# What is microbial resource management? How can we optimize the process by applying microbial resource management?

**LIFE CO2toCH4**

PPC Renewables

ARISTOTLE UNIVERSITY OF THESSALONIKI

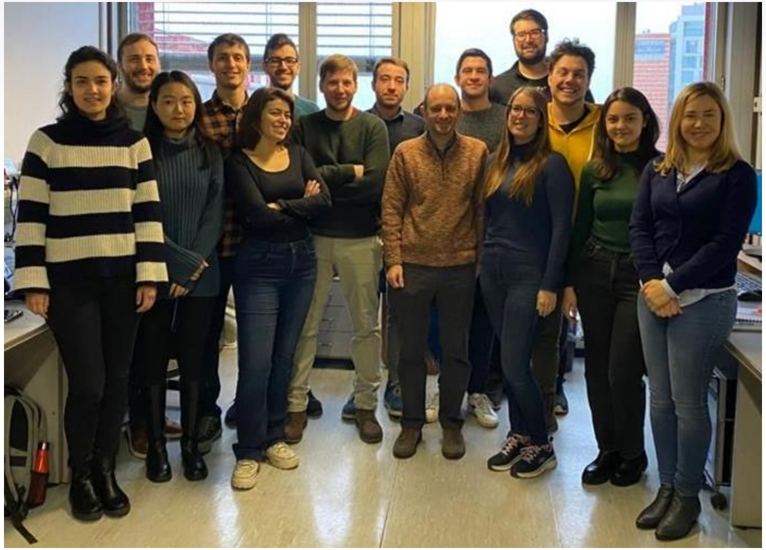
HELLENIC AGRICULTURAL ORGANIZATION- DEMETER

NEVIS INNOVATE WITH US

# Meet the UniPD partner

The **University of Padua** is one of the **oldest** in Italy and Europe, it is ranked within the **best 100 universities in Europe**. In the last evaluations of the Italian research centers, DiBio and DAFNAE ranked among the first three and the first, respectively, in their related fields.

We are affiliated to the **Genomics and Bioinformatics research unit** and the **Waste to Bioproducts-Lab.** Our research unit is equipped with **state-of-the-art technologies** for "omic analyses", that combined with microbial resource management expertise will meet the project requirements



UNIVERSITÀ DEGLI STUDI DI PADOVA

@bioinf.omics.unipd

# UniPD team on CO2toCH4

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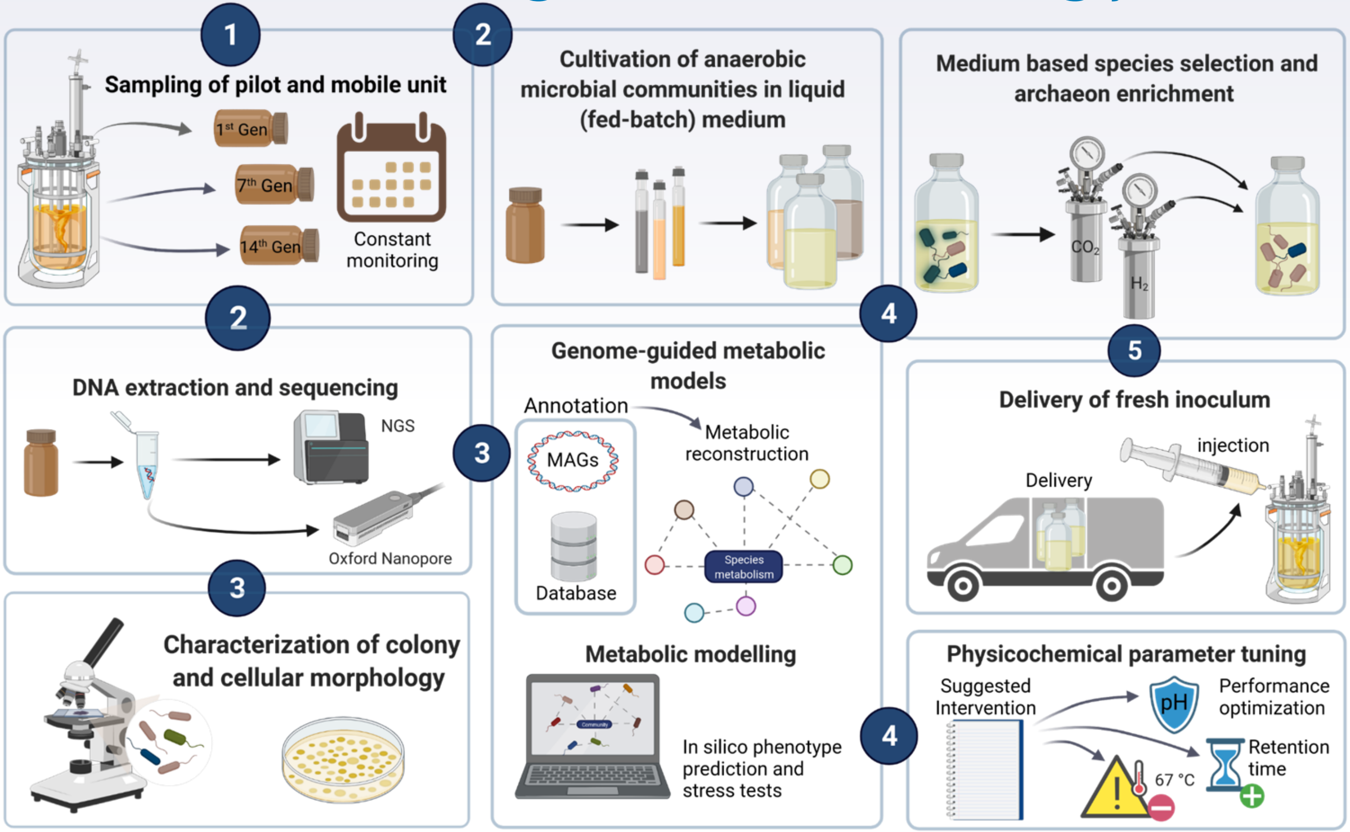
# Microbial resource management strategy

Monitor microbial community and plan strategies for process optimization:

- ***shifts in microbial composition***
- ***fine tune of working parameters***

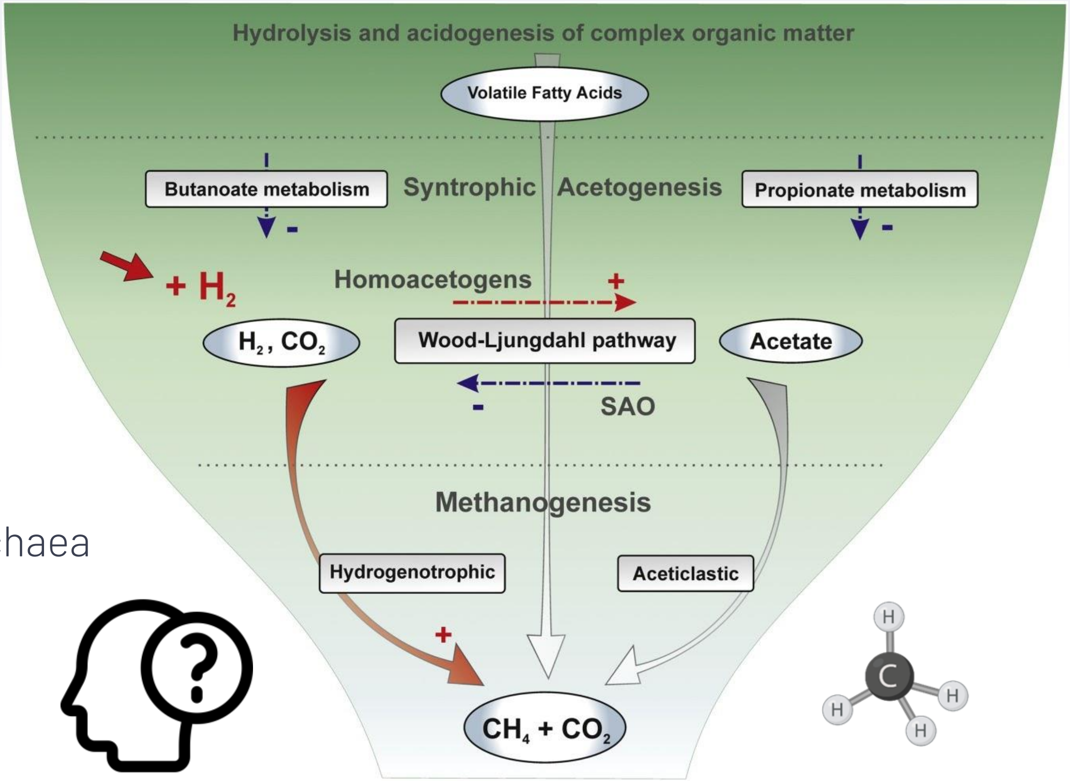


# Background: methanogenic routes

Biological fixation of $CO_2$ with the use of external $H_2$ can follow different metabolic routes:

**Hydrogenotrophic methanogenesis**
archaea directly convert $CO_2$ to $CH_4$

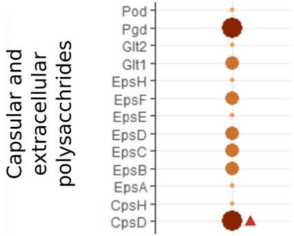
**Homoacetogenic bacteria**
convert $CO_2$ to acetate
-> **OK** if acetoclastic methanogenic archaea convert the acetate into $CH_4$
-> **NOT OK** if acetate accumulates in the system

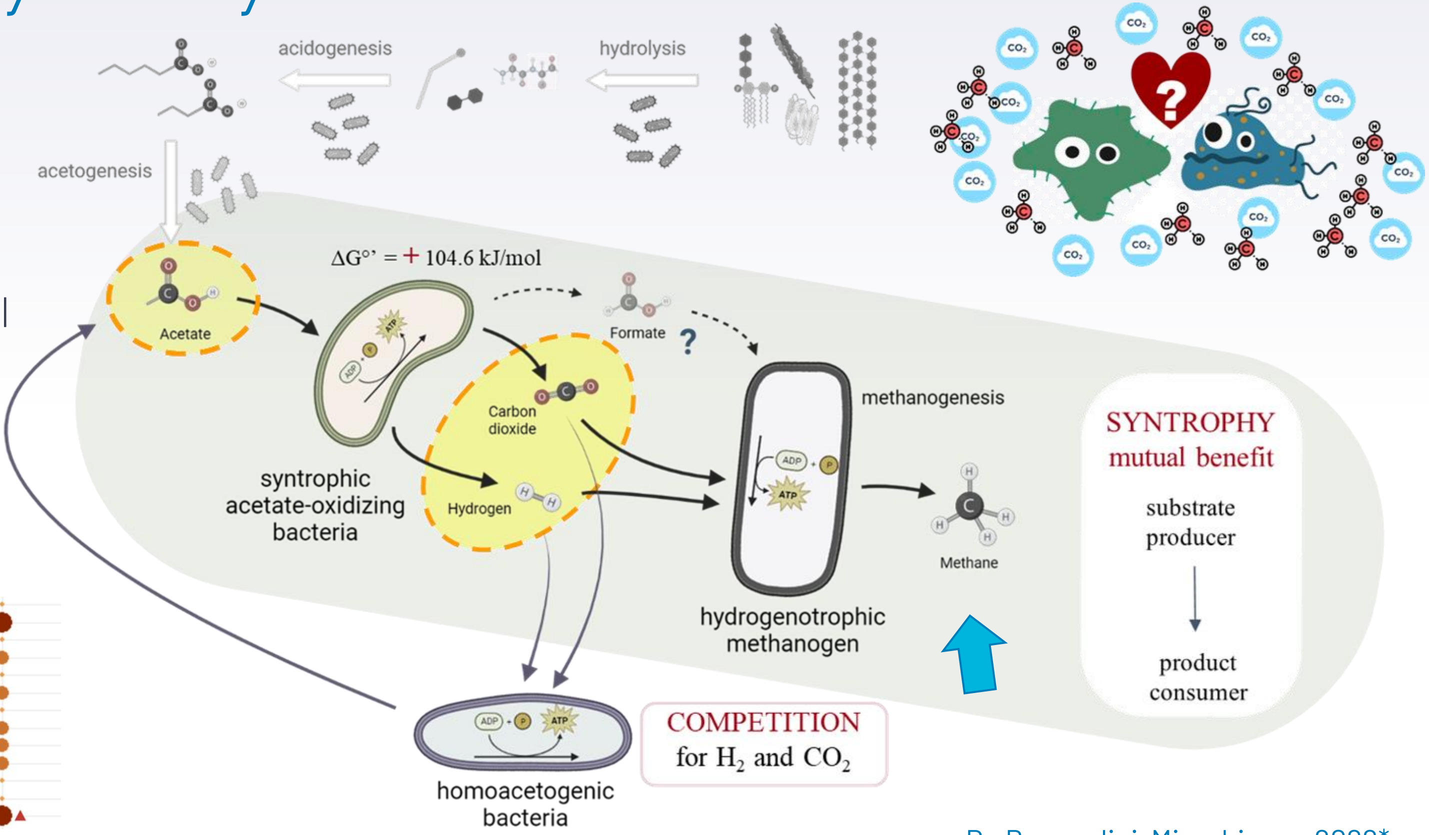



Angelidaki, Biotechnology advances, 2018

# Preliminary study: biofilm microbiome

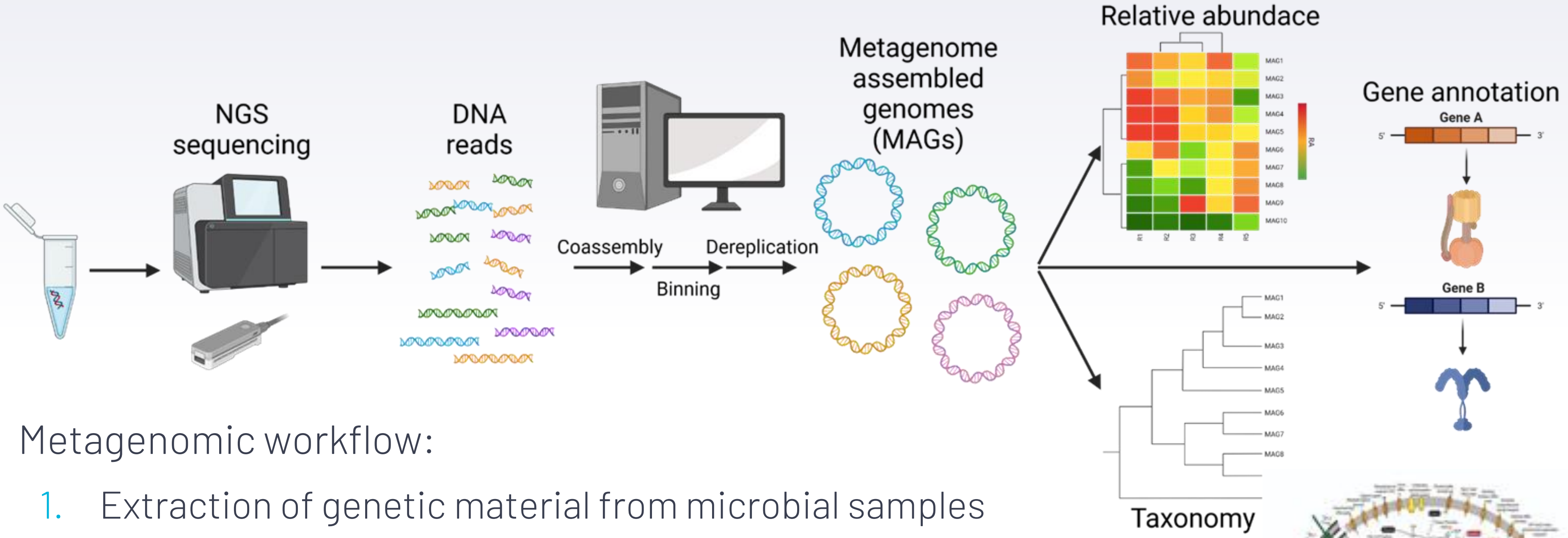
Fundamental role of bacteria in **biofilm** formation



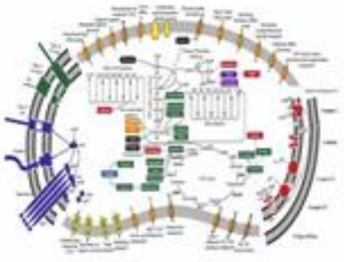

De Bernardini, Microbiome, 2022*

# Methods: microbiome characterisation



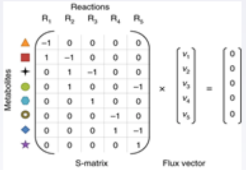

Metagenomic workflow:

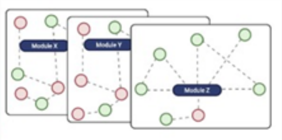
1. Extraction of genetic material from microbial samples
2. Sequencing and metagenomic analysis
3. Abundance, taxonomy and function of species

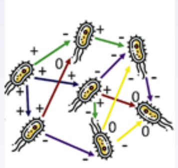


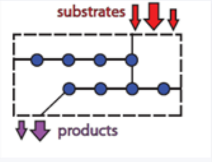
**How many - Who - What**

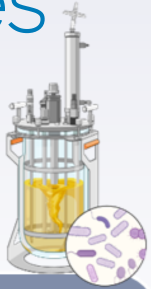
# Methods: microbial control architectures







Genome-scale metabolic models GEM reconstruction

Fill gaps in the model by improving gene annotations

*In silico* community-level simulations with defined medium

Condition-specific modelling for cross-feedings prediction

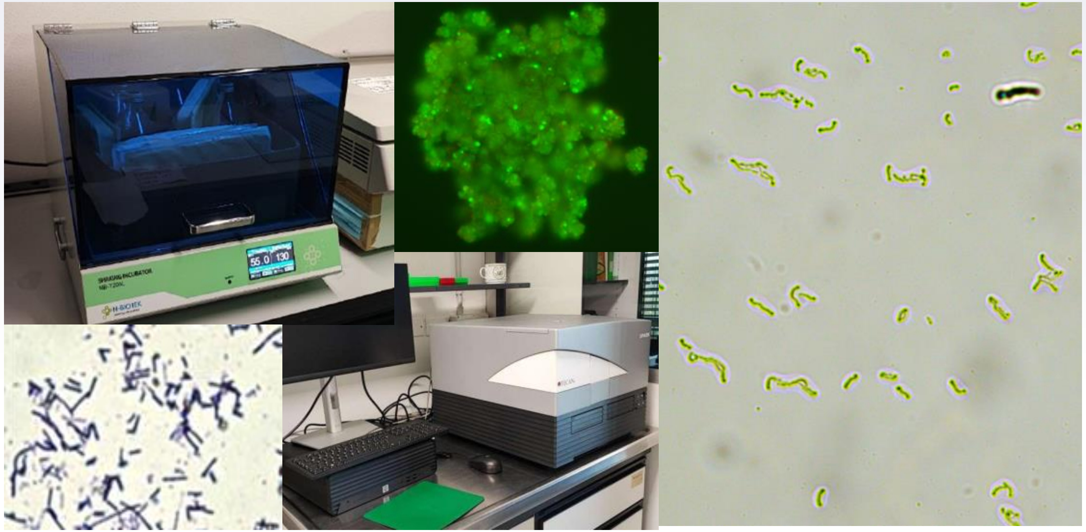
Avoid potential inhibition from process perturbation

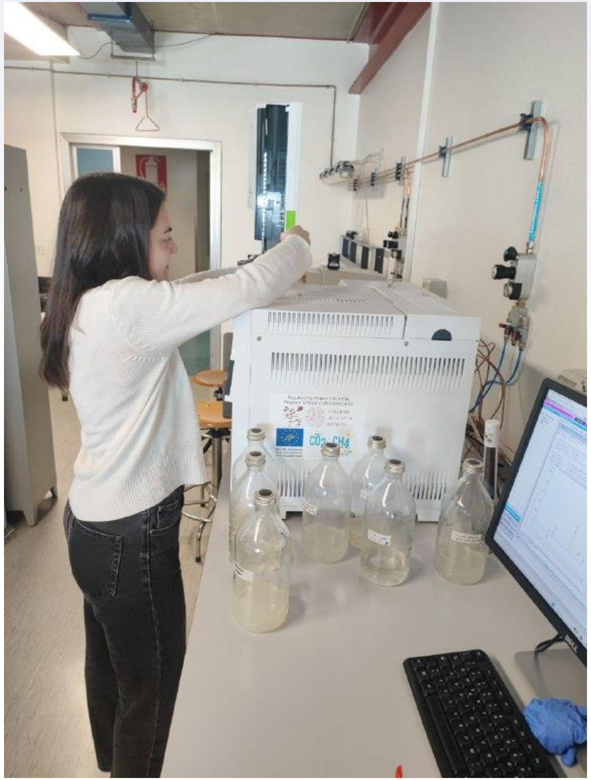
Modelling workflow:

1. Microbial metabolic **modelling for system prediction**
2. Integration of biochemical **data** from reactor operation, i.e. VFA, $CH_4$ content, pH
3. **Strategy** planning for avoiding potential system inhibition

Palù, Computational and Structural Biotechnology Journal, 2022*

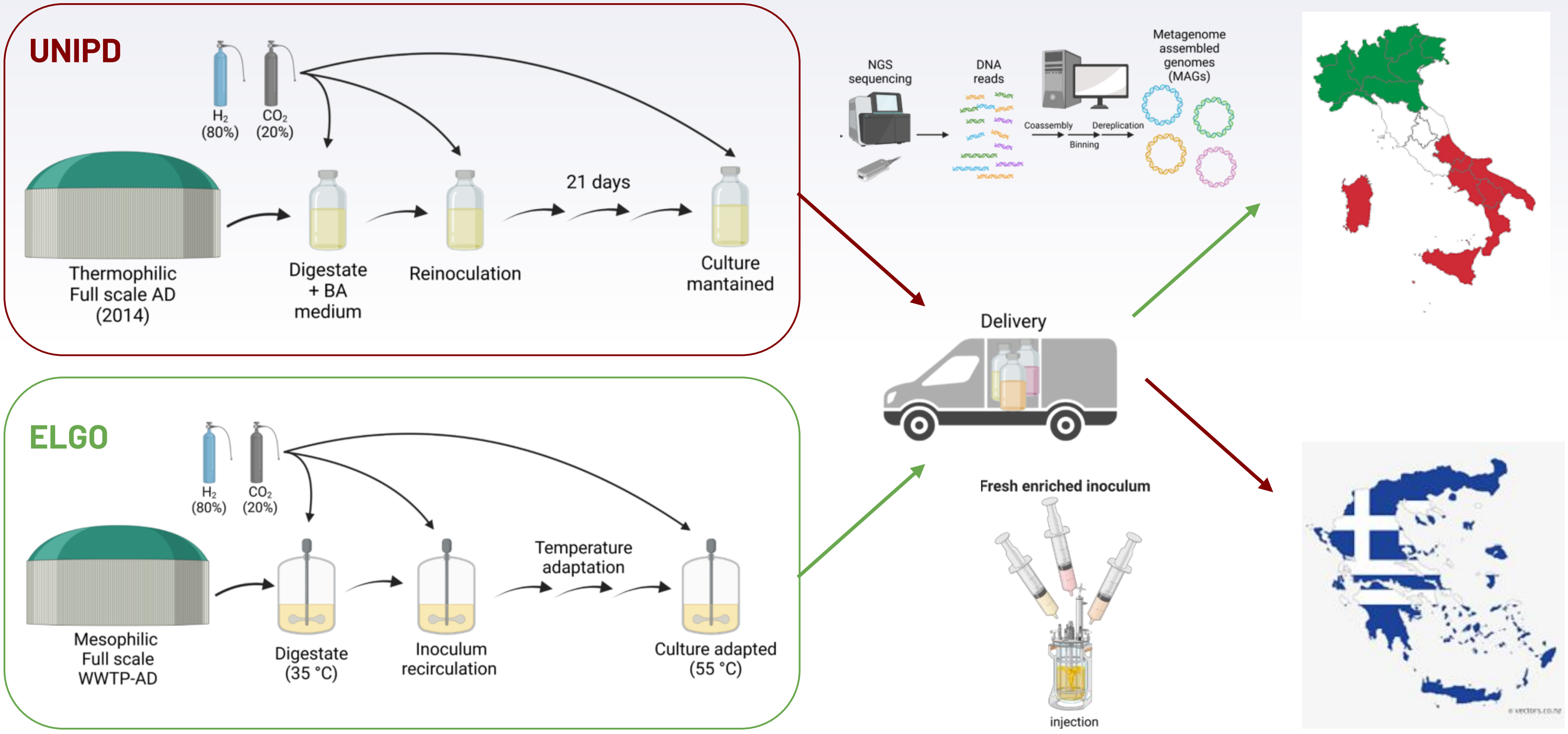
# Methods: ongoing work



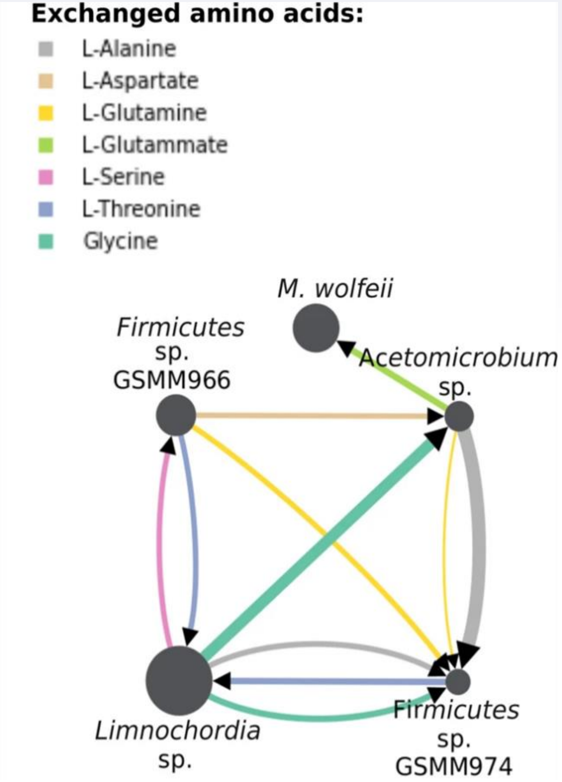


- Maintenance of anaerobic microbial **inoculum** at 55 ° C
- **Microscopy** for morphology and live characterization
- Gas chromatography and optical density are used to verify microbiota **performance** in $CO_2$ methanation

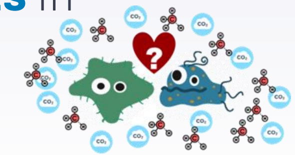
# Microbial Inocula of CO2toCH4



# Old Inoculum: flux balance analysis



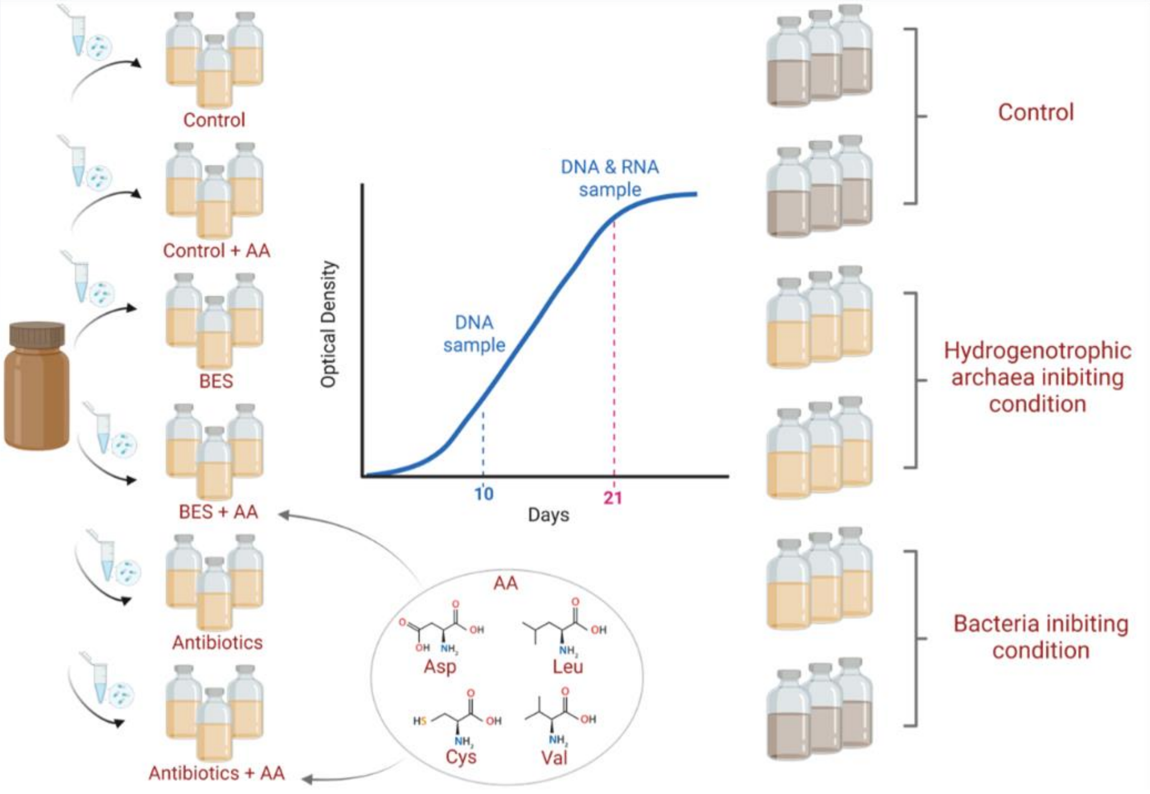

The preliminary study suggested a crucial role of **amino acids** and other **micronutrients** in syntrophic interactions during $CO_2$ methanation.



- L-Glutamate is showed as the main amino acid exchange between *Acetomicrobium* sp. (bacterium) and *Methanothermobacter wolfeii* (archaeon).
- Other amino acids were hypothesized to be important in the exchanges between the archaeon and several bacteria (e.g. Aspartate, Alanine, Leucine, Cysteine, Serine and Valine).

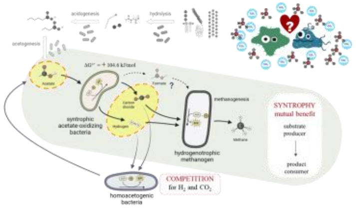
De Bernardini, Microbiome, 2022*

# Old Inoculum: test on amino acids

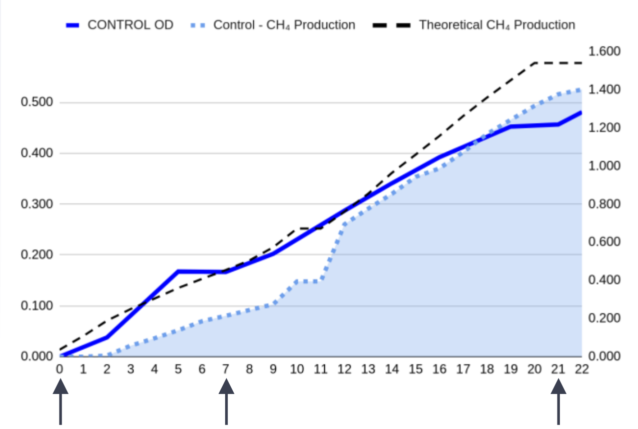


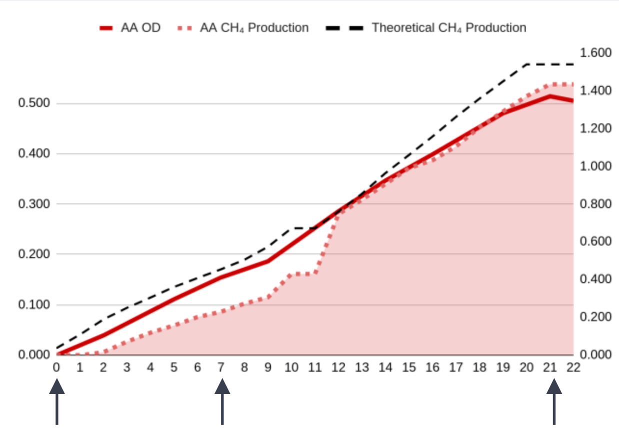

Plan of experimental setup to verify key metabolites involved in the methanation

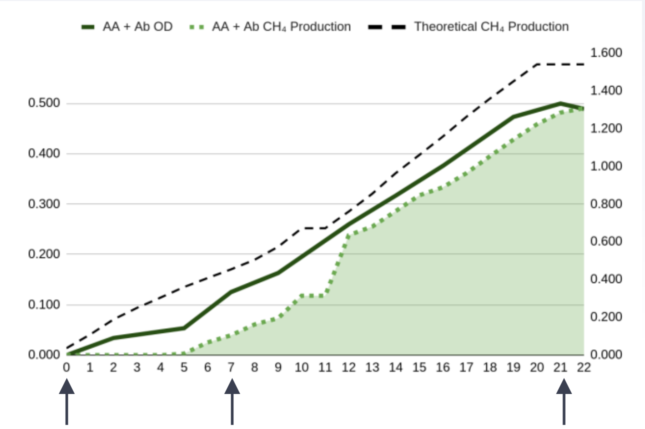
- Amino acids: Asp, Leu, Cys, Val.
- Antibiotics: Ampicillin and Penicillin
- Monitoring: gas composition, optical density , VFA and metabolites



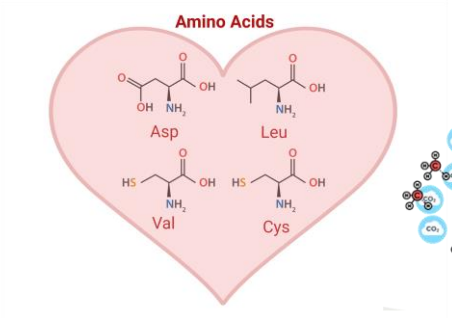
# Amino acids role in process stability

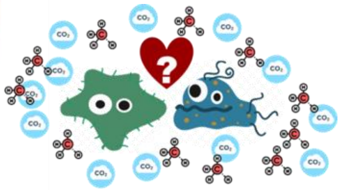


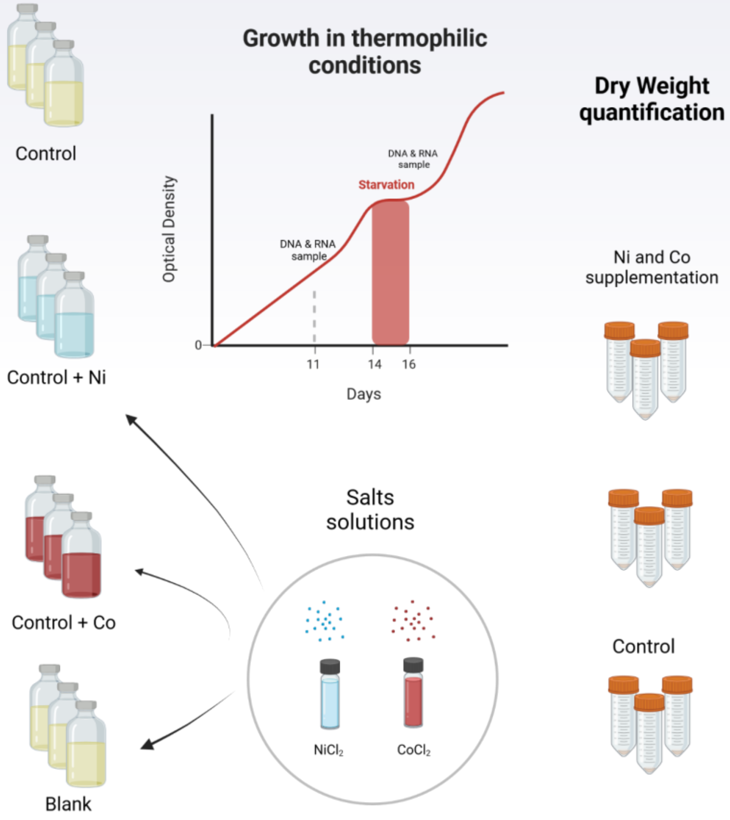




- Antibiotics without the addition of amino acids **killed the entire community**.
- The addition of amino acids maintained **stable the process** in depletion of bacteria, the microbiota could survive and produce $CH_4$



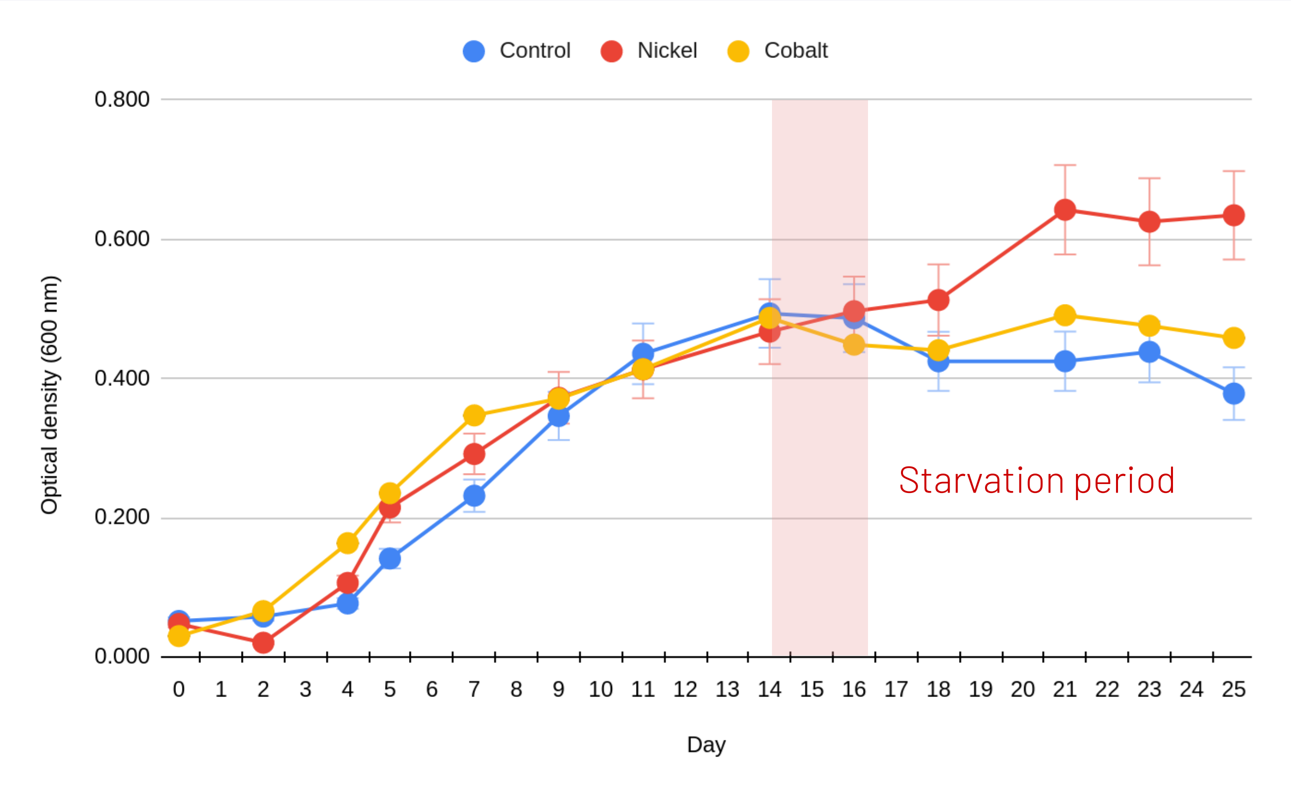
# Old Inoculum: test on micronutrients uptake




Plan of experimental setup to verify micronutrients involved in the methanation in standard feeding conditions and during $H_2$ and $CO_2$ starvation

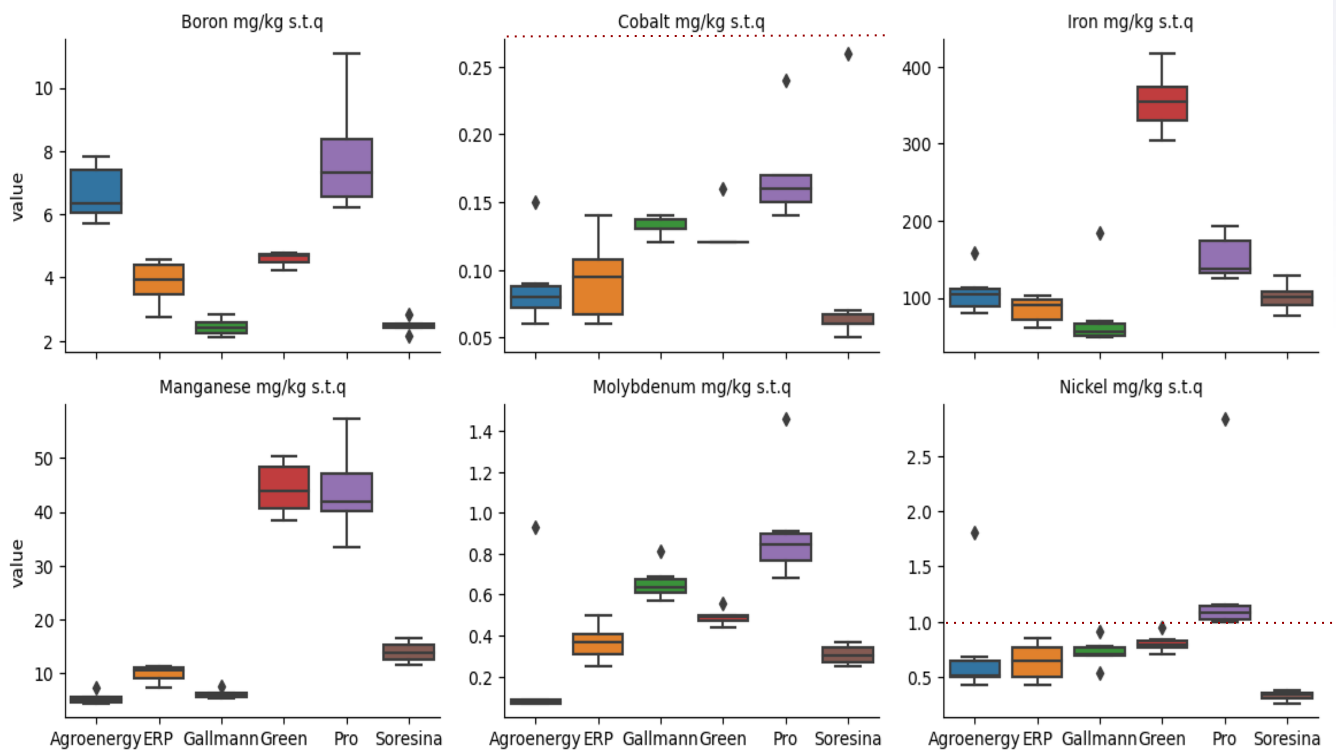
- Micronutrients: Cobalt, Nickel
- Monitoring: gas composition, optical density , VFA and metabolites

# Micronutrient role in process stability




- The addition of micronutrients (i.e. nickel and cobalt) to the feeding **improved** system performance.
- Especially **nickel** was fundamental in supporting the community **to face the stress period** of feeding starvation.

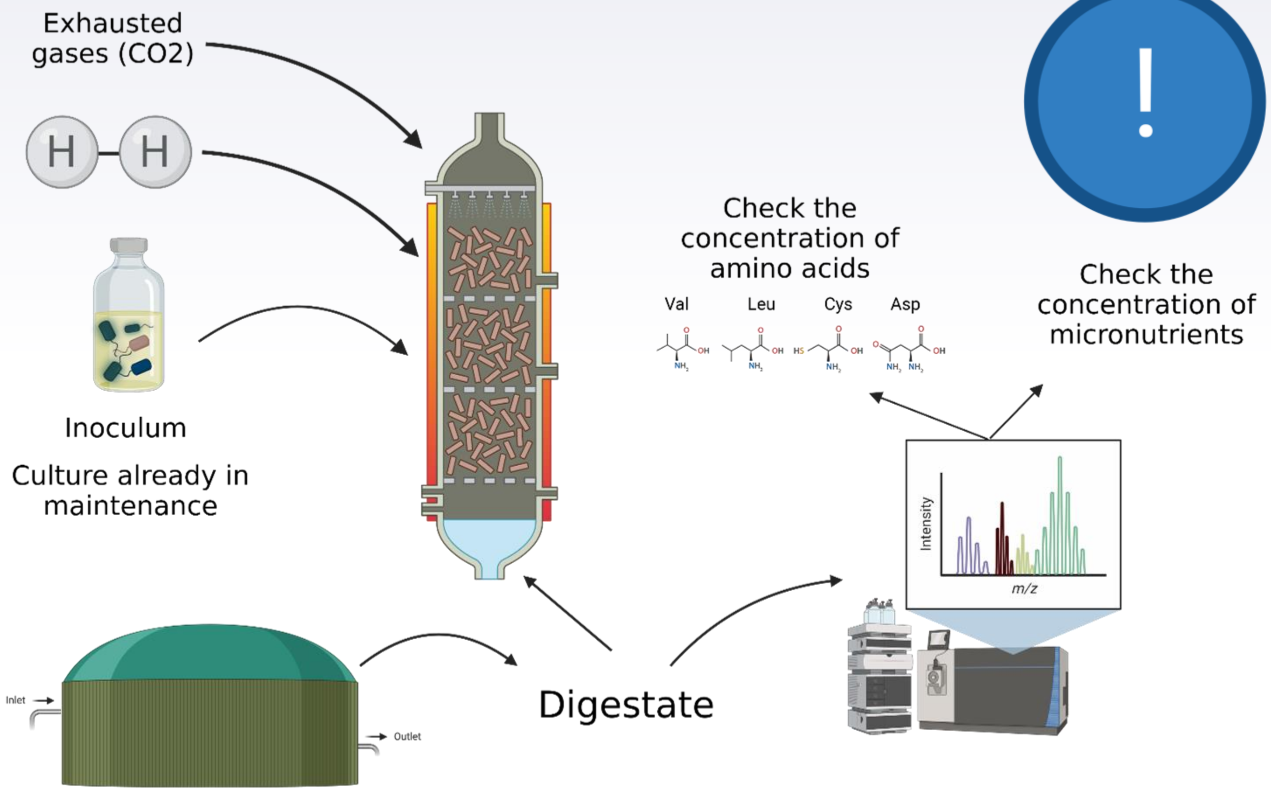
# Test for selection of suitable digestate



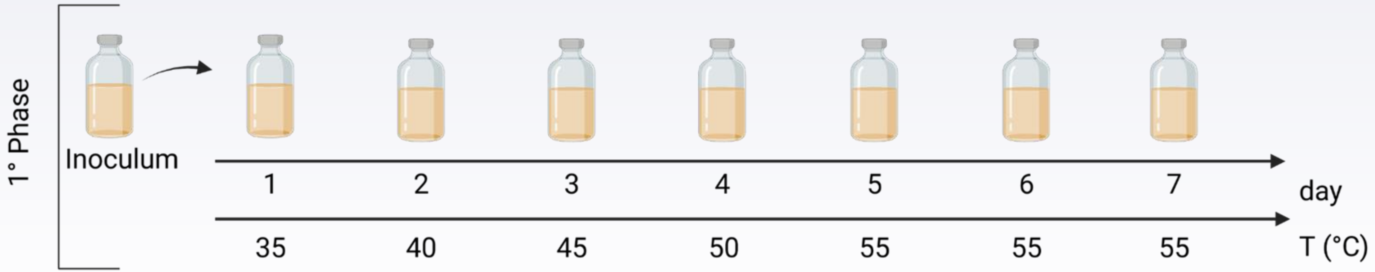

- Ni and Co were tested for threshold definition, approx. 1 mg/L
- Biogas plants (IT) monitor micronutrients concentration: not all digestates meet the requirements for optimisation
- IMPORTANT: check micronutrients concentration in trickling media for optimal efficiency!

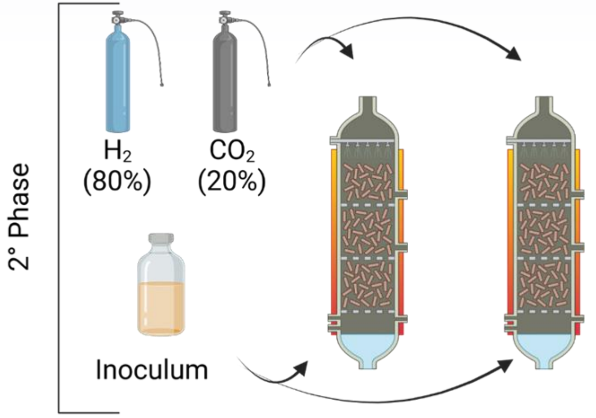
# Strategy proposal for $CO_2$ methanation

The **concentration** of micronutrients and amino acids (Valine, Leucine, Cysteine and Asparagine) must be measured in the trickling medium to **verify** the minimal requirements of the inoculum for process optimisation.



# Young Inoculum: from meso to thermophilic



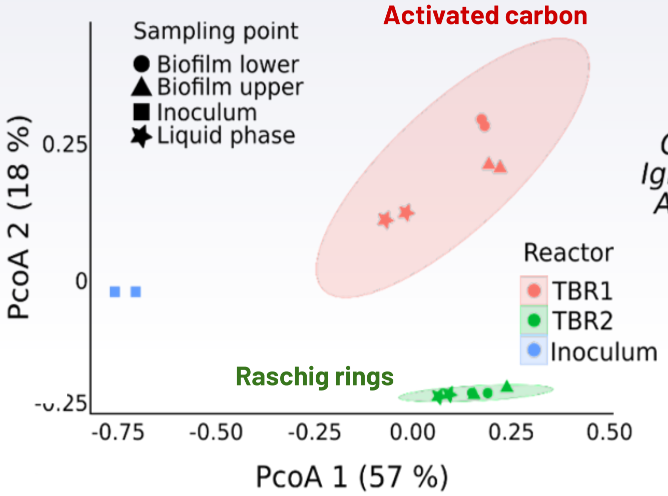

Adaptation from mesophilic to thermophilic condition

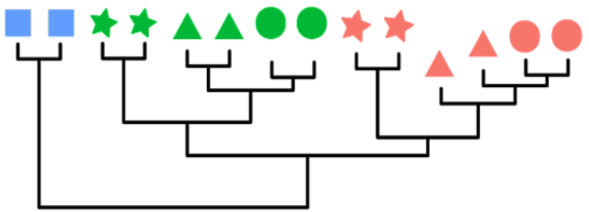
Biofilm formation test with two materials:

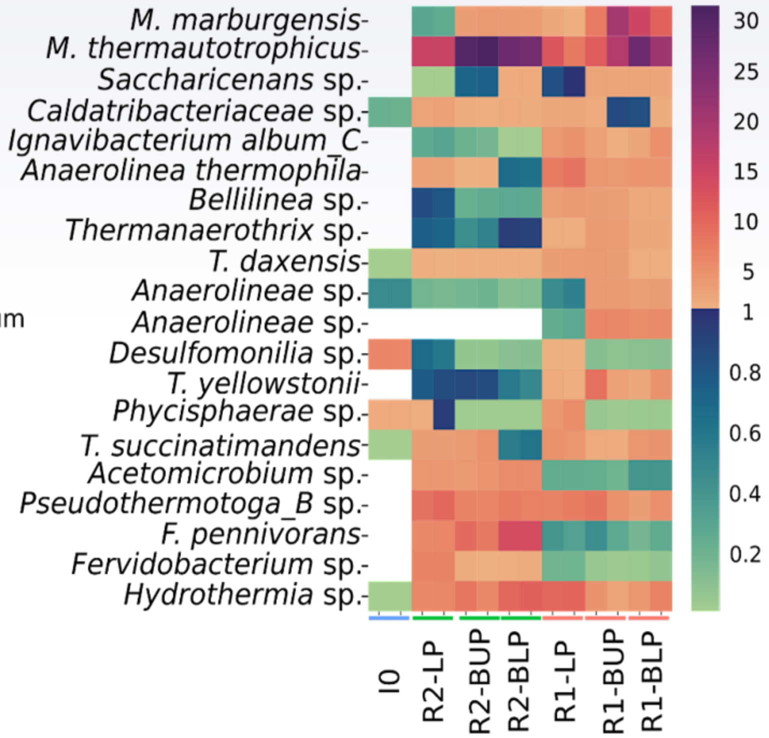
- Activated carbon (pellets)
- Raschig rings

Feeding conditions in Trickle Bed Reactors were 80% $H_2$ and 20% $CO_2$

# Young Inoculum: microbiome composition






Microbiota formed on activated carbon pellets and raschig rings were different.

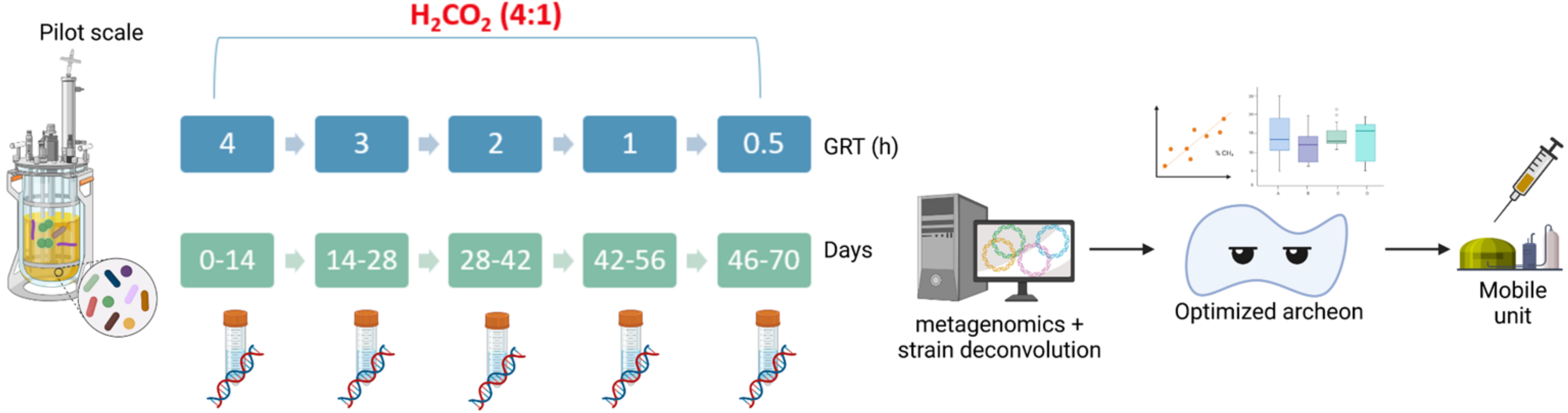
The dominant archaeon *M. thermautotrophicus* was extremely rare in the inoculum.

# Young Inoculum: GRT reduction

The young inoculum is used in the prototype unit at low GRT, subsequently increasing the inlet gas flow rate will provide higher $CH_4$ production

**PROBLEM**: risk of limiting the solubility and mass transfer of $CO_2$ and $H_2$ into the liquid

- AIM: enhance the efficiency of the biomethanation process
- OUTCOME: obtain a microbial consortia with high biomethanation potential

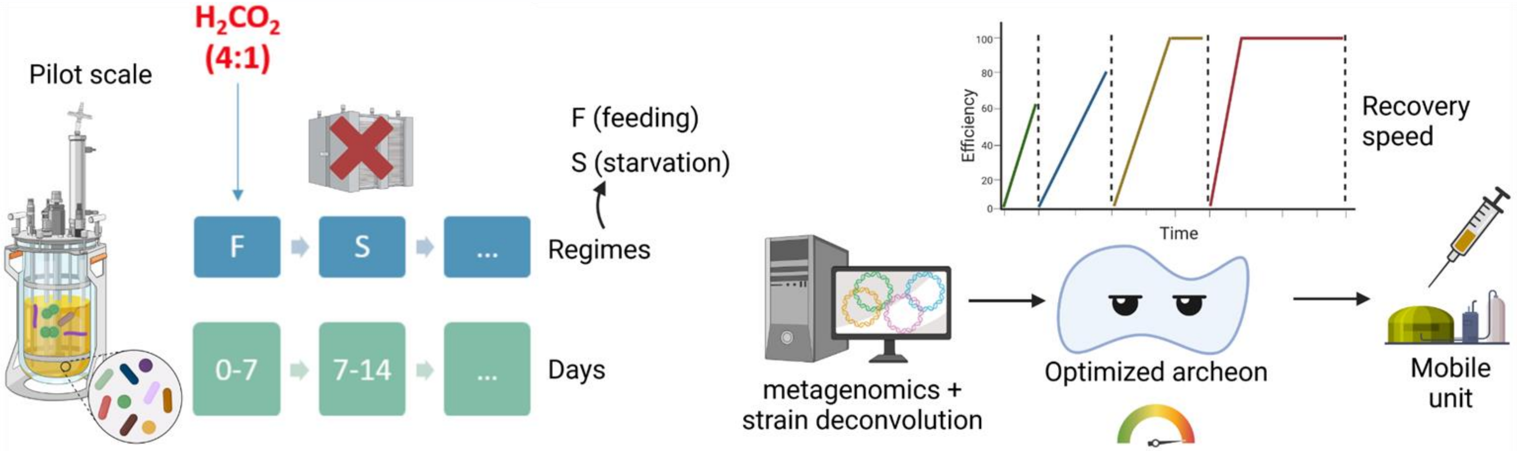


# Future perspectives: Feeding-starvation

The surplus of renewable energy is needed to produce $H_2$ through $H_2O$ electrolysis

**PROBLEM**: this surplus will not always be available → the $CH_4$ production will be stopped and the microbial community will need time to recover after a starving period

- AIM: enhance the productivity of the methanation process
- OUTCOME: obtain a microbial consortia able to recover faster from starvation



# Questions?

Thanks for your attention!