Project Overview

8th CCU congress, Nova Institute

Online congress, 24-25 March 2020

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Wageningen Food and Biobased Research, The Netherlands

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At a Glance

BIOCON-CO_2

Transforming raw CO_2 waste from the iron, steel, cement and electric power industries into value-added chemicals and plastics

Programme: EU Horizon 2020 – (BIOTEC-05-2017) Microbial platforms for CO_2 re-use processes in the low-carbon economy

Duration: January 2018 – December 2021 (48 months)

Consortium: 18 partners in 9 countries

Budget: €6.9 million

Coordinator: Acondicionamiento Tarrasense Asociación (LEITAT), Spain

Focus: Reduce greenhouse gas emissions and avoid overexploitation of natural resources

Impact: Convert CO_2 from industrial activity into valuable commodities

Core activities: Develop and validate, in an industrially relevant environment, a flexible strategy to biologically transform CO_2 into value-added chemicals and plastics

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BIOCON-CO2:18 partners from 9 countries (5 SMEs, 5 large industries, 4 research organisations, and 4 universities)

2020 research and innovation programme
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Project Objectives

BIOCON-CO_2

- **Develop and validate** flexible and versatile techniques using **biological processes** to transform raw CO_2 waste from the iron, steel, cement and electric power industries into value-added chemicals and plastics
- **Generate new knowledge for** commercially viable strategies for reducing Europe's dependency on fossil resources
- **Increased sustainability** of the chemical industry, providing support for European leadership in CO_2 re-use technologies

Unsplash © Patrick Hendry

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Project Structure

BIOCON-CO_2

WP1: Management

WP2: CO_2 capture, mixtures and solubilisation strategies

- Gas composition
- Gas requirements
- CO_2 solubilisation strategies

WP3: *Clostridia* fermentative process for C3-C6 alcohols production

WP4: *Oligotropha* fermentative process for 3HP production

WP5: One-pot biocatalytic systems for formic acid and lactic acid production

WP6: Downstream and validations

WP7: Demonstration in industrial relevant context

WP8: Socio-economic evaluation and environmental sustainability

WP9: Dissemination, exploitation and public acceptance

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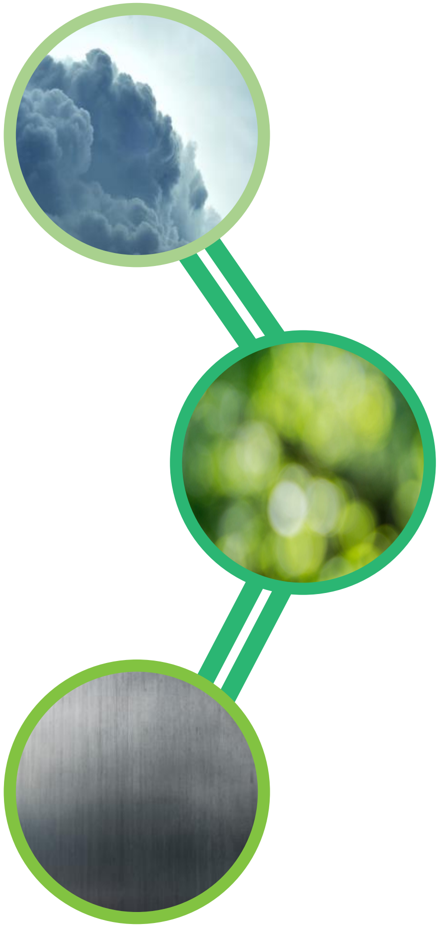
Expected Results

- Assessment and validation of **three low-energy microbial processing systems** capable of converting CO_2 emissions from the iron and steel industry into valuable industrial products
- Production of **four chemical building blocks** produced using CO_2 re-use technologies that have application in the food/feed, chemical (acrylates, polymers, surfactants) and plastic industries
- **Pilot installation** in an industrial setting upon project completion which demonstrates and validates the effectiveness of four chemical building blocks produced using CO_2 re-use technologies
- **Improved public perception** of CO_2 re-use technologies through transparent and responsible communication, knowledge transfer and exploitation of project outcomes



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Target Audiences

BIOCON-CO_2

Industry CO_2 Suppliers

Positive environmental gains

Regulators /Policy Makers

Policy framework to implement CO_2 transformation technologies efficiently

Scientific Community

Continuous research and uptake of BIOCON-CO_2 results

Biotech Industry

Cost-efficiency & environmental impacts of using BIOCON-CO_2 technologies

Public and Private Investors

Continuous development & future industrial exploitation of BIOCON-CO_2 technologies

Related H2020 Initiatives

Collaboration with other ongoing projects in the same field

Chemical/ Food & Feed/ Plastic Industries

Main end-users of target products, key for market uptake

Local Authorities /Decision Makers

Implementation of a CO_2 capture plant and pilot/industrial installation for BIOCON-CO_2 implementation

General Public

Impacts of BIOCON-CO_2 technologies on the economy & environment

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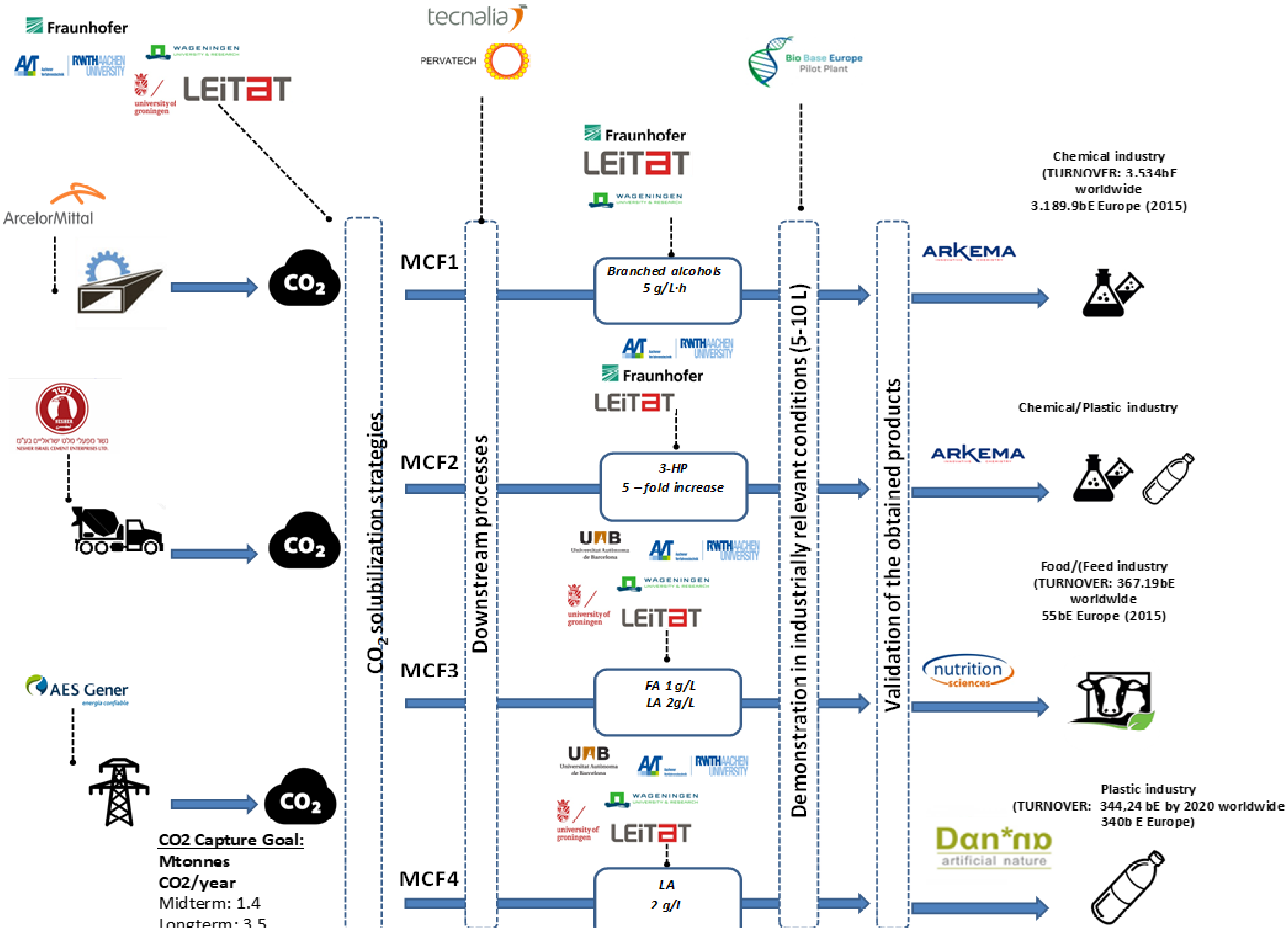
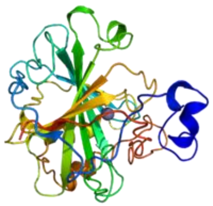
Approaches



MCF1
MCF2
MCF3
MCF4
CO2 solubilization strategies
Downstream processes
Demonstration in industrially relevant conditions (5-10 L)
Validation of the obtained products
Branched alcohols
5 g/L·h
3-HP
5 – fold increase
FA 1 g/L
LA 2g/L
LA
2 g/L
Chemical industry
(TURNOVER: 3.534bE
worldwide
3.189.9bE Europe (2015)
Chemical/Plastic industry
Food/(Feed industry
(TURNOVER: 367,19bE
worldwide
55bE Europe (2015)
Plastic industry
(TURNOVER: 344,24 bE by 2020 worldwide
340b E Europe)
CO2 Capture Goal:
Mtonnes
CO2/year
Midterm: 1.4
Longterm: 3.5

Improving CO_2 solubility
The carbonic anhydrase enzyme (CA)

Carbonic anhydrase (CA) catalyses the hydration of CO_2 into bicarbonate and a proton.



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

Advantages

- ✓ High turnover rate (k cat = 10^6 s^{-1})
- ✓ Mild operative conditions

Disadvantages

- ❑ Possible inhibition at operating conditions & impurities
- ❑ High cost of the comercial enzyme (1 mg = 500 €)
- ❑ Reported low stability

LEITAT managing technologies

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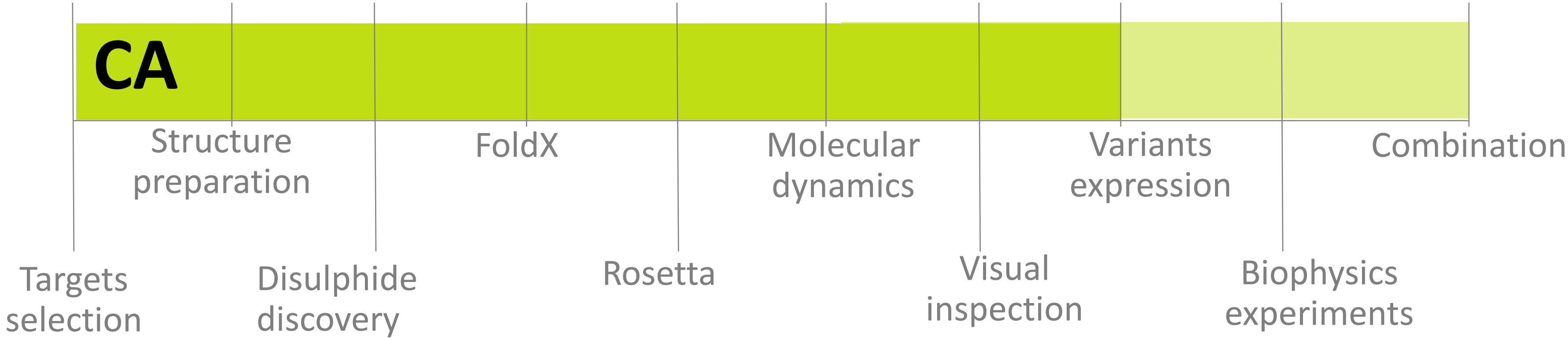
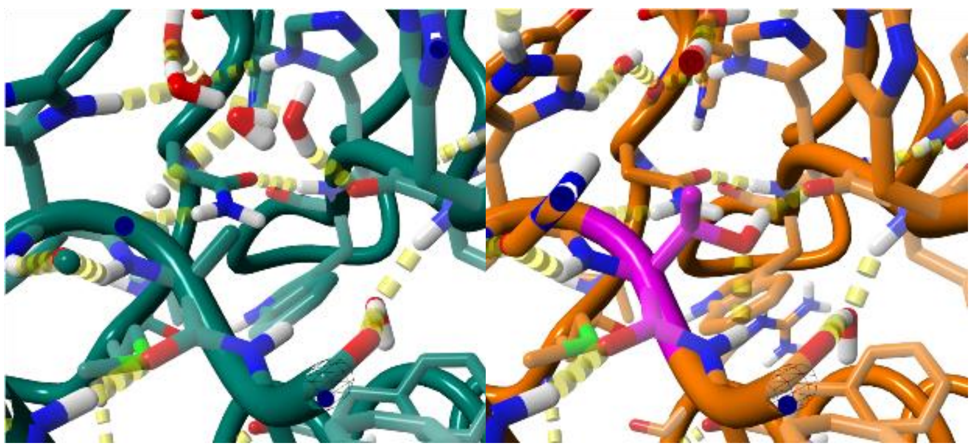
hCAII engineering (RUG)





755 screen mutations

49 selected mutations

8 mutation screened

21 mutation for screening

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MCF1: Clostridium – a versatile genus for basic and applied research

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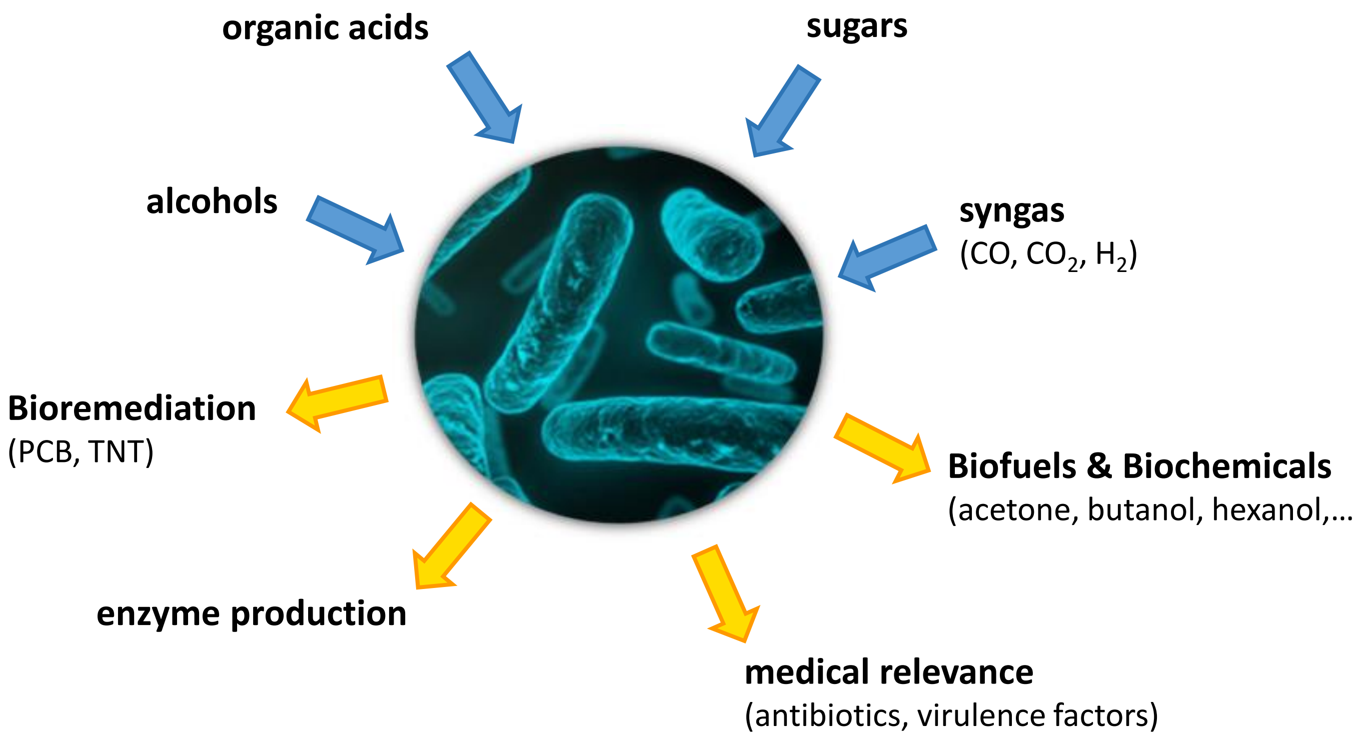
Fraunhofer IME

- Gram positive, anaerobic bacteria
- High relevance in medical, ecological and biotechnological sciences



- Small genomes; > 100 strains are sequenced
- Increasing set of tools for genetic manipulation

picture: www.sbi.uni-rostock.de

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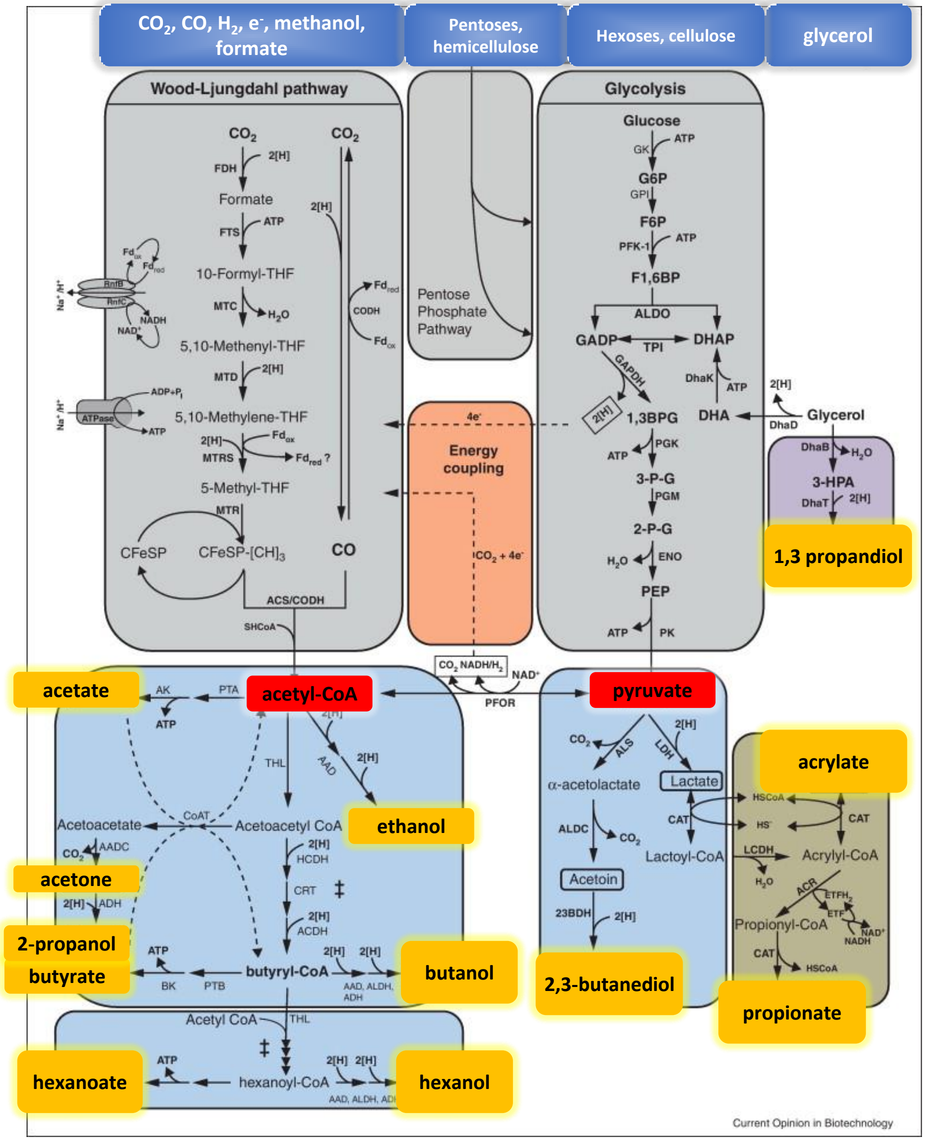
Metabolism

- Wood-ljungdahl pathway and glycolysis are typical pathways
  - Syngas, biological waste
- acetyl-CoA and pyruvate are central intermediates and starting substrates for heterologous pathways
- Genus with a broad product spectrum (native & foreign)
  - e.g. farnesene, isoprene, PHB, isobutene,…



Tracy et al. 2012

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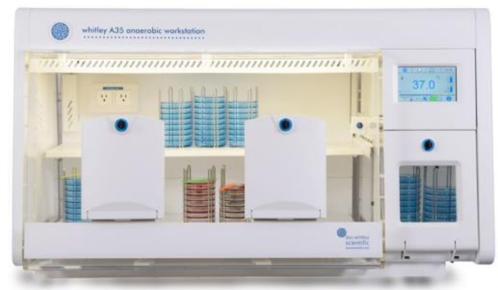
Working under anaerobic conditions

Fraunhofer IME

- Anaerobic jars, e.g:
  - Anaerocult A to remove oxygen

- Anaerobic work chambers, e.g:
  - A) Whitley A35 workstation (don whitley scientific)
  - B) Bactron-600 (Shellab)
  - C) Type A anaerobic chamber (Coy laboratory products)

A



C

B

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Cultivation methods

- Syngas fermentation unit

Fraunhofer

- Parallel fermentation system (3 x 3.7L) suitable for syngas
- Gas mixing station (N_2, CO_2, CO, H_2)
- Control of pH, temperature, stirrer speed…

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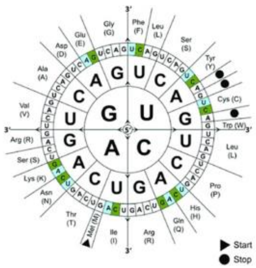
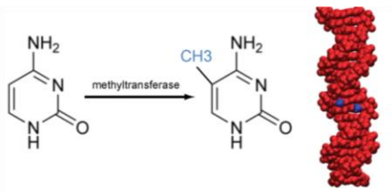
Metabolic engineering methods

- Challenges when using clostridia for metabolic engineering



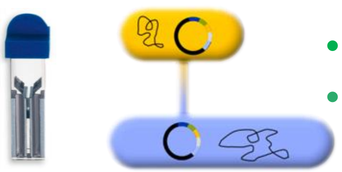
- The genome of clostridial species is very AT-rich with a very biased codon usage

⇨ Genes can be synthesized with an adapted codon usage



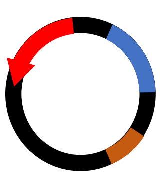
- The restriction-modification system in Clostridia can massively reduce transformation of Clostridia with foreign DNA

⇨ use of *in vivo* or *in vitro* methylation or generation of knock-out strains



- Electroporation is the common method to transfer DNA into bacteria, but:
- the efficiency decreases by size and large gene-clusters are difficult to transfer

⇨ Conjugation as rather size independent method



- Traditionally episomal vectors are used for heterologous expression of the foreign clusters, but these are not stably maintained without antibiotics

⇨ Genomic integration for stable expression appropriate for large scale fermentation

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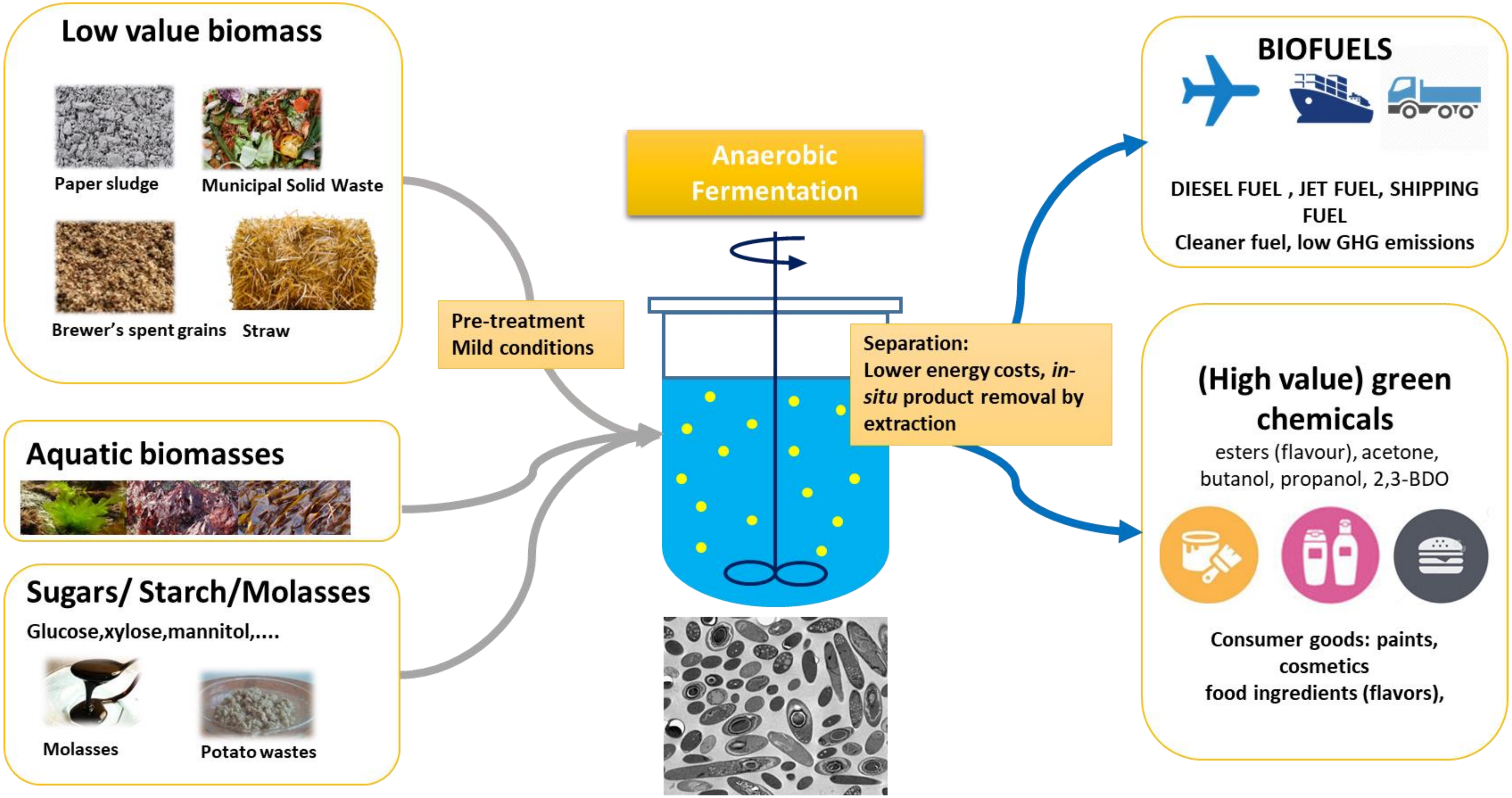
Solventogenic Clostridia

Clostridium acetobutylicum, C. beijerinckii



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Process improvement

BIOCON-CO2

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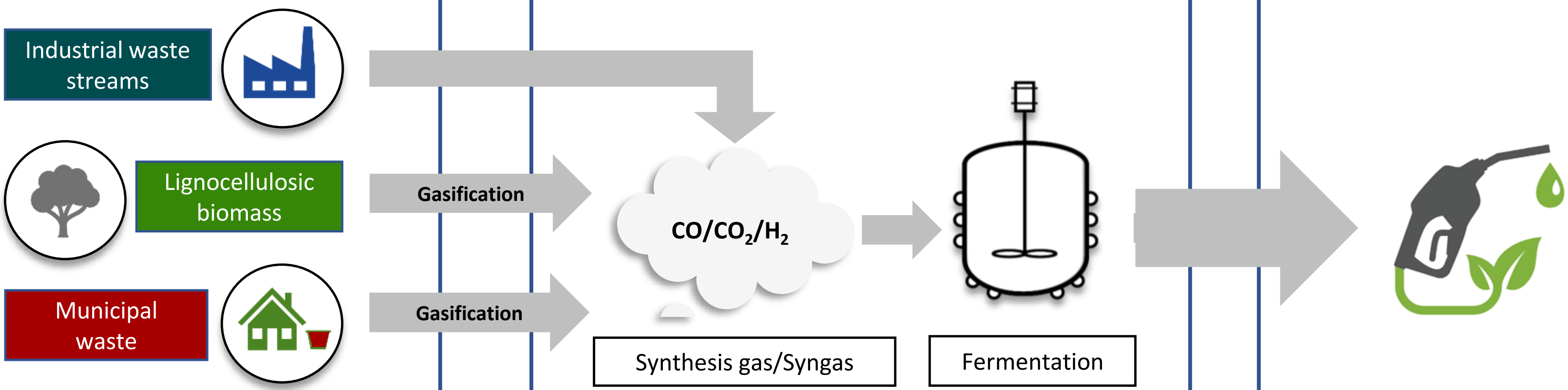
Great need for effcient screening and process development methods



- Non-eatable feedstock
- Feedstock flexibility
- Utilizes all biomass components (Lignin!)

- Mild reaction conditions
  → Energy & cost saving
- Microbial catalysts are more resistant to syngas contaminations than metal catalysts

Wide product range

- A multitude of new potential process routes
  - Biofuels
  - Intermediates
  - ...

Liew et al. (2016), Bengelsdorf and Dürre (2017)

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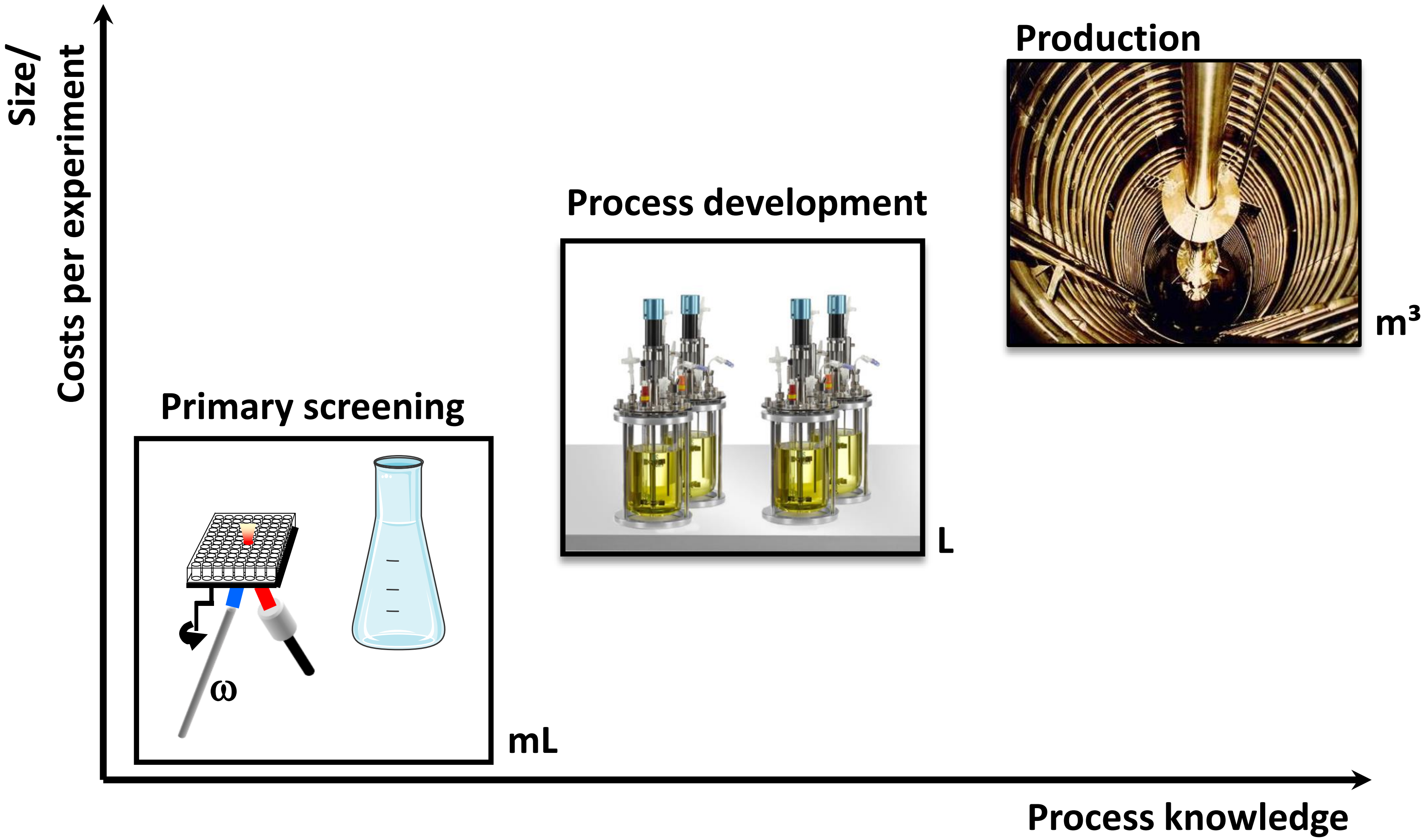
Bioprocessdevelopment



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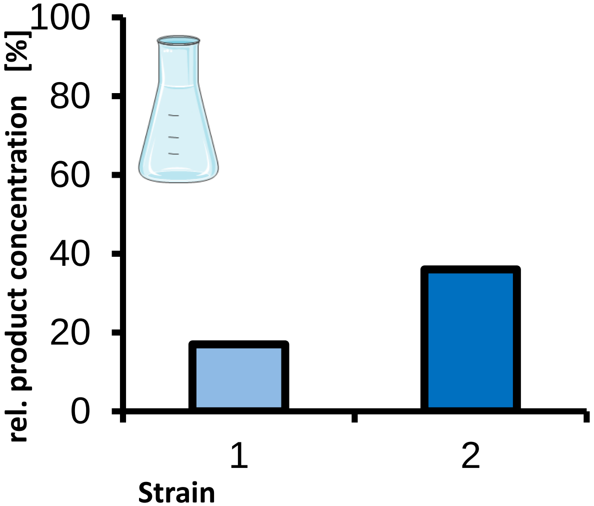
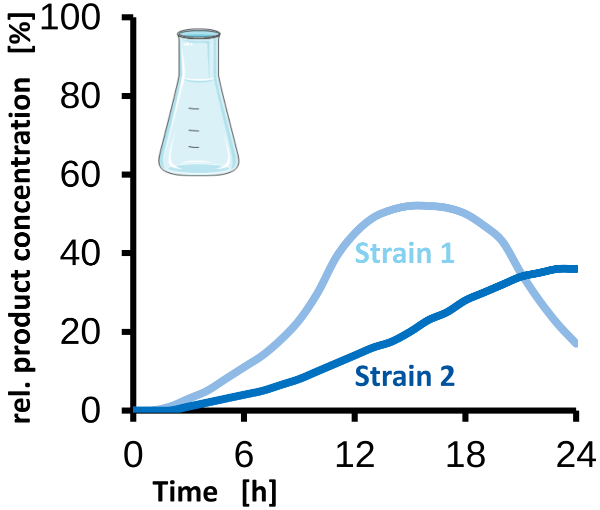
Endpoint determination vs. online monitoring

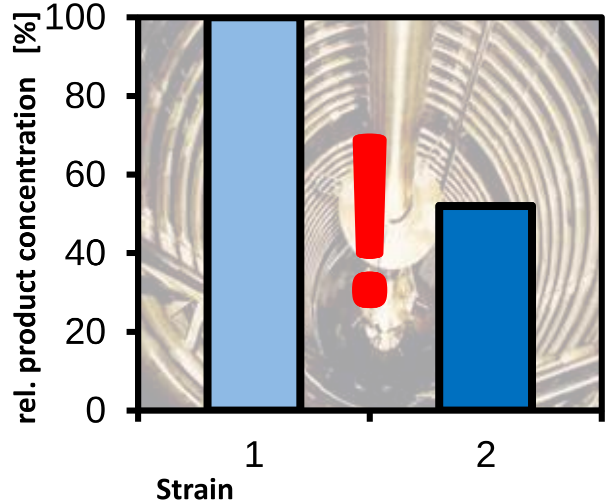
Primary screening with endpoint determination



Primary screening with online-monitoring



Final production process



- Wrong decisions in primary screening cannot be compensated
- Detailed **process understanding** on a **small scale** is required

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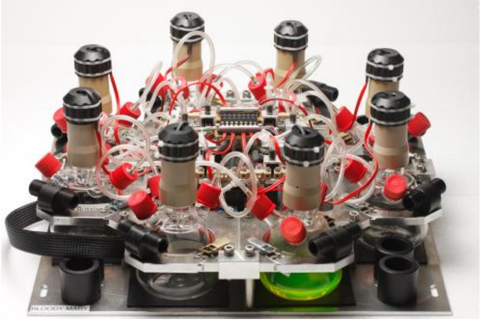
AnaRAMOS - Implementation of anaerobic cultivation in shake flask

- RAMOS System „**R**espiration **A**ctivity **Mo**nitoring **S**ystem“
  - Online measurement of OTR and CTR possible

- Sucessful application for anaerobic cultivation on solid carbon sources
  - AnaRAMOS System
  - Online measurement of CO_2TR and HTR possible
  - Measuring the CO_2 and H_2 production



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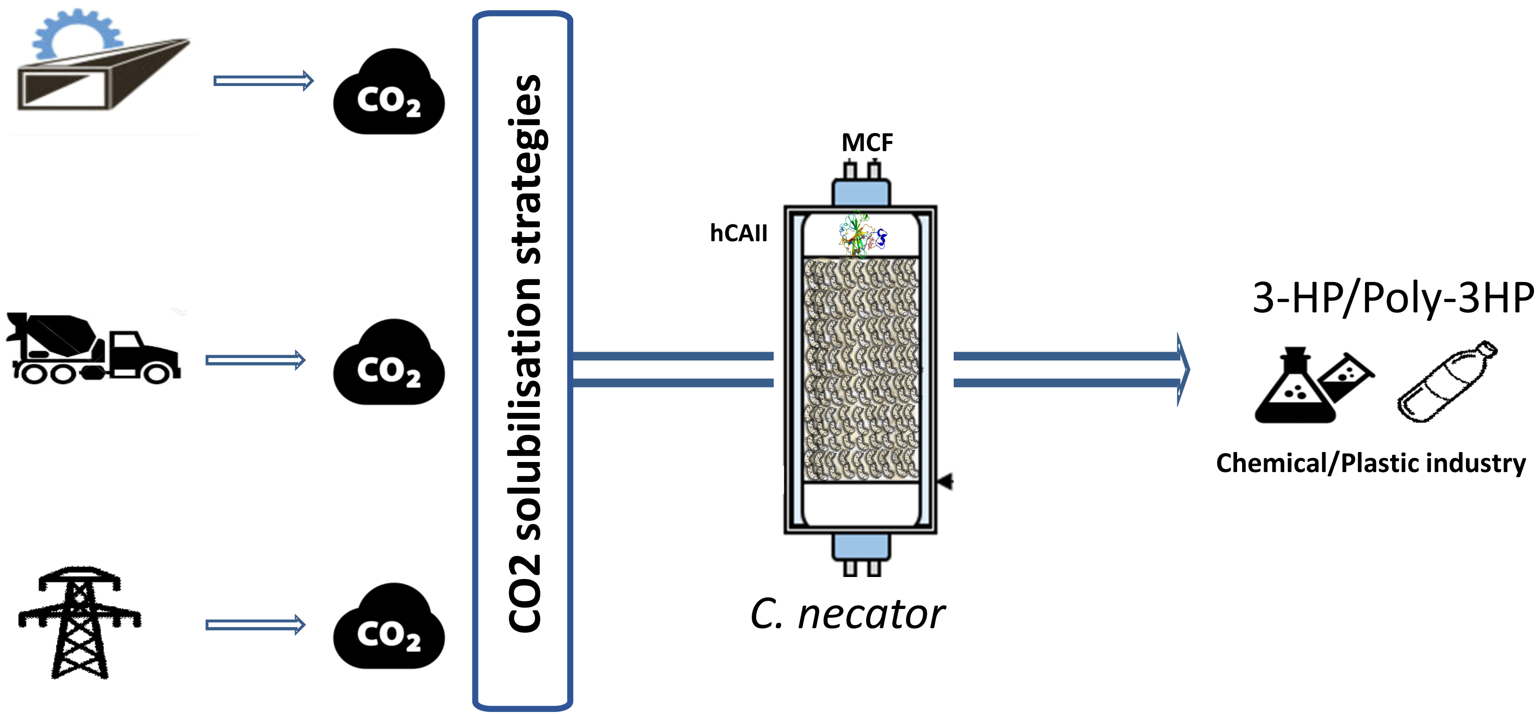
MCF 2: *Cupriavidus necator* to produce 3-HP



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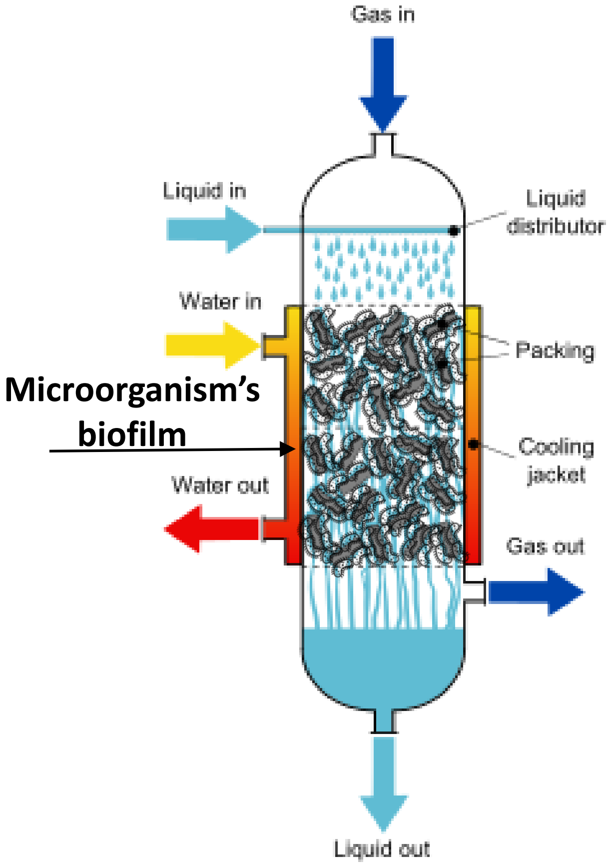
BIOFILM TBR

In a biofilm TBR the packing material is used as a support to facilitate the cell attachment and growth of the microorganisms, catalysing the reaction.

Advantages

- Higher biomass density and operation stability
- Easy product recovery
- Relatively low costs for construction and operation
- Withstand load changes and toxicity
- Versatility with different functionalized packing materials



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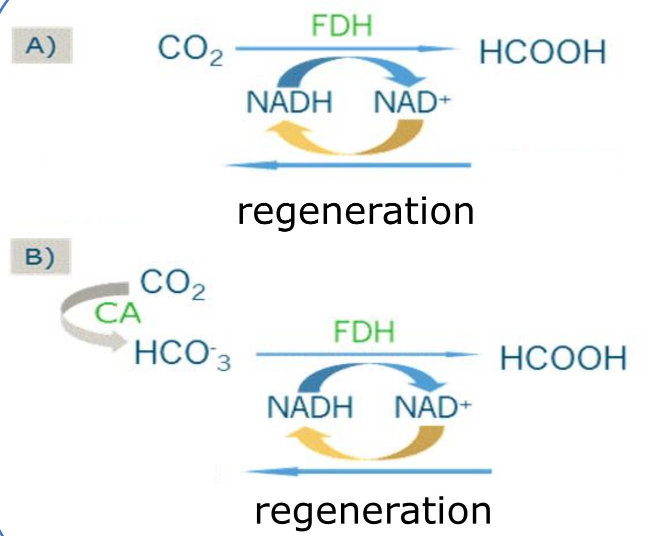
MCF 3: Enzymatic production of formic acid from industrial CO2-rich streams

Approach

Gas source

Industry



Research

Robust & efficient enzymes

Enhance CO_2 levels

Artificial reducing agents (NADH free process)

Process optimization

Gas composition

Compatibility with biocatalysts

WAGENINGEN UNIVERSITY & RESEARCH

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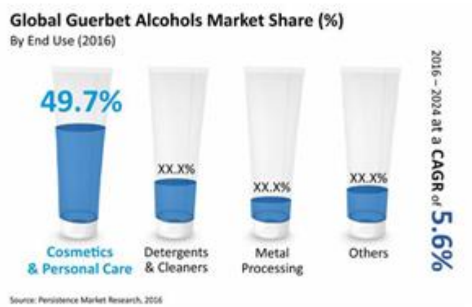
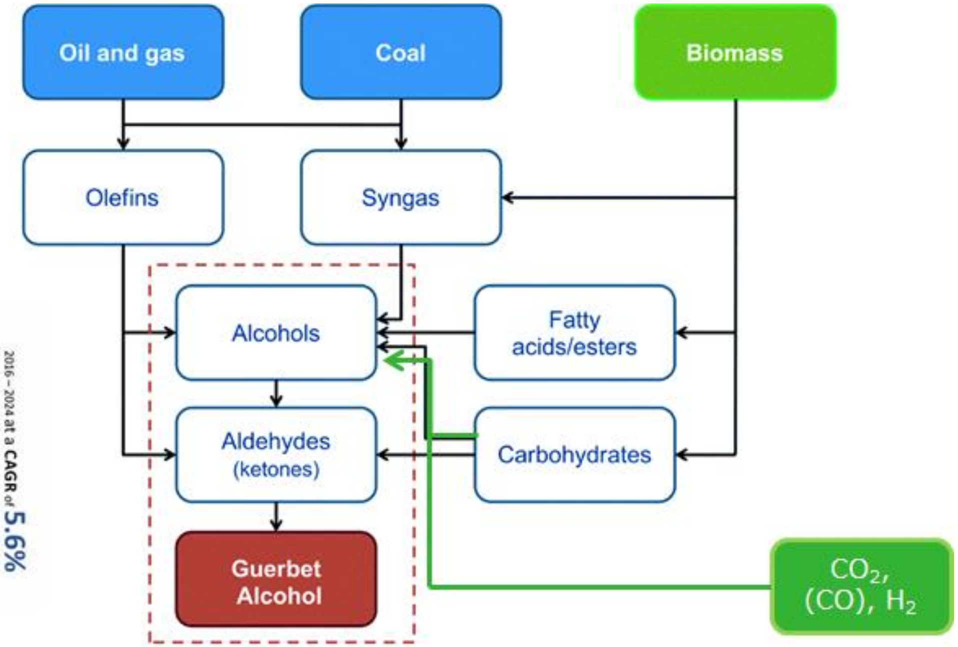
Production of Guerbet alcohols





Biolubricants

Chemoenzymatic cascade

Ethanol
n-propanol, iso-propanol
n-butanol, 2-butanol
Pentanol, hexanol

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Validation at BBEPP
Pressurized Fermentation

- Installation of **4x1 L gasfermentation system** @ BBEPP
- Fully independent
- Screening and optimization
- High-pressure up to 10 barg
- 5 gasses CO, CO2, O2, H2, air, N2
- Safety system being installed

Operational since Q2 2019

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Upscaling @ BBEPP
Design and construction of a mobile plant

- Motives of building/installing mobile bioreactor
  - Compression of gas in high-pressure cylinders: no-go
    - Pumps erode
    - Compounds condensate
  - Mimic off-gas in high-pressure cylinders
    - Practically impossible
    - Expensive
  - Most reliable and practical approach

➡ **Bring plant to emission source**

➡ **Mobile gasfermentation unit containing 10 L CSTR, 10 L trickle bed and 100 L CSTR (10 bar)**

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Summary

- Aerobic and anaerobic fermentation of CO_2-containing gases to chemicals is under development, from the molecular level to a pilot scale mobile plant
- New technologies for process engineering (TBR) and for screening of gas utilisation at small scale are developed
- Enzymatic production of formic acid can be efficient using enzyme cascading
- Combining biological production with enzymatic and chemical approaches can result in new products

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Invitation

BIOCON-CO_2

2nd Co2oling the Earth Summer School

29 Sept – 2 Oct 2020 in Athens (if Covid-19 allows)
hosted by BIOCON-CO_2

Main session topics

- CO_2 Capture & Sequestration technologies
- Chemical CCU: state of the art & future prospects
- Microbial CCU: state of the art & future prospects
- Enzymatic CCU: state of the art & future prospects
- Genetic engineering tools for CCU
- Electrochemical & electrobiocatalytic CCU applications
- Industrial CCUS experiences
- CCS & CCU retrospective, legislation, EU targets

Invited speakers (more to be announced)

- Ana Lopez - Contreras, Wageningen
- Carmen Boeriu, Wageningen
- Guiomar Sanchez, Leitat
- Gabriele Philips, Fraunhofer
- Aline Hüser, AVT
- George Skevis, CERTH
- George Romanos, Demokritos
- Marios Katsiotis, TITAN

Registration (re)opens soon on: cmt.eurtd.com/groups/profile/64195/biocon-co2-community

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Acknowledgments

The BIOCONCO2 Partners

Thank you for your attention

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