Metagenomic analysis on hydrogen assisted carbon dioxide fixation for biomethane production

M. Gaspari¹, A. Chatzis¹,², E. Orellana³, L. Treu³, K. Kontogiannopoulos¹, S. Campanaro³, A. Zouboulis², P.G. Kougias¹

¹Soil and Water Resources Institute, Hellenic Agricultural Organization Dimitra, Thermi, 57001, Greece
²Laboratory of Chemical & Environmental Technology, Dept. of Chemistry, Aristotle University of Thessaloniki, Greece
³Department of Biology, University of Padova, Padova 35121, Italy

p.kougias@swri.gr  @kougias  0000-0003-4416-2135
Biofuels as a source of biogenic emissions

Market value of biofuels worldwide in 2020 and 2021, with a forecast until 2030 (in billion U.S. dollars)

Source: Precedence Research © Statista 2023

Additional Information:
Worldwide; 2020 and 2021

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 matter in the absence of oxygen. This process leads to the production of biogas as an end product.

**Main biogenic emissions:** CH₄, CO₂ and N₂O

## 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:** CO₂

## 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:** CO₂ and CO
H$_2$ assisted carbon dioxide fixation for biomethane
Important aspect for efficient biomethanation

Biological fixation of CO₂ with the use of external H₂ can follow different metabolic routes:

- **Hydrogenotrophic methanogenesis**
  - archaea directly convert CO₂ to CH₄

- **Homoacetogenic bacteria**
  - convert CO₂ to acetate
    - ✔️ if acetoclastic methanogenic archaea convert the acetate into CH₄
    - ✗️ 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 CO₂ purity
- Transformation of CO₂ 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:

- CH₄ 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
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

Materials and Methods

1. Sampling
2. DNA extraction & Sequencing
3. Metagenomic Analysis

- shotgun sequencing
- metabolic reconstruction
- phylogenetic composition
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 – CO₂ and H₂ efficiencies

Efficiency of CO₂ capture and H₂ 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., *Caldtribacteriaceae* 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 CO₂ and H₂.
Conclusions

- **Raschig rings** achieved higher biomethanation efficiency, resulting in CH₄ 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!
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