10th International Conference on Sustainable Solid Waste Management Chania, Greece, 21 - 24 JUNE 2023

Metagenomic analysis on hydrogen assisted carbon dioxide fixation for biomethane production

M. Gaspari[1], A. Chatzis[1,2], E. Orellana[3], L. Treu[3], K. Kontogiannopoulos[1], S. Campanaro[3], A. Zouboulis[2], P.G. Kougias[1]

[1]Soil and Water Resources Institute, Hellenic Agricultural Organization Dimitra, Thermi, 57001, Greece

[2]Laboratory of Chemical & Environmental Technology, Dept. of Chemistry, Aristotle University of Thessaloniki, Greece

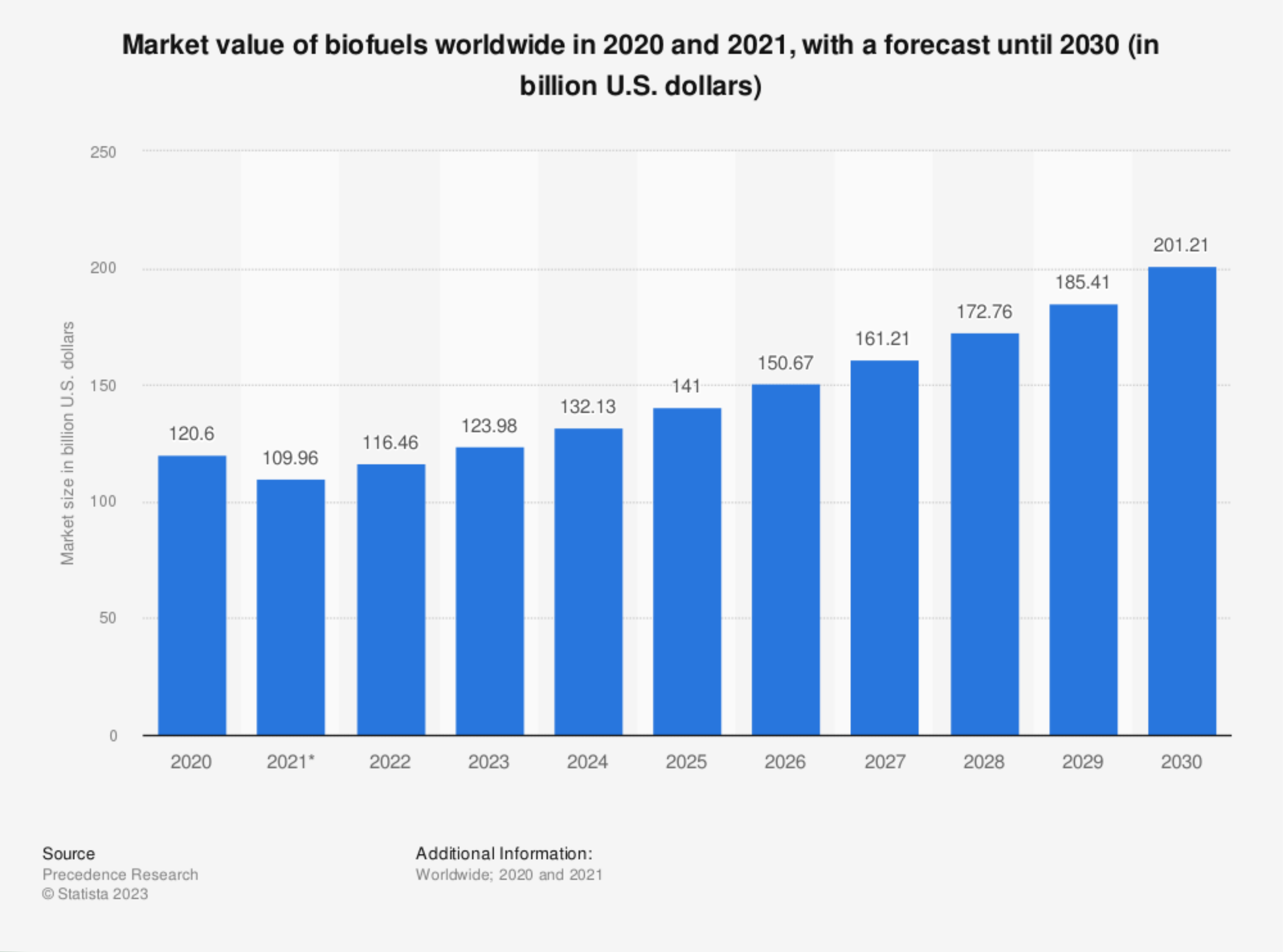
[3]Department of Biology, University of Padova, Padova 35121, Italy

p.kougias@swri.gr

@kougias

0000-0003-4416-2135

Biofuels as a source of biogenic emissions



→ **Increased Biogenic Effluent Gases**

Main routes of biogenic effluent gases production

Anaerobic digestion

Anaerobic digestion is a complex biological process in which microorganisms break down organic matterin the absence of oxygen. This process leads to the production of biogas as an end product.
Main biogenic emissions: CH_4, CO_2 and N_2O

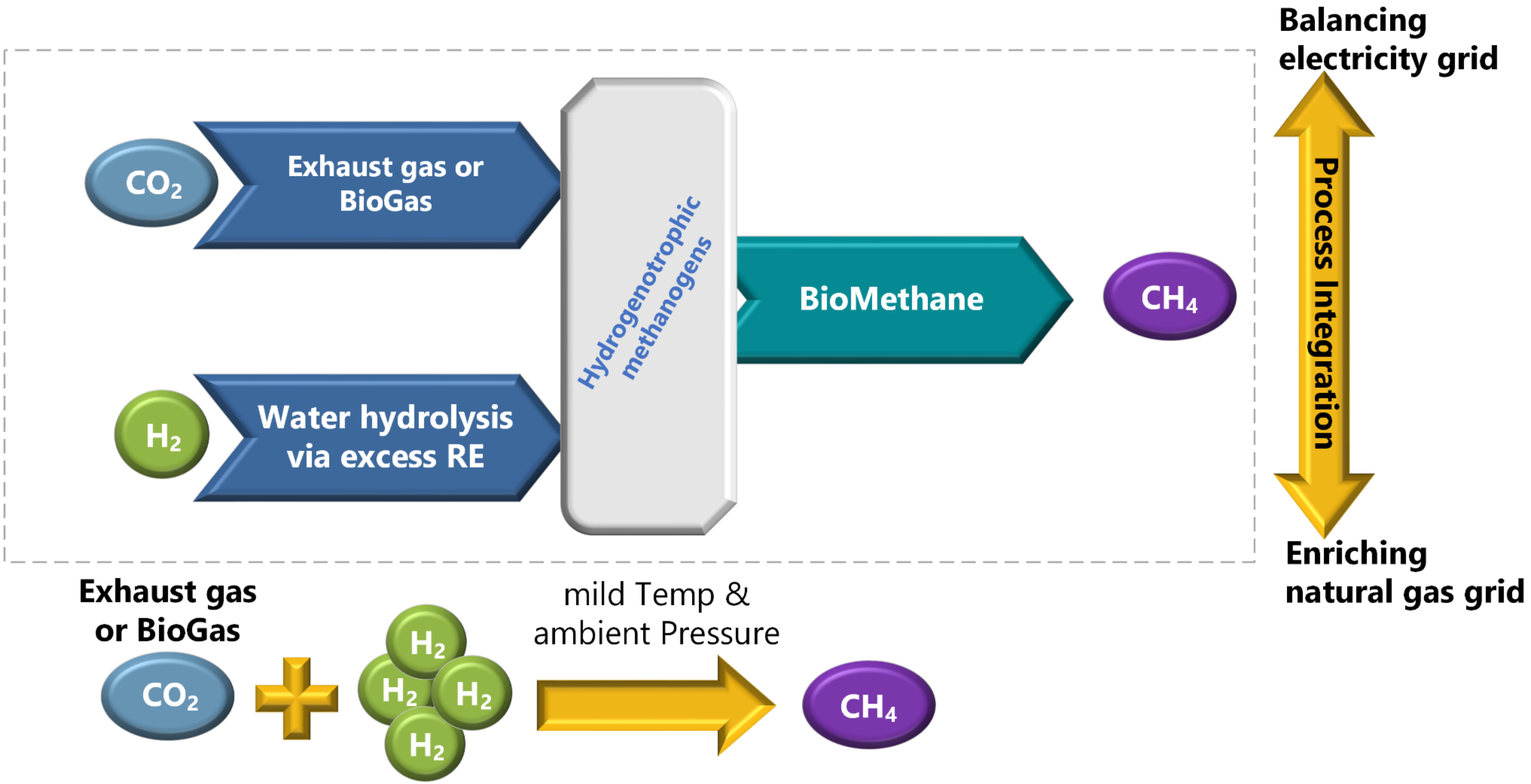
Ethanolic fermentation

Ethanolic fermentation, also known as alcohol fermentation, is a metabolic process in which microorganisms, such as yeasts, convert sugars into ethanol (ethyl alcohol) and carbon dioxide in the absence of oxygen.
Main biogenic emissions: CO_2

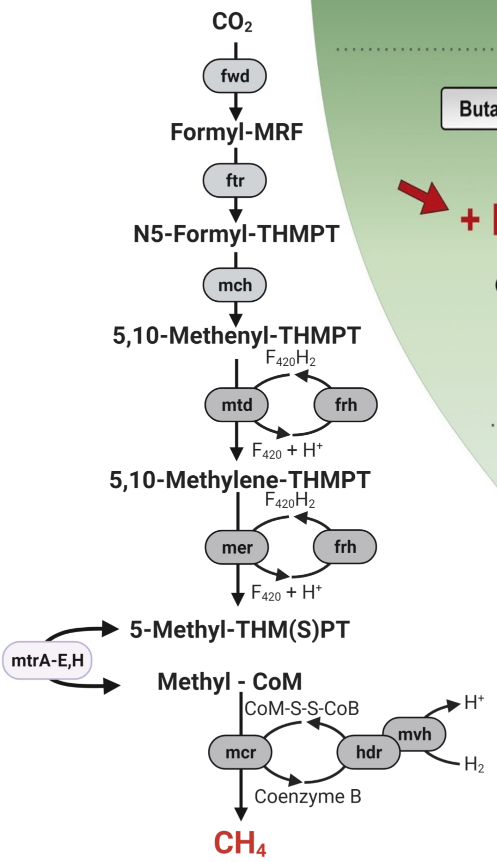
Thermochemical processes

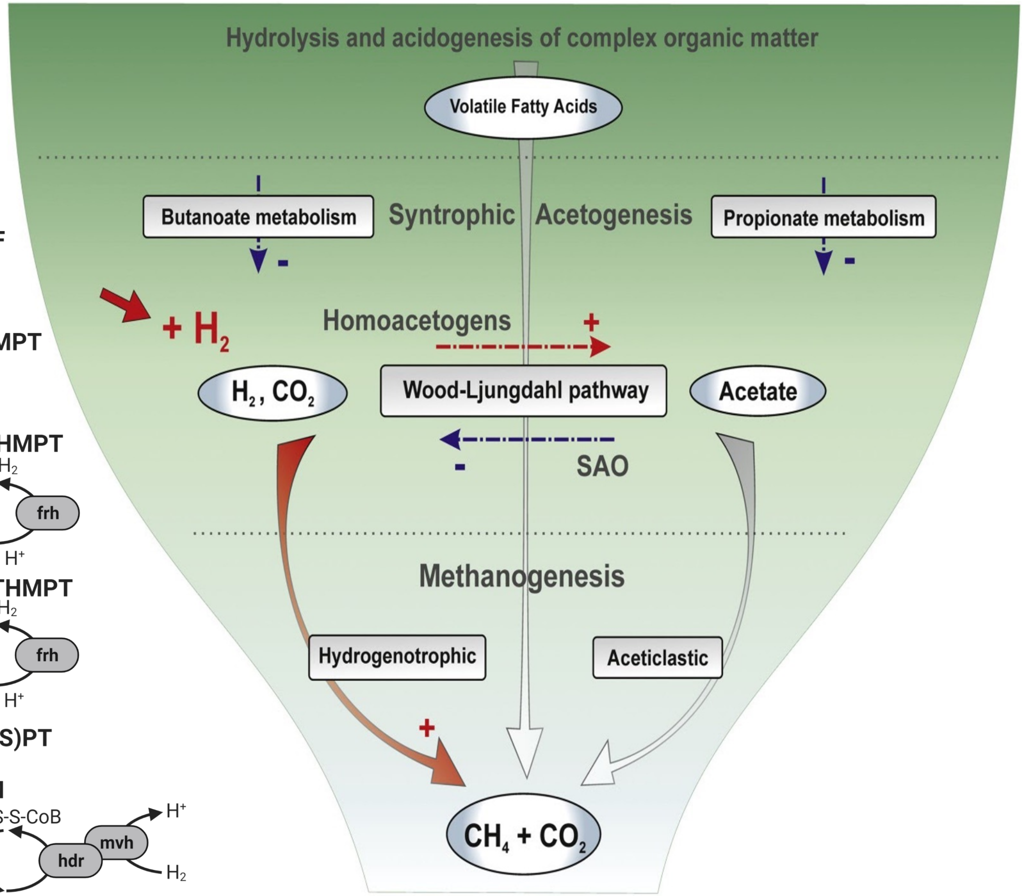
Thermochemical processes encompass various technologies, including pyrolysis, gasification, and liquefaction. The "bio" aspect in thermochemical processes comes from the use of biomass as the feedstock.
Main emissions: CO_2 and CO

H_2 assisted carbon dioxide fixation for biomethane



Important aspect for efficient biomethanation





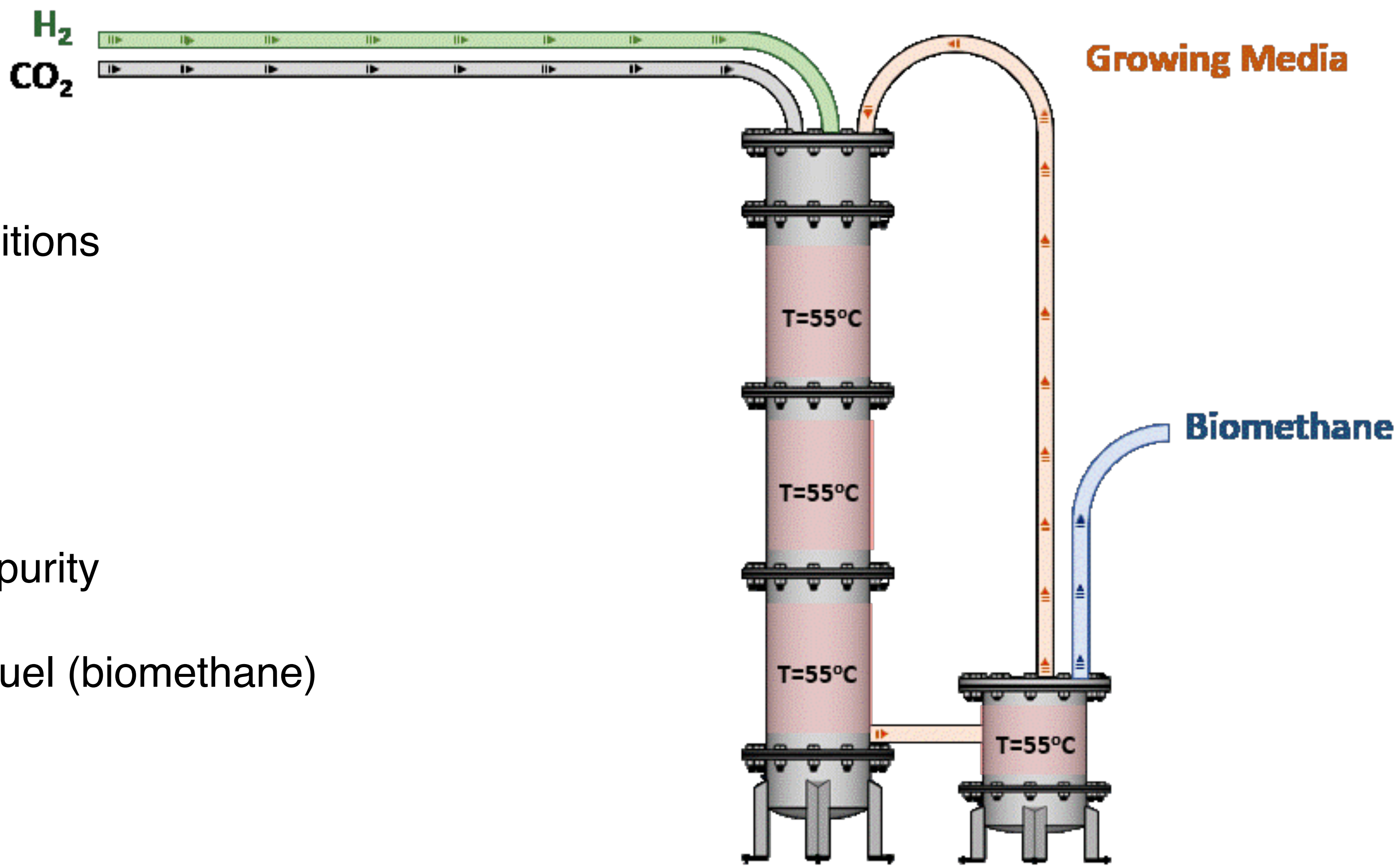
Biological fixation of CO_2 with the use of external H_2 can follow different metabolic routes:

- **Hydrogenotrophic methanogenesis** archaea directly convert CO_2 to CH_4
- **Homoacetogenic bacteria** convert CO_2 to acetate

✔ if acetoclastic methanogenic archaea convert the acetate into CH_4

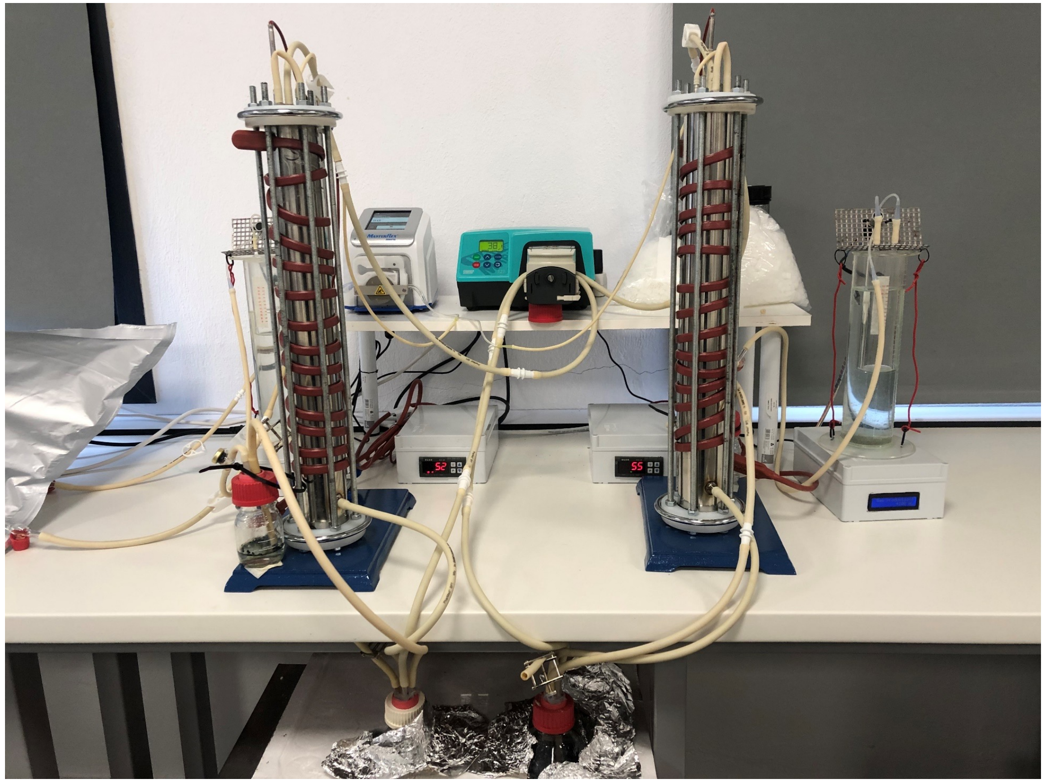
✘ if acetate accumulates in the system

The concept of using TBR for biomethanation



- ✓ Operation in mild temperature conditions
- ✓ Operation in ambient pressure
- ✓ No need for pure microbial culture
- ✓ Process is not affected by the CO_2 purity
- ✓ Transformation of CO_2 to 3-gen biofuel (biomethane)

Aim and Objectives



Assess the **biomethanation efficiency** of Trickle Bed Reactors packed with **activated carbon** or with **Raschig rings**, in terms of:

- CH_4 concentration in the output gas
- pH and the volatile fatty acids (VFA) concentrations
- Microbial community structure

Operating conditions

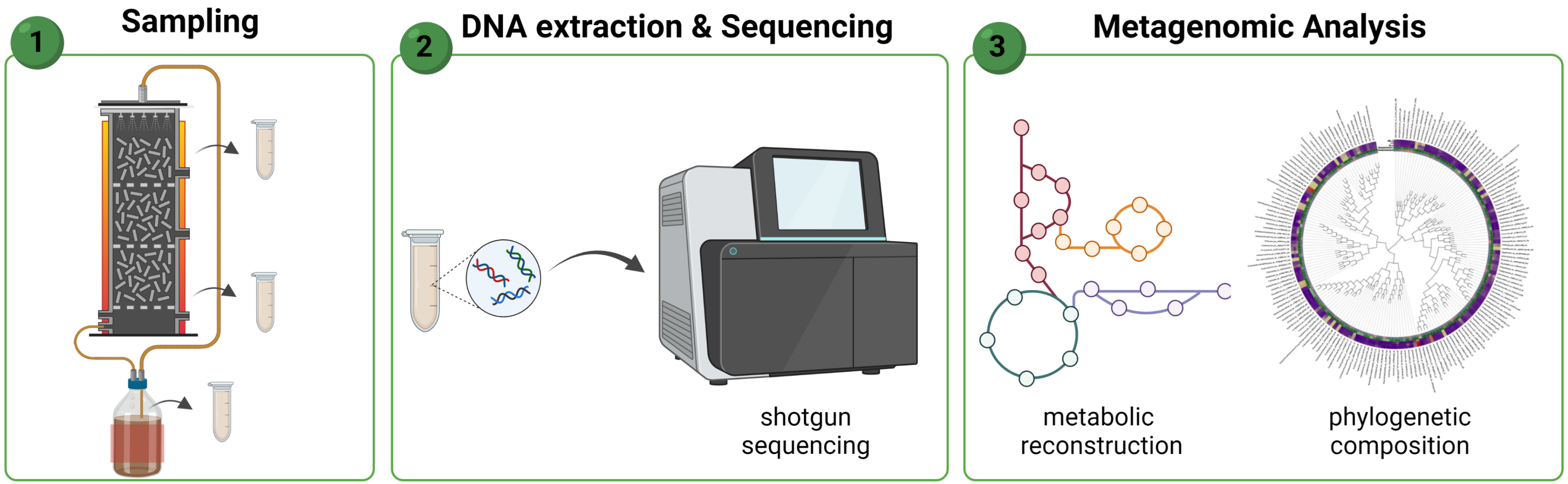
- Temp: 55°C
- GRT: 12-8-10-6-4-3-2-1 h
- Packing material
  - TBR1: activated carbon pellet
  - TBR2: raschig rings
- Metagenomic Microbial analyses

Materials and Methods

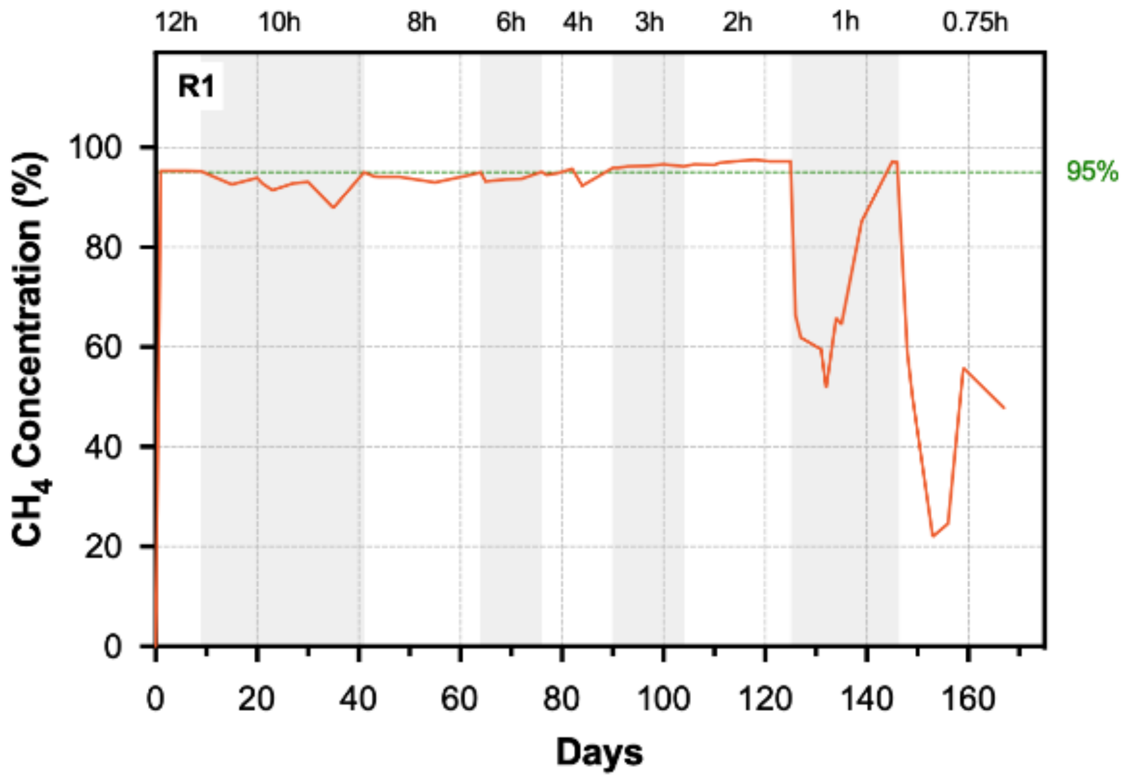
Genomic Samples:

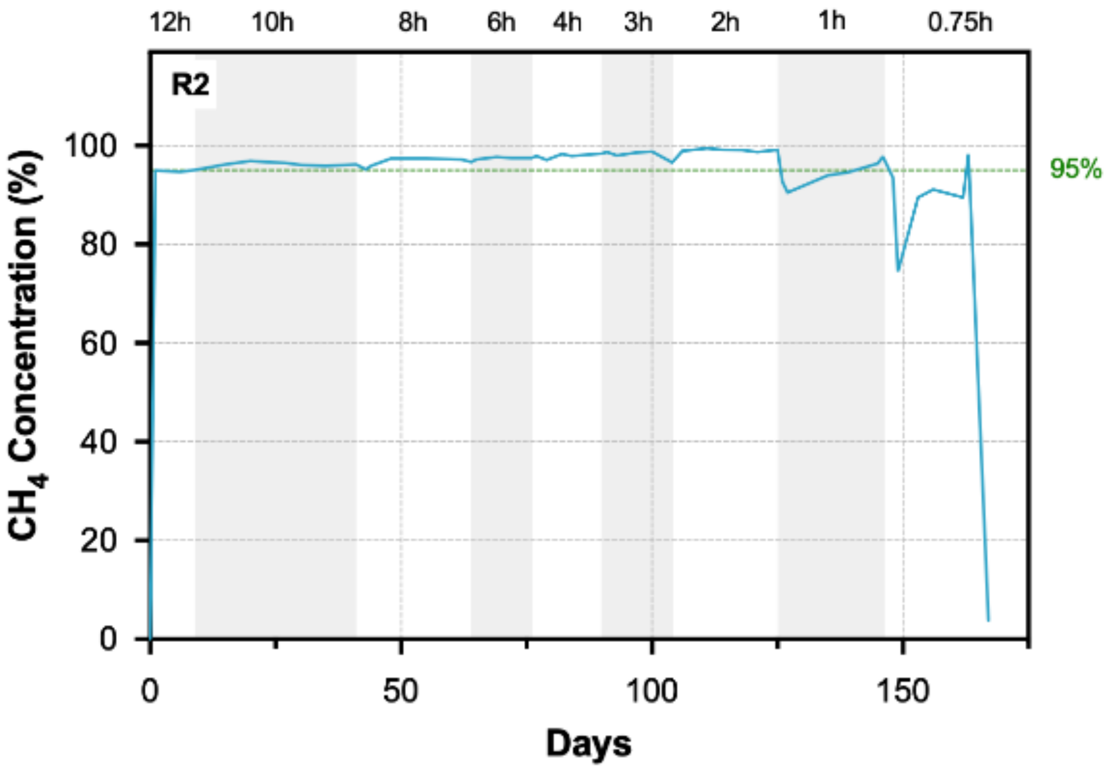
- Initial inoculum
- Biofilm in the upper part of each TBR
- Biofilm in the lower part of each TBR
- Liquid (planktonic cells) of each TBR

Under steady state conditions in the GRT of 1h



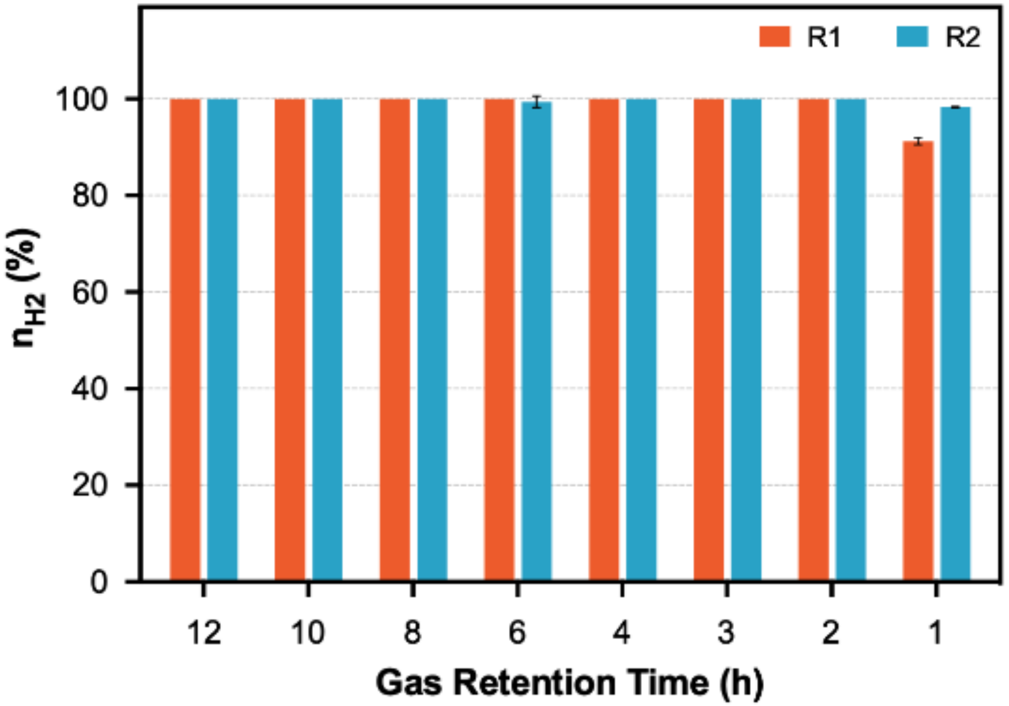
Biomethane production – CH_4 (%) in the output gas

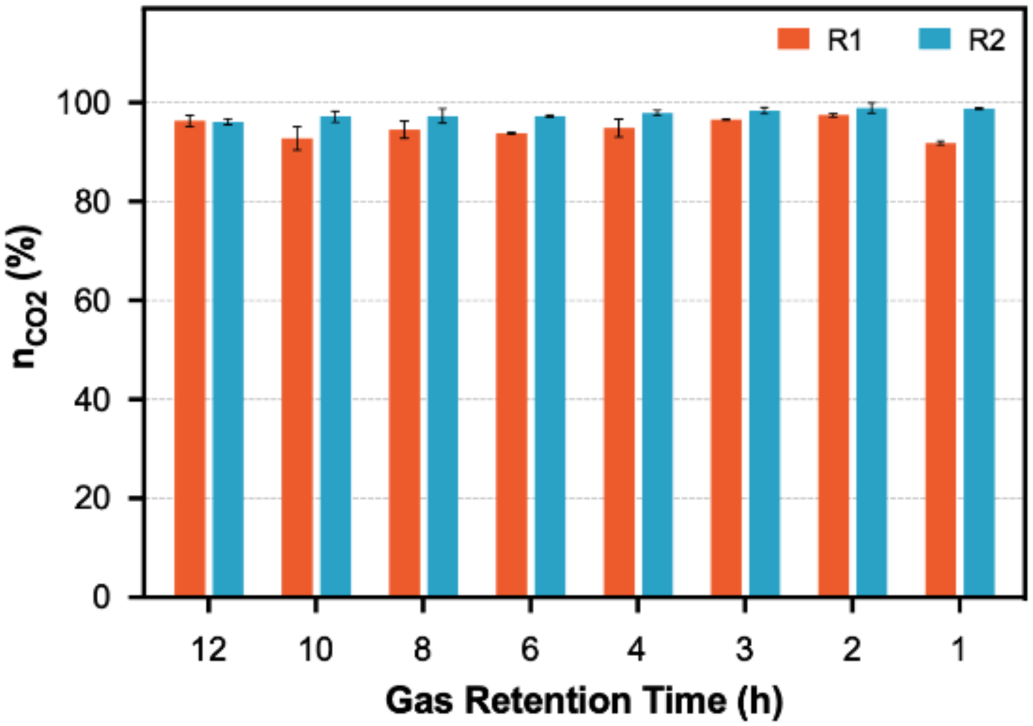




Concentration of methane at the output gas of TR1 (carbon pellets) and TR2 (raschig rings) during the different Gas Retention Times.

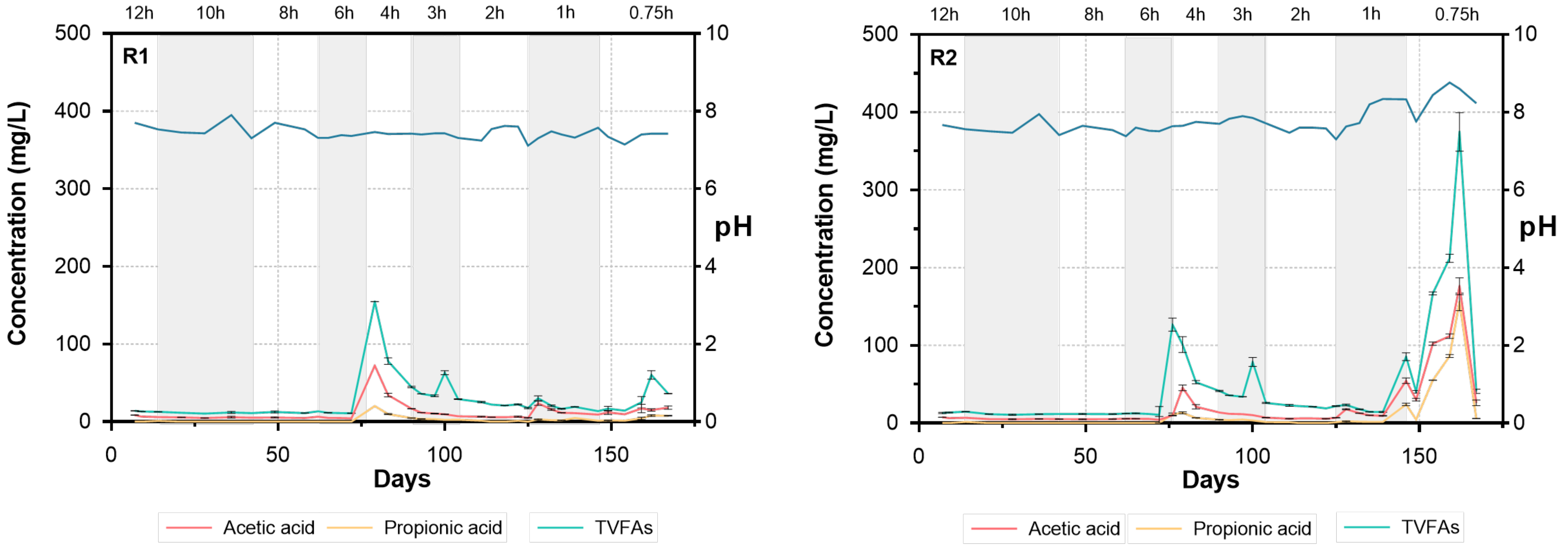
Biomethane production – CO_2 and H_2 efficiencies





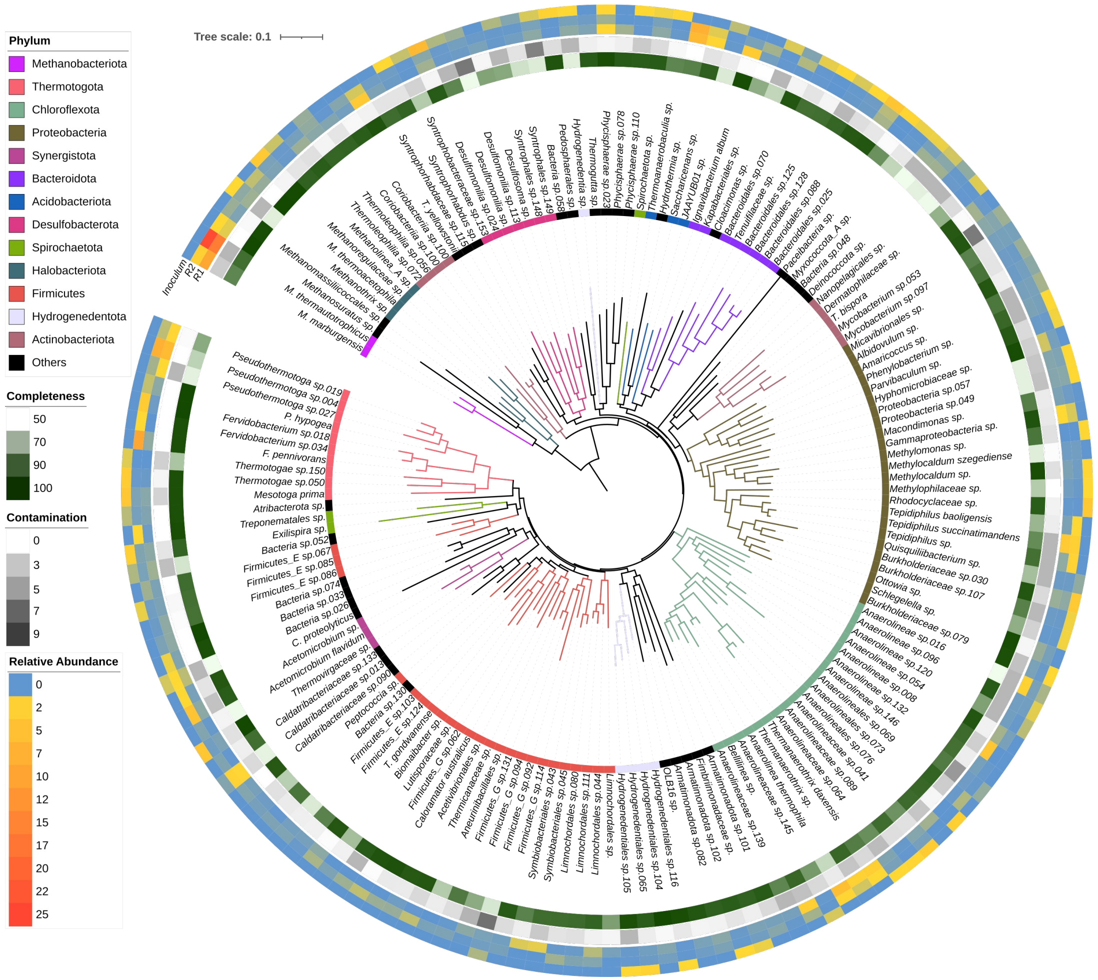
Efficiency of CO_2 capture and H_2 conversion rates in TR1 (carbon pellets) and TR2 (raschig rings) during the different Gas Retention Times.

Biomethanation performance – pH and VFA



pH and VFA concentrations of (carbon pellets) and TR2 (raschig rings) during the different Gas Retention Times.

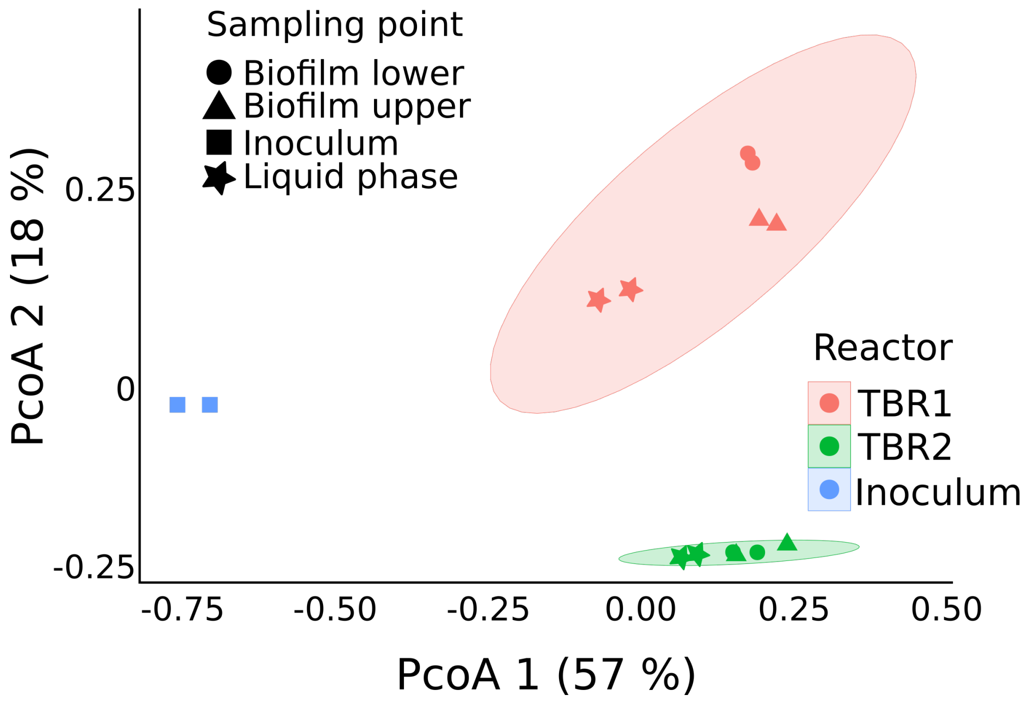
Overview of microbial community

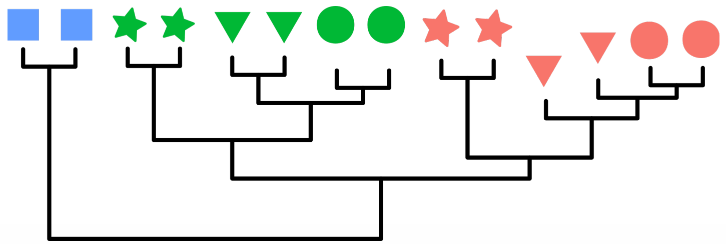


- 156 Metagenome Assembled Genomes
- 35 Phyla
- *Firmicutes* (16%) and *Proteobacteria* (15%) the dominant phyla
- Methanogenic representatives from 4 phyla
- Bacteria represented the majority of microbial community:
  - Inoculum: 95%
  - Liquid phase: 82-89%
  - Biofilm: 56-80%

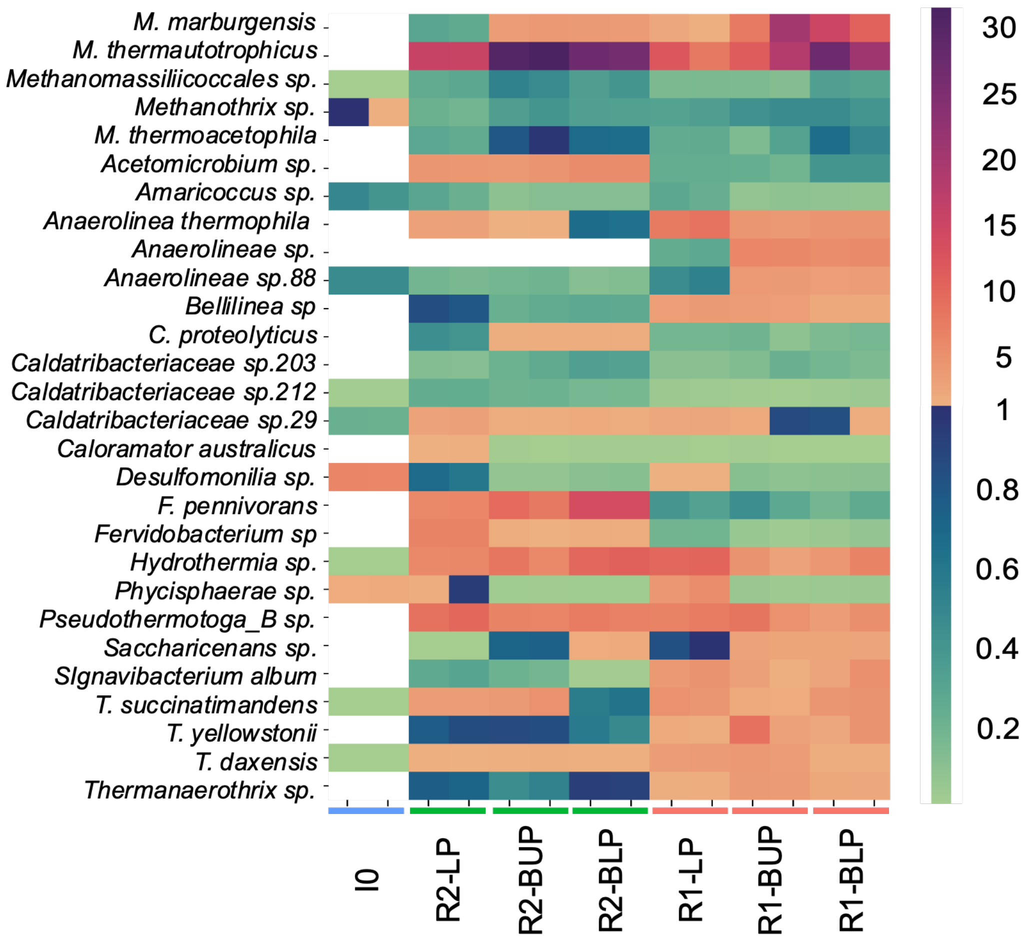
Microbial community structure

- PCoA analysis showed **distinct behavior** between the samples from the inoculum and those from the reactors
- **Biofilms presented greater separation**, indicating higher diversity compared to liquid phase
- **TBR1 presented greater variation between the lower and the upper part** (dissimilarity bray curtis being between 0.19 and 0.3) compared to TBR2 (dissimilarity bray curtis being between 0.15 and 0.2)
- HCA suggested a **stronger separation between the lower and the upper part of biofilm in TBR1**, compared to TBR2



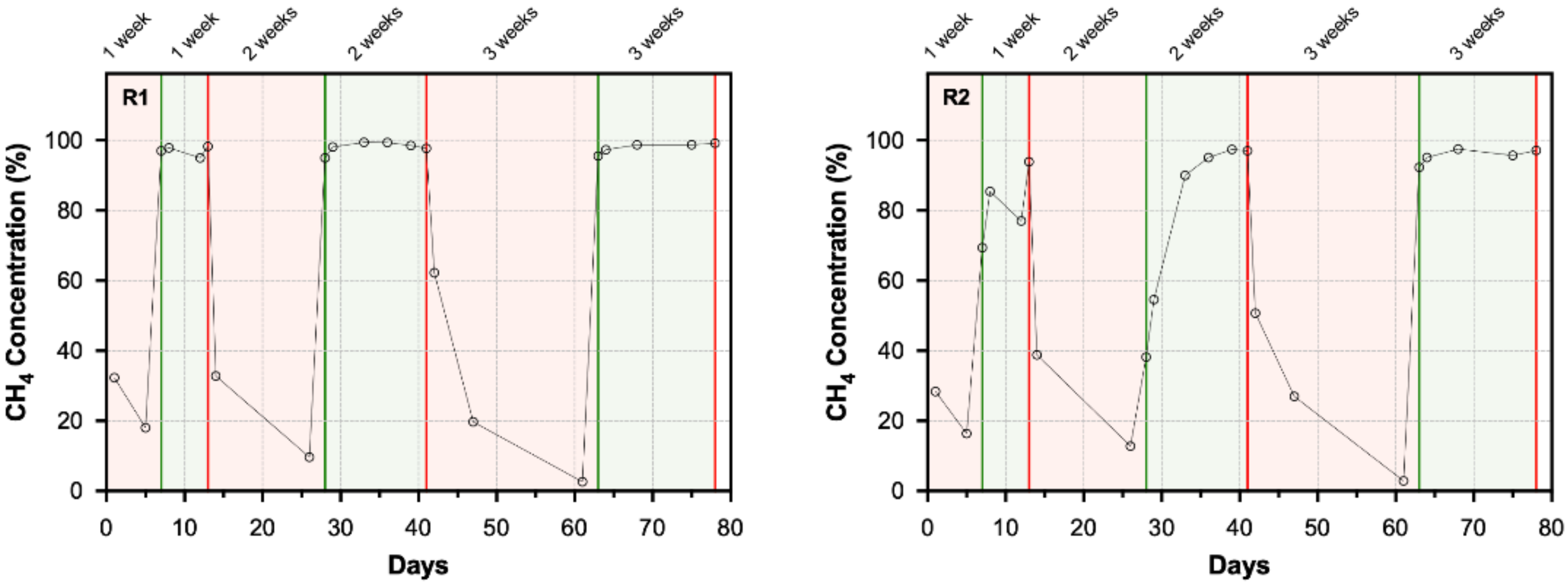


Microbial community synthesis



- *Methanothermobacter thermautotrophicus* dominance in both reactors
- *Methanothermobacter thermautotrophicus*, *Methanothrix_B thermoacetophila* and *Methanomassiliicoccales* sp. were more present in the upper part of the reactors
- Distinct preference of some microorganisms for one of the two materials
- A plethora of syntrophic bacteria were present in both reactors (e.g., *Caldatribacteriaceae* sp., *Coprothermobacter proteolyticus*, *Anaerolineaceae* sp. and *Symbiobacteriales* sp.)

Sneak peek on the current experimental work



Process performance of TR1 (carbon pellets) and TR2 (raschig rings) under intermittent provision of CO_2 and H_2.

Conclusions

- **Raschig rings** achieved higher biomethanation efficiency, resulting in **CH_4 purity of >95%** for GRTs 10-2h.
- GRT of 0.75h was the critical point for **process failure**.
- Biofilm formation can be significantly affected by **the flux of gasses from the top to the bottom** of the reactor.
- The biofilm communities in both reactors were predominantly dominated by the methanogen called ***Methanothermobacter thermautotrophicus***.
- Certain microorganisms displayed a **clear preference for one of the two materials**.

Acknowledgements

This work was financially supported by the project “Harnessing potential of biological CO2 capture for Circular Economy (CooCe) (Project No 327331) which is co-funded by the European Commission (receiving funding from European Union’s Horizon 2020 Grant agreement No. 691712)) and national funds (General Secretariat for Research and Innovation GSRI, Greece, MIS 5168516/ T12EPA5-00078) under the ERA-Net Cofund ACCELERATING CCUS TECHNOLOGY (ACT).

Thank you for your attention!

10th International Conference on Sustainable Solid Waste Management Chania, Greece, 21 - 24 JUNE 2023

Metagenomic analysis on hydrogen assisted carbon dioxide fixation for biomethane production

M. Gaspari[1], A. Chatzis[1,2], E. Orellana[3], L. Treu[3], K. Kontogiannopoulos[1], S. Campanaro[3], A. Zouboulis[2], P.G. Kougias[1]

[1]Soil and Water Resources Institute, Hellenic Agricultural Organization Dimitra, Thermi, 57001, Greece

[2]Laboratory of Chemical & Environmental Technology, Dept. of Chemistry, Aristotle University of Thessaloniki, Greece

[3]Department of Biology, University of Padova, Padova 35121, Italy

p.kougias@swri.gr

@kougias

0000-0003-4416-2135