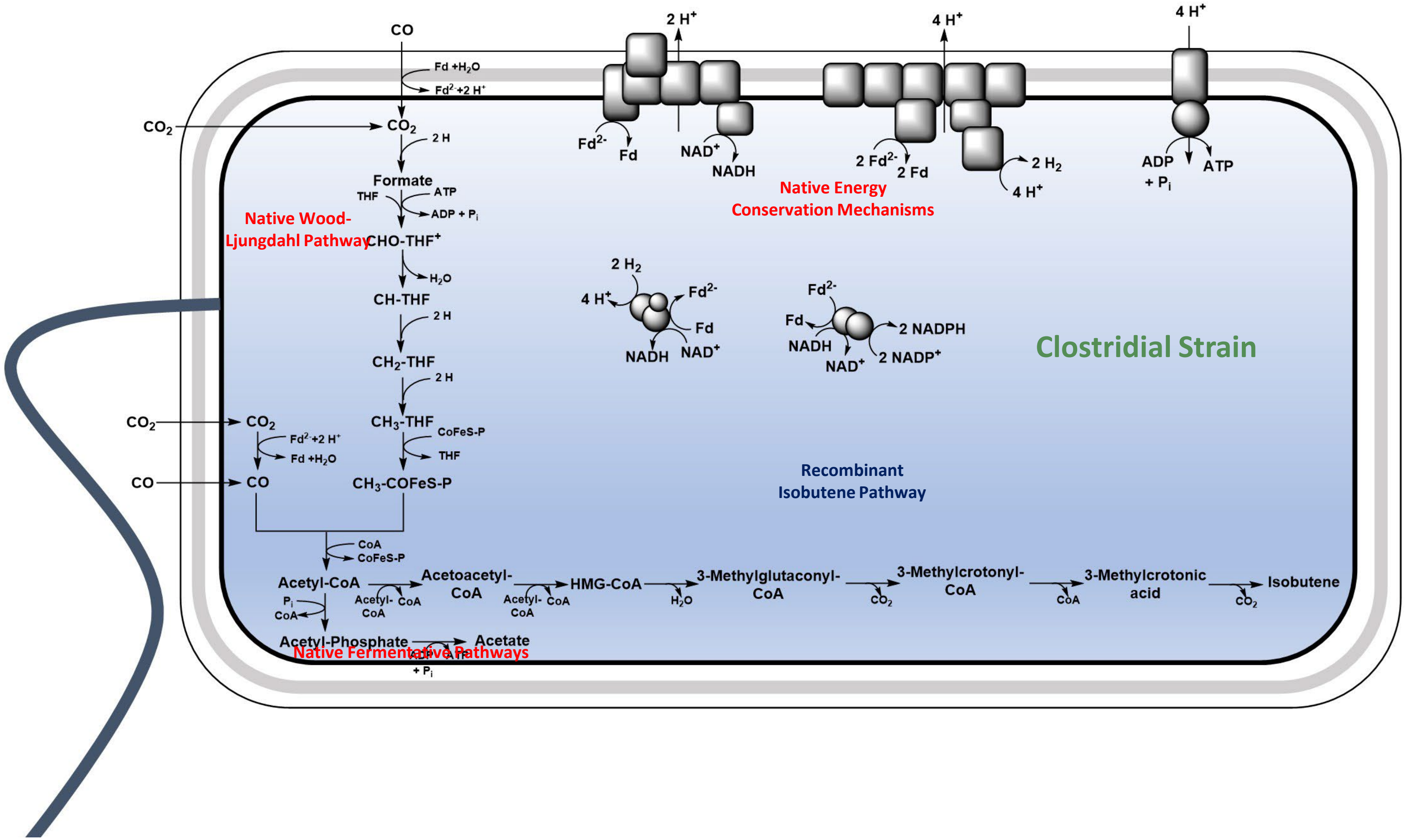
- Integration of ***immobilized improved epCA variants*** with the developed ***novel hybrid solvent*** for ***large scale absorption*** using ***flue gas*** resulting in a concentrated gas stream of at ***least 95% CO_2***

Microbial CO_2 conversion

- 3 microbial platforms

Microbial platforms		T range	O_2 tolerance	Target product	Partner
Autotrophic	Clostridial strain	Mesophilic	Anaerobic	Isobutene	Global Bioenergies
	Cupriavidus necator	Mesophilic	Aerobic	Lactate	Enobraq
Capnophilic	*Thermotoga neapolitana*	Hyper-thermophilic	Strictly anaerobic	Lactate + H_2	Consiglio Nazionale delle Ricerche

Wood-Ljungdahl and Isobutene Pathway

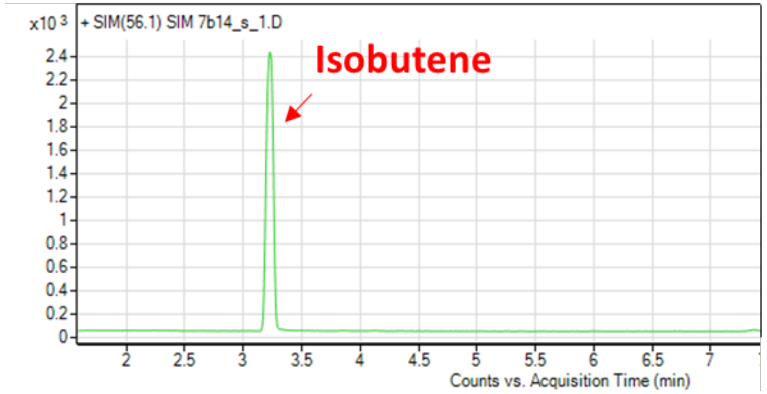


- WLP is the most efficient carbon fixation pathway
- Isobutene is derived from central metabolic precursor acetyl-CoA
- Isobutene pathway is Redox-neutral and does not require directly ATP

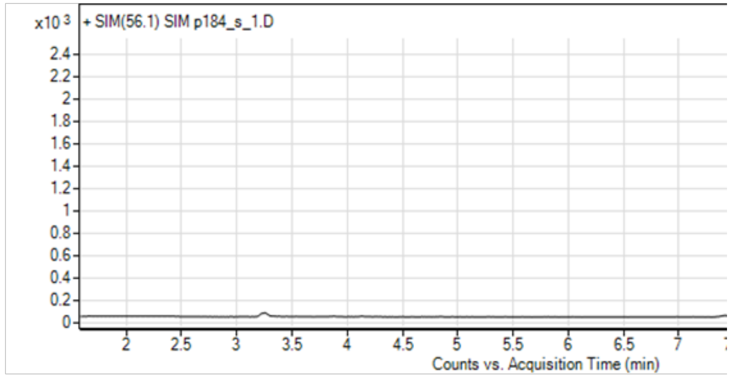
Isobutene production under autotrophic conditions

- Successful implementation of Isobutene pathway proven on protein level
- Isobutene production from CO, CO_2 and H_2 mixture and from CO_2/H_2 mixture
- Large improvement of production during the BioRECO$_2$VER project

With IBN Pathway



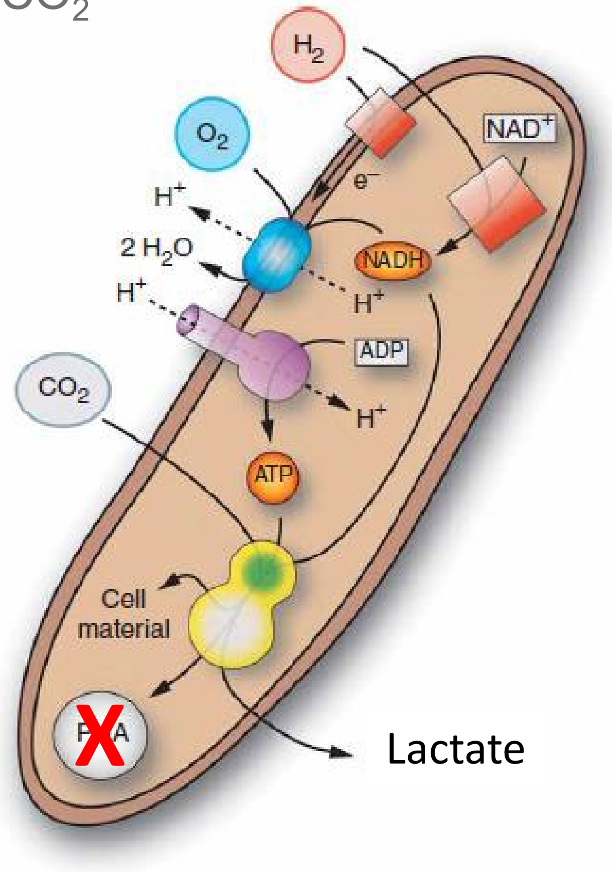
Without IBN Pathway



Aerobic lactate production

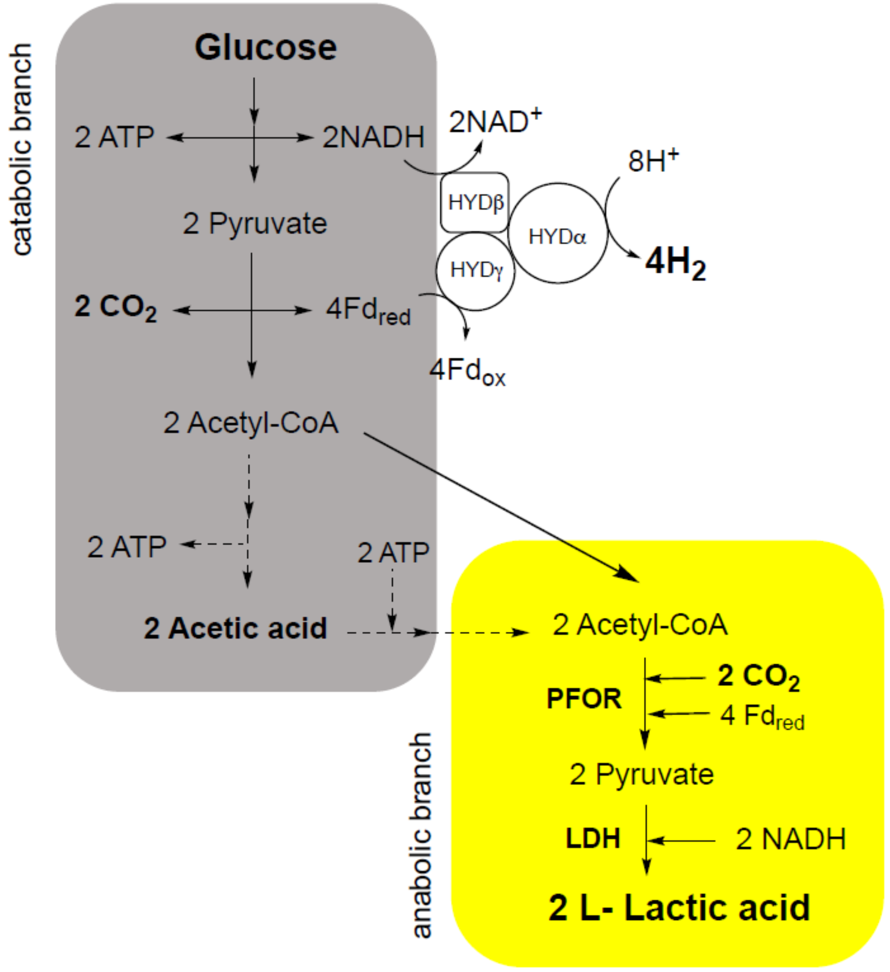
Metabolic engineering of *Cupriavidus necator* strain to improve lactate production from CO_2

1. **Improve lactate dehydrogenase (LDH) activity**
   - LDH activity increased by a factor 180
   - Specific lactate production rate increased by a factor 4
2. **Increase pyruvate availability by deletion of competitive pathways**
   - Different competitive pathways deletions evaluated
3. **Block lactate re-consumption**
   - Unexpected pathway identified by transcriptomic study
   - Lactate re-consumption issue solved by deletion work
   - Patent application filed



Lithoautotrophic metabolism

Capnophilic lactate production



catabolic branch

Glucose

2 ATP

2NADH

2 Pyruvate

$2\ CO_2$

$4Fd_{red}$

2 Acetyl-CoA

2 ATP

2 Acetic acid

$2NAD^+$

$8H^+$

HYDβ

HYDα

HYDγ

$4H_2$

$4Fd_{ox}$

2 ATP

anabolic branch

2 Acetyl-CoA

PFOR

$2\ CO_2$

$4\ Fd_{red}$

2 Pyruvate

LDH

2 NADH

2 L- Lactic acid

Capnophilic Lactic Fermentation (CLF) pathway:
Thermotoga neapolitana-based platform to gain value from CO_2 and waste by production of L-lactate & H_2

- Newly discovered pathway (ChemSusChem, 2014, 7, 2678-2683)
- Dissection of anabolic branch of CLF
- Increase metabolic flow from CO_2 & acetate to lactate
- Proof of concept net CO_2 fixation in lactic acid
- Explore feeding of exogenous acetate as C2 unit

Capnophilic lactate production

- Selection of two model strains with increased CLF productivity
    - ***T. neapolitana* subsp. *capnolactica*** (DSM33003) is a mutant of DSMZ 4359^T strain and has been generated in our laboratory under saturating concentrations of CO_2
    - **RQ7** is a *Thermotoga* sp. strain isolated from hot sea-floor Ribeira Quente (the Azores)

→ Complete genome sequences are available

ACETATE + **exogenous CO_2** → **L-LACTATE*** **(95% e.e.)**

228 mg L^{-1} | **167 mg L^{-1}** | **340 mg L^{-1}**

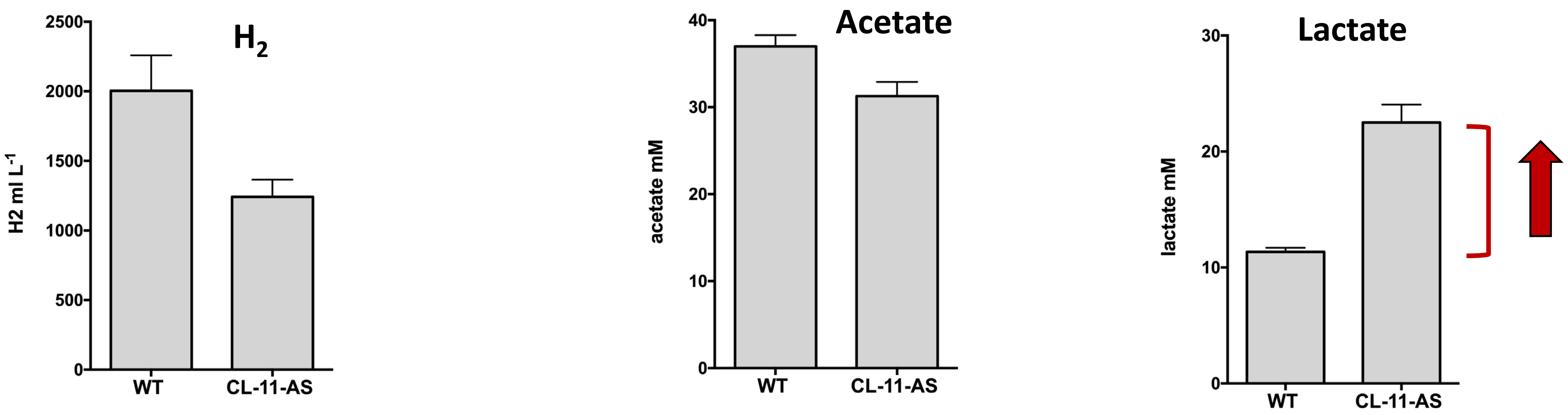
*100 % more than reference strain DSMZ 4359^T

Capnophilic lactate production

DSM33003

- heterologous expression AcCoA synthetase (*acs*) of *Thermus thermophilus* (**mutant CL-11-AS**)

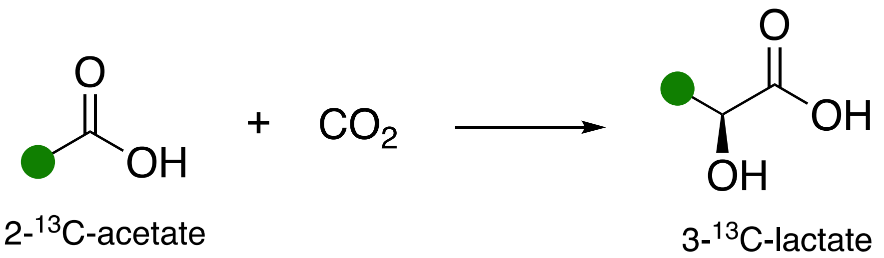
*CLF performances under standard medium **+ 20 mM acetate***



- Comparable growth curves
- Same glucose consumption rate

- Decrease H_2 production
- 100% increase lactate production

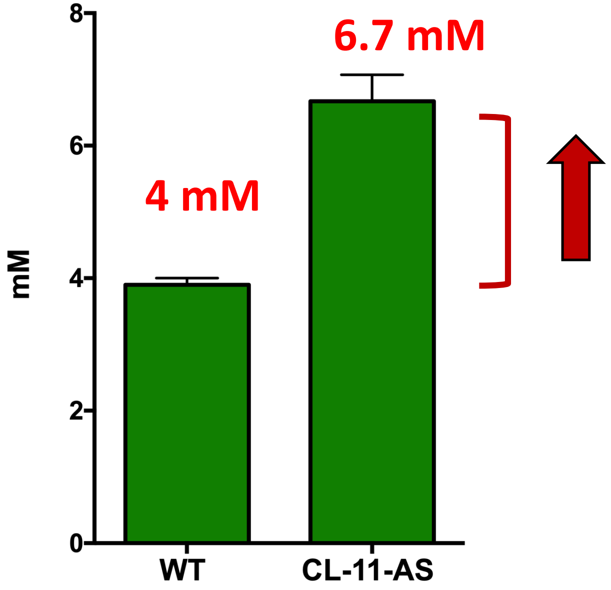
Capnophilic lactate production



2-^{13}C-acetate

3-^{13}C-lactate

3-^{13}C-Lactate



Increase of CO_2-derived lactate of 70%

UPTAKE

Wild type

CH_3CO_2Na +	CO_2	$\longrightarrow$	Sodium lactate
0.8 ± 0.020 g L^{-1}	**0.4 ± 0.01 g L^{-1}**		**1.1 ± 0.03 g L^{-1}**

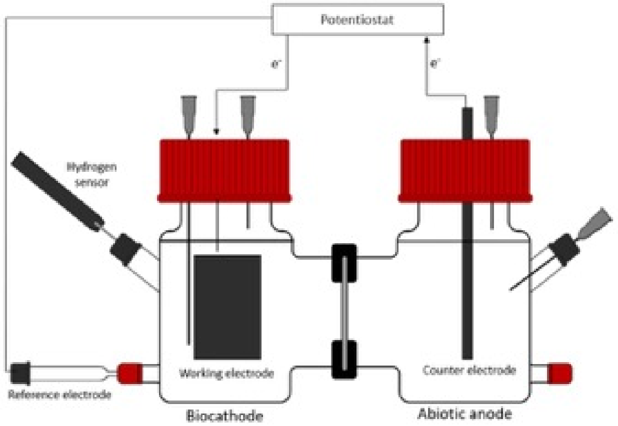
CL-11-AS

CH_3CO_2Na +	CO_2	$\longrightarrow$	Sodium lactate
1.4 ± 0.08 g L^{-1}	**0.7 ± 0.04 g L^{-1}**		**1.9 ± 0.1 g L^{-1}**

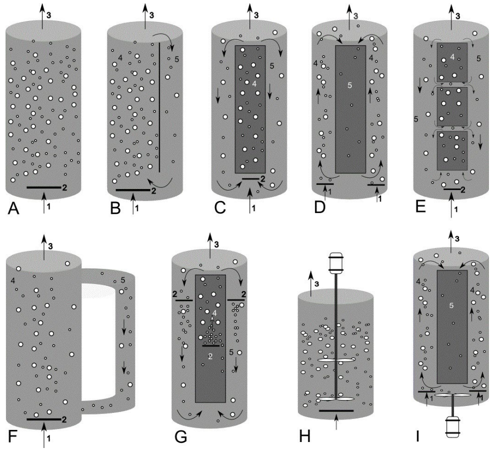
Microbial CO_2 conversion

- **2 technologies**

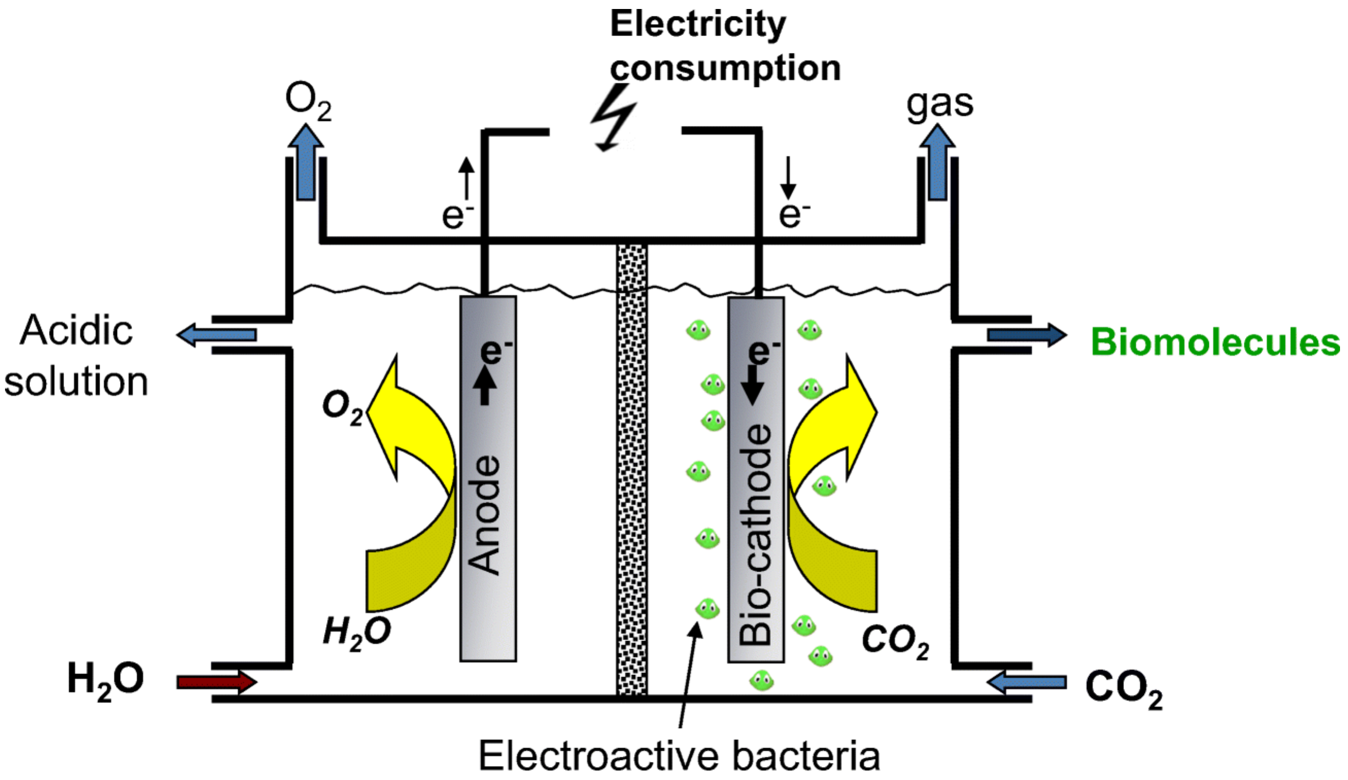
Bioelectrochemical systems



[High pressure] fermentors



Bioelectrochemical systems (BES)



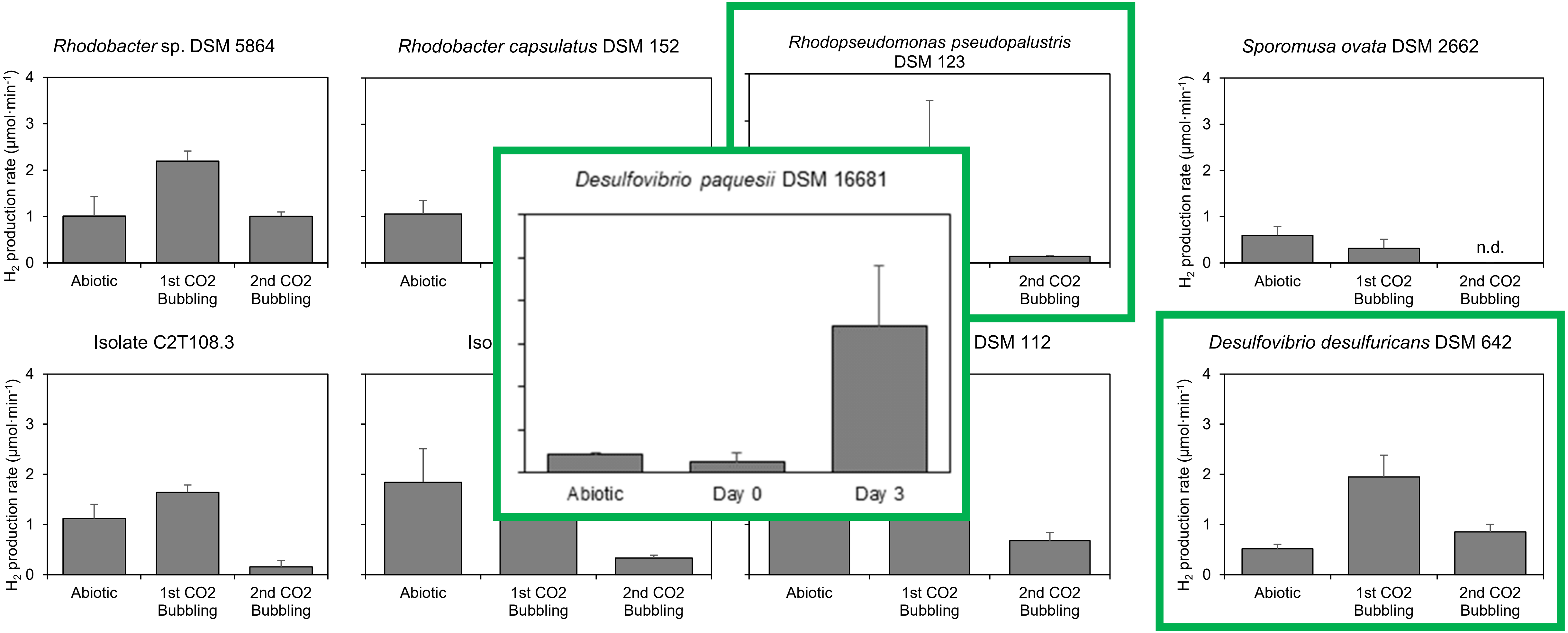
Cell configurations

***Clostridium* platform:**
Double chamber
- Anaerobic conditions

***Cupriavidus* platform:**
Single chamber
- Membraneless system
- Coexistence of O_2 and H_2

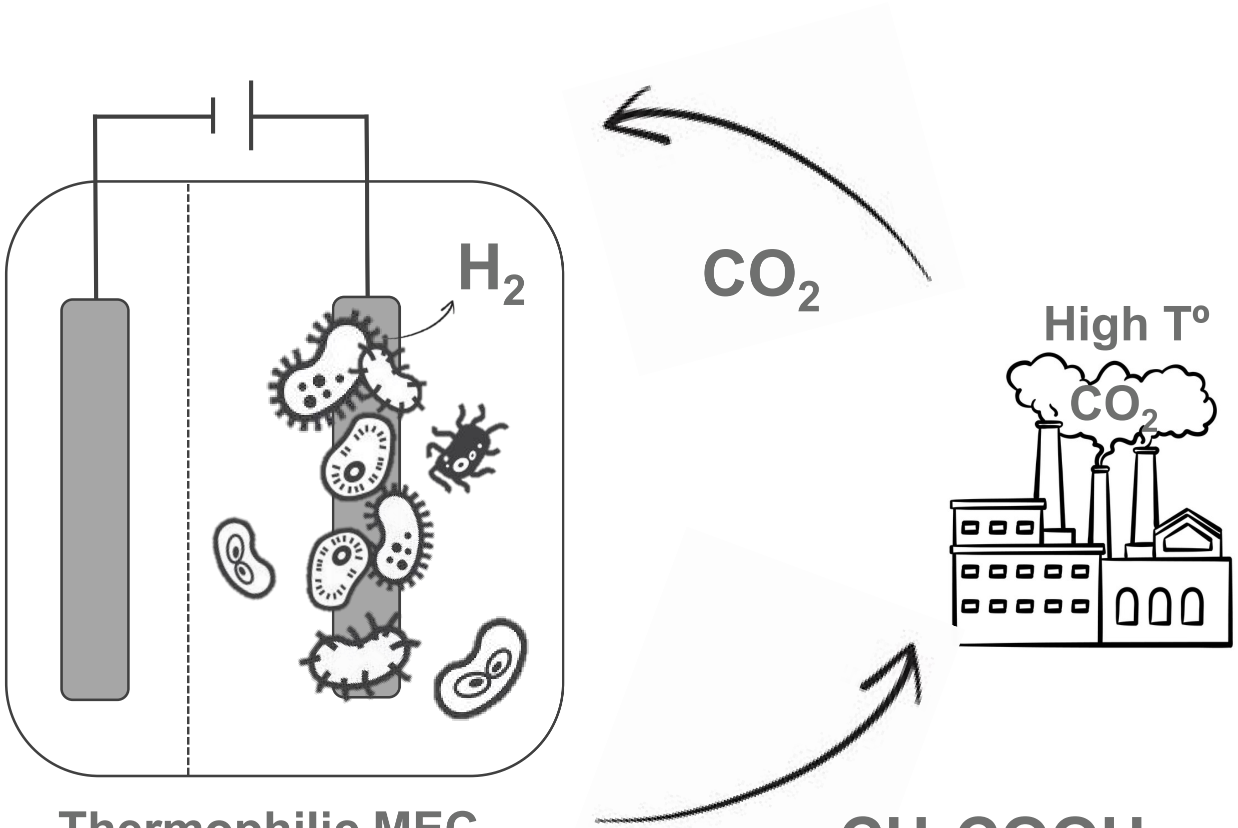
O_2 30ºC

Maximize *in situ* H_2 production



O_2 50ºC

Thermophilic process



H_2

CO_2

High Tº

CO_2

Thermophilic MEC

CH_3COOH, …

High Tº

- Higher reaction rates
- Less risk of contamination
- More product specificity

O_2 50ºC

Thermophilic process

Set-up of mild thermophilic systems

Chronology

Reactors 1 and 2 (280 d operation)

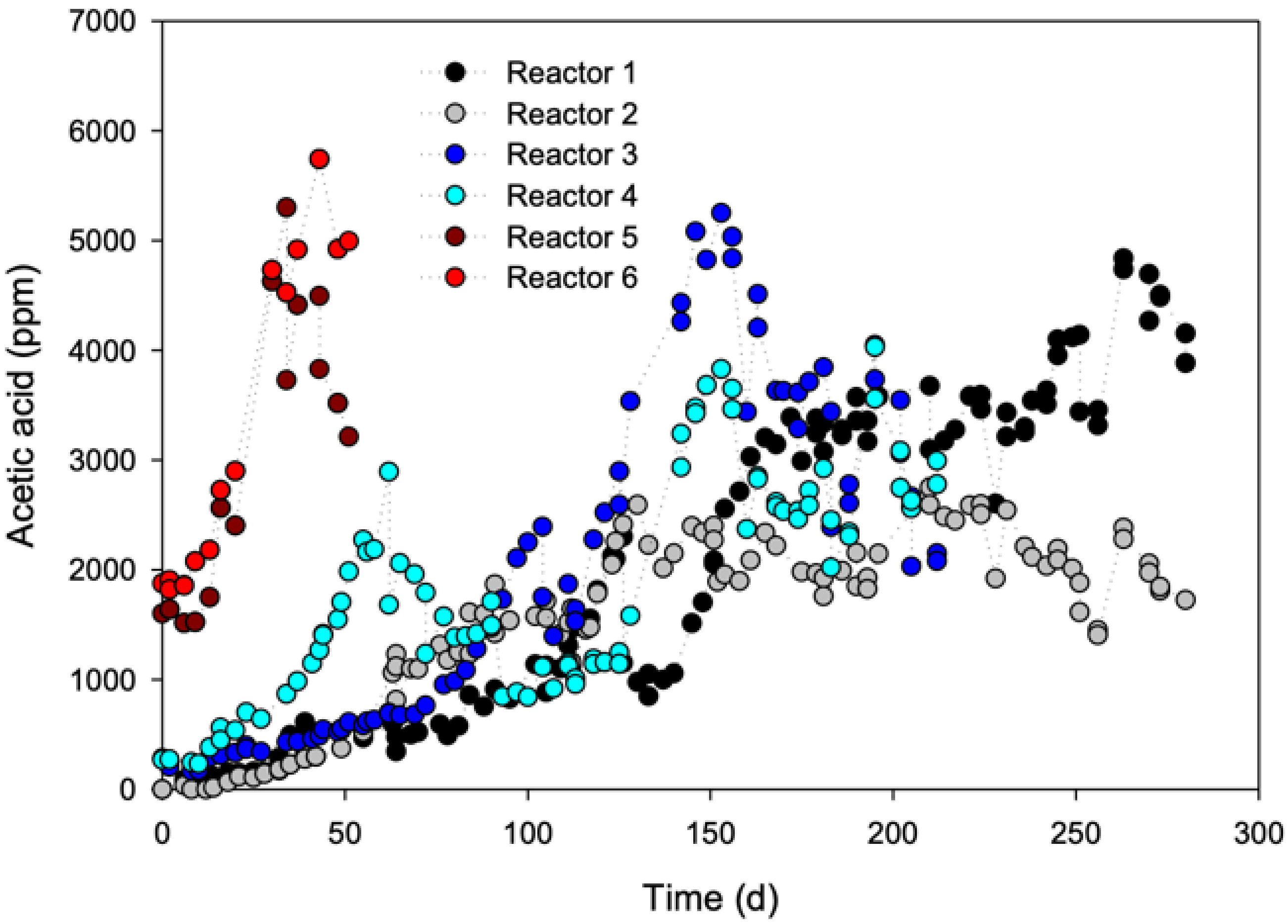
After 70 d, inoculation of

Reactors 3 and 4 (210 d operation)

After 160 d, inoculation of

Reactors 5 and 6 (50 d operation)

- Max production rate: 28 g acetate m^{-2} d^{-1}
- Coulombic efficiency: 80-90%



Aerobic process

- *Cupriavidus* lactate fermentation in a single chamber BES
  - 2-stage process
  - Expected stoichiometries

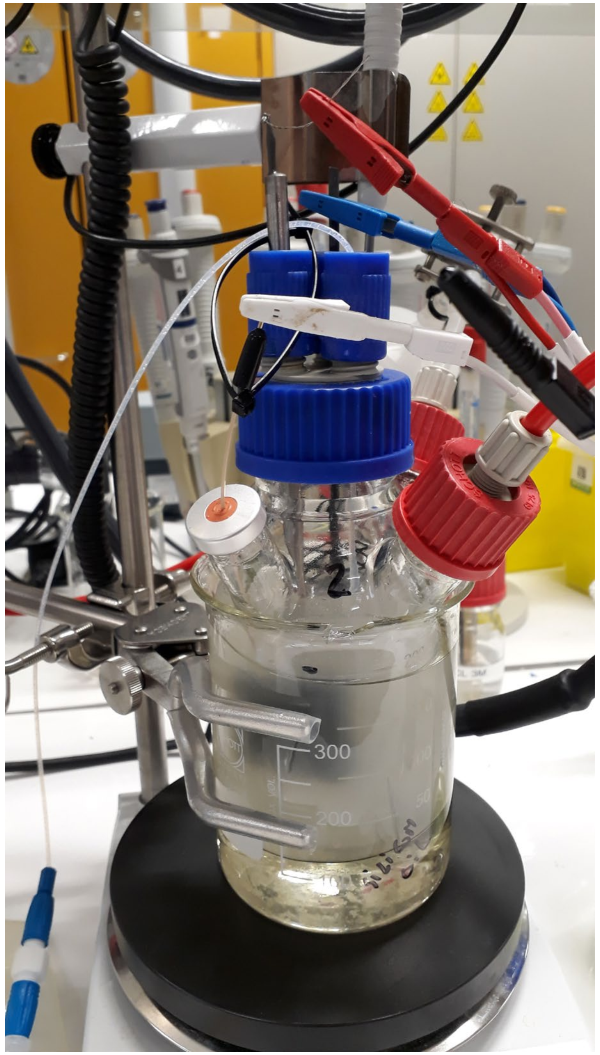
Biomass production *Cupriavidus*

$$4{,}09\ CO_2 + 6{,}21\ O_2 + 21{,}36\ H_2 + 0{,}76\ NH_4^+ \rightarrow C_{4,09}H_{17}O_{1,89}N_{0,76} + 18{,}7\ H_2O + 0{,}76\ H^+$$

Gas mix $CO_2/O_2/H_2$ = 16/20/64 (literature)

Lactate production

$$3\ CO_2 + 3.5\ O_2 + 13\ H_2 \rightarrow C_3H_5O_3^- + 10\ H_2O + H^+$$



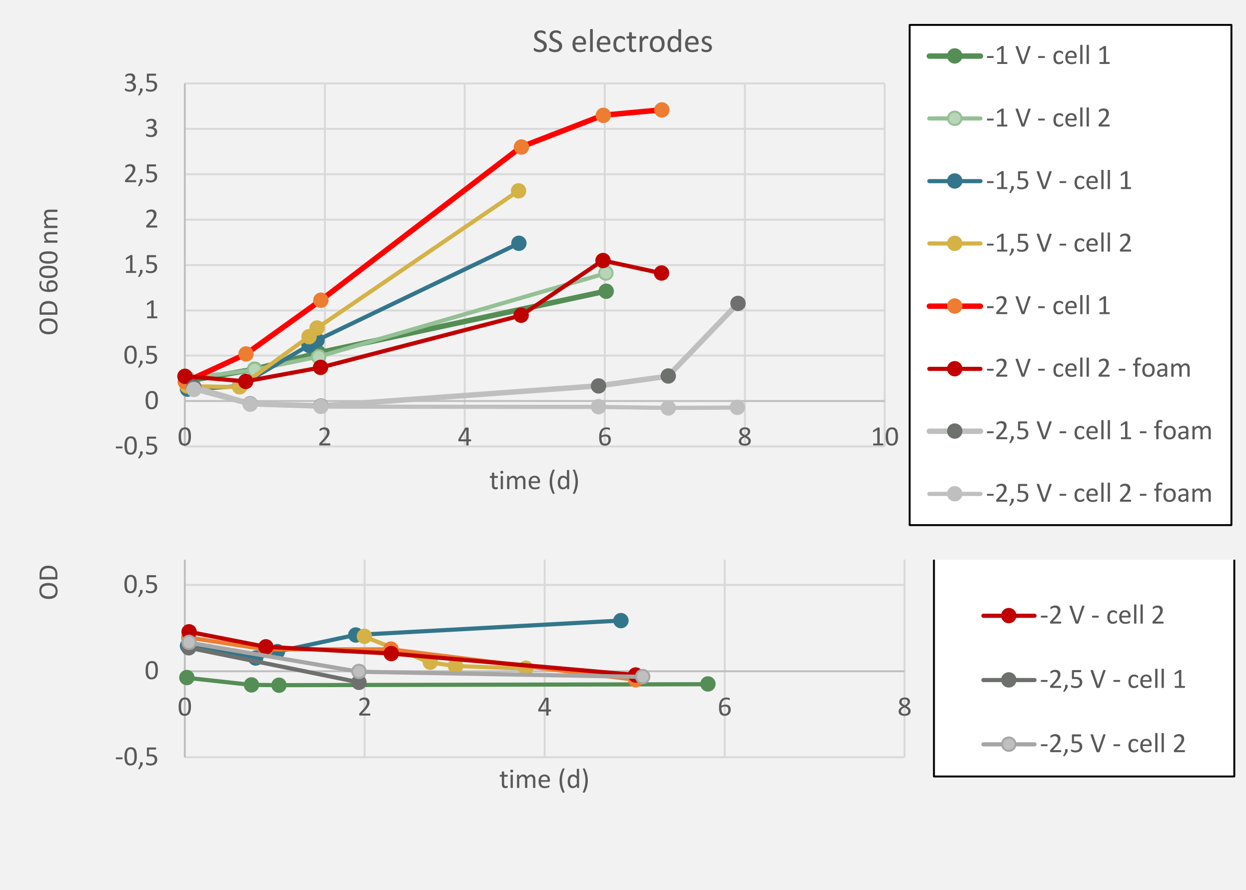
Aerobic process

- Growth of wild type *Cupriavidus* strain
  - Stainless steel electrodes result in OD increase while graphite ones do not

    (SS: higher H_2 evolution)

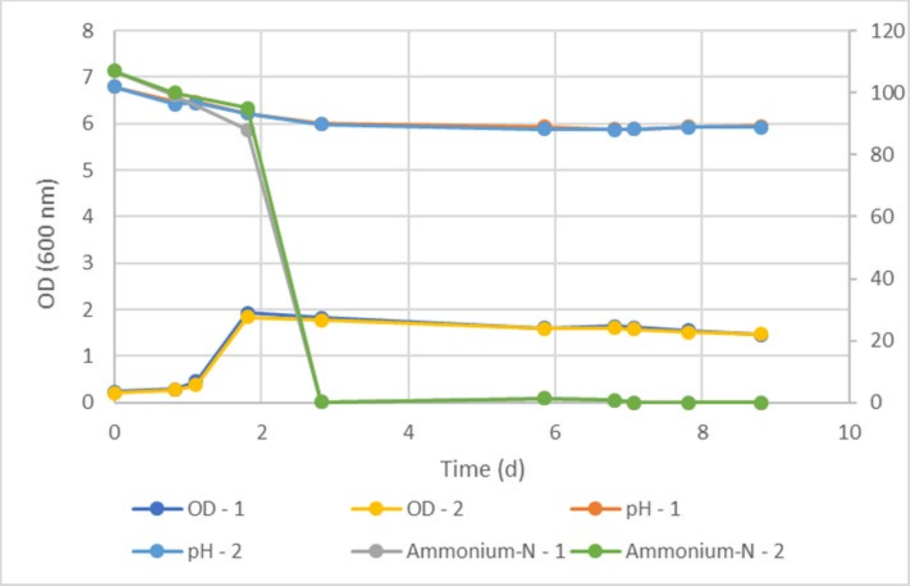
    (Graphite: sorption - peroxide)

  - Too negative voltage results in slower growth
    (foam formation)



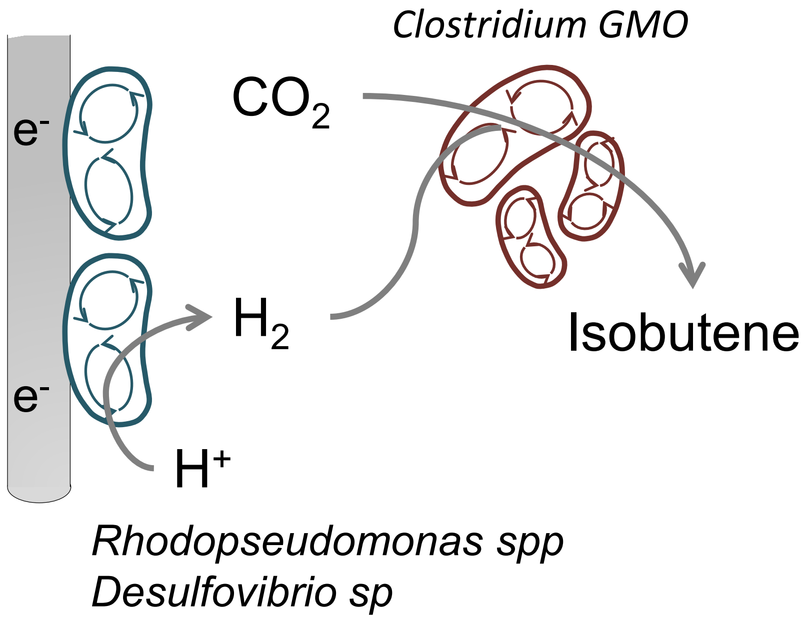
Aerobic process

- Tests with modified *Cupriavidus* strain
  - Conditions
    - Stainless steel electrodes
    - Avoid O_2 inhibition enzymes
    - Apply different voltages
    - Test different electrode surface/liquid ratio
  - Conclusions
    - Faster growth in reactors with higher electrode surface area
    - Fixed ratio O_2/H_2 from water electrolysis has risk O_2 inhibition or H_2 limitations
    - Headspace composition to be optimized

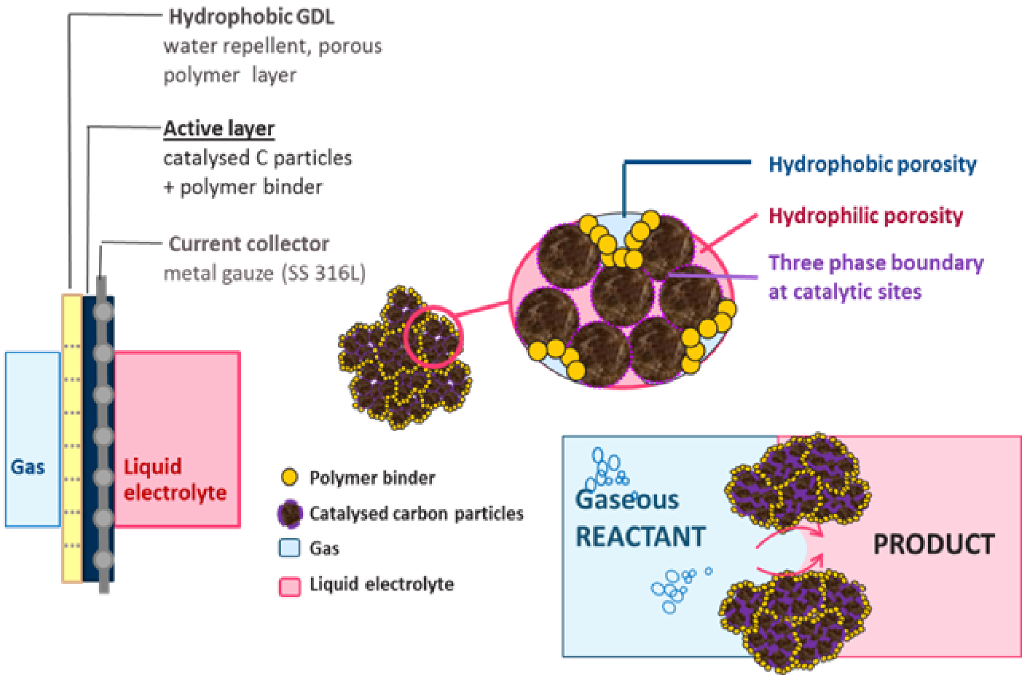


Bioelectrochemistry

- Next steps
  - Co-culture tests



- Benchmark with proprietary gas diffusion electrode



High pressure fermentation

- Features
  - Operating pressure = 1 to 10 bara
  - H_2 and CO_2 as process gas; also for gas mixtures with O_2, CH_4 and CO, real offgases
  - Online GC to monitor headspace composition
  - Separate electrical cabinet controlling battery of mass flow controllers for gas addition
  - Design allows 100% gas consumption
  - Integrated membrane filtration unit for cell retention
  - *In situ* product recovery

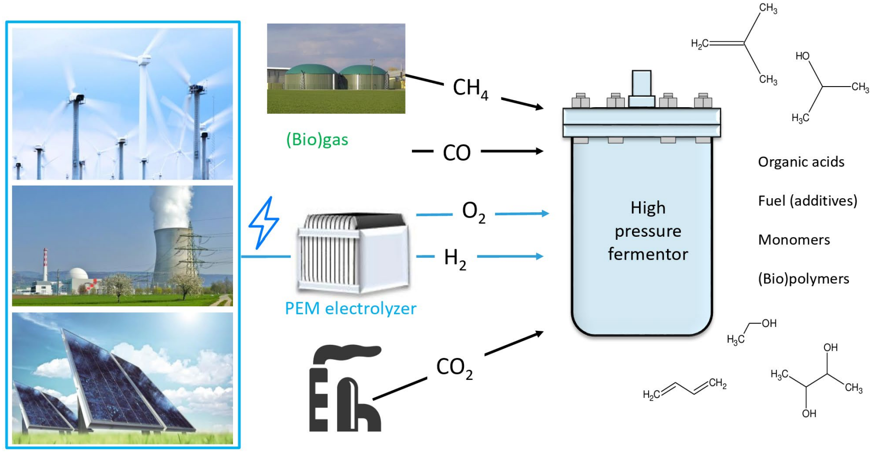
- ATEX compliance
- Fully automated – Labview
- Commissioned December 2019 – January 2020

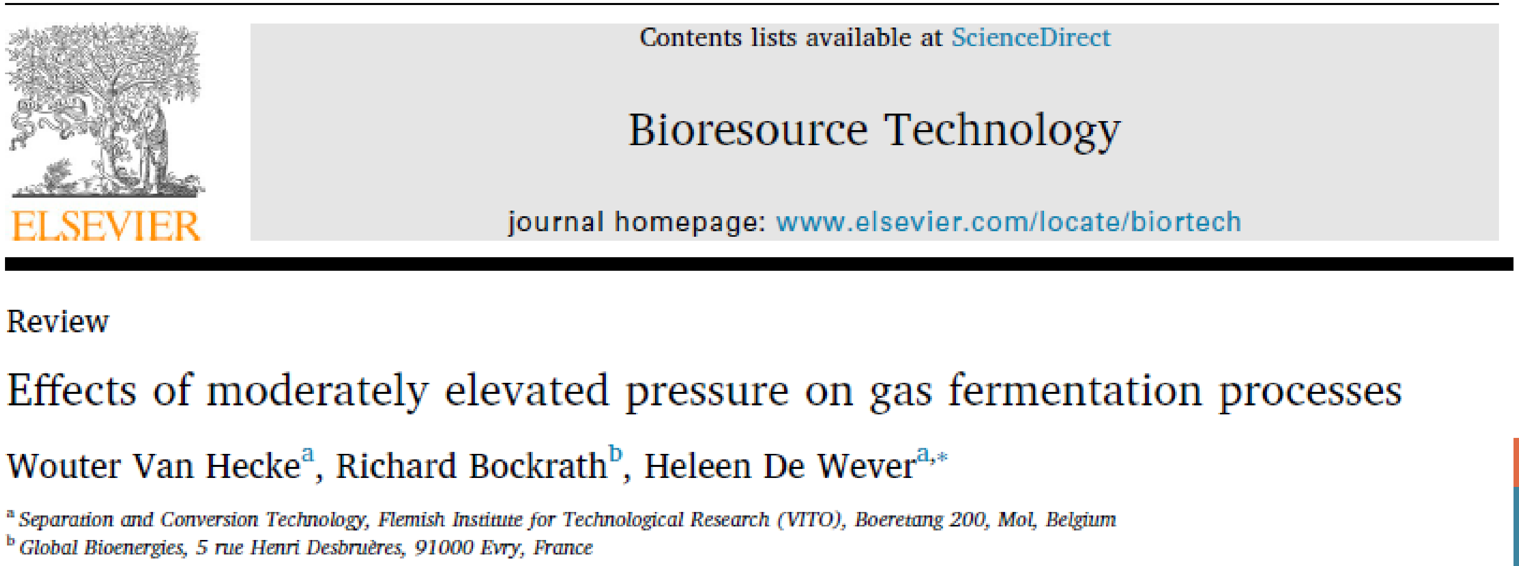
Online GC

Fermentor skid

High pressure fermentation

- Open-access review paper (see project website)
  - Different types of reactors discussed
  - H_2 and CO sensors in liquid: troublesome
  - Titers and productivities need to be improved significantly to allow industrialization
  - Gas to ethanol most advanced
  - More optimization work required to produce non-native chemicals from C1 substrate gases





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Review

Effects of moderately elevated pressure on gas fermentation processes

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Microbial conversions

- **Conclusions**
  - Established manipulation and genetic toolbox for *C. ljungdahlii* and implemented the isobutene pathway
  - Isobutene production under autotrophic conditions
  - Lactate re-consumption issue solved within *Cupriavidus* and lactate production improved by lactate dehydrogenase overexpression
  - Selection of two model strains of *Thermotoga neapolitana*, DSM33003 and RQ7
    - productivity (increase lactic acid molarity)
    - genetic tools (transformable strains and amelioration of target steps)
  - Test work with bioelectrochemical and high pressure set-ups initiated
  - Further optimizations to improve titers and productivity

Acknowledgements

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Thank you for your attention!

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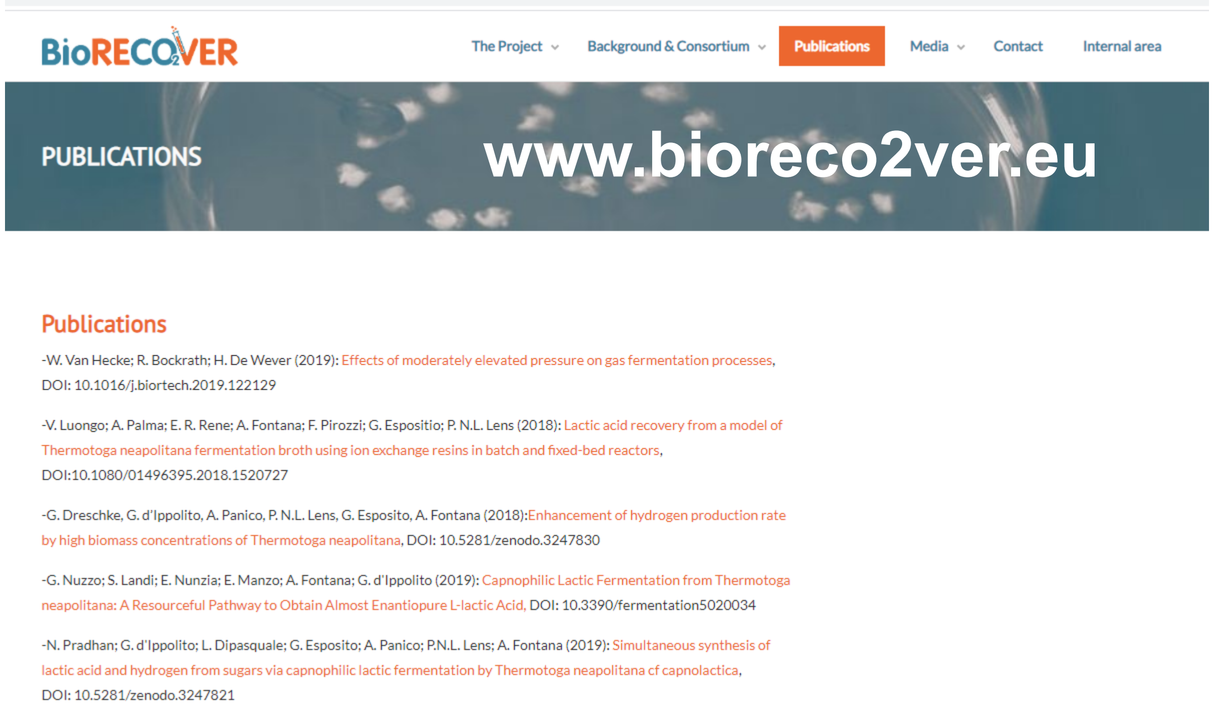

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Publications

-W. Van Hecke; R. Bockrath; H. De Wever (2019): Effects of moderately elevated pressure on gas fermentation processes, DOI: 10.1016/j.biortech.2019.122129

-V. Luongo; A. Palma; E. R. Rene; A. Fontana; F. Pirozzi; G. Espositio; P. N.L. Lens (2018): Lactic acid recovery from a model of Thermotoga neapolitana fermentation broth using ion exchange resins in batch and fixed-bed reactors, DOI:10.1080/01496395.2018.1520727

-G. Dreschke, G. d'Ippolito, A. Panico, P. N.L. Lens, G. Esposito, A. Fontana (2018):Enhancement of hydrogen production rate by high biomass concentrations of Thermotoga neapolitana, DOI: 10.5281/zenodo.3247830

-G. Nuzzo; S. Landi; E. Nunzia; E. Manzo; A. Fontana; G. d'Ippolito (2019): Capnophilic Lactic Fermentation from Thermotoga neapolitana: A Resourceful Pathway to Obtain Almost Enantiopure L-lactic Acid, DOI: 10.3390/fermentation5020034

-N. Pradhan; G. d'Ippolito; L. Dipasquale; G. Esposito; A. Panico; P.N.L. Lens; A. Fontana (2019): Simultaneous synthesis of lactic acid and hydrogen from sugars via capnophilic lactic fermentation by Thermotoga neapolitana cf capnolactica, DOI: 10.5281/zenodo.3247821

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