TRANS FORMATE

Project TRANSFORMATE

Combined process development of electro-chemical CO_2-reduction and synthetic biology for production of the biopolymer PHB and crotonic acid

Frank Kensy, Project Coordinator

1. Status Conference CO_2-WIN

9th June 2021

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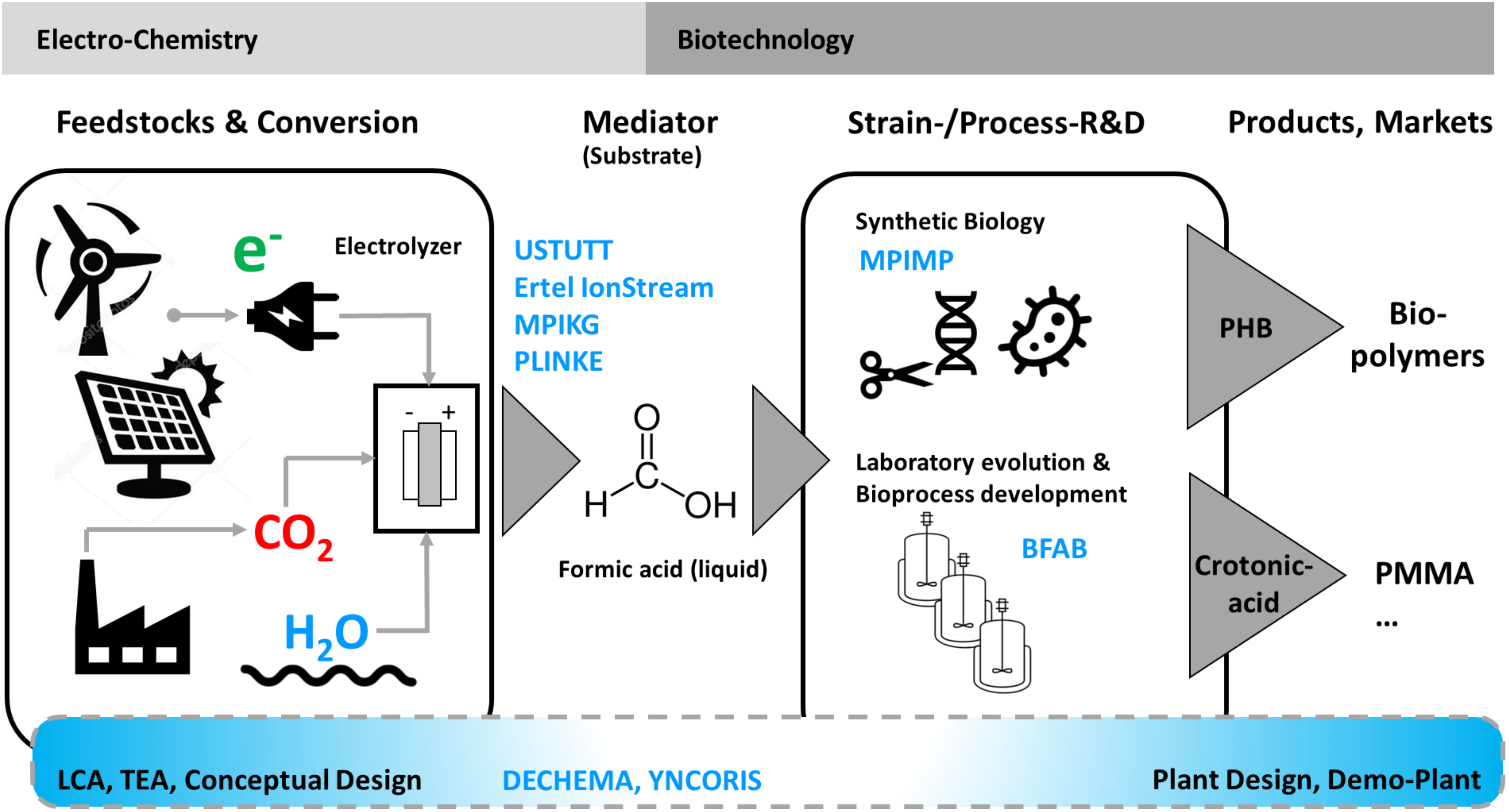
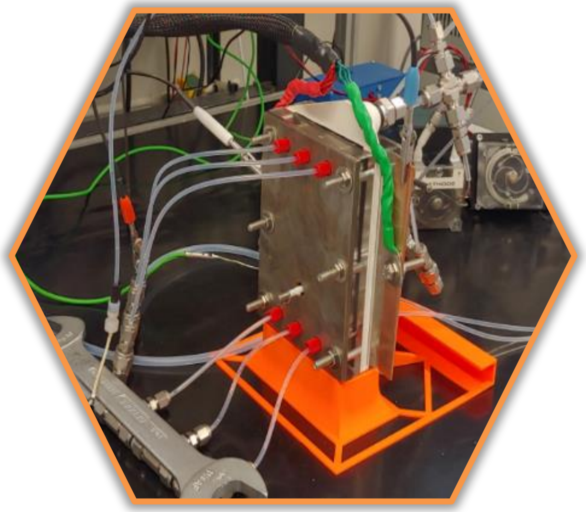
Federal Ministry of Education and Research

Project Overview



Why CO_2 Reduction to Formic Acid?



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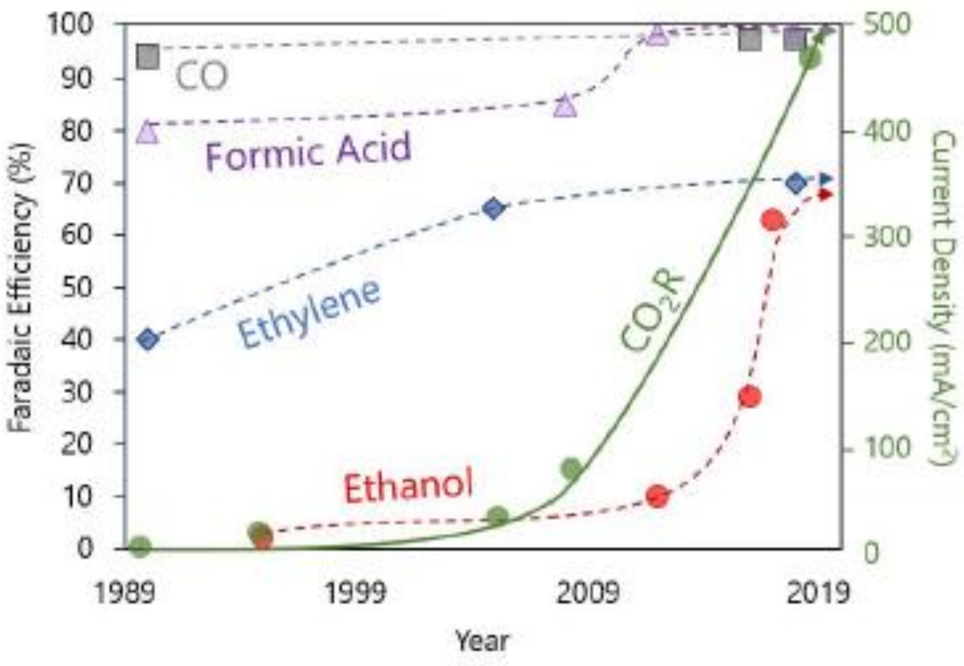


- CO_2 and H_2 storage in a liquid
- High energetic efficiency (FE > 90%, EE > 30-50%)
- High current densities (normally 200-300 mA/cm^2, max. 1 A/cm^2 reached)
- No mass transfer limitation in fermentation (fully soluble)

De Luna et al., Science 364, 350 (2019)

Biological Transformation through Synthetic Biology

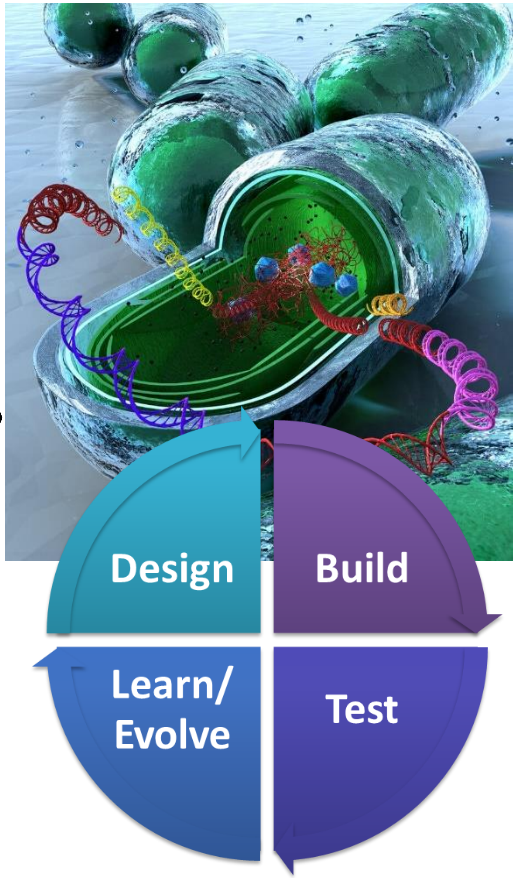
PAST → FUTURE

Ethanol

Biopolymers

Insulin

Synthetic Biology



Proteins

Spider Silk

Flavours

...

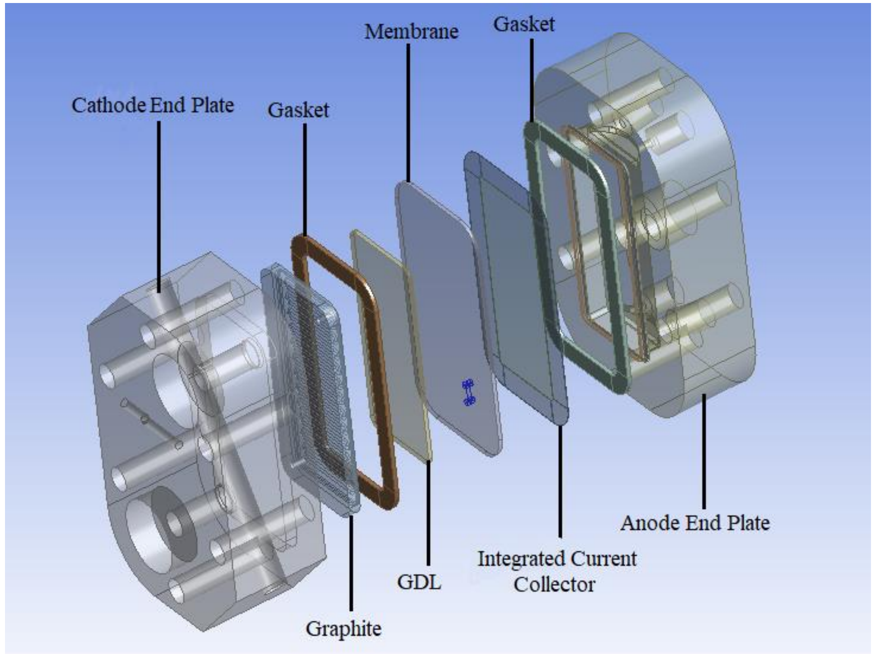
Goals of the project

Specific project goals:

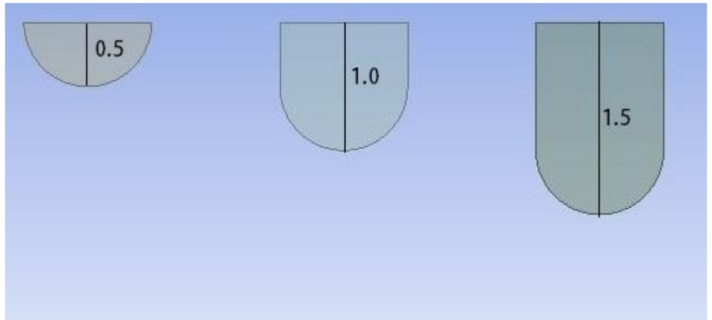
1. CO_2-electrolyzer for the efficient production of formic acid from CO_2 and water
2. Platform strain *Cupriavidus necator* which is capable to growth on formate via rGP
3. Production strain and -process for PHB
4. Production strain and -process for crotonic acid
5. Engineering and plant concept for industrial implementation (including LCA & TEA)

Laboratory CO_2-Electrolyzer

- Anode
- Cathode
- Flow Field
- Membrane, Gas Diffusion Layer and Sealing Gaskets



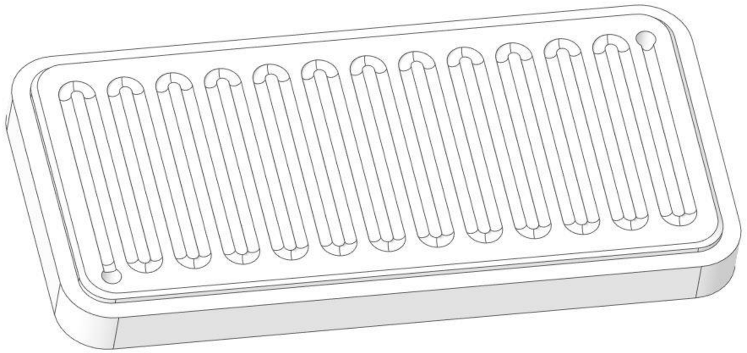
Lab Electrolyzer: explosion view



Flow Field channels with different depth, measured in mm

Manufactured Flow Fields:

- Channel depths, 0.5, 1.0 and 1.5 mm
- Two different graphite grades

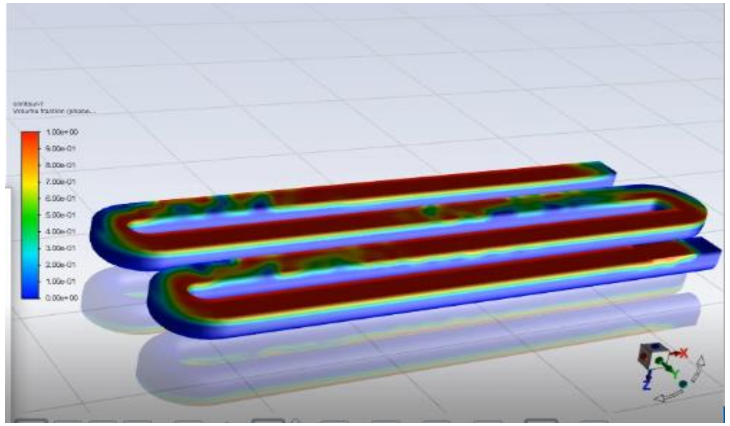


Graphite Flow Field 27 mm × 56 mm

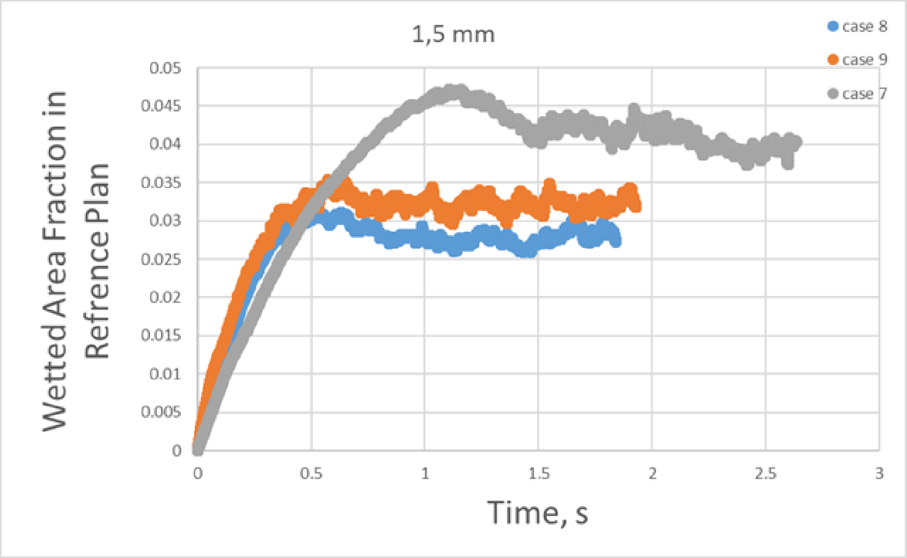
Flow Simulation

Parameters Studied in Simulation

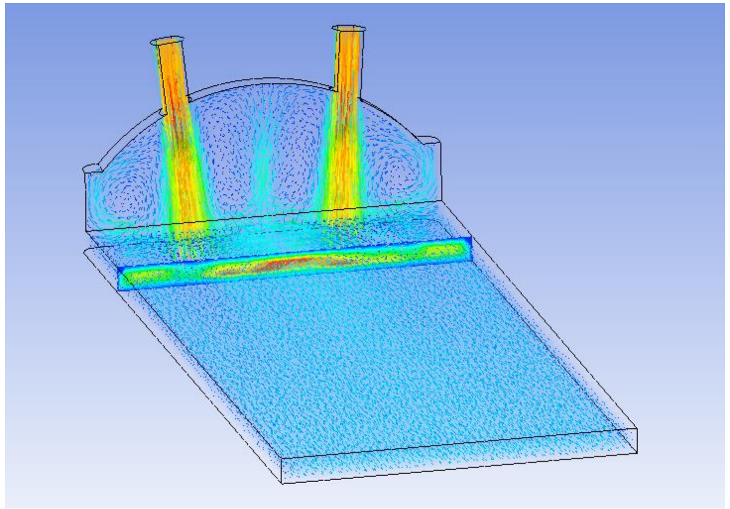
- Hydrophobicity of the Flow Filed
- Inclination Angle
- Flow Rate of Gas and Liquid in Cathode
- Channel Depth
- Channel Shape
- Anode Inlet/Outlet Manifolds



Two-phase Flow Simulation in Channels



Simulation Result Analysis



Flow Simulation in Anode End-Plate

MEA Design with PIL modified Catalytic Layer

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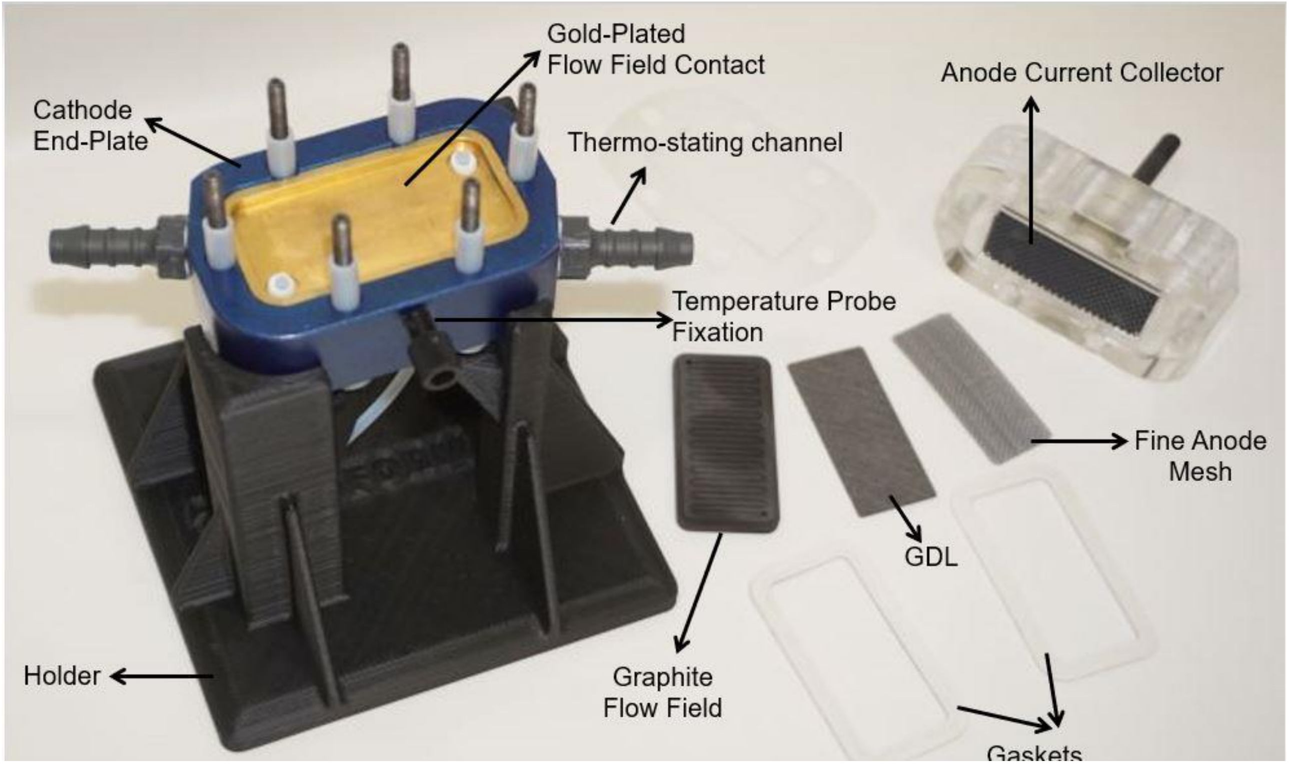
Membrane Electrode Assembly Design (MEA Design)
Zero Gap Design



Polymeric Ionic Liquids (PILs)
can be used as…
… binders in the CL
… stabilisers for metal clusters

in order to
… enhance metal dispersion
… control ion transport
… pH
… enhance CO_2 flux by
selective adsorption

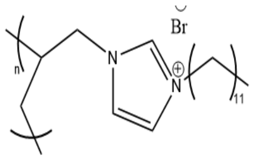
Testing of Binder and Catalyst

- AA 39 – GDL
- BC 39 – GDL + MPL
- 35% of PILs for each sample
- SnO2@AB Catalyst
- 1 mg cm-2 of SnO2 synthesized by homogeneous precipitation
- 64% of AB (carbon)

PILs synthesized by:

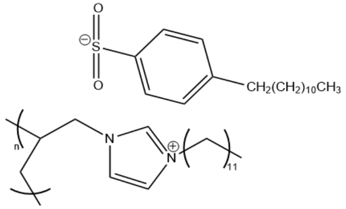
Max Planck Institute of Colloids and Interfaces

- PIL3: PIL-Imid-C12 Br



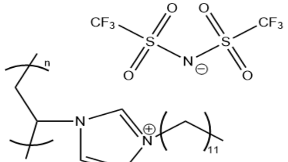
PIL-Imid-C_{12} BR

- PIL5: PIL-Imid-C12 DBS

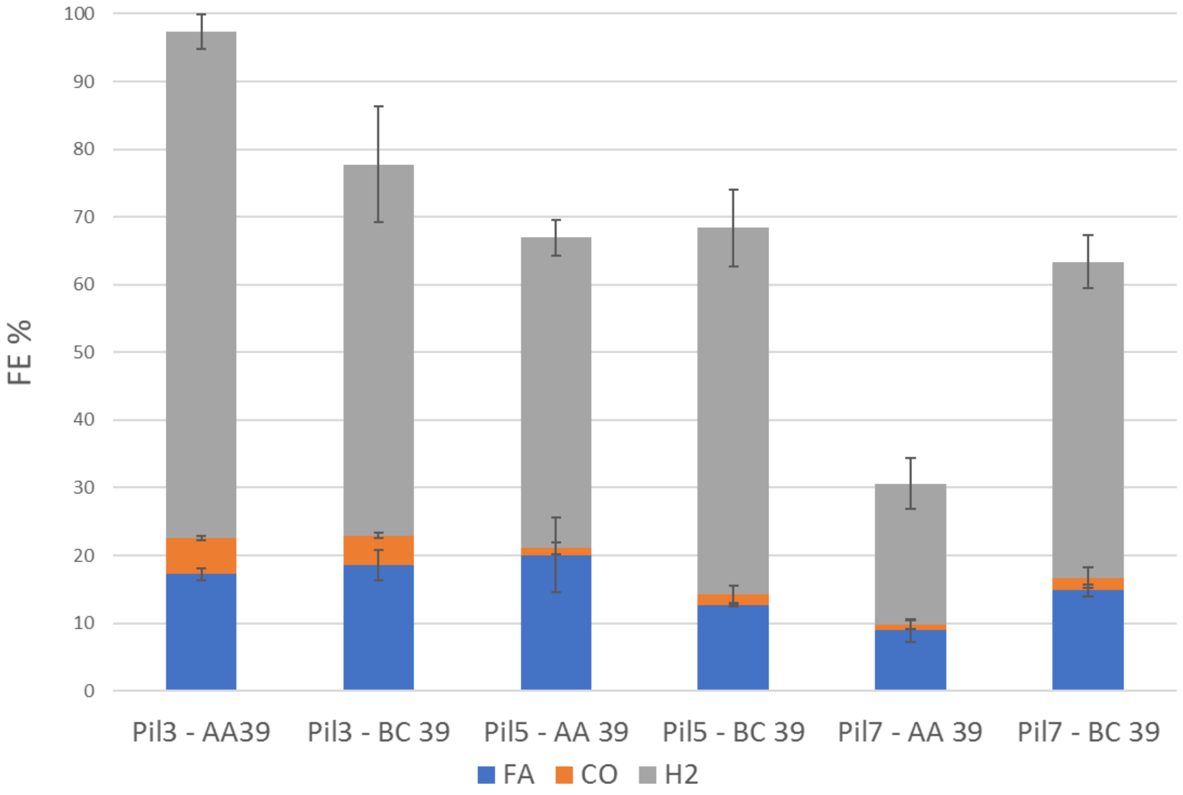


PIL-Imid-C_{12} DBS

- PIL7:PIL-Imid-C12 TFSI



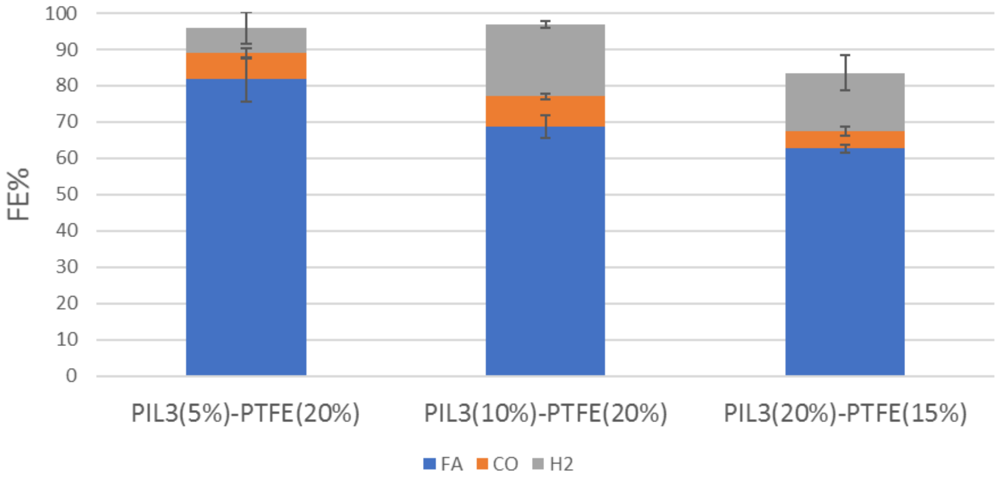
PIL-Imid-C_{12} TFSI



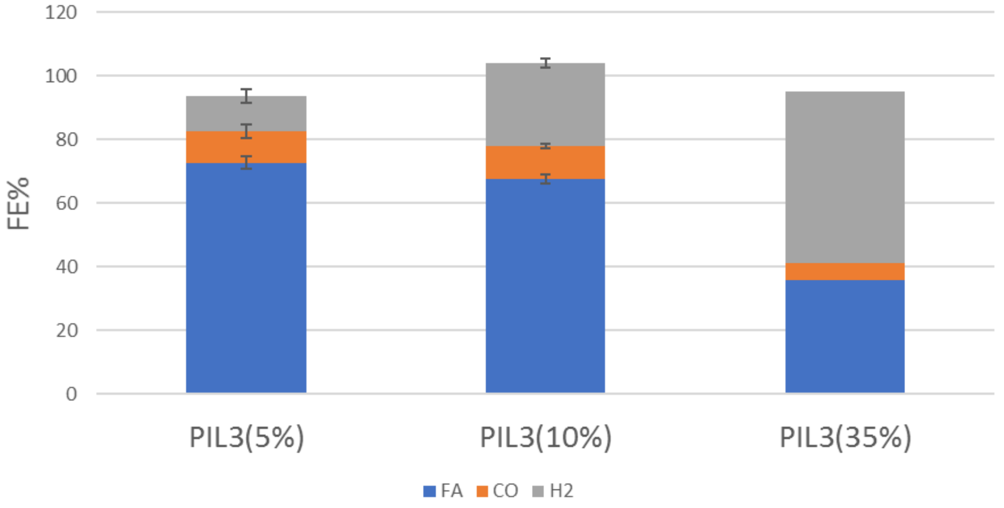
GSS analysis conditions: : j = -200 mA cm^{-2}, T = 50°C, 0.5M $KHSO_4$, pH = 2.5 (H_2SO_4), Ir/Ti anode in H_2O

Testing of Binder and Catalyst

- AA 39 – GDL
- J – C01 CL: 1 mg cm^{-2} of commercial SnO2 supported in AB

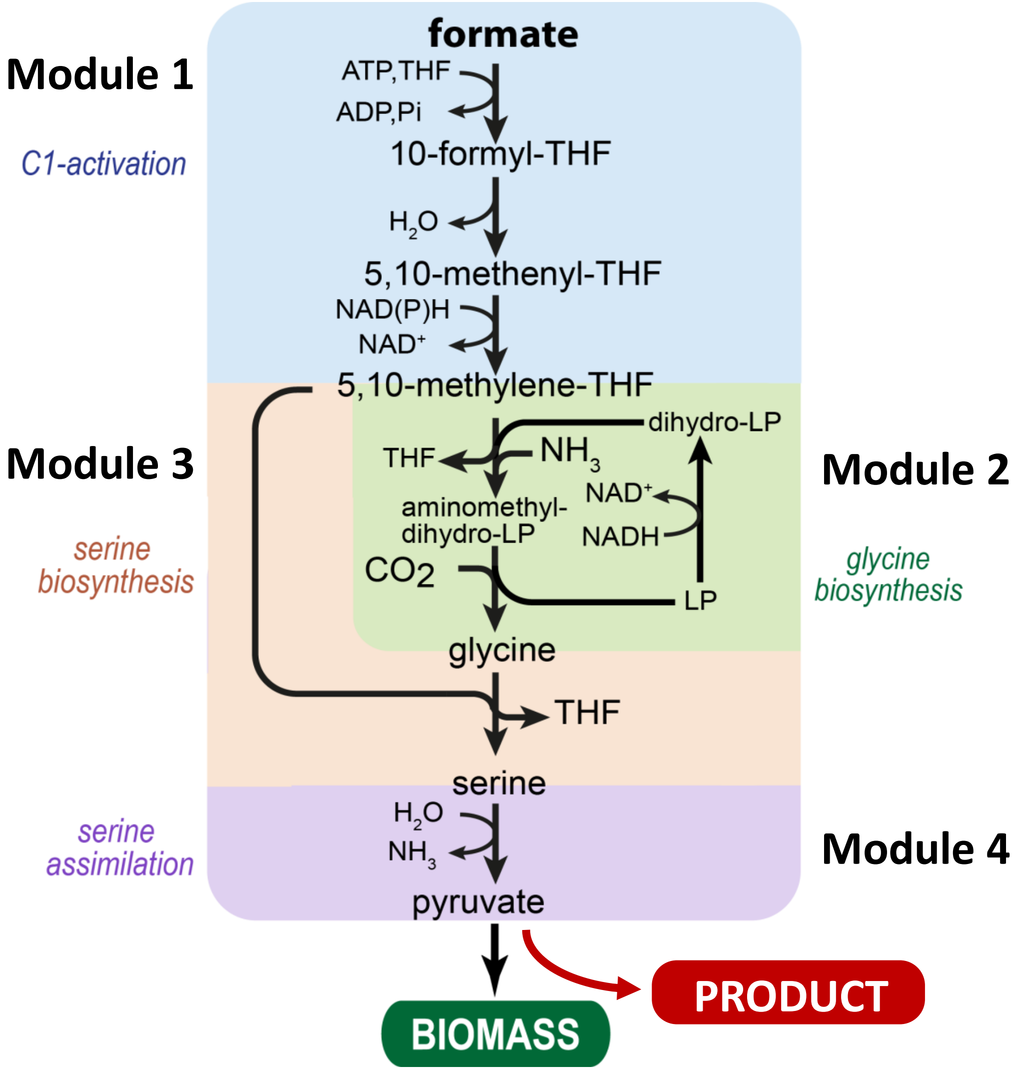


- Decreasing amount of PILs increases FE for FA and HER highly suppressed

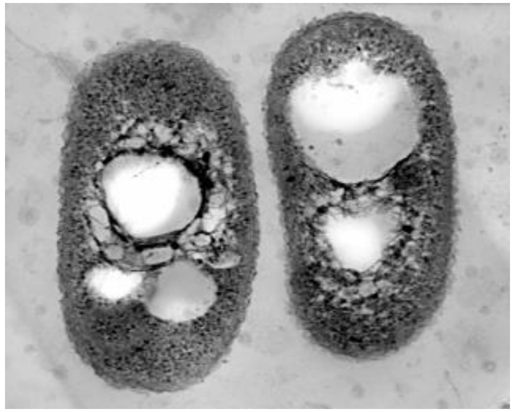


GSS analysis conditions: : j = -200 mA cm^{-2}, T = 50°C, 0.5M $KHSO_4$, pH = 2.5 (H_2SO_4), Ir/Ti anode in H_2O

Cloning of the Reductive Glycine Pathway



Cupriavidus necator



Alternative names:
Alcaligenes eutrophus,
Ralstonia eutropha,
Wautersia eutropha and
Hydrogenomonas eutropha

- capable to grow on formate via Calvin Cycle
- natural PHB producer
- versatile host
- basic genetic tools available
- industrial proven microorganism

Genome Integration of 3 Modules of the rGP

Max-Planck-Institut für Molekulare Pflanzenphysiologie

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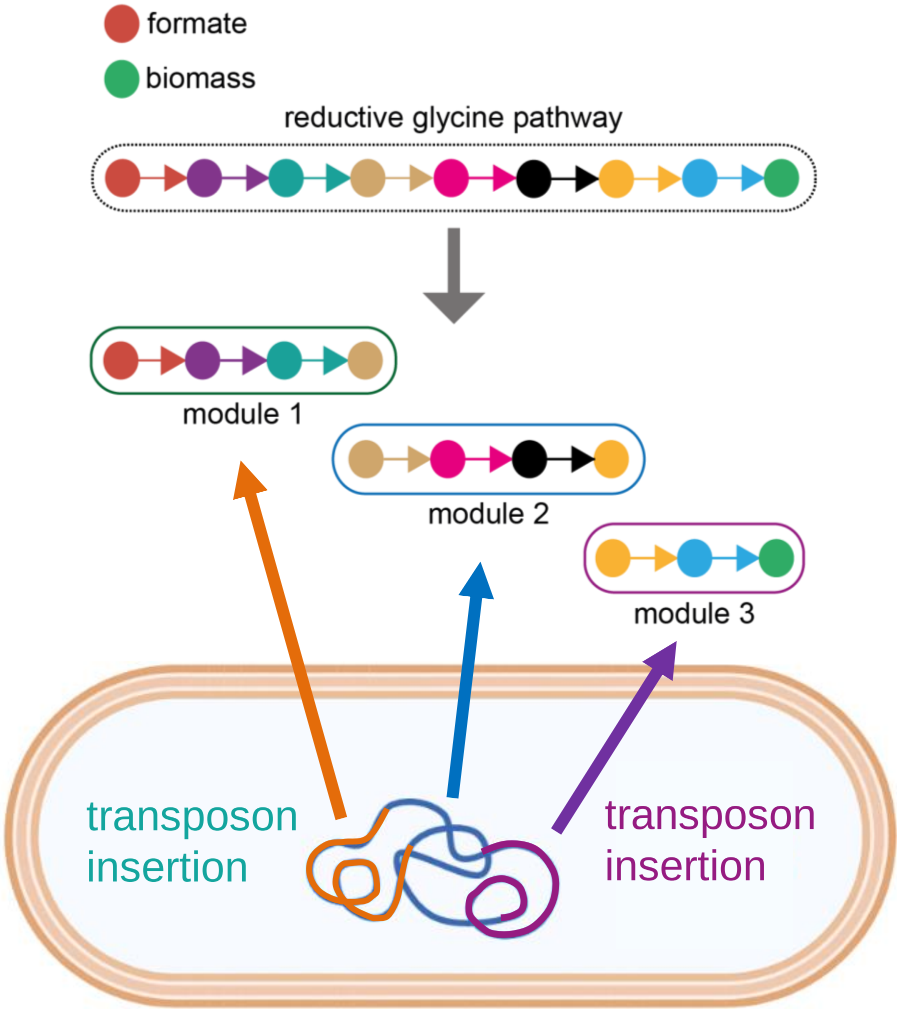
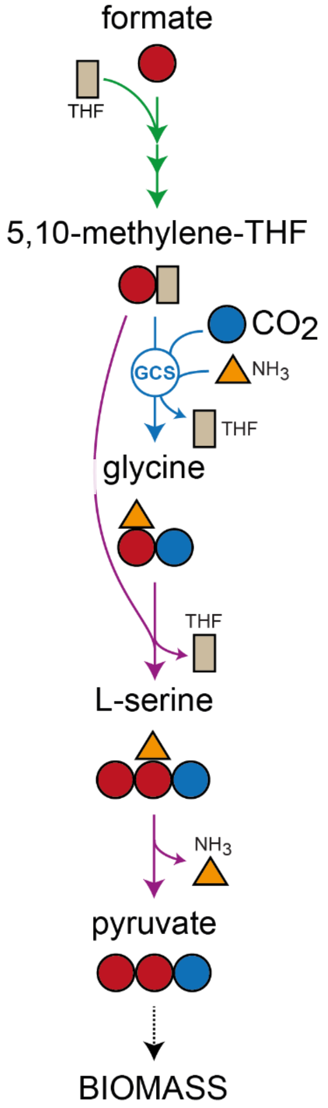
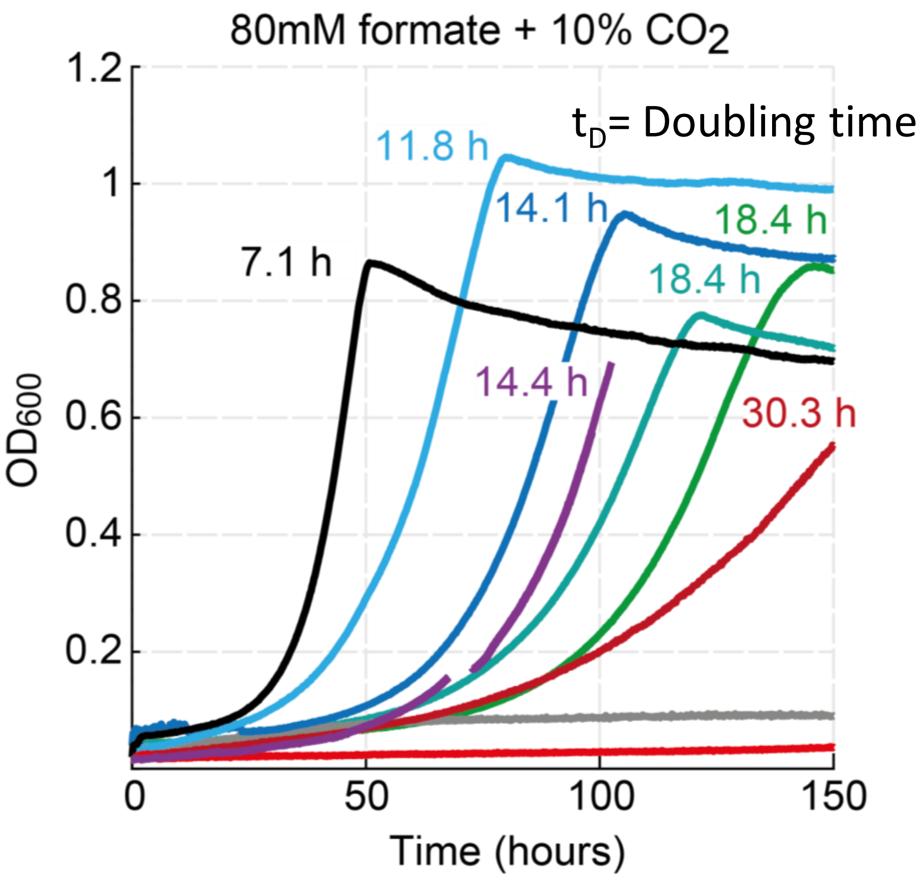


C. necator ΔcbbL

Fully genomic rGP





2nd passage shown

Product Synthesis via Plug and Play Modules

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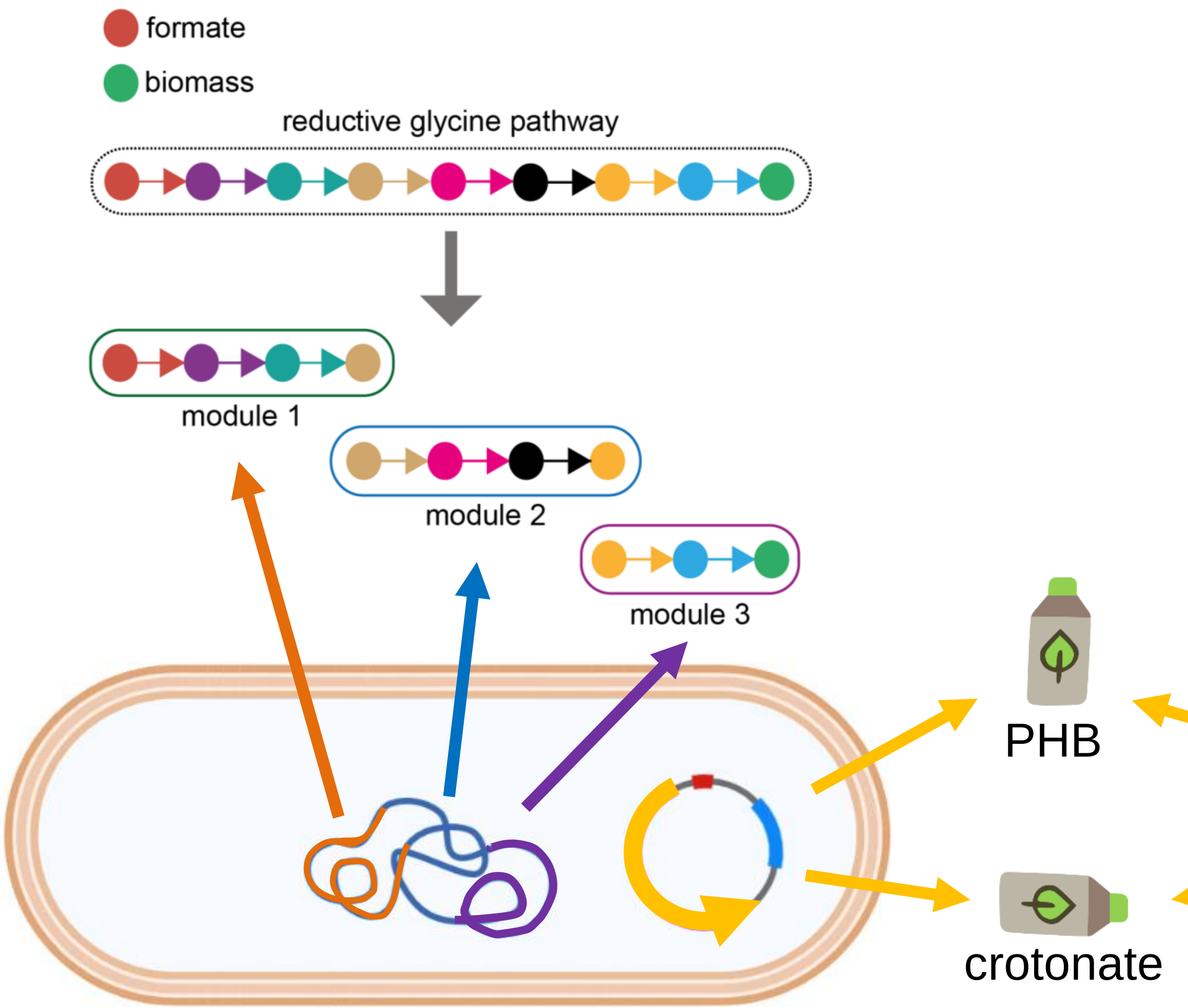
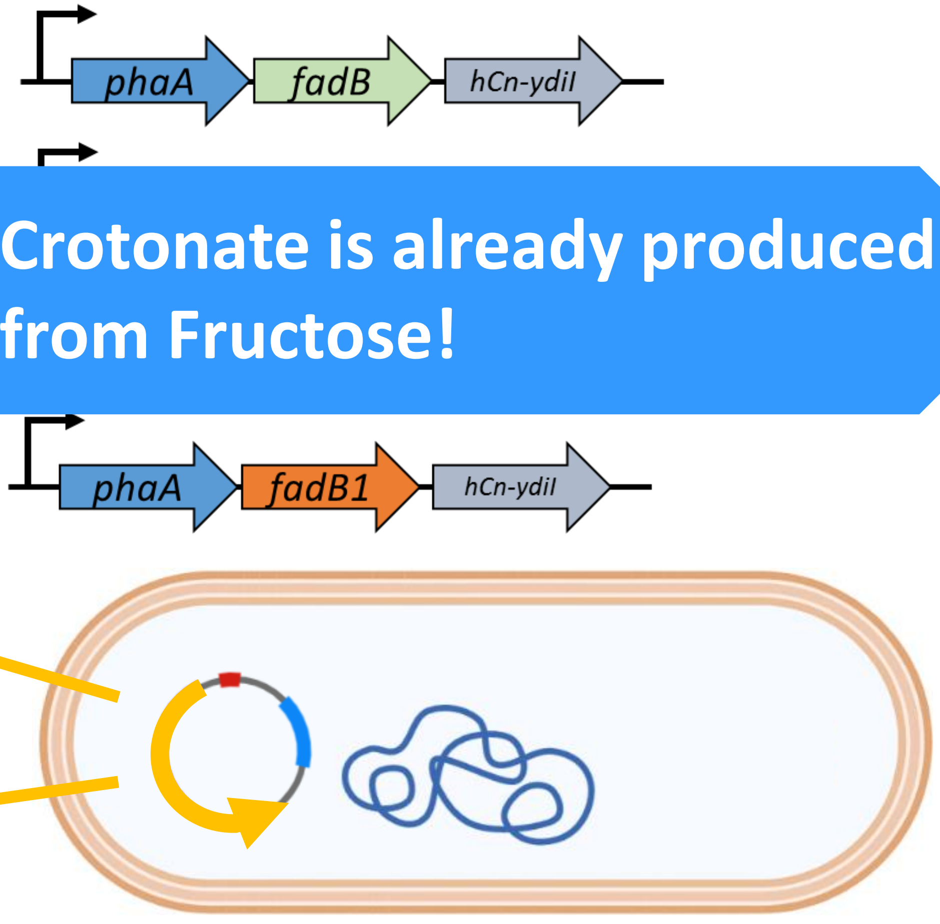


C. necator H16 with rGP

Test different crotonate operons



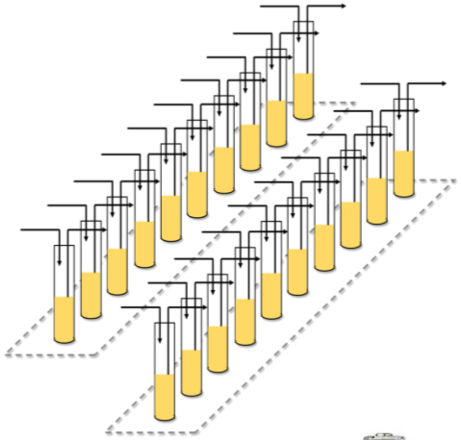
Crotonate is already produced from Fructose!

C. necator H16 with Calvin cycle

Next steps in Bioprocess Development

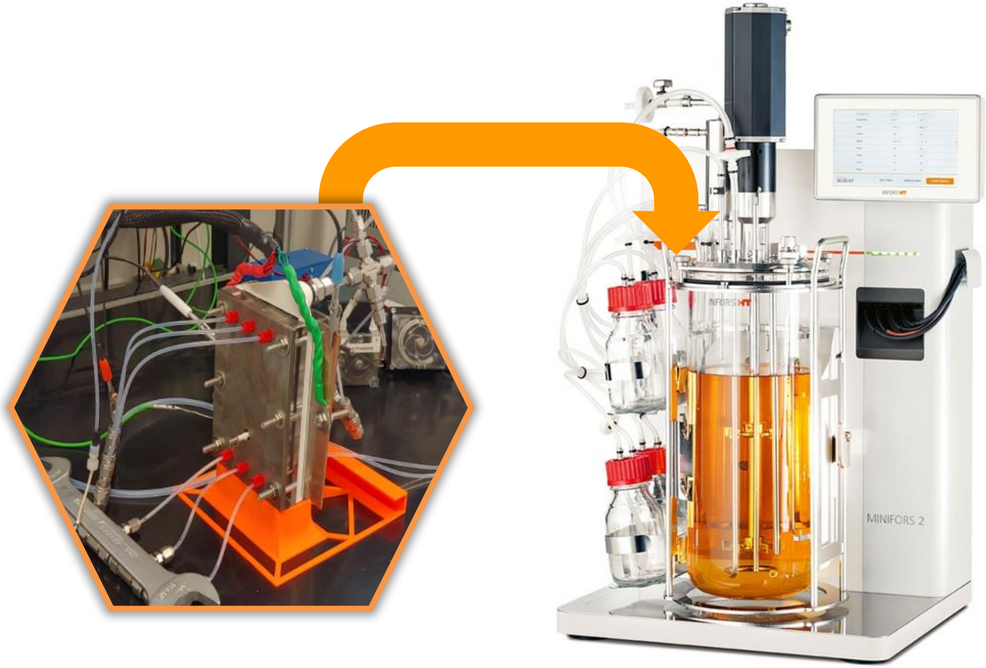
Evolution of synthetic strains for faster growth

Minibioreactors (5-10mL)
Adaptive laboratory evolution

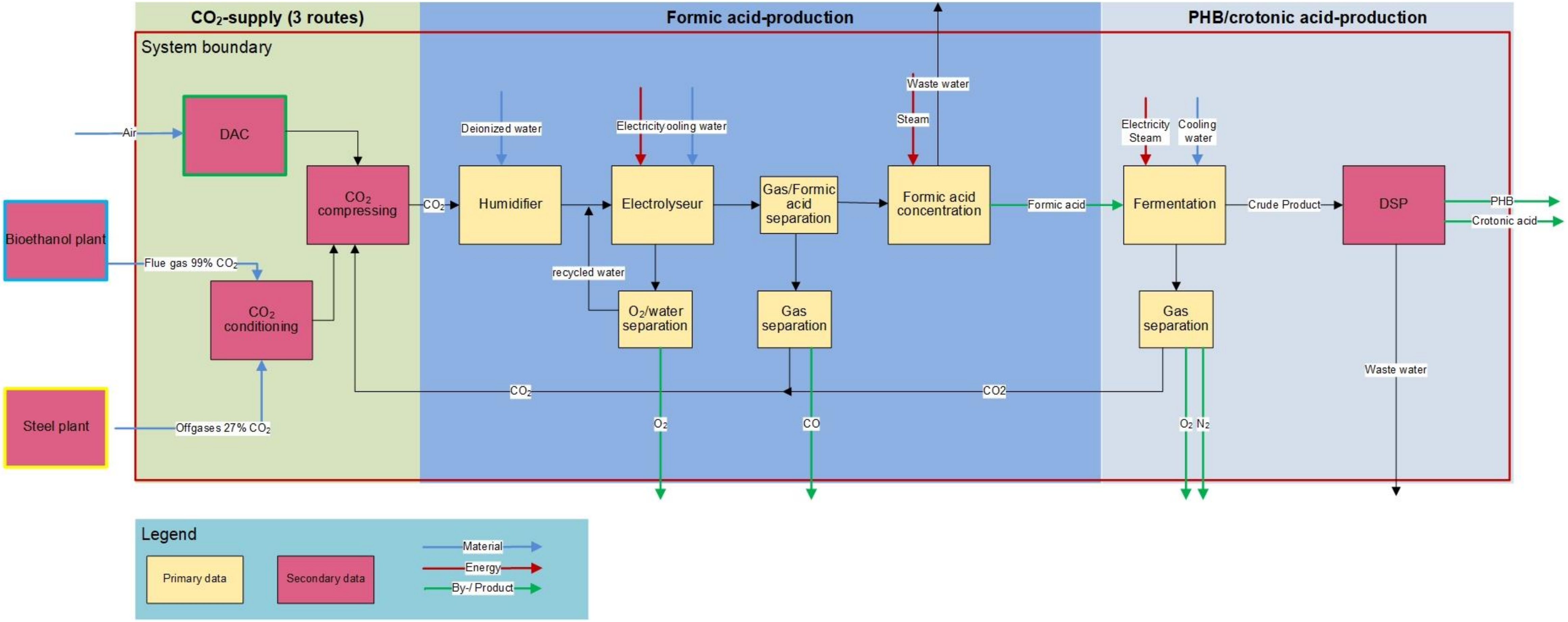


Bioprocess Characterization & Optimization

Prozess Integration - Electrolyzer & Bioreactor at laboratory scale (2L)

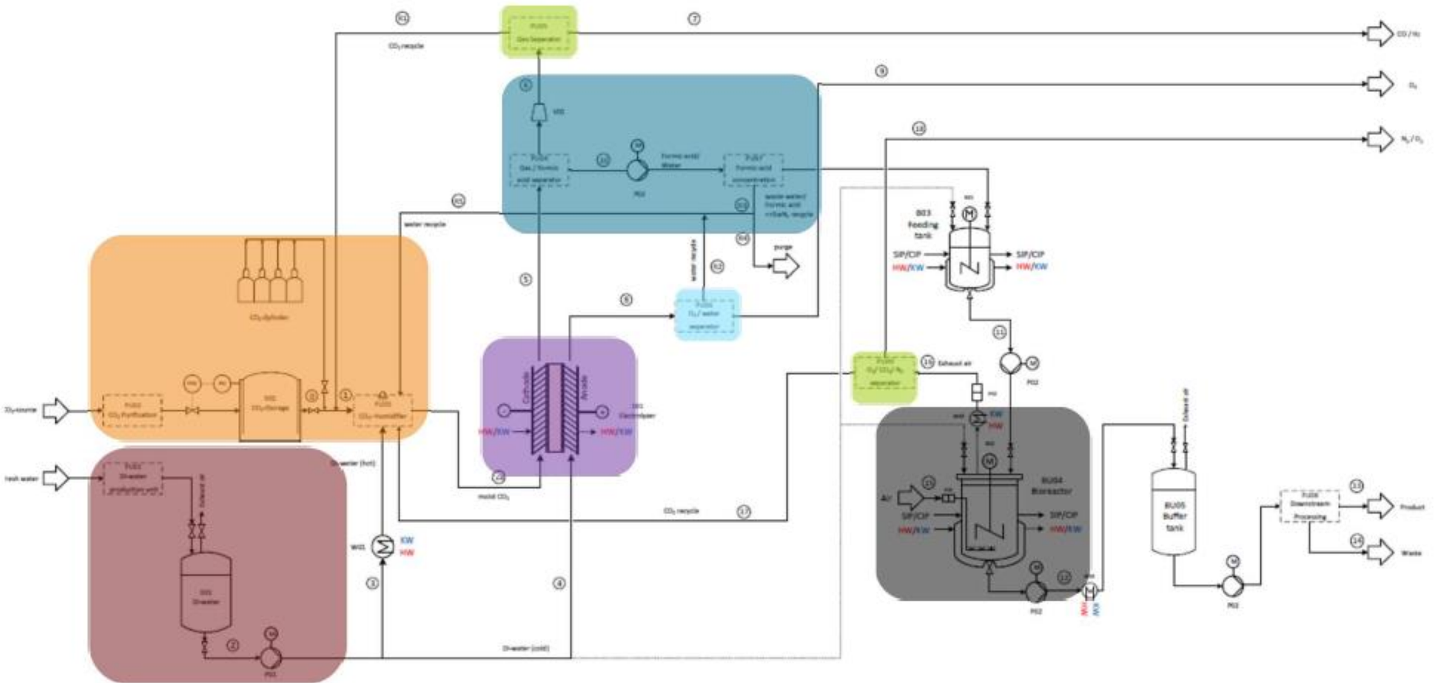


LCA - System Boundaries of TRANSFORMATE Process

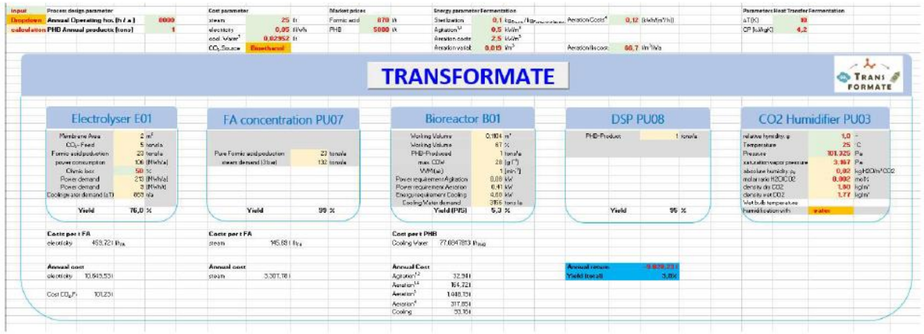


TEA & Conceptual Design

Process Flow Diagram



Dashboard - Mass and Energy Balance



Summary

- First laboratory CO_2-Electrolyzer built and operated
- 7 different PILs synthesized and tested
- Electrolyzer MEA design is very promising for selective production of formic acid
- Synthetic pathway (rGP) established in *Cupriavidus necator*
- First product (crotonate) synthesized in *Cupriavidus necator*
- LCA model and system boundries fixed
- Process flow diagram and process dashboard established

Outlook

- Design and construction of a stackable MEA electrolyzer
- Selection of the best PIL for formic acid production
- Optimization and stabilization of electrolyzer performance
- Improvement of cell growth on synthetic pathway via evolution
- Implementation of product synthesis (PHB and crotonate) on new pathway
- Process integration of electrolyzer and bioreactor
- Establishment of a full LCA and TEA

Thank you for your attention!

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