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► **To cite this version:**

Laure Deschamps, Nabila Imatoukene, Julien Lemaire, Mahamadou Mounkaila, Rayen Filali, et al. In-situ biogas upgrading by bio-methanation with an innovative membrane bioreactor combining sludge filtration and H₂ injection. *Bioresource Technology*, 2021, 337, pp.125444. 10.1016/j.biortech.2021.125444 . hal-03272414

HAL Id: hal-03272414

<https://hal.science/hal-03272414v1>

Submitted on 28 Jun 2021

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Title

In-situ biogas upgrading by bio-methanation with an innovative membrane bioreactor combining sludge filtration and H₂ injection

Authors

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Abstract

A bioreactor using membrane technologies was used to demonstrate the feasibility of *in-situ* bio-methanation coupled to industrial wastewater treatment for biogas upgrading. High biogas productivity ($1.7 \text{ Nm}^3_{\text{Biogas}}/\text{m}^3_{\text{Bioreactor}}/\text{day}$) with high CH_4 content (97.9%) was reached. *In-situ* bio-methanation did not affect the COD removal efficiency of anaerobic digestion (>94%). Process resilience has been tested for both substrate overload and H_2 intermittence injection. Recovery of high CH_4 content after 7 days without H_2 injection occurred within few hours. Influence of microbial community has been studied showing that both hydrogenotrophic and homoacetogenic-acetoclastic pathways were involved.

Keywords

In-situ bio-methanation

Membrane bioreactor

Biogas up-grading

Power-to-Gas

1. Introduction

Anaerobic digestion has the potential to become net producers of renewable energy.

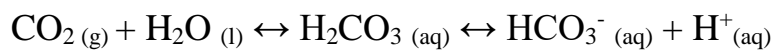
However, it leads to biogas production containing CO₂ which must be eliminated prior to injection into the gas-grid. Researches on new upgrading process is needed while it is an expensive step of anaerobic digestion plant (Muñoz et al., 2015). Biological conversion of CO₂ and H₂ to CH₄, called bio-methanation (Braga Nan et al., 2020; Wise et al., 1978), is a promising way to upgrade biogas (Luo and Angelidaki, 2013a).

During the first three steps of anaerobic digestion process, bacterial population, transform large organic polymer into acetic acid, CO₂ and H₂. Finally, two paths of methane production take place during methanogenesis. The major way is methane production from acetic acid conversion by acetoclastic methanogens. The second way corresponds to the bio-methanation reaction performed by hydrogenotrophic methanogens according to the following reaction: $4 \text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2 \text{H}_2\text{O}$.

In anaerobic digestion, the bio-methanation reaction is limited by H₂ amount leading to high CO₂ content in biogas. Bio-methanation can be promoted by injecting H₂ directly into anaerobic digester, this is *in-situ* bio-methanation (Luo and Angelidaki, 2013b). It is also possible to use another bioreactor fed with H₂ and CO₂ (Díaz et al., 2015), known as *ex-situ* bio-methanation. Bio-methanation is considered as a value chain of power-to-gas technologies when hydrogen is produced by water electrolysis from electricity excess from intermittent renewable energies (Götz et al., 2016).

Ex-situ methanation has the advantages of a greater rate of methane produced: methane purity can exceed 95% with a biogas flowrate up to 288 m³/m³_{reactor}/day (Peillex et al., 1990) against only 0.39 m³/m³_{reactor}/day (Luo and Angelidaki, 2013b) in the case of *in-situ* methanation. The lower biogas flowrate observed in *in-situ* methanation is mainly

due to the limit of biogas produced and thus CO₂ produced from anaerobic digestion itself. Compared to *ex-situ* configuration, *in-situ* methanation is cheaper as it does not need an additional reactor by using the anaerobic digestion bioreactor for biogas upgrading. However, its microbial consortium is complex and imbalance can occur. Thus, *in-situ* bio-methanation can alter the microbial community and affect performances of anaerobic digestion. For instance, increase of H₂ partial pressure is known to have negative effect on certain population involved in anaerobic digestion, mainly hydrolytic bacteria (Cazier et al., 2015) and acetogenic bacteria (Fukuzaki et al., 1990), which could lead to anaerobic digestion imbalances such as VFA accumulation. Moreover, *in-situ* bio-methanation tends to increase pH of fermentation and can lead to a pH out of the optimal range of anaerobic digestion consortium (6.5-7.5) (Luo and Angelidaki, 2013a; Wang et al., 2013). Indeed, dissolved CO₂ consumption leads to H⁺ consumption according to the following acid-base equilibrium:



In this study, a membrane for sludge filtration was used in order to keep a high microorganism content in the bioreactor and to prevent previously described imbalances due to H₂ addition. Acetoclastic microorganisms have low growth rate and their washout can occur and lead to imbalance and VFA accumulation during *in-situ* bio-methanation. It will also prevent washout of H₂ sensitive bacteria in case of H₂ partial pressure increase. Moreover, adapted microbial community to H₂ addition will be kept and concentrated in the bioreactor which can increase progressively H₂ consumption rate. The technology used is called an anaerobic membrane bioreactor.

A major bio-methanation limiting factor is H₂ dissolution as the latter is poorly soluble in water but must be dissolved to be metabolized by microorganisms. Moreover, it

should not exceed the inhibiting partial pressure (10^{-5} atm for VFA degradation - Moletta, 2015) and not be present in the upgraded biogas. Few membrane technologies have been developed to enhance H_2 gas-to-liquid transfer. Some studies used biofilms formed on the membrane surface (Luo and Angelidaki, 2013a; Wang et al., 2013) or produced microbubbles with possible biogas recycling (Alfaro et al., 2019; Díaz et al., 2015). However, both technologies have major drawbacks. Biofilm technologies required large surface area due to the progressive gas-to-liquid transfer decrease because of the biofilm (Luo and Angelidaki, 2013a) and microbubbles technologies lead to residual hydrogen in upgraded biogas (Alfaro et al., 2019). In this work, a hydrophobic microporous membrane for hydrogen transfer was investigated. Anaerobic digestion medium recirculated through the tubular membrane and hydrogen was injected in the other side of the membrane. Ojeda et al. (2017) also used an external module for gas injection into an anaerobic bioreactor and showed that this technology was efficient for performing hydrogenotrophic fermentation. Moreover, they showed that gas-to-liquid transfer depended on operating conditions such as gas feed pressure. Membrane surface or pore size are also parameters to be considered.

This study aims at investigating the performance of an innovative anaerobic membrane bioreactor for biogas upgrading with high CH_4 productivity by *in-situ* bio-methanation. It combines different microporous membranes for H_2 injection and microorganism retention. Moreover, the effect of organic matter and H_2 overloads as well as intermittent H_2 injection was studied. The evolution of microbial community was investigated to evaluate the influence of *in-situ* bio-methanation on anaerobic digestion metabolisms.

2. Material and methods

2.1 Membrane bioreactor description

The membrane bioreactor setup is shown in Fig.1. The bioreactor was connected to two external membranes modules with a liquid recirculation pump. The first membrane, used for sludge filtration, was a tubular ceramic module with 19 channels (Orelis Environnement - KLEANSEP™ BW). Its surface area was 0.25 m² and pore size 0.1 µm. Permeate flowrate was controlled by a peristaltic pump which also controlled feed flowrate by natural level adjustment. The bioreactor was fed continuously with substrate in the reserve kept at 5 °C to prevent any biodegradation. No sludge was discarded during the experiment except for sampling.

The second membrane used for H₂ injection is similar to the first one but has been submitted to a hydrophobic surface treatment (fluorinated silanes coating). This membrane was used to dissolve H₂ in the bioreactor medium. H₂ was produced continuously by a water electrolysis device (Peak scientific 450cc) and hydrogen flowrate was regulated by a H₂ flowmeter (Bronks SLA5850).

Bioreactor mixing was ensured by liquid recirculations. Recirculation in the membrane was 700 L/h. A pH and temperature probe (JUMO 201020) and a sludge sample point were placed in another recirculation loop. The temperature was set at 37 °C with a water bath and coil in the bioreactor. No pH adjustment was done in the bioreactor.

2.2 Sludge inoculum

The pilot scale anaerobic membrane bioreactor was inoculated with effluent from an anaerobic packed bed bioreactor treating the same wastewater used in this study as described previously (Deschamps et al., 2021).

2.3 Wastewater

The substrate used as nutrient feedstock was a wastewater of an industrial distillery which produces bioethanol from sugar beet and wheat (Cristanol, Bazancourt, France). It was a blend of evaporation condensate and cleaning water of tanks. Suspended solids represented 3 ± 1 g/L, total nitrogen 180 ± 90 mg/L and sulfate 220 ± 80 mg/L. A new batch of fresh wastewater was collected every two to three weeks. Initial pH of the wastewater was 4.5 ± 0.5 and was adjusted to 7 ± 0.2 while working in anaerobic digestion process only. No pH adjustment was done when H₂ was added. Average total COD concentration of the wastewater was 12 ± 2 g_{COD}/L.

2.4 Experimental setup

The bioreactor was running for 5 months in anaerobic digestion process only before starting *in-situ* bio-methanation experiment.

First, during transition phase between phase 0 and phase 1, hydrogen flowrate was slowly increased in order to prevent H₂ inhibition of microorganisms starting at 0.8 NL/h. During this transition, H₂ flowrate was doubled when less than 1% of H₂ was detected in the biogas until it reached the stoichiometric flowrate (molar ratio of 4 H₂/1 CO₂). Flowrate of H₂ was manually controlled according to flowrate of remained CO₂ in the biogas. Then, bio-methanation ramp-up was composed of 3 phases. In the first phase, H₂ was added stoichiometrically with CO₂ produced by anaerobic digestion. Hydraulic retention time (HRT) was fixed at 3.3 days, corresponding to an organic loading rate (OLR) equal to 2.7 kg_{COD}/m³/day. Then, during the second phase, OLR was increased to 3.9 kg_{COD}/m³/day by decreasing HRT to 2.8 days in order to increase process productivity. H₂ was increased as well, stoichiometrically to the CO₂ produced.

During the third phase, liquid pressure in the injection membrane was increased in order to enhance H₂ gas-to-liquid transfer.

Then, during the fourth phase, high organic content substrate (15 g_{COD}/L) and increase of 60 % of OLR within 2 days was used to mimic an overloading situation and evaluate its effect on *in-situ* bio-methanation. When process performances were recovered, H₂ injection was stopped for 7 days in order to evaluate the feasibility of intermittent H₂ addition for *in-situ* bio-methanation (phase 5).

2.5 Analytical procedure

During all operations, buffer capacity (TAC) and biogas composition were recorded daily. Volatile fatty acids (VFA) and chemical oxygen demand (COD) were measured 3 times a week.

Online data acquisition was carried out every 10 min for the weight of produced permeate (with OHAUS balance type DEFENDER 5000), the volume of produced biogas (with drum gas meter RITTER TG 0.5) and the pH value. All these devices were connected to a computer for reading, processing, synchronizing and displaying data in real time. The balance had a RS232 communication port which was connected directly to the computer. The gas meter had a rotary encoder associated with a pulse generator that produced one pulse (TTL signal from 0.7 to 3.7V) for each 0.0025 NL of produced biogas. The pH probe was connected to the JUMO ecoTRANS pH transmitter, which had an analog output of 2 to 10V depending on the pH value from 0 to 14. The analog outputs of the gas meter and the pH transmitter were connected to inputs of an ad hoc 16-bit analog-to-digital converter (ADC). The ADC was connected to the computer through USB interface. Reading, processing, recording and displaying of all the data was done with the LabVIEW software. A sliding average and a median were carried out

on the signal from the balance in order to reduce noise. A software was developed to count pulses from the gas meter and convert them into gas volume.

TAC value was measured by pH titration using 0.1 N H₂SO₄ based on the Nordmann method (Lili et al. 2011). It was determined through titration of 20 mL of sample up to pH 5. COD analysis was performed using Spectroquant® cell tests (1.14541) with Spectroquant® Multy photometer.

VFA analysis was performed using HPLC (Ultimate 3000 Thermo Fisher) with an Aminex HPX-87H (7.8 x 300 mm, 9 μm) column at 50 °C with UV detection. The mobile phase was 8 mM H₂SO₄ at 0.8 mL/min, and 20 μL of sample was injected for analysis. Acetic, butyric, and propionic acids were quantified.

Biogas analysis was carried out on micro-GC 490 from Agilent Technologies. Biogas sample was introduced through a heated line at 100 °C. Three columns were used: a molecular sieve 5 Å (MS-5A, 10 m) to quantify H₂ using Argon as the carrier gas, another molecular sieve 5 Å (MS-5A, 10 m) to separate O₂, N₂ and CH₄ and a PoraPLOT U (PPU, 10 m) to quantify CO₂, both columns used Helium as the carrier gas. Separation was achieved at 100 °C, 100 °C and 60 °C with a backflush of 26 s, 4.7 s and 16 s, respectively. Columns pressure was 29 psi. The detector was a Thermal Conductivity Detector (TCD). Total run lasted 80 s. Calibration was performed using standard gases (Air Liquide) and diluted using flowmeters from Bronkhorst. Quantification was achieved using peaks area with an external calibration (concentration range from 0.05 to 100%).

2.6 Microbial analysis

Microbial analysis was performed by qPCR in order to quantify total archaea (ARC) and bacteria (BAC) in the reactor. Quantification was also carried out for the following

hydrogenotrophic methanogen archaea: Methanococcales (MCC), Methanomicrobiales (MMB), Methanobacteriales (MBT) and, acetoclastic methanogen archaea *Methanosaetaceae* (Mst) (Garrity et al., 2001). Methanosarcinales (MSL) were quantified as well. Regarding bacterial community, the following *phylum* were quantified: Sulfate-reducing bacteria (SRB) and Bacteroidetes (BDT).

50 mL of sludge samples were collected during each phase and during transition phase and were centrifuged (4000 g, 15 min). DNA was extracted from pellets with PowerSoil® DNA Isolation Kit (MO BIO, Qiagen) according to manufacturer's instructions. Plasmids including the target sequences (pET-15b, GenScript) were used as reference sample excepted for total bacteria where DNA was extracted from pure culture of *Bacillus subtilis* and then was used as standard. Serial dilutions of reference sample were prepared to establish the qPCR calibration curve.

qPCR was performed by targeting 16S rRNA coded by 16S rDNA except for SRB which was performed by targeting *DsrB* gene. Ssoadvanced universal sybr green supermix (Bio-Rad) was used with C1000 touch thermal cycler CFX96 (Bio-Rad). qPCR mix were composed of 10 µL of master mix, 1 µL of DNA and 2 µL of each primer (2 µM) for each target, except for BDT where 1 µL of primers was used, for a final volume of 20 µL.

Primer specificity were validated for each target with pure DNA and sample DNA by performing analysis of the amplicon melting curves which showed one peak corresponding to one amplicon. They were established by increasing the temperature from 72 °C to 95 °C and by measuring the fluorescence intensity after amplification cycles.

3. Results and discussion

3.1 Bioreactor performance

Performance of anaerobic digestion and bio-methanation are shown in Table 1 for the first three phases. Bioreactor performances of all experiment are shown in Fig. 2.

During the phase 0, when only anaerobic digestion was performed, methane content of 74.7% was reached which is common for distillery wastewater (Moletta, 2005).

Moreover, high digestion performance was observed. A previous study focusing on membrane filtration performance and anaerobic digestion was published showing how this technology leads to high OLR, COD removal efficiency and biogas production (Deschamps et al., 2021).

First, H₂ flowrate was slowly increased in order to prevent H₂ inhibition of microorganisms starting at 0.8 NL/h. During this transition, H₂ flow rate was doubled every 4 days on average, when less than 1% of H₂ was detected in the biogas until it reached the stoichiometric flowrate. 20 days after the beginning of H₂ addition, H₂ was added stoichiometrically to CO₂ produced. Then, during the first phase, 98.9% of H₂ added was consumed when HRT was fixed at 3.3 days, corresponding to OLR equal to 2.7 kg_{COD}/m³/day. This led to high CH₄ content in the biogas (97.4%) which is high enough for biogas gas-grid injection. pH increased from 7.3, without H₂ addition, to 7.6 although feed pH was decreased to limit this pH increase by using wastewater without pH adjustment. Then, during the second phase, OLR was increased to 3.9 kg_{COD}/m³/day by decreasing HRT to 2.8 days in order to increase the process productivity. H₂ consumption yield decreased to 84.3% which led to a lower CH₄ content in the biogas (82.8%). During the third phase, H₂ gas-to-liquid transfer was improved significantly by increasing the pressure of the liquid size of the injection membrane: H₂ consumption

yield was 99.2% and CH₄ biogas content reached 97.9% with a higher OLR (4.4 kg_{COD}/m³_{Bioreactor}/day). Thus, higher productivity of biogas was reached: 10.8 NL/h which correspond to 1.7 Nm³_{Biogas}/m³_{Bioreactor}/day. During the first three phases, COD removal efficiency remained high (> 94%) and VFA remained below quantification limit (< 0.02 g/L).

These results confirmed that anaerobic digestion consortium is able to catalyze the methanation reaction. Moreover, the high COD removal efficiency showed that the *in-situ* bio-methanation did not affect the anaerobic digestion process. Despite pH increase all along the experiment, from 7.6 in phase 1, 7.7 in phase 2 and up to 7.9 in phase 3, it was still within the optimal range of anaerobic digestion without any pH control. pH increase is explained by CO₂ consumption which led to displacement of carbonate-bicarbonate equilibrium in the liquid phase. Indeed, buffer capacity (TAC) decreased from 1866 ± 155 mg_{CaCO3}/L before H₂ addition to 1122 ± 74 mg_{CaCO3}/L during the first phase. pH increase up to 8.3 was also observed in other studies without affecting the anaerobic digestion performance (Luo and Angelidaki, 2013b, 2013a). Keeping pH within optimal range for anaerobic digestion was possible in the present study because low pH wastewater was used. Luo and Angelidaki (2013b) also showed that feeding the bioreactor with acidic substrate was a way to keep pH optimal for anaerobic digestion during *in-situ* bio-methanation.

During the first phase, high H₂ consumption yield and CH₄ content were reached showing that the membrane technology used was able to dissolve high amount of hydrogen with very low H₂ residual content (1.1%). However, OLR was relatively low during this phase compared to phase 0 because of low COD content of the wastewater batch used (8.8 g_{COD}/L).

Therefore, OLR was increased during the second phase by using a new wastewater batch with higher COD content (10.8 g_{COD}/L), as well as decreasing HRT to 2.8 days. However, increasing both OLR and H₂ injection led to a CH₄ content decrease (82.8%) because of lower bio-methanation efficiency which led to high H₂ residual content (12.9%) and CO₂ residual content (4.3%). It could be the results of either non-dissolved H₂ or non-consumed H₂ by microorganisms. Dissolved but not consumed H₂ would lead to H₂ concentration increase in the liquid phase. However, dissolved H₂ accumulation is known to be toxic for the bacterial community during anaerobic digestion (Cazier et al., 2015; Fukuzaki et al., 1990) and would conduct to a decrease of COD removal efficiency. As COD removal efficiency remained high during the second phase, it is assumed that H₂ residual content was mainly due to non-dissolved H₂. This demonstrated the need to increase the limit of H₂ gas-to-liquid transfer to improve overall process performance. Yasin et al., 2015 suggested that gas-to-liquid transfer could be improved by either increasing injection membrane surface area, liquid flow-rate or gas pressure.

H₂ pressure in the membrane was adjusted by the gas flowmeter to deliver the desired flowrate. As H₂ pressure depends on liquid pressure in the injection membrane, to improve H₂ effective dissolution, liquid pressure was increased in the membrane from 0.4 to 0.6 bar_g in the third phase. It led to a higher H₂ consumption yield (99.2%) than second phase despite the higher H₂ feed flowrate (11.6 NL/h) required to meet stoichiometric CO₂ demand at higher OLR (4.4 kg_{COD}/m³/day). This result confirmed that liquid pressure is a crucial parameter for effective H₂ dissolution. Bioreactor pressure had already been shown to affect H₂ gas-to-liquid transfer for bio-methanation by Díaz et al., 2020 who increased methane purity from 69.4% to 92.9% by increasing

bioreactor pressure from 0.2 bar_g to 0.3 bar_g. Increasing bioreactor pressure led to a H₂ solubility increase according to Henry's law which says that "the amount of a given gas that dissolves in a given type and volume of liquid is directly proportional to the partial pressure of that gas in equilibrium with that liquid":

$$H_2 \text{ saturation concentration} = H_2 \text{ Henry's constant} \times H_2 \text{ pressure}$$

Moreover, the flux of H₂ transfer from gaseous phase to liquid phase can be calculated according to the following formula (Jensen et al., 2021):

$$H_2 \text{ flux} = H_2 \text{ volumetric mass transfer coefficient} \times (H_2 \text{ saturation concentration} - H_2 \text{ concentration in liquid phase})$$

This shows the correlation between pressure of H₂ and flux of H₂. However, pressure can also affect the H₂ volumetric mass transfer coefficient, at higher pressure, medium viscosity increased which can cause surface tension decrease, and consequently led to the formation of smaller bubbles, increasing gas-to-liquid transfer inside the bioreactor (Lau et al., 2004).

During the third phase, it was feasible to produce up to 1.7 Nm³_{Biogas}/m³_{Bioreactor}/day with a CH₄ content up to 97.9%, which corresponds to a higher heating value compatible with gas-grid injection (10.88 ± 0.05 kWh/Nm³_{Biogas}). As initial composition of biogas with the treated wastewater is about 75% of CH₄ content and 25% of CO₂ content, it can be assumed that, during the third phase, about 75% of the CH₄ is produced from the wastewater treatment and 25% of the CH₄ is produced from H₂ and CO₂.

Compared to similar recent studies, such productivity and CH₄ content were higher and quite outstanding. Other studies on *in-situ* bio-methanation used injection membrane module to improve H₂ dissolution are presented in Table 2. Díaz et al., 2015 showed that micro-bubbling through porous hollowfiber membranes was a way to reach very

high k_{La} value for H_2 gas-to-liquid transfer (up to $430h^{-1}$). However, when used directly in the bioreactor for *in-situ* bio-methanation, it led to a significant H_2 residual content (> 7.2%) in the produced biogas (Alfaro et al., 2019) despite a lower H_2 feed flowrate of $7.8 NL_{H_2}/m^2_{membrane}/h$, 5.9-times lower than the present study. Biofilm formation by using dense membrane is a way to prevent any H_2 residue in the produced biogas (Luo and Angelidaki, 2013a; Wang et al., 2013). However, these technologies required high specific membrane area: $119 m^2/m^3_{Bioreactor}$ (Luo and Angelidaki, 2013a) and $58 m^2/m^3_{Bioreactor}$ (Pratofiorito et al., 2021) while only $1.7 m^2/m^3_{Bioreactor}$ was used in the present study. Moreover, biofilm technology provided lower methane production rate: $1.2 Nm^3/m^3_{Bioreactor}/day$ (Pratofiorito et al., 2021) and $0.9 Nm^3/m^3_{Bioreactor}/day$ (Luo and Angelidaki, 2013a).

The technology developed in this work combined the advantage of lower H_2 residue in the produced biogas and lower specific membrane area required. However, high recirculation flowrate was needed in the present study for H_2 dissolution which can lead to high operational cost at industrial scale. The results showed that H_2 gas-to-liquid transfer can be improved by increasing liquid pressure. Thus, bioreactor pressure could be naturally increased during biogas production which does not require additional energy. Nevertheless, further studies are required to have a better understanding of the influence of recirculation rate and liquid pressure on H_2 gas-to-liquid transfer and H_2 residual content in the produced biogas.

3.2 Process resilience

3.2.1 Phase 4: Overload organic matter and H_2

At the end of the third phase, a new batch of wastewater with high COD content (15.0 g_{COD}/L) and an increase of OLR up to 7.3 kg_{COD}/m³_{Bioreactor}/day was used to mimic an overload situation which is a common cause of anaerobic digester failure. H₂ flowrate was fixed at 15 NL/h in order to be added stoichiometrically with estimated CO₂ produced with this new batch and was not changed during this period. The effect of *in-situ* bio-methanation during an overload was studied. Biogas production (including unconsumed H₂), pH and VFA concentration measured during this period are shown in Fig. 3.

Acidification started at day 74 leading to a progressive biogas production shortage. pH decrease was due to VFA accumulation and significant reduction of biogas production was observed. Decrease of biogas production led to excess of H₂ added compared to CO₂ produced. The turning point where H₂ was added in excess can be seen in Fig. 3 at day 77. From this point, pH stopped to decrease and start to increase which means that CO₂ was fully consumed. Indeed, during anaerobic digestion, pH is stabilized by carbonate-bicarbonate buffer (Murphy and Thanasit Thamsiriroj, 2013). Thus, when CO₂ was consumed, pH increased despite feeding with wastewater at low pH and VFA accumulation in the bioreactor. This suggests that hydrogenotrophic activity was still high compared to acetogenic activity which confirms that hydrogenotrophic methanogen have higher consumption rate or higher growth rate than acetogenic methanogen in these experimental conditions. Hydrogenotrophic methanogen are known to have higher growth rate than acetoclastic methanogen (Murphy and Thanasit Thamsiriroj, 2013).

Moreover, at the same time, apparent biogas production flowrate stabilized around 10.5 NL/h whereas it would have been expected that biogas production kept on decreasing.

Actually, biogas production decreased but was counterbalanced by the excess of unconsumed H₂ in headspace which was recorded with the biogas produced. A plateau was observed because anaerobic digestion of this wastewater usually produces a biogas with 75% of CH₄ and 25% of CO₂. Thus, one volume of biogas which was not produced led to one volume of H₂ not consumed, which resulted in an apparent stabilized biogas production.

Biogas production variation occurred when pH exceeded 8 (day 78) suggesting that microbial community and metabolic pathways changed either because of high pH or high H₂ partial pressure. At this point, VFA content in the bioreactor was 1.41 g/L of acetic acid, 0.55 g/L of propionic acid and 0.97 g/L of butyric acid. Compared to day 75, acetic acid concentration did not increase while longer chain VFA accumulation was observed, suggesting that acetogenesis was inhibited at this stage due to increase H₂ partial pressure or high pH. VFA accumulation had also been observed in other study operating at high H₂ partial pressure and had been shown to be dependent on initial microbial community used (Braga Nan et al., 2020). Moreover, propionate biodegradation by acetogenesis bacteria had been shown to be inhibited by high H₂ partial pressure (Fukuzaki et al., 1990). High pH had also been shown to have high effect on VFA production during anaerobic fermentation (Horiuchi et al., 2002). At day 78, headspace was composed of 50% CH₄ and 50% H₂ confirming that CO₂ was totally consumed.

H₂ addition was stopped when pH reached 9 (day 78), then pH value dropped below 6.5 in only 2 hours. This quick decrease suggests CO₂ production by the bacterial community which was still able to perform first steps of anaerobic digestion despite high pH. H₂ injection was restarted when pH decreased below 6.5 (day 78) in order to

prevent any further pH decrease which would lead to methanogenesis inhibition. Stopping H₂ addition in excess led to consumption of butyric and propionic acids confirming the previous inhibition of bacteria involved in their degradation because of high H₂ partial pressure. However, high acetic acid concentration was still observed (> 2 g/L) 5 days after H₂ injection restart showing that acetoclastic methanogen community content was not high enough to consume all acetic acid produced by the first step of methanogenesis. Thus, decreasing both OLR and H₂ was needed to recover a good productivity.

These results showed that H₂ addition has a huge impact on the bioreactor pH and on metabolic pathways. Thus, H₂ flowrate has to be controlled accurately and continuously based on CO₂ production and pH in order to keep the process in optimal condition for anaerobic digestion as well as high biogas CH₄ content.

3.2.2 Phase 5: Intermittent H₂ addition

Feasibility of *in-situ* bio-methanation is considered as economically and environmentally sustainable if it uses H₂ produced from water electrolysis with renewable electricity excess such as intermittent wind or solar energy (Fu et al., 2020). Thus, in the fifth phase, H₂ addition was stopped for 7 days then upturned to mimic the effect of intermittent H₂ addition due to intermittent electricity production from renewable energy. Results showed a quick effect of H₂ addition interruption on methane purity which returned to 75% with 25% of CO₂ the day after H₂ injection was turned off. Interestingly, methane content went back to high content (93%) with no H₂ residue detected in biogas less than 24h after H₂ addition resumption. Moreover, continuously

monitored biogas flowrate suggests that H₂ was directly consumed, within few hours, after H₂ resumption.

These outcomes demonstrate the resilience of the developed *in-situ* bio-methanation membrane bioreactor. It appears that hydrogenotrophic methanogens activity was not affected by stopping H₂ addition for 7 days. One week of total H₂ shortage is a much longer period than expected with intermittent renewable energy. Therefore, intermittent H₂ addition is biologically feasible without affecting the hydrogenotrophic activity of the flora present in the *in-situ* bio-methanation bioreactor. Moreover, a quick restart of H₂ injection to 100% of CO₂ stoichiometry demand did not affect the COD removal efficiency showing that H₂ was directly used by the hydrogenotrophic microorganisms without any accumulation in liquid and gas phases. A slight decrease of COD removal efficiency (88%) was observed after H₂ stop but came back to high level (>90%) within 2 days. It can be explained by a volume, representing 4% of the total bioreactor volume, taken out for analysis reducing the total microbial community in the bioreactor.

3.3 Microbial community analysis

MCC were lower than quantification limit in all samples. *Mst* represented almost 100% of MSL in all samples, thus, only *Mst* results are shown. Results of microbial community analysis are shown in Fig. 4.

Both total archaea and bacteria populations increased between the anaerobic digestion initial phase and the transition phase (6 days after with progressive H₂ injection).

Among archaea phylum, both *Mst* and MMB population increased. Proportion of *Mst* was 1.2-fold higher during the transition phase and MMB 2.7-fold higher suggesting that both homoacetogenic-acetoclastic and hydrogenotrophic pathways co-existed.

Then, during the first phase, MMB increased 3-fold and *Mst* decreased and came back

to its initial proportion. These results suggest that *in-situ* bio-methanation is feasible with few adaptations of microbial community by first using mainly the homoacetogenic-acetoclastic pathway. Then, hydrogenotrophic MMB growth was stimulated by H₂ addition and was faster than *Mst* growth. Thus, adaptation of microbial community occurred after 3 weeks of H₂ injection in the bioreactor and increased the use of hydrogenotrophic pathway.

From the first and second phases, *in-situ* bio-methanation was limited by low H₂ gas-to-liquid transfer, then H₂ supply was not sufficient to meet the demand due to OLR increase. It resulted in a 2.5-fold decrease of total bacteria despite OLR increase. It suggests that bacteria played an important role on bio-methanation during the first phase and thus that homoacetogenic-acetoclastic pathway was still important for bio-methanation.

Moreover, comparison of microbial community between the second and third phases showed that increasing of H₂ gas-liquid transfer led to a direct consumption of H₂ with only few changes of microbial community (MMB proportion by 1.4-fold increase).

Wang et al., 2013 also reported that both ways of methane production co-exist during *in-situ* biogas upgrading by bio-methanation and that hydrogenotrophic archaea were stimulated by H₂ addition.

Analysis of microbial community after an organic matter overload, which resulted in excess of H₂, showed a significant increase of bacterial community and MMB proportion (see Fig. 4). Increasing of bacterial community was most probably due to COD overload than indirect H₂ excess. Indeed, when an anaerobic digester is overloaded with organic matter, acidification occur because bacterial community involved in acidogenesis and acetogenesis steps have higher growth rate than

acetoclastic archaea leading to accumulation of acetic acid in the bioreactor. Moreover, the excess of H₂ added led to 6.3-fold increase of MMB proportion while *Mst* did not increase despite acetic acid accumulation. This result confirmed that hydrogenotrophic archaea have a higher growth rate than acetoclastic archaea in the experimental conditions.

Analysis of SRB shows no significant influence of H₂ injection because its population increased 4-fold during the transition phase, but remained between 1 and 1.6 10⁶ copies/mL during next phases. So, SRB seemed to not be stimulated by H₂ addition, despite competition of SRB, acetogens and methanogens can occur during anaerobic digestion (Dar et al., 2008). Wahid et al., 2019 showed a decreasing of SRB under high H₂ partial pressure suggesting that H₂ added was mainly used by hydrogenotrophic methanogen and homo-acetogenic bacteria.

BDT decrease among bacterial community was observed during the experiment. BDT are responsible of hydrolytic and acidogenic activity (Jaenicke et al., 2011). However, the relative decrease of BDT did not affect the COD removal efficiency showing that it did not affect hydrolytic and acidogenic activity. Relative decrease can be explained by the increasing of homoacetogenic bacteria.

These results showed that initial microbial community of an anaerobic digester was able to catalyze the bio-methanation reaction by first using mainly the homoacetogenic-acetoclastic pathway. Then, addition of H₂ in the anaerobic digester led to community adaptation to the hydrogenotrophic pathway of CH₄ production due to faster growth rate of hydrogenotrophic archaea. However, both pathways seemed to co-exist at each phase.

4. Conclusion

In-situ biogas upgrading up to 97.9% CH₄ content by bio-methanation has been shown feasible at pilot-scale with high biogas productivity up to 1.7 Nm³_{Biogas}/m³_{Bioreactor}/day. Moreover, anaerobic digestion performances were not affected by the *in-situ* bio-methanation. Both hydrogenotrophic and homoacetogenic-acetoclastic pathways have been shown to be involved in methane production. However, improvement is needed to reduce energy used for H₂ gas-to-liquid transfer in order to be economically feasible which could be done by using a pressurized bioreactor or a submerged membrane for H₂ injection.

Supplementary material

E-supplementary data of this work can be found in online version of the paper.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgements

This work was supported by the French Environment and Energy Management Agency (ADEME), Cristal-Union, and GRTgaz. Authors also would like to thank ARD (Pomacle, France) for sharing their expertise on anaerobic digestion.

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Table. 1 *In-situ* bio-methanation performances.

Table 2: Performance comparison of study using membrane bioreactor for bio-methanation process. ^aEstimated from available data, ^bNot precised but absence of bubbling through the membrane suggest a yield closed to 100%

Fig. 1: Schematic diagram of the pilot scale anaerobic membrane bioreactor developed for bio-methanation.

Fig.2: Process performance evaluation during *in-situ* bio-methanation. Phase 0: Set-up phase of anaerobic digestion without H₂ addition. Phase 1: *In-situ* bio-methanation with low OLR (2.7 kg_{COD}/m³/day). Phase 2: *In-situ* bio-methanation with high OLR (3.9 kg_{COD}/m³/day). Phase 3: *In-situ* bio-methanation with high OLR (4.4 kg_{COD}/m³/day) and high pressure to enhance gas-to-liquid transfer. Phase 4: Overload organic matter and H₂. Phase 5: Intermittent H₂ addition.

Fig. 3: Phase 4: Effect of high H₂ injection during acidification on biogas production and pH

Fig. 4: Effect of *in-situ* bio-methanation on microbial community. ARC: archaea, BAC: bacteria, MMB: Methanomicrobiales, MBT: Methanobacteriales, Mst: *Methanosaetaceae* and BDT: Bacteroidetes. Phase 0: Set-up phase of anaerobic digestion without H₂ addition. Phase 1: *In-situ* bio-methanation with low OLR (2.7 kg_{COD}/m³/day). Phase 2: *In-situ* bio-methanation with high OLR (3.9 kg_{COD}/m³/day). Phase 3: *In-situ* bio-methanation with high OLR (4.4 kg_{COD}/m³/day) and high pressure to enhance gas-to-liquid transfer. Overload: Fast increase of COD concentration substrate and H₂

Tables

	Phase 0	Phase 1	Phase 2	Phase 3	
HRT (days)	3.4 ± 0.6	3.3 ± 0.1	2.8 ± 0.1	2.4 ± 0.2	
OLR (kg _{COD} /m ³ _{Bioreactor} /day)	3.5 ± 0.5	2.7 ± 0.1	3.9 ± 0.1	4.4 ± 0.4	
H ₂ Flowrate (NL/h)	0	7.2	9	11.6	
Biogas composition	CH ₄ (%)	74.7 ± 0.9	97.4 ± 0.6	82.8 ± 1.6	97.9 ± 1.1
	CO ₂ (%)	25.3 ± 0.9	1.5 ± 0.8	4.3 ± 0.9	1.4 ± 0.5
	H ₂ (%)		1.1 ± 0.5	12.9 ± 1.0	0.7 ± 1.3
Liquide membrane pressure (bar _g)	0.3	0.4	0.4	0.6	
COD removal efficiency (%)	94.8 ± 3.0	96.0 ± 2.0	96.8 ± 0.4	95.7 ± 0.2	
Biogaz flow (NL/h)	8.6 ± 1.1	7.5 ± 0.2	10.9 ± 0.3	10.8 ± 0.3	
pH	7.3 ± 0.1	7.6 ± 0.1	7.7 ± 0.1	7.9 ± 0.2	
TAC (mg _{CaCO3} /L)	1866 ± 155	1122 ± 74	1491 ± 11	1453 ± 90	
H ₂ consumption yield (%)	-	98.9	89.3	99.2	

	In-situ/Ex-situ	Membrane type	Microbubbles /Biofilm	Pores (μm)	Temperature	Surface/volume (m^2/m^3)	CH ₄ production rate $\text{Nm}^3/\text{m}^3_{\text{Bioreactor}}/\text{day}$	H ₂ feed flowrate ($\text{NL}/\text{m}^2/\text{h}$)	CH ₄ content (%)	H ₂ consumption yield (%)
Díaz et al., 2015	Ex-situ	PVDF	Microbubbles	0.45	55°C	30	8.8	56	80% ^a	95%
Diaz 2019	In-situ	Polymeric	Microbubbles	0.4	35°C	4.7	0.54	7.8	73.1%	93.9%
Pratofiorito et al., 2021	Ex-situ	hydrophobic tubular	Biofilm	0.2	37°C	57.9	1.2	3.1	97%	100% ^b
Luo and Angelidaki, 2013a	In-situ	Polyurethane + Polyethylene	Biofilm	Non-porous	55°C	119	0.9	0.6	96.1%	100%
Present study	In-situ	Ceramic tubular	Diffusion and microbubbles	0.1	37°C	1.7	1.7	46	97.9%	99.2%

Figures

