Biological CO₂ fixation in up-flow reactors via exogenous H₂ addition

Kougias, P. G.; Tsapekos, Panagiotis; Treu, L.; Kostoula, M; Campanaro, S; Lyberatos, G; Angelidaki, Irini

Published in:
Journal of Biotechnology

Link to article, DOI:
[10.1016/j.jbiotec.2020.05.012](https://doi.org/10.1016/j.jbiotec.2020.05.012)

Publication date:
2020

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Kougias, P. G., Tsapekos, P., Treu, L., Kostoula, M., Campanaro, S., Lyberatos, G., & Angelidaki, I. (2020). Biological CO₂ fixation in up-flow reactors via exogenous H₂ addition. *Journal of Biotechnology*, 319, 1-7. <https://doi.org/10.1016/j.jbiotec.2020.05.012>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Biological CO₂ fixation in up-flow reactors via exogenous H₂ addition

P.G. Kougias^{b,1}, P. Tsapekos^{a,1,*}, L. Treu^d, M. Kostoula^c, S. Campanaro^d, G.
Lyberatos^c and I. Angelidaki^{a*}

^aDepartment of Environmental Engineering, Technical University of
Denmark, Kgs. Lyngby, DK-2800, Denmark

^bHellenic Agricultural Organisation Demeter, Institute of Animal Science,
58100 Paralimni, Greece

^cSchool of Chemical Engineering, National Technical University of Athens,
Zografou Campus, Athens 15780, Greece

^dDepartment of Biology, University of Padova, Via U. Bassi 58/b, 35121,
Padova Italy

¹ These authors contributed equally to the present work.

*Corresponding authors:

Irini Angelidaki, Department of Environmental Engineering, Technical University of
Denmark, Bld 115, 2800 Lyngby, Denmark, iria@env.dtu.dk, Tel.: +45 45251429

Panagiotis Tsapekos, Department of Environmental Engineering, Technical University
of Denmark, Bld 115, 2800 Lyngby, Denmark, ptsa@env.dtu.dk, Tel.: +45 45251404

Abstract

Gas fermentation for the production of building block molecules and biofuels is lately gaining attention as a means to eliminate the greenhouse gases emissions. Especially CO₂ capture and recycling are in focus. Thus, the biological coupling of CO₂ and H₂ is of high interest. Therefore, the focus of the present work was to evaluate the performances of two up-flow reactors for CO₂ and H₂ assimilation. Process monitoring showed that the gas-liquid H₂ transfer was highly affected by reactor design. A reactor filled with Raschig rings could lift up gases utilization leading to a CH₄ content of 81% at 6 h gas retention time and 8.8 L/L_R.h gas recirculation rate. In contrast, limited biomethanation was achieved in the absence of Raschig rings highlighting the positive role of packing material to the performance of up-flow-reactors. Additionally, high-throughput 16S rRNA sequencing revealed that the microbial community was ultimately resided by *Methanothermobacter* methanogens.

Keywords

CO₂ fixation; packed column reactors; hydrogenotrophic methanogenesis; homoacetogenesis; anaerobic digestion

1. Introduction

In EU countries, the upgraded biogas is injected in the gas grid or used as transportation fuel depending on the national legislation (Browne et al., 2011). Nowadays, various commercial upgrading technologies exist, and several new concepts are under development. Conventional methods for biogas upgrade using high pressure

(e.g. pressure swing adsorption), water (e.g. water physical scrubbing) and/or chemicals (e.g. amine chemical scrubbing) are available (Baena-Moreno et al., 2020). Despite the high efficiency of these technologies, the increased costs initiated a further research for alternative options (Bassani et al., 2017; Sun et al., 2015). Recently, the biological process coupling of CO₂ and H₂ is a rapidly growing platform since it is less energy demanding and retains the efficiency of the physicochemical methods (Angelidaki et al., 2018). Moreover, biological upgrading is considered as second generation upgrading since CO₂ is not only deposited or released to the atmosphere but is coupled with H₂ by the action of hydrogenotrophic methanogens forming additional amounts of CH₄ (Bassani et al., 2015; Luo and Angelidaki, 2012; Vo et al., 2018). Therefore, biological biogas upgrading can be considered as CO₂ capturing and recycling technology. In addition, H₂ derived from inexpensive renewable electricity in periods with intense wind peak loads for wind mills and sunny days for photovoltaics ensures no energy wasting, while it maintains a stable electricity grid and improves sustainability (Alfaro et al., 2018; De Vrieze et al., 2019; Strübing et al., 2018). Hence, it can be considered as energy storage technology.

Biological biogas upgrading can be conducted either simultaneously with the anaerobic digestion (AD) process or in a separate consecutive step. In the first option, which is called “in-situ process” (Kougias et al., 2017), there is a risk of exceeding the acceptable pH range for AD (i.e. pH of 6.5-8.5) due to capturing the endogenously produced CO₂, which will lead to irreversible inhibition for the methanogenic communities (Bassani et al., 2016). In contrast, during “ex-situ processes” H₂ and CO₂ are externally provided in a separated chamber and therefore the pH is not affected (Kougias et al., 2017). However, in both processes, the increased H₂ partial pressure

might provoke volatile fatty acids (VFA) accumulation and subsequently, inhibition of the methanogenic archaea (Kougias et al., 2017).

Previous research works reported that the injection of H₂ into hydrogenotrophic up-flow reactors enhanced tremendously the CH₄ content from 23% to 96% during an ex-situ process (Bassani et al., 2017). Specifically, the up-flow reactors were filled with either alumina ceramic sponges or membranes and were associated with high biomethanation efficiency, despite the stepwise reductions of gas retention time (GRT) from 15 to 4 h. High CH₄ output at low GRT ensures high production capacity which is very important in order to ensure economic feasibility at scaling-up (Sieborg et al., 2020). However, the previously used materials can be easily clogged and also, are rather expensive. Thus, they are linked to rather inconvenient daily operation/maintenance and also, reduced potential investments in real-life.

Among crucial parameters, the H₂ transfer from gas to liquid phase is the most important bottleneck to succeeding a high biomethane content (Dupnock and Deshusses, 2019). In addition, an increased contact area between liquid and gas flow and modulation of gas recirculation rate are key factors for high efficiency. Specifically, the contact area between the gas and liquid phases is markedly increased when filling the reactor with packing material (Bassani et al., 2017) and there is a variety of literature examining different carriers. For example, Lee *et al.* (2012) examined reticulated polyester urethane sponge as packing material which led to efficient biological uptake of gases at 3.8 h while the bioconversion was decreased at 2 h. Similarly, Burkhardt and Busch (2013) exploited the Bioflow 40 from Rauschert that was associated with high H₂ conversion at 4 h but lower performance was observed after that point. In addition, Rachbauer *et al.* (2016) filled a trickle-bed reactor with

polypropylene packing rings as carrier material leading to efficient bioconversion efficiencies up to 2 h GRT. Despite the high efficiency of trickle-bed reactors, the usage of submerged bed carriers in up-flow reactors could potentially be advantageous as no liquid recirculation will be needed avoiding extra operational costs. Furthermore, the introduction of gas recirculation seems to positively affect the gas conversion (Voelklein et al., 2019; Yun et al., 2017). Despite the positive effect of both parameters (packing and recirculation), the exact effect that each factor has on methane formation is still not clear.

Biological upgrading is mediated by dynamic microbial communities that can progressively shape during H₂ injection periods (Treu et al., 2018). Among archaea, hydrogenotrophic methanogens belonging to *Methanoculleus* or *Methanothermobacter* genera are commonly abundant during well-performing ex-situ processes (Bassani et al., 2015; Kougias et al., 2017). However, the increased H₂ partial pressure caused by the injection of exogenous H₂, can change the thermodynamic equilibrium promoting other metabolic pathways that act as sink of H₂ utilization. Hence, the establishment of archaeal members could be challenging.

The aim of the present work was to investigate the effect of packing material on CO₂ fixation in up-flow reactors fed with exogenous H₂. The purpose of using packed column reactors was to increase the gas-liquid mass transfer by achieving a more efficient dispersion of CO₂ and H₂. It was not attempted to optimize biomethanation, but it was aimed to monitor the end-products from the CO₂ hydrogenation and to compare the reactor configuration with a control system that was operating in the absence of packing material. Moreover, the microbial community changes upon exogenous CO₂ and H₂ addition were identified by performing high throughput 16S rRNA gene

amplicon sequencing.

2. Materials and methods

2.1. Inoculum and nutrient media

An active mixed hydrogenotrophic culture was collected from the effluent tank of well-performing biogas upgrading reactor and used as inoculum (Bassani et al., 2015). Fully degassed digestate was collected from the effluent tank of the Snertinge biogas plant (Denmark) and used for providing necessary nutrients for the microbial consortia in the upgrading reactor. The digestate was filtered through a 2 mm net to remove large particles and stored at a thermophilic incubator 55 ° C for more than two months in order to achieve complete decomposition of the degradable organic matter and to ensure the absence of biogas production. Before usage, orthophosphoric acid droplets were added to the digestate to decrease the pH from 9.6 to 8.0 and keep it within the optimal range for methanogenesis (Weiland, 2010). The chemical composition of the hydrogenotrophic inoculum and the degassed digestate are presented in Table 1.

2.2. Reactors configuration and operation

The experimental setup consisted of two cylindrical up-flow reactors with a working volume of 1.4 L. Enriched hydrogenotrophic inoculum was used to fill the control reactor (R_c) prior to start-up. Apart from inoculum, Raschig rings were added as packing material to the second reactor (R_{r-r}) in order to increase the gas-liquid contact and improve H_2 transfer to the liquid phase providing a surface area of 1.3 m². Both reactors were fed twice a day with 50 mL of degassed digestate as nutrient feedstock. The reactors were operated at thermophilic conditions (55 ± 1 °C) by recirculating hot

water. The glass walls were covered with insulating material to prevent heat loss and avoid light penetration. Reactors mixing was achieved by continuous gas recirculation. The reactors were provided with a gas mixture of 62% H₂, 15% CO₂ and 23% CH₄, (AGA A/S, Denmark), sparged through two stainless steel diffusers with pore size of 2.0 µm. The Q_{RC} was set to approximately 42 times the gas injection rate as previously described (Kougias et al., 2017). The experiment was divided into 5 distinguished periods, in which, either the gas injection rate (L/L_R/day) was increased in order to decrease the gas retention time (GRT) from 8 to 5 hours or the gas recirculation rate (Q_{RC}) was increased from 5.3 to 8.8 L/L_R/day in order to evaluate the effect of these two important operating conditions on CO₂ and H₂ utilization. The utilisation efficiency of H₂ and CO₂, η_{CO_2} and η_{H_2} , respectively, were determined in each period.

2.3. Analytical methods

Analyses of pH, TS, VS, TKN and NH₄-N were conducted according to Standard Methods for the examination of water and wastewater (APHA, 2005). The individual VFAs were quantified by gas chromatograph (GC Shimadzu) with flame ionisation detector (FID) based on Kougias *et al.* (2015). Biogas composition (CH₄, CO₂, H₂) was determined twice per week by gas chromatography equipped with thermal conductivity detectors (GC-TCD) as previously described (Bassani et al., 2017). H₂ gas transfer coefficient (day⁻¹) was calculated according to the following equation:

$$r_t = 22.4k_La(H_{2gTH} - H_{2l})$$

where, r_t (L/(L.day)) represents the H₂ gas to liquid mass transfer rate, 22.4 (L/mol) is the gas volume to mole ratio at STP, k_La (day⁻¹) represents the gas transfer coefficient, H_{2gTH} (mol/L) represents the H₂ content in the gas phase while H_{2l} (mol/L) represents

the H₂ dissolved in the liquid phase.

2.4. Microbial community composition

Samples were obtained at the end of the experiment to ensure microbial community stability. Genomic DNA was extracted from reactors' liquid samples, in triplicates, with PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA). The followed procedure was previously described by Bassani *et al.* (2015). NanoDrop (ThermoFisher Scientific, Waltham, MA) and Qubit™ fluorimeter (Life Technologies, Carlsbad, CA) were used for quality and quantity control of the extracted DNA. Universal primers were used for 16S rRNA gene V4 hypervariable region amplification (Caporaso *et al.*, 2012). The 16S rRNA genes were sequenced using Illumina MiSeq sequencing technology. The obtained reads were submitted to the NCBI sequence read archive database (SRA) under the BioProject ID PRJNA607234. Detailed sample IDs are presented in Supplementary Data. The 16S rRNA gene sequences were analyzed according to Kougias *et al.* (2017) and subsequently, additional taxonomic verification was conducted using BLASTN against NCBI 16S rRNA. Alpha diversity was calculated from the number of operational taxonomic units (OTUs), while beta diversity was calculated with the Bray-Curtis matrix and represented as Principal Coordinate Analysis (PCoA). The relative abundance and fold change of most relevant OTUs are presented in heat map drawn using Multiexperiment Viewer software (MeV) (Howe *et al.*, 2010).

2.5. Statistical analysis

Descriptive statistics were performed for all data, mean values and standard

deviations were calculated using Graphpad Prism 5 program (Graphpad Software, Inc., San Diego, CA). Anova Analysis with a Post Hoc test (Tukey HSD) was carried out to compare and determine significant differences ($p < 0.05$) among the different experimental periods. Concerning the AD microbiome, the significant abundance differences for each microorganism were conducted using STAMP.

3. Results and Discussion

3.1. Overview of reactors performance

Coupling CO₂ and H₂ was examined under five separately distinguished operating conditions. Experimental data from operation of the control reactor (R_c) and the reactor filled with packing material (R_{r-r}) are summarized in Table 2. With respect to CO₂ fixation, H₂ utilization and CH₄ production; the performance of both reactors was increasing from the beginning of the experiment since the end of the second experimental period. Subsequently, the efficiency of R_c steadily decreased, reaching the lowest performance at the end of the experiment, when the gas injection rate was set at a GRT of 5 h. Indeed, the microbiome did not manage to sufficiently metabolize the extra CO₂ and thus, the overall efficiency of the reactor decreased. On the contrary, R_{r-r} retained a relatively more stable performance during the whole experiment. The added packing material increased the surface area that was served for both biofilm formation (Maegaard et al., 2019) and also, as a means for bubbles disruption of the injected gas (Burkhardt and Busch, 2013). The comparison between the two reactors under all experimental phases is further discussed in the following sections.

Regarding the pH, relatively unchanged values (8.53 ± 0.06) were measured during the first two experimental periods. In contrast, different levels of VFAs and especially,

acetate were detected in two reactors in the same interval (Fig. 1). The detected accumulation indicated that the injected CO₂ and H₂ were not fully utilized by hydrogenotrophic methanogens but by homoacetogens, producing acetate instead of methane (Bassani et al., 2017). Consequently, the pH dropped from the end of period III until the end of period IV. In parallel, a second trend of acetate accumulation was observed during period IV (Fig. 1). In accordance, η_{CO_2} values were increased and η_{H_2} remained at high levels. Hence, we assumed presence of homoacetogenic microbes for CO₂ and H₂ fixation (Ragsdale and Pierce, 2008). This hypothesis was validated by the microbial analysis as shown in the following sections. Acetogenic CO₂/H₂ fermentation is lately gaining increased attention. Acetate can be produced as sole molecule containing trace by-products which makes the economic feasibility of its biological production route promising (De Vrieze et al., 2019). Hence, acetate production can be an alternative to biomethanation, offering flexibility to the gas fermentation system.

3.2. The role of packing materials

During the first experimental period, the performance of both reactors revealed a similar behaviour and an immediate start of CO₂ and H₂ utilisation for CH₄ production. At the end of period I, the control reactor had slightly higher CH₄ content (R_c: 62%) compared to the reactor filled with packing material (R_{r-r}: 60%). In the beginning of the experiment, the volume of the hydrogenotrophic inoculum was higher in R_c than R_{r-r} which was partially filled with packing materials. Hence, the enriched microbial community could have positively affected the biomethanation due to the already high abundance of specialized microbes harbouring the key metabolic pathways to conduct the specific biological conversion (Herrero and Stuckey, 2015). In contrast, the

microbiome in R_{r-r} might have needed an adaptation period to create biofilm around the Raschig rings where the microorganisms are immobilised and boost the biomethanation at higher levels. On this topic, it has been previously observed that a tremendously more prolonged adaptation period (>150 days) was necessary also under thermophilic conditions to build-up adequate and stable amount of anaerobic biomass that could efficiently bioconvert CO_2 and H_2 (Pokorna et al., 2019).

Subsequently, the performance of R_{r-r} was significantly improved ($p < 0.05$) during the second period, indicating that sufficient biofilm was developed and consisted of specialized microbes at increased abundance to enhance the biological absorption of gases (Kim and Deshusses, 2003). Specifically, during period II, H_2 utilization was significantly enhanced ($p < 0.05$) from 73.9% to 90.6% (Table 2) and the CH_4 concentration was 81%. Indeed, methanogenic archaea are growing rather slowly and hence, a lag phase was needed in order to be amplified around the carrier material in sufficient colonies for H_2 utilization (Siegert et al., 2014).

In accordance, the improved performance of the R_{r-r} containing Raschig rings was revealed during the following experimental periods (III-V). Higher CO_2 fixation efficiency was achieved in R_{r-r} (61.2-68.1%) in comparison with R_c (27.2-55.3%). Moreover, the H_2 transfer coefficient was always higher in R_{r-r} compared to R_c (Table 2). In fact, it was previously shown that packing units can boost the performance of biological conversion processes, providing extra surface area for microbial activity (Singh and Perna, 2009) and inducing the gas-liquid transfer (Billet and Schultes, 1993). The superiority of the R_{r-r} with the Raschig rings was more clearly detected at the final experimental period in which the most intense experimental conditions were applied (i.e. lowest GRT, highest Q_{RC}). Although the CH_4 concentration of R_{r-r} was

decreased at the end of period V, the packing materials managed to retain the CH₄ productivity at higher levels compared to the previous experimental periods. In contrast, the biomethanation process performance in R_c was dramatically dropped, supporting the hypothesis that the presence of packing materials with increased surface is prerequisite in order to have efficient gas-liquid mass transfer (Bassani et al., 2017) and create an immobilized biofilm to efficiently carry out the hydrogenotrophic methanogenesis (Sieborg et al., 2020). In fact, the η_{CO_2} and η_{H_2} had the lowest values during the whole experiment 30.5% and 64.9%, respectively, in the up-flow R_c without packing materials.

3.3. Effect of gas retention time and recirculation rate

Reactors monitoring showed that GRT reduction did not influence positively the biomethanation efficiencies. While the performance of R_{r-r} remained at the same levels in the first periods (i.e. period II to III) with subsequently reduced GRTs, the efficiency of the control reactor R_c clearly deteriorated at low GRT. In contrast, the biomethanation process of both reactors failed in the final period with the lowest GRTs applied (i.e. period IV to V).

The GRT was altered by changing the input gas flow rate (i.e. feeding rate). However, increasing the gas feeding rate could negatively affect a previously well-performing upgrading community (Rachbauer et al., 2016). Thus, the decreased efficiency can be ascribed to the non-optimal operational conditions (e.g. low gas liquid mass transfer for the microbiome. Alteration of GRT was previously examined for ex-situ CO₂ and H₂ fixation (Burkhardt and Busch, 2013). In accordance, it was shown that at GRT lower than 6 h, the biomethanation efficiency was negatively affected as

advised by the lower H_2 degradation rates. Despite the limited biomethanation, the CO_2 convection rate of R_{r-r} increased during the last GRT decrease. In agreement, Lee *et al.* (2012) found that high conversion CO_2 rate can be achieved with a GRT up to approximately 4 h. In parallel, in the same period there was a peak in acetate production, highlighting CO_2 and H_2 partial utilization for homoacetogenesis instead of methanogenesis.

Regarding the impact of the gas recirculation rate (Q_{RC}), distinct impacts were observed for both reactors during the first increase (i.e. period I to II). The methane production rate was increased by 52% and 35% in R_c and R_{r-r} , respectively. The positive effect of Q_{RC} was also detected in a recent biogas upgrading study (Alfaro *et al.*, 2019). Interestingly, in the cited study a positive effect was revealed upon changing the Q_{RC} (i.e. from 5.3 to 7.0 L/L_R.h) to similar levels compared to the present work (i.e. from 4.2 to 8.3 L/L_R.h). The second Q_{RC} increment (period III to IV) contributed to η_{CO_2} increase, while retaining the η_{H_2} at high levels for both R_c and R_{r-r} . Indeed, the gas absorption rate is enhanced through increased recirculation (Jacob-Lopes *et al.*, 2009) and accordingly, CO_2 fixation was finally induced. Regarding R_{r-r} , the increased recirculation rate did not manage to boost the biomethanation process. In agreement, Dupnock and Deshusses (2019) concluded that increasing Q_{RC} has not a clearly visible impact in reactors filled with packing materials. In contrast, intense Q_{RC} is a prerequisite to achieving high CH_4 purity in well-mixed reactors, by enhancing H_2 holdup and increasing the gas-liquid interfacial area.

3.4. Microbial diversity and dynamicity

Alpha diversity based on the number of OTUs and the rarefaction curves showed

that the sequencing depth was adequate to deliver information about the microbial species richness (Supplementary Data). PCoA analysis was also performed to evaluate composition and variability of microorganisms (Supplementary Data).

In both reactors, the most abundant microorganisms belonged to the *Pseudomonadaceae* family. *Pseudomonas caeni* 1 had a relative abundance of 11.29% and 18.61% in R_c and R_{r-r}, respectively. Apart from *Pseudomonas caeni* 1, additional members within *Pseudomonas* genus were detected (i.e. *Pseudomonas* sp. 4, 10, 11 and 30). This is in accordance with Kougias *et al.* (2017) who found that *Pseudomonas* genera were remarkably enriched in the liquid phase of different ex-situ upgrading reactor systems. Likewise, members of the genus *Pseudomonas* were detected during biologically mediated CO₂ reduction containing 656 mg_{-acetate}/L (Porté *et al.*, 2019). Moreover, the ability of *Pseudomonas* spp. to tolerate much higher concentrations (up to 10 g_{-acetate}/L) was lately shown (Yang *et al.*, 2019). Thus, the proliferation of these acetate utilizers is in agreement with the high concentrations of the carboxylic acid observed in R_c (743 mg_{-acetate}/L) and R_{r-r} (539 mg_{-acetate}/L). In contrast, *Porphyromonadaceae* sp. 5 was 1.9-fold higher in R_c compared to R_{r-r} and on top of this, diverse species of *Porphyromonadaceae* family (*Porphyromonadaceae* sp. 7, 21, 28, 37, 38 and 46) were detected in both reactor systems. The high abundance of members within either *Pseudomonadaceae* or *Porphyromonadaceae* family in acetate-rich media could be associated to a potential presence of homoacetogenic and syntrophic bacteria within these families. Specifically, the function of *Pseudomonas* genera to accelerate electron transfer was suggested as the reason for their co-habitation with archaea during efficient methanogenesis (Yuan *et al.*, 2020). On the other hand, representatives of *Porphyromonadaceae* family dominated the bacterial community

during efficient hydrogenotrophic methanogenesis driven by *Methanobacteriaceae* archaea (Granada et al., 2018).

On this topic, the only highly abundant methanogen in both reactors was the *Methanothermobacter* sp. 12 which is known to create syntrophies with acetate oxidizers (Treu et al., 2018). Also, it should be noted that *Methanothermobacter* sp. 12 was 1.5-fold higher in R_{r-r} which is explained by the higher upgrading efficiency of the reactor filled with Raschig rings. Hence, the colonization of acetate-utilizing bacteria could be favoured with the proliferation of *Methanothermobacter* species. The preferential utilisation of acetate via syntrophic oxidation pathway, instead of direct acetate methanation, is further supported by the absence of known aceticlastic methanogens, such as members of the genus *Methanothrix* (Karakashev et al., 2006) (Supplementary Data). Furthermore, the existence of syntrophic acetate oxidising (SAO) bacteria and hydrogenotrophic archaea during ex-situ methanation is known (Corbellini et al., 2018). As expected, potential SAO were also detected in the present work. According to the results of the BLASTn search, *Peptococcaceae* sp. 25 was closely related to *Desulfonisporea thiosulfatigenes* with a sequence similarity of 93%. The identified OTU was 6.3-fold higher in R_c in which higher acetate concentrations were detected. Similarly, *Sulfurospirillum* sp. 29 was detected in both systems and was more abundant in the control operation. Despite the fact that H₂S was not monitored in the present study, it is known that the presence of sulphate reducing bacteria can deteriorate process performance and is reducing sulfate to H₂S. In addition, the metabolic role of *Sulfurospirillum* genera is uncertain; however, it was lately indicated that their growth could rely on acetate oxidation by SAO (Westerholm et al., 2018). Finally, *Thermoanaerobacteraceae* sp. 18 (90% similarity with *Moorella thermoacetica*

and *Moorella thermoautotrophica*) was 2.8-fold higher in R_c. Within this family well-known homo- acetogenic species producing acetate from H₂ and CO₂ are available (Angelidaki et al., 2018). Despite the fact that potential SAO candidates were available in both systems, higher relative abundances were detected in control operation in which higher concentrations of acetate were measured.

4. Conclusions

The current research showed that in order to achieve an improved biological biogas upgrading by coupling CO₂ with H₂, the addition of packing material is crucial for up-flow reactors. Challenges were achieved decreasing the gas retention time. Intense gas recirculation rate can partially favour the performance of ex-situ biogas upgrading. The obtained results can be further interpreted as engineering data to use alternative reactors design that can efficiently work at shorter retention times. Results from the microbial analysis showed that *Methanothermobacter* species dominated completely the archaeal population.

Acknowledgement

The present work was supported by the Energinet under the project framework ForskEL ‘‘BioUpgrade- Ex-situ biogas upgrading through biologically mediated CO₂ reduction’’.

References

Alfaro, N., Fdz-Polanco, M., Fdz-Polanco, F., Díaz, I., 2019. H₂ addition through a submerged membrane for in-situ biogas upgrading in the anaerobic digestion of

sewage sludge. *Bioresour. Technol.* 280, 1–8.

Alfaro, N., Fdz-Polanco, M., Fdz-Polanco, F., Díaz, I., 2018. Evaluation of process performance, energy consumption and microbiota characterization in a ceramic membrane bioreactor for ex-situ biomethanation of H₂ and CO₂. *Bioresour. Technol.* 258, 142–150.

Angelidaki, I., Treu, L., Tsapekos, P., Luo, G., Campanaro, S., Wenzel, H., Kougias, P.G., 2018. Biogas upgrading and utilization: Current status and perspectives. *Biotechnol. Adv.* 36, 452–466.

APHA, 2005. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, Washington, DC, USA.

Baena-Moreno, F.M., le Saché, E., Pastor-Pérez, L., Reina, T.R., 2020. Biogas as a Renewable Energy Source: Focusing on Principles and Recent Advances of Membrane-Based Technologies for Biogas Upgrading, in: Zhang, Z., Zhang, W., Lichtfouse, E. (Eds.), *Membranes for Environmental Applications*. Springer International Publishing, Cham, pp. 95–120.

Bassani, I., Kougias, P.G., Angelidaki, I., 2016. In-situ biogas upgrading in thermophilic granular UASB reactor: key factors affecting the hydrogen mass transfer rate. *Bioresour. Technol.* 221, 485–491.

Bassani, I., Kougias, P.G., Treu, L., Angelidaki, I., 2015. Biogas Upgrading via Hydrogenotrophic Methanogenesis in Two-Stage Continuous Stirred Tank Reactors at Mesophilic and Thermophilic Conditions. *Environ. Sci. Technol.* 49, 12585–12593.

Bassani, I., Kougias, P.G., Treu, L., Porté, H., Campanaro, S., Angelidaki, I., 2017. Optimization of hydrogen dispersion in thermophilic up-flow reactors for ex-situ

biogas upgrading. *Bioresour. Technol.* 234, 310–319.

Billet, R., Schultes, M., 1993. Predicting mass transfer in packed columns. *Chem. Eng. Technol.* 16, 1–9.

Browne, J., Nizami, A.S., Thamsiroj, T., Murphy, J.D., 2011. Assessing the cost of biofuel production with increasing penetration of the transport fuel market: A case study of gaseous biomethane in Ireland. *Renew. Sustain. Energy Rev.* 15, 4537–4547.

Burkhardt, M., Busch, G., 2013. Methanation of hydrogen and carbon dioxide. *Appl. Energy* 111, 74–79.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., Knight, R., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* 6, 1621–1624.

Corbellini, V., Kougias, P.G., Treu, L., Bassani, I., Malpei, F., Angelidaki, I., 2018. Hybrid biogas upgrading in a two-stage thermophilic reactor. *Energy Convers. Manag.* 168, 1–10.

De Vrieze, J., Verbeeck, K., Pikaar, I., Boere, J., Van Wijk, A., Rabaey, K., Verstraete, W., 2019. The hydrogen gas bio-based economy and the production of renewable building block chemicals, food and energy. *N. Biotechnol.* 55, 12–18.

Dupnock, T.L., Deshusses, M.A., 2019. Detailed investigations of dissolved hydrogen and hydrogen mass transfer in a biotrickling filter for upgrading biogas. *Bioresour. Technol.* 290, 121780.

Granada, C.E., Hasan, C., Marder, M., Konrad, O., Vargas, L.K., Passaglia, L.M.P., Giongo, A., de Oliveira, R.R., Pereira, L. de M., de Jesus Trindade, F., Sperotto,

R.A., 2018. Biogas from slaughterhouse wastewater anaerobic digestion is driven by the archaeal family Methanobacteriaceae and bacterial families Porphyromonadaceae and Tissierellaceae. *Renew. Energy* 118, 840–846.

Herrero, M., Stuckey, D.C., 2015. Bioaugmentation and its application in wastewater treatment: A review. *Chemosphere* 140, 119–128.

Howe, E., Holton, K., Nair, S., Schlauch, D., Sinha, R., Quackenbush, J., 2010. MeV: MultiExperiment Viewer, in: Ochs, M.F., Casagrande, J.T., Davuluri, R. V (Eds.), *Biomedical Informatics for Cancer Research*. Springer US, Boston, MA, pp. 267–277.

Jacob-Lopes, E., Revah, S., Hernández, S., Shirai, K., Franco, T.T., 2009. Development of operational strategies to remove carbon dioxide in photobioreactors. *Chem. Eng. J.* 153, 120–126.

Karakashev, D., Batstone, D.J., Trably, E., Angelidaki, I., 2006. Acetate oxidation is the dominant methanogenic pathway from acetate in the absence of Methanosaetaceae. *Appl. Environ. Microbiol.* 72, 5138–5141.

Kim, S., Deshusses, M.A., 2003. Development and experimental validation of a conceptual model for biotrickling filtration of H₂S. *Environ. Prog.* 22, 119–128.

Kougias, P.G., Boe, K., Einarsdottir, E.S., Angelidaki, I., 2015. Counteracting foaming caused by lipids or proteins in biogas reactors using rapeseed oil or oleic acid as antifoaming agents. *Water Res.* 79, 119–127.

Kougias, P.G., Treu, L., Benavente, D.P., Boe, K., Campanaro, S., Angelidaki, I., 2017. Ex-situ biogas upgrading and enhancement in different reactor systems. *Bioresour. Technol.* 225, 429–437.

Lee, J.C., Kim, J.H., Chang, W.S., Pak, D., 2012. Biological conversion of CO₂ to CH₄

using hydrogenotrophic methanogen in a fixed bed reactor. *J. Chem. Technol.*

Biotechnol. 87, 844–847.

Luo, G., Angelidaki, I., 2012. Integrated biogas upgrading and hydrogen utilization in an anaerobic reactor containing enriched hydrogenotrophic methanogenic culture.

Biotechnol. Bioeng. 109, 2729–2736.

Maegaard, K., Garcia-Robledo, E., Kofoed, M.V.W., Agneessens, L.M., de Jonge, N.,

Nielsen, J.L., Ottosen, L.D.M., Nielsen, L.P., Revsbech, N.P., 2019. Biogas

upgrading with hydrogenotrophic methanogenic biofilms. *Bioresour. Technol.* 287,

121422.

Pokorna, D., Varga, Z., Andreides, D., Zabranska, J., 2019. Adaptation of anaerobic

culture to bioconversion of carbon dioxide with hydrogen to biomethane. *Renew.*

Energy 142, 167–172.

Porté, H., Kougias, P.G., Alfaro, N., Treu, L., Campanaro, S., Angelidaki, I., 2019.

Process performance and microbial community structure in thermophilic trickling

biofilter reactors for biogas upgrading. *Sci. Total Environ.* 655, 529–538.

Rachbauer, L., Voithl, G., Bochmann, G., Fuchs, W., 2016. Biological biogas upgrading

capacity of a hydrogenotrophic community in a trickle-bed reactor. *Appl. Energy*

180, 483–490.

Ragsdale, S.W., Pierce, E., 2008. Acetogenesis and the Wood-Ljungdahl pathway of

CO₂ fixation. *Biochim. Biophys. Acta - Proteins Proteomics* 1784, 1873–1898.

Sieborg, M.U., Jønson, B.D., Ashraf, M.T., Yde, L., Triolo, J.M., 2020. Biomethanation

in a thermophilic biotrickling filter using cattle manure as nutrient media.

Bioresour. Technol. Reports 9, 100391.

Siegert, M., Li, X.F., Yates, M.D., Logan, B.E., 2014. The presence of

hydrogenotrophic methanogens in the inoculum improves methane gas production in microbial electrolysis cells. *Front. Microbiol.* 5, 778.

Singh, S.P., Prerna, P., 2009. Review of recent advances in anaerobic packed-bed biogas reactors. *Renew. Sustain. Energy Rev.* 13, 1569–1575.

Strübing, D., Moeller, A.B., Mößnang, B., Lebuhn, M., Drewes, J.E., Koch, K., 2018. Anaerobic thermophilic trickle bed reactor as a promising technology for flexible and demand-oriented H₂/CO₂ biomethanation. *Appl. Energy* 232, 543–554.

Sun, Q., Li, H., Yan, J., Liu, L., Yu, Z., Yu, X., 2015. Selection of appropriate biogas upgrading technology-a review of biogas cleaning, upgrading and utilisation. *Renew. Sustain. Energy Rev.* 51, 521–532.

Treu, L., Kougias, P.G., de Diego-Díaz, B., Campanaro, S., Bassani, I., Fernández-Rodríguez, J., Angelidaki, I., 2018. Two-year microbial adaptation during hydrogen-mediated biogas upgrading process in a serial reactor configuration. *Bioresour. Technol.* 264, 140–147.

Vo, T.T.Q., Wall, D.M., Ring, D., Rajendran, K., Murphy, J.D., 2018. Techno-economic analysis of biogas upgrading via amine scrubber, carbon capture and ex-situ methanation. *Appl. Energy* 212, 1191–1202.

Voelklein, M.A., Rusmanis, D., Murphy, J.D., 2019. Biological methanation: Strategies for in-situ and ex-situ upgrading in anaerobic digestion. *Appl. Energy* 235, 1061–1071.

Weiland, P., 2010. Biogas production: Current state and perspectives. *Appl. Microbiol. Biotechnol.* 85, 849–860.

Westerholm, M., Müller, B., Singh, A., Karlsson Lindsjö, O., Schnürer, A., 2018.

Detection of novel syntrophic acetate-oxidizing bacteria from biogas processes by

continuous acetate enrichment approaches. *Microb. Biotechnol.* 11, 680–693.

Yang, S., Li, S., Jia, X., 2019. Production of medium chain length polyhydroxyalkanoate from acetate by engineered *Pseudomonas putida* KT2440. *J. Ind. Microbiol. Biotechnol.* 46, 793–800.

Yuan, T., Bian, S., Hac, J., Liu, J., Shi, X., Xu, Q., 2020. Exploring the roles of zero-valent iron in two-stage food waste anaerobic digestion. *Waste Manag.* 107, 91–100.

Yun, Y.M., Sung, S., Kang, S., Kim, M.S., Kim, D.H., 2017. Enrichment of hydrogenotrophic methanogens by means of gas recycle and its application in biogas upgrading. *Energy* 135, 294–302.

Tables

Table 1. Characteristics of degassed digestate and hydrogenotrophic inoculum

	Digestate	Inoculum
pH	8.00±0.10	8.28±0.04
Total solids (TS), g/L	23.1±1.2	25.5±1.3
Volatile solids (VS), g/L	12.0±0.6	17.8±0.9
Total Kjeldahl Nitrogen (TKN), g/L	4.9±0.2	2.01±0.03
NH ₄ -N, g/L	3.6±0.2	1.67±0.01
Total volatile fatty acids (TVFA), mg/L	136.6± 6.8	290.1±13.6
Acetate, mg/L	64.9±3.2	239.2±11.2
Propionate, mg/L	39.9±2.0	26.8±0.4
Iso-butyrate, mg/L	17.9±0.9	7.9±1.4
Butyrate, mg/L	2.0±0.1	1.5±0.2
Iso-valerate, mg/L	11.3±0.6	14.7±1.2
Valerate, mg/L	0.3±0.0	0.1±0.0
n-hexanoate, mg/L	0.3±0.0	0.1±0.0

519 **Table 2.** Reactors' upgrading performance under different experimental periods

Period	GRT, h	Q _{RC} , L/L.R.h	Reactor	PCH ₄ , LCH ₄ /L.R.d	η_{CO_2} , %	η_{H_2} , %	k _{La} , 1/day
I	8	5.3	R _c	0.29±0.02	48.3±5.3	80.4±2.6	8.95*10 ²
			R _{r-r}	0.32±0.03	54.8±1.4	73.9±0.8	9.22*10 ²
II	8	7.0	R _c	0.44±0.02	63.0±4.1	94.7±0.7	2.89*10 ³
			R _{r-r}	0.42±0.04	55.2±3.5	90.6±1.3	2.24*10 ³
III	6	7.0	R _c	0.48±0.01	55.3±13.5	81.8±2.4	1.29*10 ³
			R _{r-r}	0.52±0.03	61.2±10.3	87.3±2.6	2.40*10 ³
IV	6	8.8	R _c	0.43±0.01	62.9±0.5	74.5±0.5	9.59 *10 ²
			R _{r-r}	0.54±0.07	64.1±4.6	86.1±7.0	2.11*10 ³
V	5	8.8	R _c	0.38±0.17	30.5±6.6	64.9±2.9	1.59*10 ³
			R _{r-r}	0.67±0.17	72.5±8.1	74.7±3.8	2.57*10 ³

GRT: gas retention time, Q_{RC}: gas recirculation rate, η_{CO_2} : CO₂ utilisation efficiency, η_{H_2} : H₂ utilisation efficiency, k_{La}: H₂ gas transfer coefficient

520

Figures captions

Fig. 1. VFA concentration and pH in R_C (a) and R_{r-r} (b)

Fig. 2. Biogas composition at the outlet of R_C (a) and R_{r-r} (b)

Fig. 3. Heat maps of relative abundance (left side of the panel) and fold change in microbial composition (right side of the panel). In the relative abundance heat map, the colour scale varies from low abundance (blue) to high abundance (red); in the heat map of fold changes, an increase trend is reported in red, while a decrease is expressed in green. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)