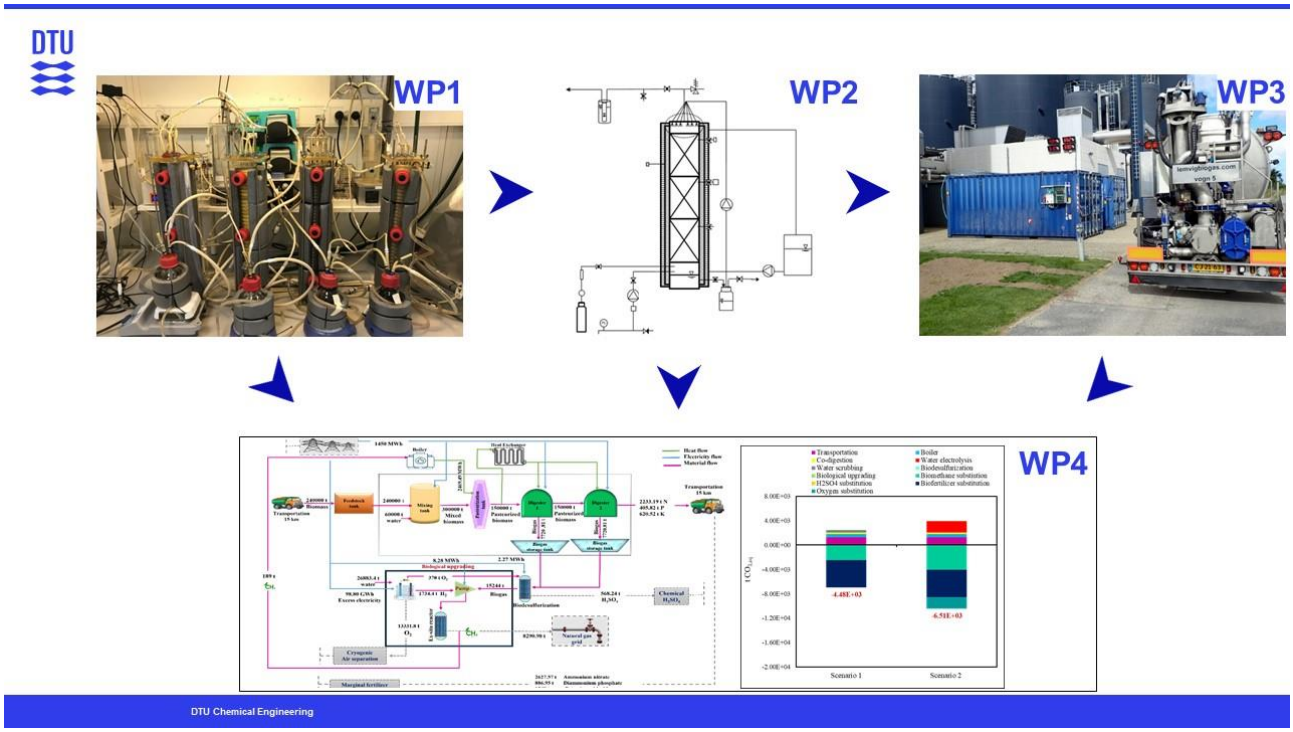
Final report

ForskEL 2016-1-12465

BioUpgrade- Ex-situ biogas upgrading through biologically mediated CO_2 reduction



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1. Project details

Project title	BioUpgrade- Ex-situ biogas upgrading through biologically mediated CO_2 reduction
File no.	2016-1-12465
Name of the funding scheme	ForskEL
Project managing company / institution	DTU Kemiteknik, Department of Chemical and Biochemical Engineering, Technical University of Denmark, Søltofts Plads 228A 2800 Kgs. Lyngby, Denmark, Phone: +45 4525 2822, Email: kt@kt.dtu.dk, www.kt.dtu.dk
CVR number (central business register)	30060946
Project partners	DTU KT, Lemvig Biogasanlæg Amba, LSH-BIOTECH aps
Submission date	21 June 2021

2. Summary

English: The main objective of this project was to first develop at lab-scale a novel biogas upgrading technology with high CO_2 and H_2 conversion rates and then, up-scale the technology at operational environment to validate process efficiency closer to real-life application (i.e. full-scale biogas plant). Microbial community dynamics and syntrophic interactions helped to optimize process efficiency and stability. Technological, environmental and economic perspectives of the biological upgrading process was defined using life-cycle assessment (LCA) methodology. Different reactor configurations were explored, and the superiority and suitability of trickle bed reactor (TBR) was revealed. As a result, a TBR was exploited at higher scale. The main outcome of the project was an integrated pilot-scale unit able to operate at raw biogas and digestate from biogas plant. The LCA unveiled that biological biogas upgrading can be a sustainable process to be implemented in full-scale facilities.

Danish: Hovedformålet med dette projekt var for det første at udvikle en ny biogasopgraderingsteknologi i lab med høje CO_2- og H_2-konverteringshastigheder, og derefter validere effektivitet af processen i driftsmiljøet, dvs. fuldskala biogas anlæg. Viden om mikrobiel-sammensætnings dynamik og syntrofiske interaktioner har givet den grundlæggende viden for at optimere effektivitet og stabilitet af processen. Teknologiske, miljømæssige og økonomiske perspektiver ved den biologiske opgraderingsproces blev defineret ved hjælp af livscyklusvurderingsmetoden (LCA). Forskellige reaktor konfigurationer blev oprindeligt undersøgt, og overlegenhed og egnethed af modstrøms ”trickling bed reactor” (TBR) blev valideret. En pilot skal TBR blev designet og bygget og dernæst testet i Lemvig biogasanlæg. Resultatet af projektet var en integreret pilot anlæg, der kunne med succes opgradere biogas til biomethan. Miljøeffekterne af opgraderings processen blev vurderet mht fremtidig implementering i fuld skala anlæg.

3. Project objectives

BioUpgrade aimed to develop an innovative, efficient and robust technology for biological biogas upgrade, utilising the indigenous hydrogenotrophic cultures of the anaerobic digestion (AD) process, and achieve a biogas methane concentration equivalent to natural gas (>95% CH_4). The project had the following sub-objectives:

- Define the optimum reactor configuration that can lead to a robust biogas upgrading process, maximizing the H_2 utilization efficiency and CO_2 conversion into CH_4.
- Identify the metabolic pathways and decipher the dynamics of microbial community composition and activity due to the effect from H_2 addition in order to optimize the process efficiency and stability.
- Validate the developed upgrading technology in pilot scale conditions and demonstrate the operation of a prototype unit in connection with a full-scale biogas plant.
- Assess the proposed technology from a technological, environmental and economic perspective using life-cycle assessment (LCA) methodology.

Ex-situ biogas upgrading through biologically mediated CO_2 reduction was firstly developed in the lab and secondly, demonstrated in operational environment using real biogas in Lemvig biogas plant. To demonstrate the energy technology the following scientific and technological challenges were solved:

- Development of novel reactor configuration to establish a technical solution for efficient H_2 injection to increase coupling with CO_2 from the biogas and capturing in the form of CH_4 above 95%.
- Elucidate syntrophic interactions and microbial community dynamics for ex-situ biogas upgrading process to ensure long-term process reliability and high bioconversion rates.
- Test and demonstrate the proposed system under pilot-scale in full-scale biogas to boost commercialization of the concept.
- Define environmental impacts of the proposed process compared to other commercial biogas upgrading technologies.

4. Project implementation

4.1 Project evolvement

Staff members involved in the current project are affiliated in the Department of Chemical and Biochemical Engineering, Technical University of Denmark (DTU-KT). Irini Angelidaki managed the whole project. Panagiotis Tsapekos was involved in the design and execution of project's research work as Work Package leader, distribution of project tasks, guidance and supervision of other researchers.

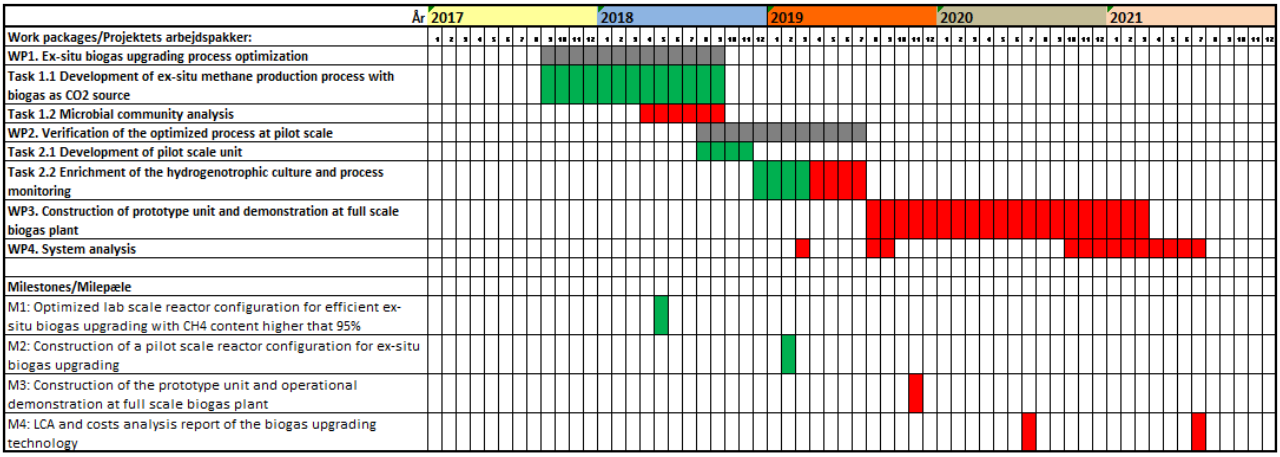

Table 1. Gantt chart of project activities

In order to address the objectives and the hypotheses of this project, the research project was divided in four major work packages (WP). The initial duration of the project was 3 years; however, due to delays on hiring employees and also, findings' significance related to green transition, the project was extended for 2 more years. The project has been successfully fulfilled as it shown in the following section.

4.2 Risks

Two major risks were associated with the project execution. The first one considered the finding of skilled and experienced researchers to efficiently set-up, operate and evaluate different reactor configurations in the lab and conduct the demonstration tests. Meanwhile, the second risk related to the upscaling process concerned the possibility of incurring delays or complications during construction or operation of the setup.

4.3 Project development

In general, the project met the targeted milestones and the flow of project delivery is depicted in Table 1. However, few minor changes were conducted compared to the initial project outline. A modification to the original plan was the extension of the overall project period. This was deemed necessary due to changes in project personnel and the inability of the demonstration to be conducted on initially scheduled time due to extended ATEX considerations. Meanwhile, the missing process information related to LCA was collected from additional pilot- and laboratory-scale experiments carried out at DTU, along with experimental data reported in the scientific literature.

4.4 Unexpected challenges

The challenges faced during project execution were associated with the timely management of the project tasks, given the complications with the pilot-scale reactor construction acceptance based on highest safety standards. Nevertheless, these technical challenges were considered during the risk assessment of the project (see section 4.2), therefore it cannot be listed in unexpected challenges. Lab-scale operation had to be prolonged to ensure that the obtained results will be reliable for the scale-up operation. Nevertheless, lab-tests prolongation did not cause any significant delay to the overall project. An unexpected challenge was related to changes in personnel at the final phase of project execution. However, hired employees contributed to finishing the remaining tasks and the project was successfully completed.

5. Project results

5.1 WP1

WP1 was focused on the examination of different reactor set-ups to define optimal reactor configuration that can increase the biologically coupling of CO_2 with H_2 and define microbial community dynamics during ex-situ biogas upgrading process. Different reactor systems have already been examined in DTU as for as example continuous stirred tank reactors (CSTRs)[1] and hollow fiber membranes[2]. Despite the promising results achieved (>90% CH_4), the previously examined systems are facing multiple challenges. For example, a big disadvantage of CSTRs is the demand of high-volume reactors increasing the investment costs. On the other hand, hollow fiber membranes can easily clog due to formed biofilm on outer surface of the membrane increasing the operational expenditure for regular maintenance. Moreover, the above-mentioned systems were not associated with low gas retention times (GRT < 4h) which has a drawback on the CH_4 production capacity and so, there was a need for finding a more efficient system. During BioUpgrade execution four different systems were explored: Hybrid system, up-flow reactors, ceramic membranes, and trickle bed-reactors (Fig. 1).

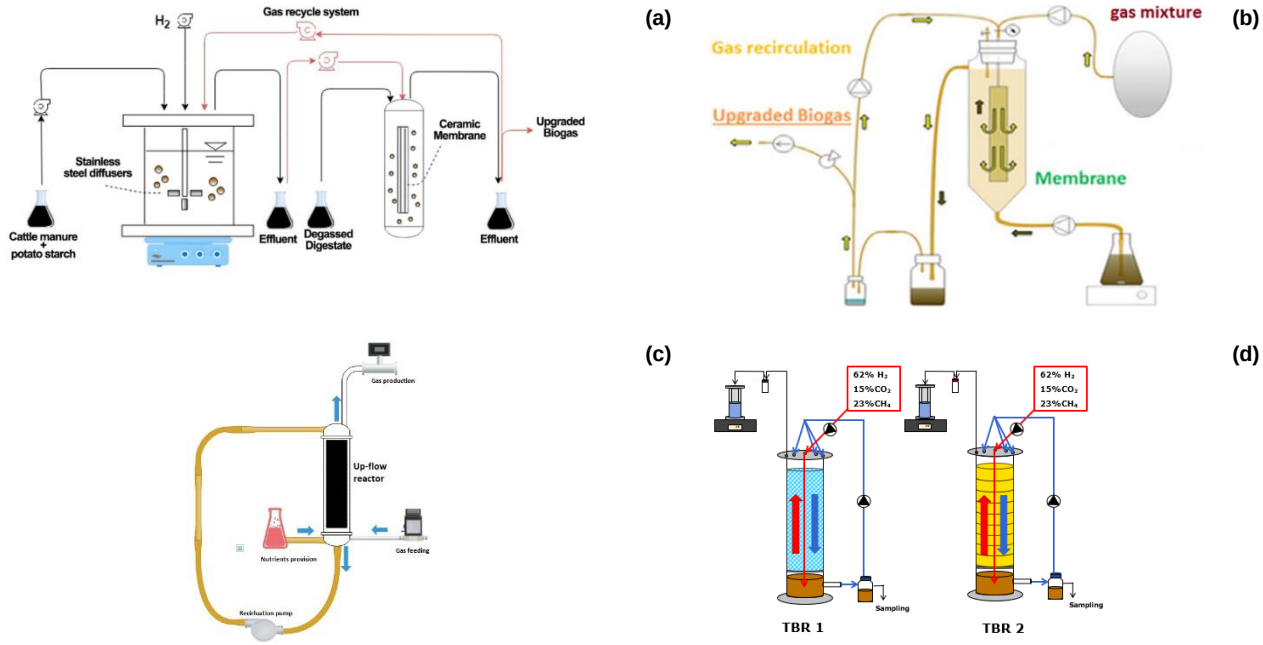

Fig. 1. Different configurations: hybrid system (a), membrane filled reactor (b), up-flow set-up (c) and trickle bed reactor (d)

In the hybrid system, H_2 was initially injected in the first stage of a CSTR via stainless steel diffusers and then, the partially upgraded biogas was injected in a second stage reactor[3] (Fig. 1a). The process was consisted of 3 operational periods: a) the configuration was operated as a conventional anaerobic digester (Period I); b) H_2 was directly injected in the first stage reactor and the output gas from the first reactor (in-situ biogas upgrading) was subsequently transferred to the second reactor (ex-situ upgrading) (Period II); c) the injection of H_2 was

[1] Bassani, I., Kougias, P.G., Treu, L. and Angelidaki, I., 2015. Biogas upgrading via hydrogenotrophic methanogenesis in two-stage continuous stirred tank reactors at mesophilic and thermophilic conditions. *Environmental science & technology*, *49*(20), pp.12585-12593.

[2] Luo, G. and Angelidaki, I., 2013. Hollow fiber membrane based H_2 diffusion for efficient in situ biogas upgrading in an anaerobic reactor. *Applied microbiology and biotechnology*, *97*(8), pp.3739-3744.

[3] Corbellini, V., Kougias, P.G., Treu, L., Bassani, I., Malpei, F. and Angelidaki, I., 2018. Hybrid biogas upgrading in a two-stage thermophilic reactor. *Energy Conversion and Management*, *168*, pp.1-10.

stopped and the system worked with the same operating conditions as in Period I and the two reactors were connected by a gas recirculation system (Period III). Despite the promising results achieved at the 1st and 2nd stage equal to 87% and 95% CH_4, respectively; a clear drawback of the system was the remarkable volatile fatty acids (VFA) accumulation and especially, acetate concentrations in the 1st stage (>5 g-VFA/L, >3 g-HAc/L) (Fig. 2). Thus, to avoid risk for inhibition due to accumulated acetate in the 1st stage (i.e. biogas reactor) the 2 step injections was not further followed. However, due to the high efficiency of ceramic membranes to highly upgrade the biogas during the 2nd stage, membranes were further explored during project execution.

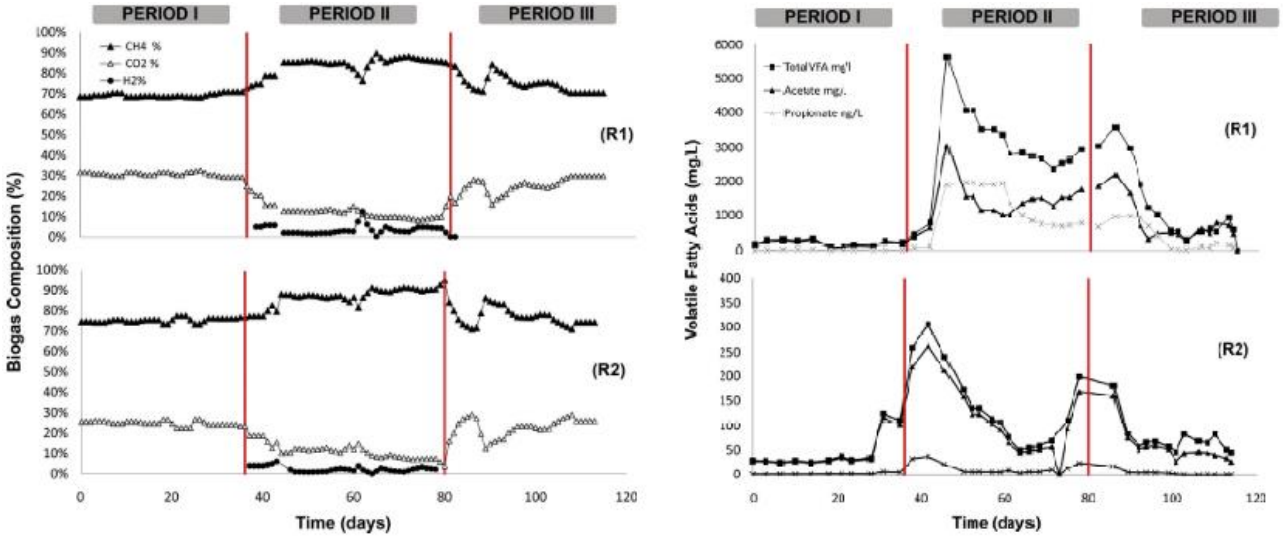

Fig. 2. Gas composition and VFA accumulation for the *in-situ* (R1) and *ex-situ* (R2) hybrid system

As a continuation of the hybrid system, 4 different ceramic membranes made of either SiC or AlO_3 were exploited at ex-situ configuration (Fig. 1b). The experiment was separated at three distinctly periods with different GRT of 10 (P I), 5 (P II) and 2.5 h (P III). Despite GRT decrease was associated with higher CH_4 production rate, $\%CH_4$ was higher at longer GRTs as defined by higher gas holdup and higher overall mass transfer coefficient. SiC membranes had improved efficiency than Al_2O_3 materials due to improve mixing and reached CH_4 concentrations up to 99% in output (Fig. 3). For GRTs higher than 2.5 h, increasing pore size of SiC diffuser from 0.5 to 7 µm resulted in higher bubble size and so, higher mixing, turbulency and biomethanation efficiency due to the improved mass transfer coefficient. The membrane with increased pore size (i.e. 14 µm) had lower overall mass transfer coefficient due to the substantial reduction of the specific interfacial area. Overall, the reactors equipped with SiC gas diffusers effectively improved CH_4 content fulfilling natural gas standards.

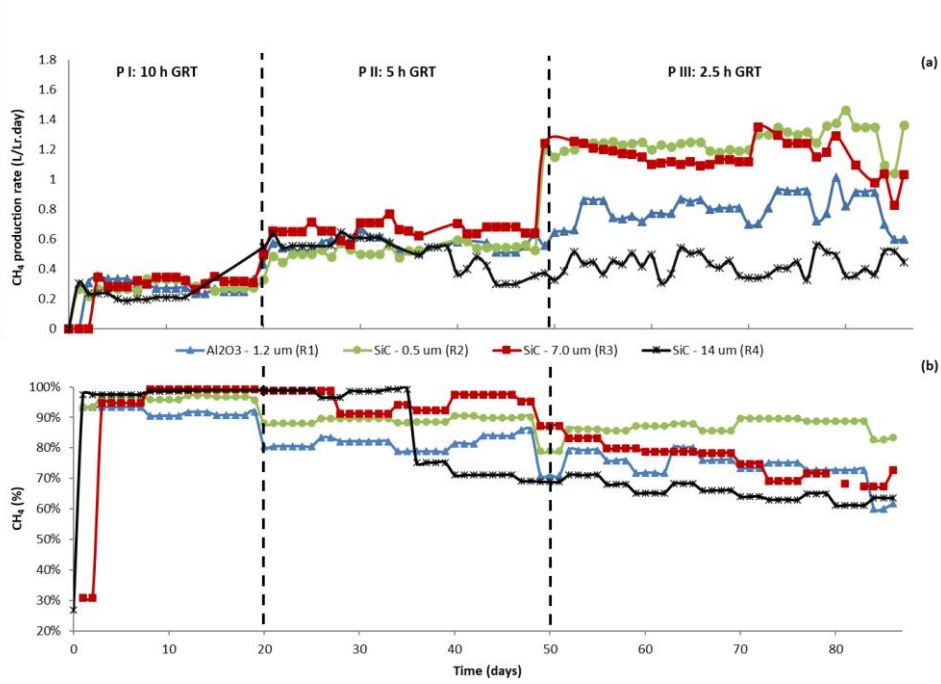

Fig. 3. Ex-situ biogas upgrading at membranes filled reactors: CH_4 production rate (a) and percentage (b)

Considering that in a recent study[4], the biological coupling of CO_2 with H_2 in up-flow reactors led to high biomethanation efficiency, up-flow reactors were examined as alternative reactor set-up[5] (Fig. 1c). The experiment was divided into five distinguished periods, in which, either the gas injection rate (L/L_R/day) was increased to decrease the GRT from 8 to 5 h or the gas recirculation rate (Q_{RC}) was increased from 5.3–8.8 L/L_R/day to evaluate the effect of these two parameters on CO_2 and H_2 utilization. In contrast to the previous study where ceramic membranes were used to sparge the gases, the reactors in BioUpgrade were filled with and without Raschig rings as packing materials without the usage of gas distributor. Results showed gas recirculation did not have a highly positive impact on biomethanation and that the reactor filled with packing materials increased gases utilization leading to a CH_4 content of 81% at 6 h GRT time (Fig. 4). On the contrary, limited biomethanation was achieved in the absence of Raschig rings, revealing the key role of packing materials in ex-situ biomethanation processes.

[4] Bassani, I., Kougias, P.G., Treu, L., Porté, H., Campanaro, S. and Angelidaki, I., 2017. Optimization of hydrogen dispersion in thermophilic up-flow reactors for ex situ biogas upgrading. *Bioresource technology*, *234*, pp.310-319.

[5] Kougias, P.G., Tsapekos, P., Treu, L., Kostoula, M., Campanaro, S., Lyberatos, G. and Angelidaki, I., 2020. Biological CO_2 fixation in up-flow reactors via exogenous H_2 addition. *Journal of Biotechnology*, *319*, pp.1-7.

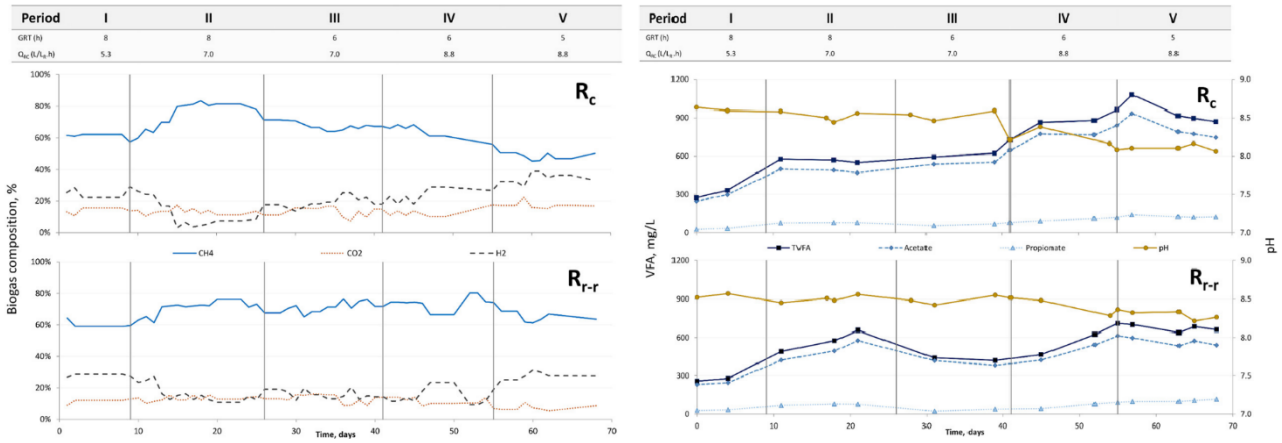

Fig. 4. Gas composition and VFA accumulation for up flow reactors R_c as control and $R_{r\text{-}r}$ filled with Raschig rings

Lately, TBRs packed with material of high specific surface area have been defined as highly efficient reactor system to support biological upgrading[6]. During BioUpgrade, the lab-scale TBRs were operated at 4 and 2 h GRT (Fig. 5). As mentioned above, promising results have been achieved at the up-flow reactors filled with packing materials. Thus, there was an evidence that packing selection is crucial to achieve high biomethanation. Hence, TBRs were constructed and equipped with different packing materials: Raschig rings and polyurethane foam. Reactors' monitoring showed that proper trickling is mandatory to wet the filling materials and create good biofilm. Specifically, at TBR filled with Raschig rings the liquid media was trickled and recycled over the packing material leading to limited moisture and nutrients provision. On the contrary, polyurethane foam helped to be formed biofilm at low trickling speed. As a result of the formed biofilm, over 95% CH_4 was detected in the output at 4 and 2 h GRT, which were the best results achieved in the lab-tests. Hence, polyurethane foam was explored as a packing material during the up-scaling operation of TBR.

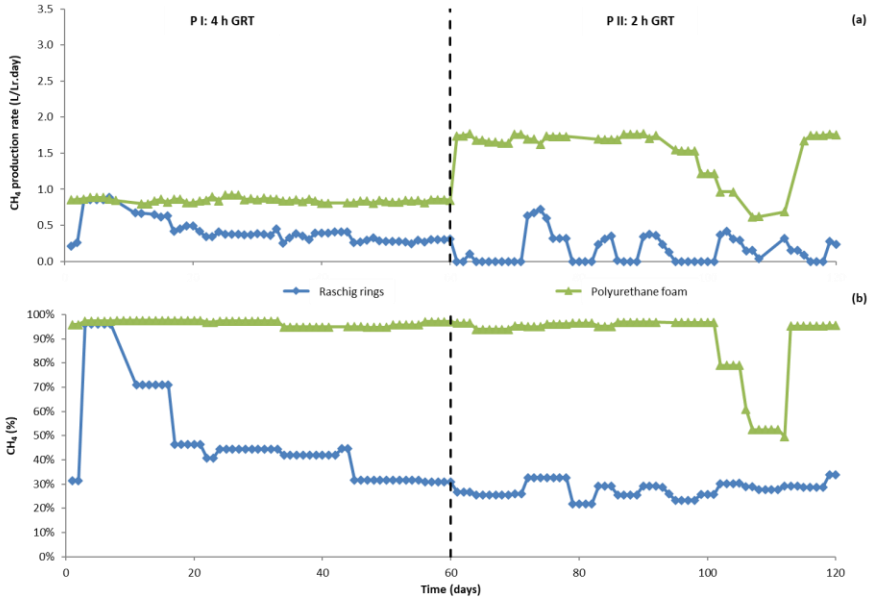

Fig. 5. Ex-situ biogas upgrading at trickle bed reactors: CH_4 production rate (a) and percentage (b)

[6] Porté, H., Kougias, P.G., Alfaro, N., Treu, L., Campanaro, S. and Angelidaki, I., 2019. Process performance and microbial community structure in thermophilic trickling biofilter reactors for biogas upgrading. *Science of The Total Environment*, *655*, pp.529-538.

During ex-situ biogas upgrading, the process can be directly mediated via hydrogenotrophic methanogens to convert CO_2 to CH_4 using external H_2 as a source of electrons (Fig. 6). Hydrogenotrophic methanogenesis is energetically favorable as shown by the negative change in Gibbs free energy[7].

$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$ $\Delta G°' = -130.7$ KJ/mol

Biomethanation can also occur indirectly via the involvement of homo-acetogenic bacteria converting CO_2 to acetate via the Wood-Ljungdahl pathway which is also an exergonic process (Fig. 6). Then, low energy gain is compensated by acetoclastic methanogens converting CH_3COOH into CH_4.

$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O$ $\Delta G°' = -104.5$ kJ/mol
$CH_3COOH \rightarrow CH_4 + CO_2$ $\Delta G°' = -31.0$ kJ/mol

Due to the important role of H_2 partial pressure on equilibrium of biochemical reactions, supplementation of exogenous H_2 has a strong impact on the microbial community shaping composition with a massive increase of both hydrogenotrophic archaea and homo-acetogenic bacteria producing acetate from H_2 and CO_2. On the contrary, external H_2 provision can also inhibit syntrophic acetogens (involved in propionate and butyrate degradation) and syntrophic acetate oxidizers (SAO).

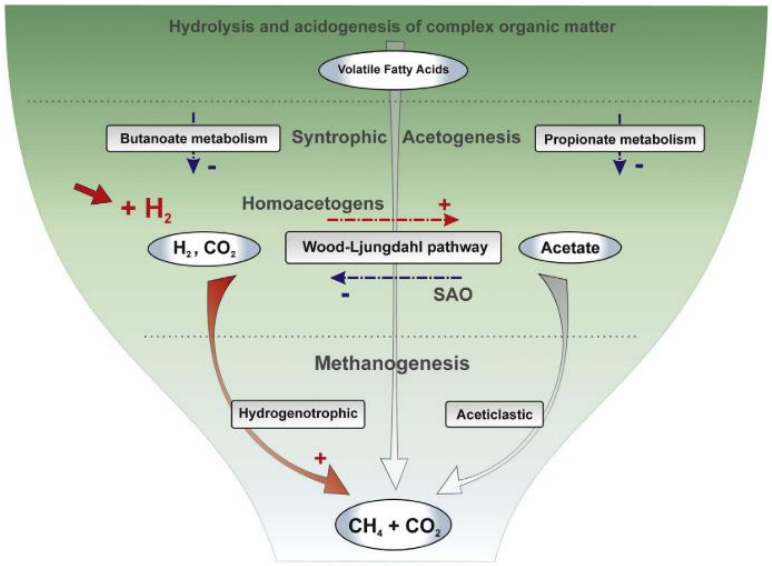

Fig. 6. Metabolic pathways for hydrogen assisted methanogenesis (Angelidaki et al., 2018)[7].

Microbial analysis performed during ex-situ biogas upgrading, showed that the most frequently detected hydrogenotrophic methanogens belonged to *Methanobacterium*, *Methanoculleus*, *Methanomicrobium* and *Methanothermobacter* genera, whereas *Methanosarcina* and other acetoclastic methanogens were presented at lower abundance. For example, a *Methanothermobacter* species (100% similar to *Methanothermobacter thermautotrophicus)* dominated the second stage of the hybrid system (Fig. 7). It should be noted that its abundance was enhanced by 45-fold from the beginning to the end of the experiment.

[7] Angelidaki, I., Treu, L., Tsapekos, P., Luo, G., Campanaro, S., Wenzel, H. and Kougias, P.G., 2018. Biogas upgrading and utilization: Current status and perspectives. *Biotechnology advances*, *36*(2), pp.452-466.

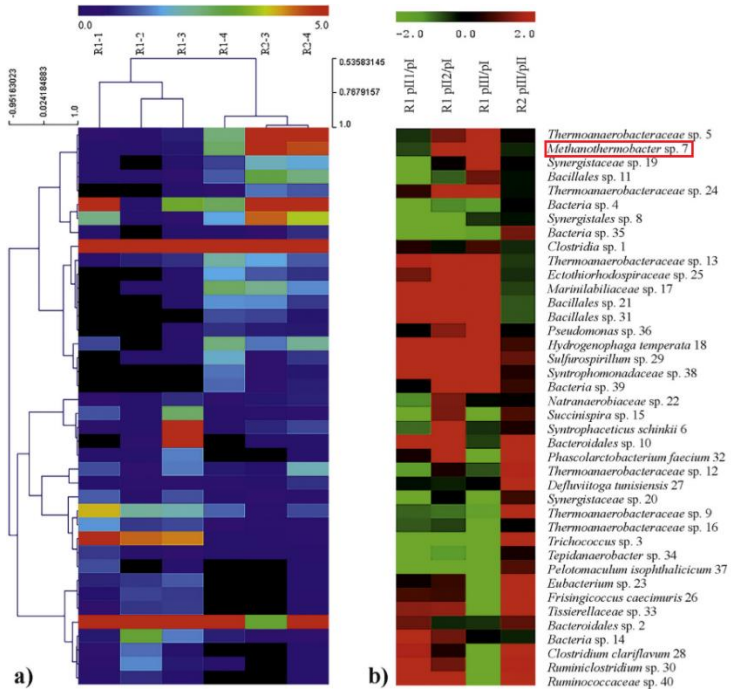

Fig. 7. Relative abundance shown as heat map for the interesting microbes in the hybrid system from samples collected at different experimental phases

Similarly, sequencing of samples collected from the up-flow reactors showed that the microbial community was ultimately resided by *Methanothermobacter* archaea (Fig. 8). Additionally, *Methanothermobacter* was 1.5-fold higher in the reactor filled with Raschig rings compared to the control indicating the importance of packing material to provide bed for archaeal proliferation[5]. On the contrary, in acetate-rich media a high abundance of members within *Pseudomonadaceae* or *Porphyromonadaceae* families was detected and can be linked with a potential presence of homo-acetogenic and syntrophic bacteria that are available in these families. The function of *Pseudomonas* genera to accelerate electron transfer was suggested as the reason for their co-habitation with archaea during efficient methanogenesis; while representatives of *Porphyromonadaceae* can dominate the bacterial community during efficient hydrogenotrophic methanogenesis driven by *Methanobacteriaceae* methanogens.

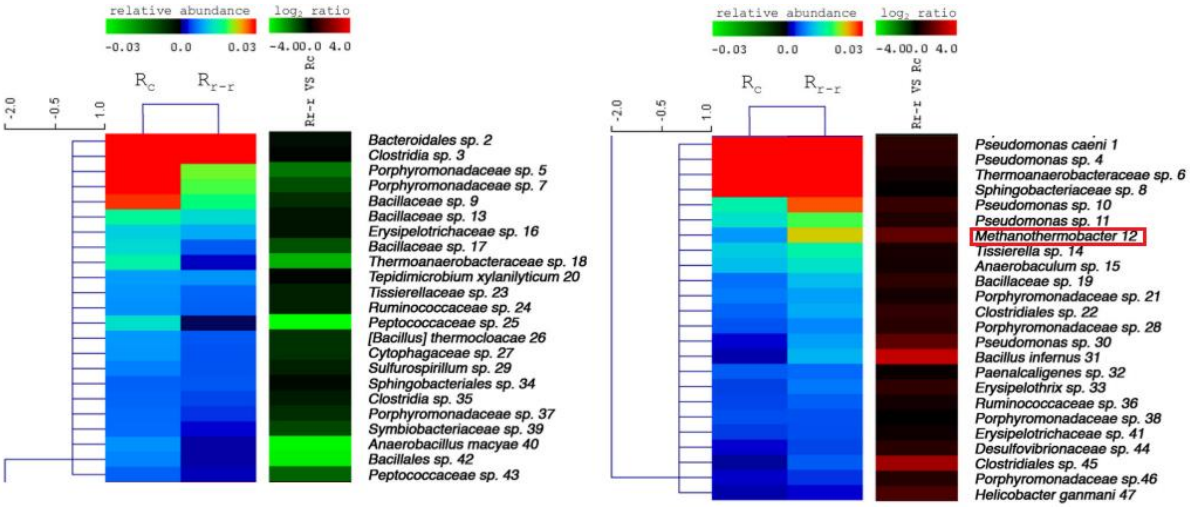

Fig. 8. Relative abundance shown as heat map for the interesting microbes for the up-flow reactors in the absence (R_c) and in the presence of Raschig rings (R_{r-r})

In accordance the most abundant archaeal genera detected in the membrane-based reactors belonged to *Methanobacteriaceae* family; while bacteria from *Thermoanaerobacteraceae* family were enriched in membrane-based reactors during periods with increased acetate accumulation (Fig. 9). The highest relative abundance of *Methanobacteriaceae* sp. was found at periods with the highest H_2 and CO_2 utilization efficiencies.

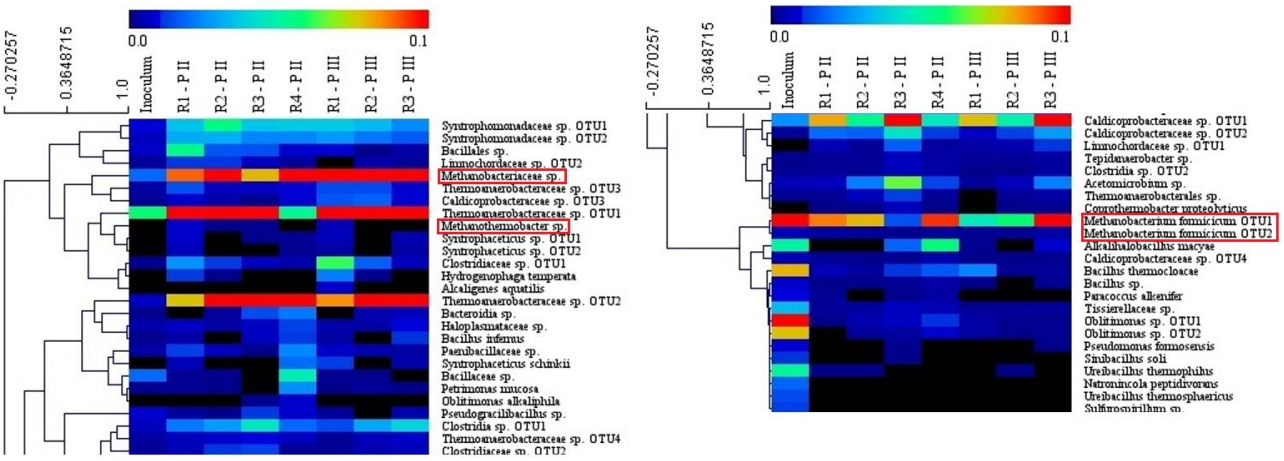

Fig. 9. Relative abundance shown as heat map for the interesting microbes for the membrane-based reactors equipped with different membranes at samples collected at different periods.

Focusing on the trickle bed reactors, high-throughput 16S rRNA results has previously revealed a clear dominance of *Methanothermobacter* sp. in the biofilm around packing material (Fig. 10). Unexplored representatives *Clostridia* class were enriched in both biofilm and liquid media and acetate utilizing bacteria resided in the liquid samples. The higher abundance of hydrogenotrophic methanogens in the biofilm could be explained as a result of higher proximity with the carbon and hydrogen source in the biofilm and possible syntrophic relationship with biofilm forming microbes. Linking biochemical and microbial data, it was clear that wetting of packing is important to achieve a good biofilm and so, increase biomethanation efficiency.

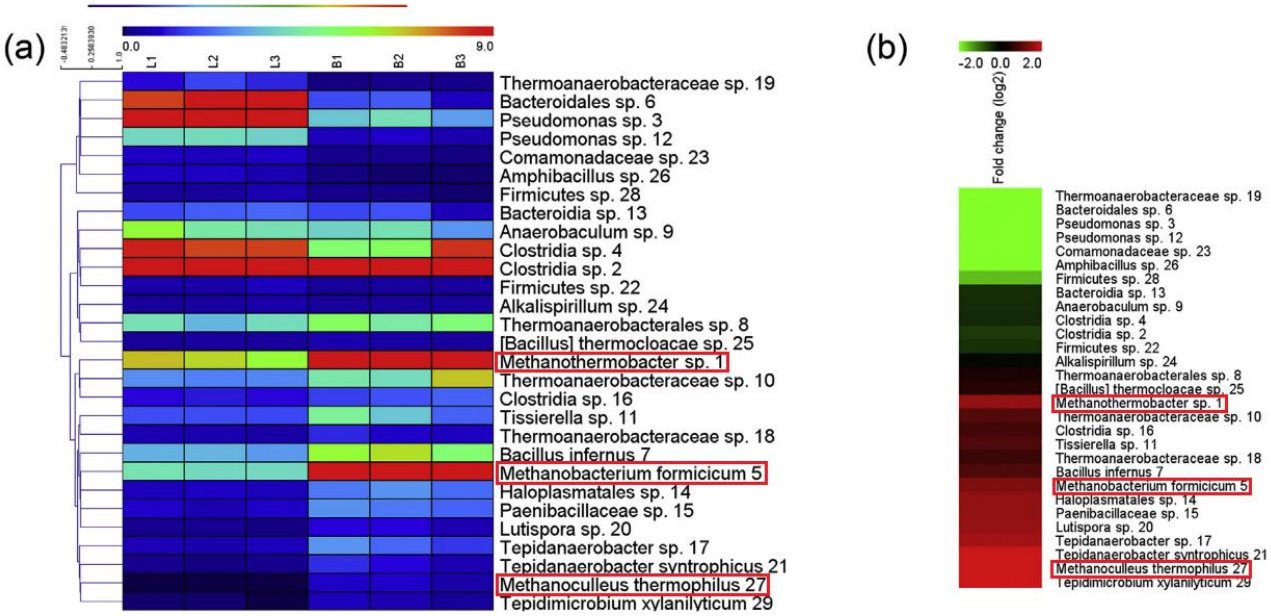

Fig. 10. Heat maps of the most abundant microbes resided in the liquid medium (a) and in the biofilm (b).

5.2 WP2

Based on the results of WP1, TBR configuration was exploited for the up-scaling tests. The TBR had a working volume of 68 L and connected with the pilot-scale biogas reactor of DTU (working volume of 500 L). The schematic diagram of the TBR, biogas reactor and ancillary equipment are depicted in Fig. 11. Initially the external upgrading reactor was inoculated with thermophilic inoculum collected from a 9.0 L CSTR operated at the lab and fed with artificial gas mixtures. The digestate from the biogas reactor was used a nutrients source. The combined CH_4:CO_2:H_2 feedstock was consisted of a 23:62:15 ratio and was added from the bottom of the upgrading reactor. Nutrients were trickled from the top of the reactor.

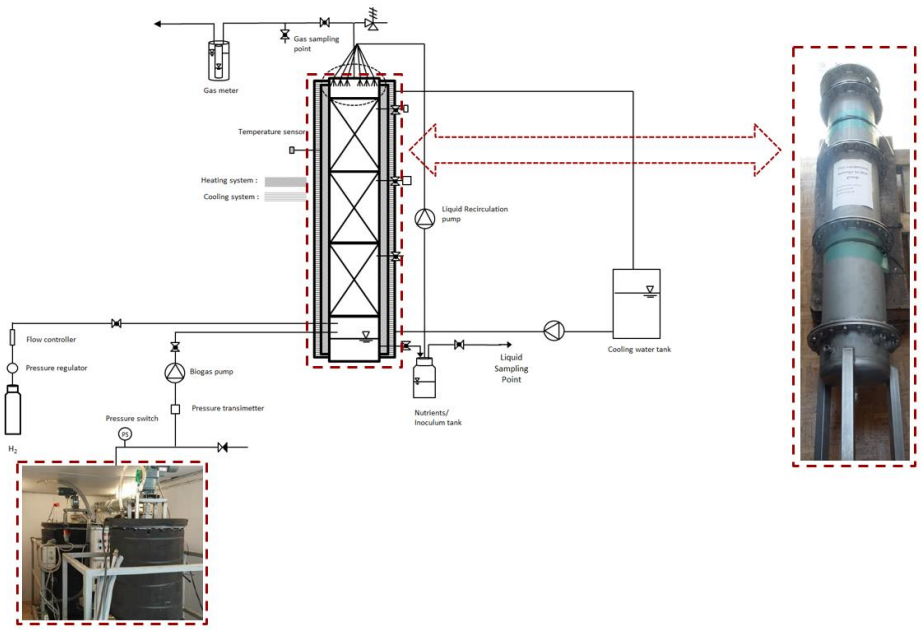

Fig. 11. Schematic diagram of the integrated pilot-scale system consisted of biogas reactor and ex-situ TBR

High biomethanation efficiency was succeeded in the first week of operation reaching more than 90% in the output (Fig. 12a). After adjusting the feedstock based on the stoichiometry (day 8), a slight drop of upgraded methane was detected (day 10 to 18). Nevertheless, at the end of P-2 and prior to standby period, during which no gases were added and trickling was paused, residual H_2 was not detected in the output and the CH_4 content was equal to 90%. The standby period was applied to evaluate reactor start-up and no significant lag phase was detected.

The highest CH_4 concentration (98%) was detected at the gas output since the beginning of the experiment at P-4. A deviation to the H_2 flow was faced from day 72 to 75 reducing the upgrading efficiency. Once the H_2 flow was adjusted to the correct value, the biomethane content reached again values higher than 95%. The biomethanation performance was deteriorated when the feeding load was doubled up to achieve a GRT of 5 h at P-5. Specifically, the CH_4 was decreased to 58% after 4 days at P-5 and stabilized at 76% at day 110. In parallel, a slight pH drop and acetate accumulation was observed indicating the presence of homo-acetogenic bacteria (Fig. 12b).

Due to the fact that polyurethane foam was a very thick filling material the contact between nutrients and gases was not optimized and thus, biofilm was not homogenously formed with the same efficiency through TBR bed. To overcome limited biomethanation, the perforated stainless-steel injectors that were used to add the gases were replaced with SiC membrane (as also dictated by WP1) as means to improve the gas-liquid contact. Indeed, the positive impact was quickly revealed and biomethane content reached a value of 98%. In parallel,

the acetate levels were not further increased validating the improved hydrogenotrophic activity compared that to previous period.

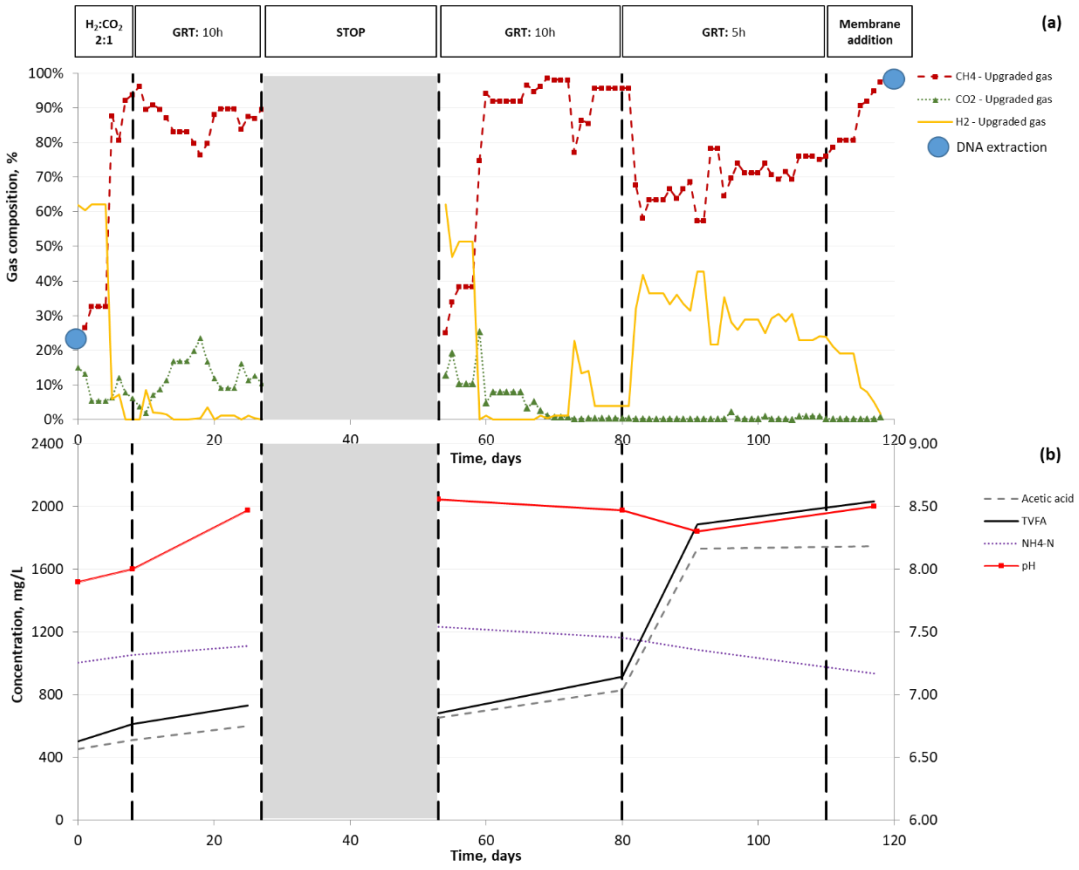

Fig. 12. Biomethane content (a), TVFA and acetic acid accumulation, NH_4-N and pH fluctuation (b)

To explore the microbial diversity and dynamicity along the TBR height, samples for genomic DNA extraction were collected from the top, middle, and low point of the packing material. Moreover, liquid sample was also extracted from the nutrients sump to define the planktonic microbiome. Inoculum microbiome was also characterized. Hence, five samples were analysed in total through metagenomic analysis.

The methanogenic archaea were represented by three Metagenome-Assembled Genomes (MAGs) assigned to the *Methanobacteriaceae* family (*Methanothermobacter wolfeii*, *Methanobacterium* sp. 1, *Methanobacteriaceae* sp. 1). The dominant bacteria in the inoculum (25%) was *Clostridia* sp. 1 which was the third most abundant (6%) in the planktonic sample, after *Firmicutes* sp. 1 and *Chromatiales* sp. 1 (both at 11%). Additionally, *Clostridia* sp. 1 was the main responsible for the bioprocess occurring in inoculum and liquid samples. In contrast, *Clostridiaceae* sp.1 was the dominant microbe (19%) at the top layer of the TBR. A stratification of microbial members throughout TBR height was clearly detected. *Methanobacterium* sp. 1 was the dominant microbe in the low and middle area of the TBR (20%) and the major microorganism found at the medium and low layer of the TBR. *Methanobacterium* sp. 1 was the main archaeon of the TBR, inoculum and nutrients sump. The other two methanogens were detected in significantly lower relative abundance; specifically, *Methanothermobacter wolfeii* was the second most abundant (0.8% average), while the abundance of *Methanobacteriaceae* sp. 1 was less than the minimal threshold considered (<0.5%).

The metabolic annotation revealed a high abundance of homo-acetogenic bacteria (for instance: *Clostridia* sp. 1 and *Clostridiaceae* sp. 1), which are able to use H_2 and CO_2 for acetate production through the Wood-Ljungdahl pathway. The finding obtained from functional annotation can potentially explain acetate accumulation (1.7 g/L) observed after 80 days of operation (Fig. 12b). The high abundance of these bacteria agrees with previous findings in the lab-scale tests fed with H_2 and CO_2 in which syntrophic association with methanogenic archaea was concluded. In addition, the three most abundant microbes of planktonic sample (*Clostridia* sp. 1, *Firmicutes* sp. 1 and *Chromatiales* sp. 1) have similar metabolic pathways.

Overall, it was revealed that methanogenesis was accomplished mainly by *Methanobacterium* sp. 1 and the complete hydrogenotrophic pathway was found in the genome of this MAG. It should be mentioned that another microorganism with high relative abundance in the samples from the low and middle layer of the TBR was *Clostridiaceae* sp. 1. Also, this MAG dominated the inoculum and it was expected that its abundance would remain stable independently from sampling points. However, the coverage of *Clostridiaceae* sp. 1 clustered with the corresponding one of *Methanobacterium* sp. 1. The fact that *Clostridiaceae* sp. 1 was found to be closely related to previously reported microbes can be strongly hypothesised that a syntropy exists due to this frequent co-occurrence.

5.3 WP3

To operate the prototype unit operation at relevant environment, the complete set-up was assembled and installed in a 20-foot container in line with ATEX regulations. Electric and mechanical equipment was connected to an electrical panel and controlled online via LabView. The complete set-up was transferred to Lemvig Biogas plant for the demonstration tests (Fig. 13).

The reactor shell was constructed by LSH Biotech using AISI 304 (ø273 x 2 mm; ID 269). The active volume was separated in three sections filled with polyurethane foam which was supported by polyester grid (h: 25 mm, ø260 mm) to ensure avoidance of packing material displacement. A screw-in resistance thermometer (JUMO Type PT 100) was placed in the middle of the TBR to monitor and control the temperature at thermophilic conditions (52 ±1 °C) using a heater cable (Fluoropolymer over jacket over tinned copper braid, EMTS2-CF). Since the biomethanation process is exothermic, a cooling system was prepared to maintain the operating temperature at the desired level. Hence, when heat was produced as advised by an online thermometer, water at room temperature was automatically recirculated using a peristaltic pump through soft copper tubing which was wrapped around the outer surface of the TBR. Inlet and outlet gas/liquid ports were available at bottom and top layer of the TBR to provide operational flexibility. The operating pressure was monitored using an analogue positive pressure gauge that was connected to the upper section of the active volume. Biogas was supplied using a peristaltic pump and H_2 was provided from commercial flasks monitored by a mass flow controller. Digestate was used as nutrient medium and trickled once per day from a nutrient sump to the top of the reactor using a peristaltic pump. Digestate was sieved to avoid clogging during operation. A cylindrical drip tray with multiple drain holes was placed at the top of the reactor to ensure proper distribution of nutrients. A pressure relief valve was placed at the top of the TBR for safety issues.

Fig. 13. Constructed prototype biogas upgrading unit and operational demonstration at Lemvig biogas plant

At Lemvig, the operation was initiated at 10 h GRT feeding the TBR with real biogas (Fig. 14). A small leakage did not allow accurate H_2 supply at the beginning of the tests. Thus, almost 30 days were needed to properly adjust the feeding regime based on the stoichiometry. Once the problem was overcome, the CH_4 at the output was higher than 90%. Consequently, by further increasing the feeding rate to reduce the GRT at 5 h more than 98.5% CH_4 was quantified in the output which was also the upper limit of produced CH_4 during the whole experiment. Adjusting the GRT at the lowest level of 2h led to a clear drop on biomethanation efficiency with a concomitant drop in pH values below 8.0. The result can be explained as the partial CO_2 pressure is in balance with the dissolved H_2CO_3 and the pH fluctuation is the result of a bicarbonate buffer system. During efficient hydrogenotrophic methanogenesis, CO_2 is coupled with H_2 and the increased pH favours HCO_3- formation. Also, the CO_2 uptake rate is enhanced at alkaline conditions. In accordance, the upgrading efficiency increased to the highest standards (>95% CH_4) when the pH increased from ~8.0 to 8.5.

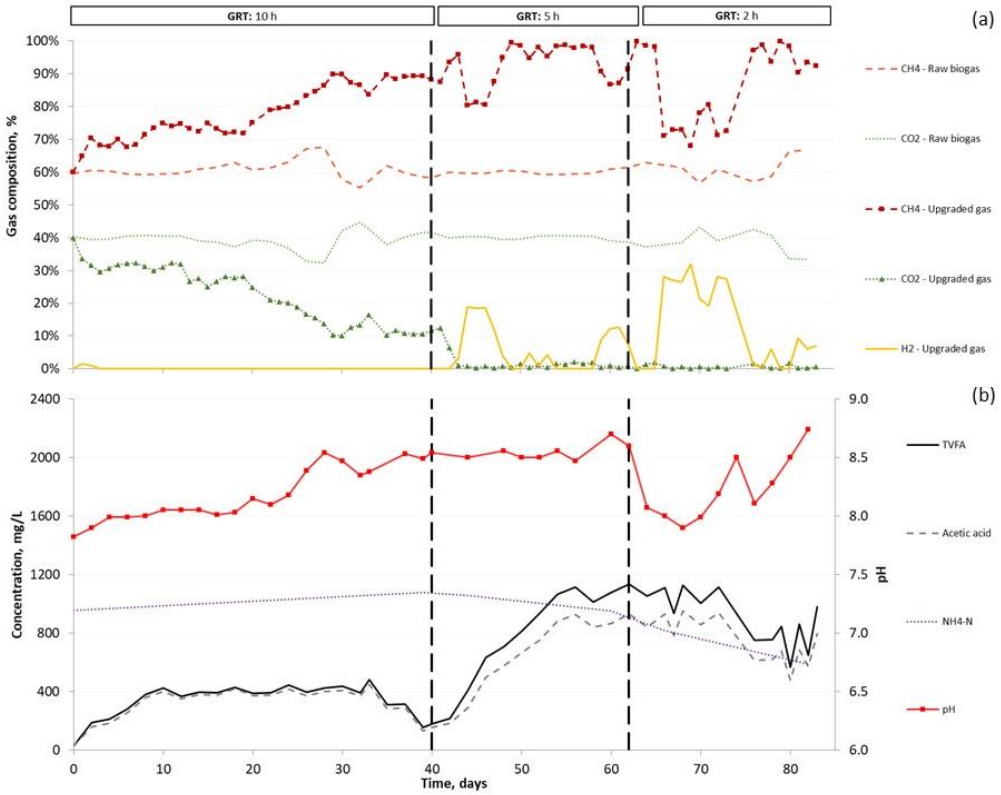

Fig. 14. Gas composition (a), TVFA and acetic acid accumulation, NH_4-N and pH fluctuation (b)

5.4 WP4

Life cycle inventory and analysis assessed the consequential life cycle of biological biogas upgrading compared to water scrubbing technology as it is the most applied technology across European Union[8]. Simultaneously, economic analysis has been conducted having Denmark as a case study. A co-digestion biogas plant located in Denmark was considered as a case study to implement the LCA and economic assessment with a waste treating capacity of 240.000 t/year as a functional unit (FU).

The results showed that biomethanation had improved environmental impacts in three damage categories (i.e., Human health, Climate change and Resources) while in Ecosystem quality damage category, water scrubbing beat biological upgrading (Fig. 15). The negative values below the zero axis indicate avoided impacts and the positive values show the induced impacts. The net balance was calculated by summing up the negative and positive values of associated unit processes (i.e., induced impacts + avoided impacts = net balance).

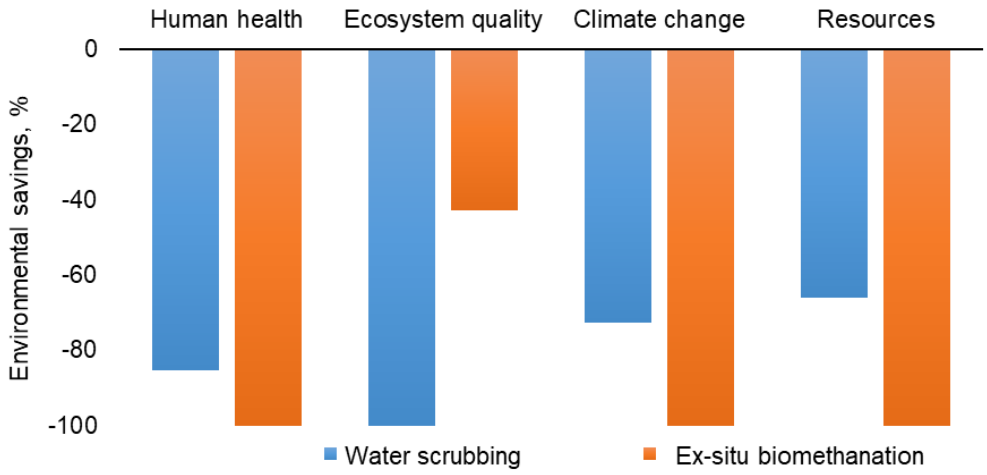

Fig. 15. Overall comparison of water scrubbing and ex-situ biomethanation in four damage categories.

To begin with human health damage category, the highest induced impact in water scrubbing originated from the transportation of feedstock to the plant and digestate to the surrounding farms. The unit for human heath damage category was disability adjusted life years (DALY), representing the years that are lost or that a person is disabled due to a disease or accident. The transportation had an impact of 1.76 DALY/FU. Despite the same impact was also imposed in biomethanation scenario in which the highest induced impact originated from water electrolysis consuming surplus wind electricity, i.e., 2.95 DALY/FU. The background impacts from the construction and manufacturing of wind turbines were responsible for the highest impacts on this damage category. Although wind electricity is a clean source of electricity with zero-direct greenhouse gas emissions, the manufacturing of wind turbines found to impose 74% of the total environmental burdens of wind electricity consumption. Emissions triggered by employing inorganic compounds in the construction of wind turbines can potentially led to substantial respiratory impacts.

The increased induced impacts caused by water electrolysis led to markedly lower net savings in ecosystem quality damage category than water scrubbing. Biological biogas upgrading could not outperform the water scrubbing technology in ecosystem damage category. However, it could still enhance avoided impacts in this damage category. Considering the impacts of curtailment or exporting surplus electricity with low/negative/zero

[8] https://www.ieabioenergy.com/blog/publications/new-iea-bioenergy-task-37-updated-list-of-biogas-upgrading-plants/

price, the integration of electrolysis and biological biogas upgrading accelerates a substantial number of environmental and economic benefits. The subsequent wind curtailment does not decrease the environmental burdens imposed on all damage categories and midpoint impact categories because the adverse impacts is imposed indirectly from the life cycle of wind farms. The higher the electricity production by wind turbines, the lower background impact per kWh electricity produced. Consequently, the implementation of biological biogas upgrading as part of the power to gas technology can decrease the negative impacts on wind electricity market and simultaneously, increase the efficiency of biogas plants by converting CO_2 into biomethane. On the contrary, water scrubbing technology releases the CO_2 into the atmosphere which is not compatible with the concept of circular bioeconomy.

Regarding climate change, both biogas upgrading technologies benefited from the production of biomethane and bio-fertilizer and so, the corresponding substitution for fossil-based products (Fig. 16a). However, biological biogas upgrading led to higher environmental savings at this damage category (i.e., -6.51 E+03 t $CO_{2,eq}$/FU *vs* -4.48 E+03 t $CO_{2,eq}$/FU). The substitution of biomethane for natural gas placed second in both biogas upgrading technologies in terms of environmental savings. However, the amounts of savings were different: -2.47E+03 t CO_{2eq} for water scrubbing compared to -4.07E+03 CO_{2eq} for biomethanation. The outcome attributes to the conversion of CO_2 of biogas into biomethane increasing the amount of recovered renewable energy. Therefore, biological biogas upgrading had higher savings from natural gas substitution.

Finally, the induced impacts revealed on the resources damage category was majorly due to the construction of fixed and moving parts of the wind turbines (i.e., 44% and 54% of total induced impacts, respectively) (Fig. 16b). Contribution analysis showed that for the construction of fixed parts, the consumption of steel and concrete was the most important contributor to the resource damage category. On the other hand, steel and glass fiber together constituted 87% of adverse impacts on resource damage category for building the moving parts. The results achieved in this study demonstrated that the environmental impacts of wind turbines were highly associated with the extraction of valuable minerals and materials used for the manufacturing and construction of wind turbines and farms.

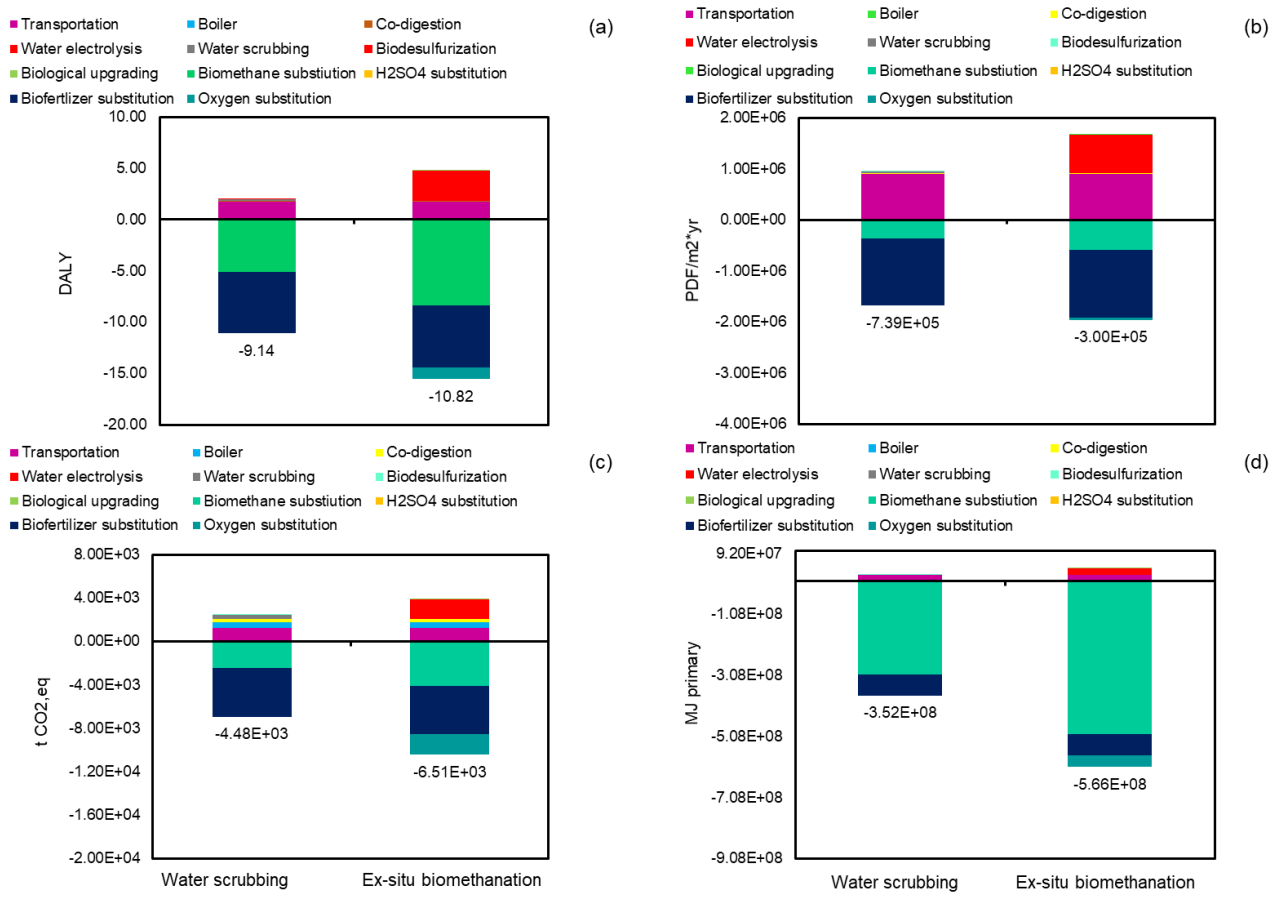

Fig. 16. Contributions of various unit processes to the following damage categories: (a) human health, (b) ecosystem quality, (c) climate change and (d) resources

The expenditures of both scenarios including the total capital expenditure (CAPEX), fixed operational expenditure (OPEX), and variable OPEX (i.e., feedstock and energy related costs) are shown in Table 2. The higher expenditures for the biomethanation process confirmed the fact that biogas plants with downstream technologies associated with biological biogas upgrading required higher investment and operational costs compared to commercial water scrubbing. The results revealed herein clearly show that among all examined infrastructures used for biological upgrading, electrolyzers had the highest CAPEX. Moreover, the total CAPEX of biological upgrading was estimated at 7.44E+6 € which was also higher compared to water scrubbing. The findings show that even if technological learning and capacity extension of the proton exchange membrane electrolyzer (PEMEL) in 2030 will reduce the CAPEX, it will still have an important impact on the investment costs to broaden the biological biogas upgrading technology.

Furthermore, VOPEX of biomethanation was approximately 2 times higher than water scrubbing due to the high quantity of electricity consumption by electrolyzers. The finding shows that although the surplus electricity can be bought at 50% of electricity price in Denmark, VOPEX highly rises the overall costs associated with biological upgrading. Electricity consumption by electrolyzers led to VOPEX above 60% of the total production. If surplus electricity is sold to the balancing market at higher price, electrolyzers' VOPEX will further rise. Hence, the pricing method and the final price for surplus wind electricity have a key role in the economic performance of biological biogas upgrading.

Table 2. Total expenditures of water scrubbing and biological upgrading

Component	Water Scrubbing			Biological upgrading		
	CAPEX, €	OPEX, €	VOPEX, €	CAPEX, €	OPEX, €	VOPEX, €
Biogas production	1.23E+07	1.23E+06	1.28E+06	1.23E+07	1.23E+06	1.28E+06
Biodesulfurization	2.70E+05	1.39E+04	6.31E+04	2.70E+05	1.39E+04	1.69E+02
Water scrubbing	4.55E+06	2.28E+05	2.83E+05	na	na	na
Electrolyzer units	na	na	na	6.00E+06	1.20E+05	2.24E+06
Hydrogen storage	na	na	na	9.00E+04	9.58E+02	na
Centrifugal pump	na	na	na	2.00E+04	1.06E+03	3.70E+02
Biological upgrading reactor	na	na	na	3.90E+06	1.95E+05	na
Gas grid injection and piping	1.02E+06	2.05E+04	na	1.37E+06	2.75E+04	na
Design, planning and engineering	5.08E+06	na	na	6.71E+06	na	na
Total, €	**2.32E+07**	**1.49E+06**	**1.62E+06**	**3.07E+07**	**1.59E+06**	**3.52E+06**

*na: not applicable

The levelized cost of energy (LCOE) for both water scrubbing and biological upgrading was calculated taking into consideration the time value of the money and the quantity of produced biomethane. Consequently, LCOE for water scrubbing was found to be higher than that of biological upgrading (i.e., 54 €/MWh for water scrubbing compared to 50€/MWh for biological upgrading). To calculate LCOE for biological upgrading, the price of surplus electricity was assumed to be 22 €/MWh meaning 50% of regular electricity price. Despite the production cost of biomethane in biological upgrading was estimated to be higher, lower LCOE was achieved for biological upgrading than water scrubbing which is a result of the production of a greater amount of biomethane via biological upgrading process. Having considered the estimated LCOE for the biomethane produced under both scenarios, the biomethane should be sold at a higher price than natural gas (e.g. 30 €/MWh) in 2030 since the LCOEs of biomethane production through both examined technologies were revealed found to be higher. This indicates that biomethane should be either sold at higher prices or should be granted with subsidy and certification.

5.5 Results in light of expectations

Overall, the project milestones were met, and the work outlined in the WPs was successfully accomplished based on the plan. Despite changes on human resources (i.e., shifts and hiring personnel) and delays with the up-scaling (i.e., construct and assemble reactor, postpone start-up due to COVID-19 lockdown), the project generated a significant amount of knowledge within bioprocess engineering area (e.g., reactor set-up, techno-economy, environmental and feasibility assessment). Biogas plant operators, gas fermentation experts, bioprocess engineers, consultants or other interested parties, can further exploit the results. Moreover, the main project objective to develop an innovative, efficient and robust technology for biological biogas upgrade, utilising the indigenous hydrogenotrophic cultures of the anaerobic digestion process, and achieve a biogas methane concentration equivalent to natural gas (>95% CH_4) was successfully realized. Combining lab- and pilot-scale and demonstration tests at relevant environment with the environmental and economic assessments during the project work, the different aspects of process efficiency and stability (e.g., biological, operational, environmental, economic etc.) were successfully examined.

Regarding the unexpected results of the project, the most important finding was related to the extremely high contribution of H_2 on process economy. Although H_2 was expected to markedly affect feasibility, it was mostly expected that the major barrier is the high-energy cost of the commercially available upgrading technologies, which add a substantial cost to the upgraded gas provision. Despite H_2 was assumed to be provided at period

of surplus electricity at lower prices, its provision still has a significant effect on the investment cost of biological biogas upgrading technology. Overall, H_2 contributes significantly to the production cost and so, process feasibility markedly relies on H_2 price. Research and development of electrolysis using surplus electricity should lead to decreased investment costs to broaden biological biogas upgrading technologies. Despite biomethanation is more expensive compared to commercial technologies in terms of production costs, it still has a better environmental performance.

5.6 Material benefits and employment

Although the demonstration at full-scale biogas plant was successful, the further development of the product requires ongoing negotiations between the project stakeholders, which would potentially concern financial implications later. However, the prototype unit can be further used in the future for demonstrating biomethanation at different sites and scales of production, providing knowledge related to CO_2 capturing and contributing to the development of the biogas industry as a competitive and environmentally friendly energy providing sector.

5.7 Dissemination of project results

Dissemination of the results generated during the project was realized in multiple ways. Firstly, the five scientific publications (Appendices) that were generated as a direct or indirect result of this project showed that the project developed in a healthy manner. In addition, five more scientific publications are going to be published. Moreover, the project work contributed to the submission of four conference abstracts and the subsequent plenary, oral or poster presentations, which increased the outward exposure of the project results and its stakeholders significantly. Finally, a constant communication between project partners about the planning and delivery of the project ensured the strengthening of existing relationships and created new connections between the academic and commercial representatives, promoting future collaborations.

6. Utilisation of project results

Generated results are expected to be utilized by biogas plants and DTU, for biogas cleaning and further research purposes, respectively. The disseminated findings of the project can be further exploited by other bioenergy plants and industrial stakeholders producing CO_2 (e.g. fermentation sites, wastewater treatment sector). The results obtained in BioUpgrade provide a good basis for roaring the full-scale implementation of biological biogas upgrading. The unique characteristics of BioUpgrade justify the necessity for full-scale applications of the developed technology due to the significant benefits that offers as defined in the system analysis. A detailed business plan can further help to reveal the need for different kinds of subsidies and incentives to increase feasibility of the developed biomethanation technology.

As mentioned above, the current project was focusing on technology development at the lab, validation in pilot-scale, demonstration at relevant environment. Additionally, system analysis of the concept was conducted. The conventional methods for biogas cleaning are mainly focused on cleaning CO_2 (i.e., water and amine scrubbing). On the contrary, in BioUpgrade the produced CO_2 was upcycled to produce more energy providing a more environmentally friendly approach. Since the climate crisis is steadily evolved, it is not enough just to reduce emissions. On the contrary, CO_2 must be actively removed from the atmosphere. This can be done most efficiently directly at point sources with high emissions and many operating hours, such as energy systems. For example, the C4 cluster in Denmark has a vision to annually reduce CO_2 emissions by 3 million tonnes; corresponding to approximately 15% of the total Danish decrease aim of 70% by 2030. In this frame, BioUpgrade concept can be further exploited in other industries except for biogas plant as for example cement and lime industries to capture the produced CO_2. The potential use of the BioUpgrade technology will allow

carbon intensive industries to improve environmental footprint, enhance productivity and consequently, profitability. Hence, BioUpgrade technology can be one a significant contributor to the green transition in Denmark.

Nowadays, the share of CH_4 coming from AD process is above 20% into the gas grid with a target of doubling it by 2025. Hence, there is a great need for novel and efficient biogas upgrading technologies. BioUpgrade technology can further enhance the interest in investment in the biogas area and boost construction activities to meet the energy targets. A highly efficient biogas sector providing clean CH_4 will allow Denmark to be less dependent on the natural gas imported from external suppliers. Through knowledge transfer process, the national target for transition to 100% renewable energy supply by 2050 could be accelerated.

The project was driven by a postdoc, a PhD student and some MSc students have been also involved in the work. Collaboration with researchers outside DTU was also established to improve BioUpgrade outcome. Some of the results were used as examples in teaching (a. 12136 Bioenergy technologies and b. 42717 Renewable gas in the energy system), where the fundamentals of anaerobic digestion process and biogas upgrading were taught. In the teaching notes the basics of biomethanation were described. Finally, BioUpgrade partners do not consider applying for any patents.

7. Project conclusion and perspective

The BioUpgrade project proposed biological biogas upgrading as a novel technical approach to establish a mixed hydrogenotrophic culture in trickle bed reactors fed with biogas and H_2. The major conclusion of the project is that it is technically possible to utilize the native AD community to form a H_2-fueled community which can increase the production capacity of anaerobic digesters. During project execution, different reactor set - up were examined at lab-scale and provide the basis for validation at higher scale. Significant scientific conclusions drawn from the experimental work have or will be included in the ISI research articles of the project. With that in mind, the main conclusions of the project are:

- Native AD community can be used as a start-up inoculum to form the H_2-fueled consortium.
- Among the tested systems, TBR showed higher potential for establishing the H_2-fueled consortium.
- The microbial ecology in the liquid phase of a TBR is significantly different from the corresponding one in the biofilm at the packing material.
- The pilot-scale operation and demonstration at relevant environment of the developed BioUpgrade technology showed that CH_4 production capacity similar to the lab-scale can be achieved.
- The developed BioUpgrade technology, if applied in full scale anaerobic reactors, could have a clearly positive environmental impact due to the reduced greenhouse gas emissions.

We strongly believe that the BioUpgrade technology can be utilized in full-scale reactors. To achieve this, the technology readiness level must be moved from the demonstration at relevant environment. Stakeholders in collaboration with researchers must create a commercially applicable process to fulfil requirements and expectations of carbon intensive industries. This commercially available biomethanation technology is the next major goal of the researchers involved in the BioUpgrade project as well as collaborators around the world that are focused on biogas upgrading in their research activities.

8. Appendices

Publications

- Kougias, P.G., Tsapekos, P., Treu, L., Kostoula, M., Campanaro, S., Lyberatos, G. and Angelidaki, I., 2020. Biological CO_2 fixation in up-flow reactors via exogenous H_2 addition. *Journal of Biotechnology*, 319, pp.1-7. https://doi.org/10.1016/j.jbiotec.2020.05.012
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Annex
The first pages of the 5 Published ISI papers.

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Biological CO_2 fixation in up-flow reactors via exogenous H_2 addition

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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 CO_2 capture and recycling are in focus. Thus, the biological coupling of CO_2 and H_2 is of high interest. Therefore, the focus of the present work was to evaluate the performances of two up-flow reactors for CO_2 and H_2 assimilation. Process monitoring showed that the gas-liquid H_2 transfer was highly affected by reactor design. A reactor filled with Raschig rings could lift up gases utilization leading to a CH_4 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.

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 CO_2 and H_2 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 CO_2 is not only deposited or released to the atmosphere but is coupled with H_2 by the action of hydrogenotrophic methanogens forming additional amounts of CH_4 (Bassani et al., 2015; Luo and Angelidaki, 2012; Vo et al., 2018). Therefore, biological biogas upgrading can be considered as CO_2 capturing and recycling technology. In addition, H_2 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 CO_2, which will lead to irreversible inhibition for the methanogenic communities (Bassani et al., 2016). In contrast, during “ex-situ processes” H_2 and CO_2 are externally provided in a separated chamber and therefore the pH is not affected (Kougias et al., 2017). However, in both processes, the increased H_2 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 H_2 into hydrogenotrophic up-flow reactors enhanced tremendously the CH_4 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

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Research review paper

Biogas upgrading and utilization: Current status and perspectives

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ABSTRACT

Biogas production is an established sustainable process for simultaneous generation of renewable energy and treatment of organic wastes. The increasing interest of utilizing biogas as substitute to natural gas or its exploitation as transport fuel opened new avenues in the development of biogas upgrading techniques. The present work is a critical review that summarizes state-of-the-art technologies for biogas upgrading and enhancement with particular attention to the emerging biological methanation processes. The review includes comprehensive description of the main principles of various biogas upgrading methodologies, scientific and technical outcomes related to their biomethanation efficiency, challenges that have to be addressed for further development and incentives and feasibility of the upgrading concepts.

1. Introduction

Biogas is the product of a biologically mediated process, which is known as Anaerobic Digestion (AD). Biogas primarily consists of methane (CH_4) in a range of 50–70% and carbon dioxide (CO_2) at a concentration of 30–50%. The relative content of CH_4 and CO_2 in biogas is mainly dependent on the nature of the substrate and pH of the reactor. Besides these two gasses, biogas additionally contains minor amounts of other compounds, such as nitrogen (N_2) at concentrations of 0–3%, which could originate from air saturated in the influent, vapour water (H_2O) at concentrations of 5–10%, or higher at thermophilic temperatures, derived from medium evaporation, oxygen (O_2) at concentrations of 0–1%, which is entering the process from the influent substrate or leakages, hydrogen sulfide (H_2S) at concentrations of 0–10,000 ppmv, which is produced from reduction of sulfate contained in some wastestreams, ammonia (NH_3) originating from hydrolysis of proteinaceous materials or urine, hydrocarbons at concentrations of 0–200 mg/m^{-3} and siloxanes at concentrations of 0–41 $mg\,m^{-3}$, originating for example from effluents from cosmetic medical industries (Muñoz et al., 2015; Petersson and Wellinger, 2009).

Apart from CH_4, all the other gasses contained in biogas are unwanted and are considered as biogas pollutants. The energy content of methane described by the Lower Calorific Value (LCV) is 50.4 MJ/kg-CH_4 or 36 MJ/m^3-CH_4 (at STP conditions). Therefore, it is well understood that the higher the CO_2 or N_2 content is, the lower the LCV in biogas. For biogas with methane content in the range of 60–65% the LCV is approximately 20–25 MJ/m^3-biogas. H_2S and NH_3 are toxic and extremely corrosive, damaging the combined heat and power (CHP) unit and metal parts via emission of SO_2 from combustion. Moreover, the presence of siloxanes in biogas, even in minor concentrations, is associated with problems. It is well known that during combustion silicone oxides generate sticky residues which deposit in biogas combustion engines and valves causing malfunction (Abatzoglou and Boivin, 2009). Nowadays, there are different treatments targeting at removing the undesired compounds from the biogas expanding its range of applications.

The first treatment is related to “biogas cleaning” and includes removal of harmful and/or toxic compounds (such as H_2S, Si, volatile organic compounds (VOCs), siloxanes, CO, and NH_3). However, it is practically only H_2S which is mainly targeted and many current biogas plants have H_2S removal units commonly based on biological H_2S oxidation by aerobic sulphate oxidizing bacteria. The second treatment is called “biogas upgrading” and aims to increase the low calorific value of the biogas, and thus, to convert it to higher fuel standard (Sun et al., 2015). In case the upgraded biogas is purified to specifications similar to natural gas, the final gas product is called biomethane (Kougias et al., 2017b). Currently, the specifications of the natural gas composition are depending on national regulations and in some countries > 95% methane content is required; however, European Commission has recently issued a mandate for determining harmonised standards for gas quality.

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Hybrid biogas upgrading in a two-stage thermophilic reactor

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ABSTRACT

The aim of this study is to propose a hybrid biogas upgrading configuration composed of two-stage thermophilic reactors. Hydrogen is directly injected in the first stage reactor. The output gas from the first reactor (*in-situ* biogas upgrade) is subsequently transferred to a second upflow reactor (*ex-situ* upgrade), in which enriched hydrogenotrophic culture is responsible for the hydrogenation of carbon dioxide to methane. The overall objective of the work was to perform an initial methane enrichment in the *in-situ* reactor, avoiding deterioration of the process due to elevated pH levels, and subsequently, to complete the biogas upgrading process in the *ex-situ* chamber. The methane content in the first stage reactor reached on average 87% and the corresponding value in the second stage was 91%, with a maximum of 95%. A remarkable accumulation of volatile fatty acids was observed in the first reactor (*in-situ*) after 8 days of continuous hydrogen injection reaching a concentration of 5.6 g/L. Nevertheless, after an adaptation period, the system managed to recover and the volatile fatty acids decreased to 2.5 g/L. No pH drop was recorded during the period characterised by increased volatile fatty acids concentration mainly due to the consumption of the endogenous carbon dioxide by hydrogenotrophic methanogens. The effect of hydrogen injection on the microbial community in both reactors was analysed by 16S rRNA gene amplicon sequencing. The results demonstrated an increment in relative abundance of hydrogenotrophic methanogens and homoacetogens in the *in-situ* reactor, while the microbial community in the *ex-situ* chamber was simpler and dominated by hydrogenotrophic methanogens.

1. Introduction

The generation of electricity from renewable energy sources (RES) is fundamental for reducing polluting emissions from fossil fuels. One implication while designing and implementing RES systems is the potential excess electricity that can be generated under certain conditions (e.g. high wind peak loads), which contributes to the increment of market volatility and frequency of sudden drops in electricity prices [1]. Unfortunately, the direct storage of the surplus energy produced from RES is still economically unfavourable. Therefore, several alternative options have been demonstrated in the concept of “Power-to-X” for transforming excess RES into power, heat, and/or gas.

In the context of Power-to-Gas (P2G), the biological biogas upgrading via hydrogenotrophic methanogenesis opens new horizons due to the more efficient exploitation of RES by integrating two renewable sources, such as biogas and wind/eolic or photovoltaic power generation [2]. From the perspective of an energy smart-grid, P2G has the inherent advantage of exploiting the existing infrastructure of the natural gas grid. Currently, this is achievable mainly via a two-step process: (1) utilisation of excess renewable energy for water electrolysis and subsequent hydrogen (H_2) production [3] and (2) conversion of H_2 by means of biological reactions with external carbon monoxide (CO) and carbon dioxide (CO_2) sources into methane (CH_4) [1].

It is widely known that biogas is typically burned in a Combined Heat and Power (CHP) unit providing thermal energy and electricity. However, the high content of CO_2 in biogas reduces its energetic value, and therefore, its conversion to CH_4 will enable the development of carbon-negative renewable energy production [4]. In order to obtain biogas with natural gas standard quality, it is necessary to increase its calorific value by removing CO_2, thus obtaining a purified gas, which is so-called “biomethane” [2]. The upgrading process allows the transformation of more than 80% of the energy content of raw biogas into new energy, as the existing biomethanation technologies consume less than 20% of biogas energy for upgrade and compression [5]. The specific requirements of biomethane for injection into natural gas grids or for exploitation as a vehicle fuel varies among different countries and

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Valorization of palm oil mill wastewater for integrated production of microbial oil and biogas in a biorefinery approach

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ABSTRACT

This work presents an integrated biorefinery concept combining two biological platforms for the valorization of palm oil mill effluents and for simultaneous production of high market value products, such as microbial oils and bioenergy. Palm oil mill effluents were aerobically fermented to produce lipids and subsequently the effluent from the fermentation was used as influent feedstock in an anaerobic digester for biogas production. It was found that pasteurization of the wastewater before fermentation by *Yarrowia lipolytica* TISTR 5151 was mandatory for efficient lipid production yielding 159.2 mg lipids/g-COD. In contrast, fermentation with untreated wastewater failed to produce lipids (3.10–43.51 mg lipids/g-COD), but instead supported the growth of several indigenous bacteria (e.g., *Asaia* sp., *Lactobacillus brevis* and *Acetobacter* sp.) with 6.57%–65.24% of relative abundance. Regarding biogas production, a maximum of 74% of the theoretical methane yield (280 mL/g-COD) in continuous reactor operation was achieved. The dominant bacteria, *Synergistales* sp. (18.19%) and *Bacteroidetes* sp. (15.96%), found at steady state period in the biogas reactor are known for acetate, butyrate, and hydrogen production. Moreover, within archaeal population, *Methanosarcina thermophila* (2.27%) and *Methanoculleus thermophilus* (1.38%) were identified as responsible for biomethane production.

1. Introduction

Palm oil mill industry has a commanding influence on the Southeast Asia region, and it is recognized as the most globally used plant oil with a total yield up to 40% compared to the other plant oils (Hansen et al., 2015). This sector has consistently encouraged economic growth however, it has been affected by environmental issues. In fact, the effluent released is approximately 26.7 million ton of palm biomass containing organic and inorganic substances and providing high strength wastes with typical chemical oxygen demand (COD) of 70,000 mg/L and biochemical oxygen demand (BOD) of 30,000 mg/L (Ahmad Farid et al., 2019; Iskandar et al., 2018). This wastewater leads to detrimental environmental impacts, and thus, requires a process to be treated before being discharged into specific recipients (Chan et al., 2012; Chan and Chong, 2019; Garritano et al., 2018). However, the compounds contained in the wastes should be considered as a valuable nutrient source, an important feedstock, and a potential renewable resource. Thus, effluent from the palm oil mill industry should play a well-defined part in the framework of a circular economy, in which biorefinery acts as a strategic mechanism (Ye et al., 2020; Venkata Mohan et al., 2019). In fact, circular economy is based on recycling and reuse of raw materials with maintaining restorative capacity of the natural resources; it represents the economic growth without simultaneous expansion in energy demand and consumption of resources (Venkata Mohan et al., 2019). Additionally, biorefinery integrates

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Modeling temperature response in bioenergy production: Novel solution to a common challenge of anaerobic digestion

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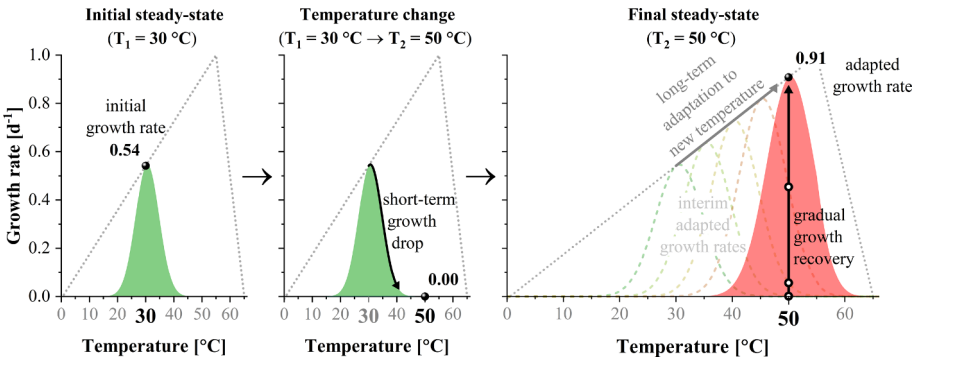
HIGHLIGHTS

- A dynamic microbial temperature-dependence function was developed for modeling.
- The novel function was implemented in an advanced anaerobic digestion model.
- Data from two experiments was used for the validation of the model simulations.
- In all cases, the simulations showed good agreement with experimental data points.
- Present extension can potentially improve other, similarly advanced models as well.

GRAPHICAL ABSTRACT



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ABSTRACT

Temperature is one of the most crucial state variables in industrial process control, which is particularly true for the biochemical conversion of biomass, as in anaerobic digestion. However, modeling the effects of temperature changes on anaerobic microbial growth are commonly considered in quasi-steady state, neglecting the timely dynamics of microbial adaptation to such phenomena. To address this inflexibility, the current work presents a new way for temperature effect calculation that improves the simulation efficiency of bioconversion models. The calculation was implemented as a function in a dynamic mathematical model of anaerobic digestion, and was validated via the simulation of experimental data from two laboratory-scale continuous experiments, involving both short- and long-term temperature changes. Model validity was further supported by 16s rRNA gene sequencing data. The bioconversion model extended with the new temperature function showed significant improvements in simulating the most important dependent variables of the digestion process, such as methane production rate and volatile fatty acid concentration during temperature variations. Finally, microbial analysis results shed light on the potential reasons for differences between simulated and experimental results. Overall, the dynamic temperature function was found to be an important addition to the reference model, allowing its user to generate more accurate simulations of digestion processes with changing temperature conditions. Furthermore, it can be seen as a step towards advanced time series forecasting, with potential benefits for

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