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## Abstract

This study aimed at studying the biomethanation process using a 100 L pilot-scale digester equipped with a dense membrane for hydrogen injection. Hydrogen mass transfer was characterized and the impact of hydrogen flowrate, agitation rate and of the co-injection of $CO_2$, on biogas production and composition, was precisely studied. A linear relationship between $H_2$ flowrate and the $CO_2$ and $CH_4$ rates in biogas was found but no impact on biogas flowrate was shown. It was also noticed that, without exogenous $CO_2$ injection, and for high $H_2$ injection flowrates, residual $H_2$ could be found at the digester outlet due to local $CO_2$ limitation. Thus, this study suggested that biogas production in biomethanation process at the pilot scale was probably rather limited by the dissolved $CO_2$ transport within the liquid phase than by the hydrogen mass transfer itself.

*Keywords:* biomethanation, permeation membrane, pilot-scale digester, methanization

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## 1. Introduction

Anaerobic digestion is a natural biological process that transforms organic matter into biogas, consisting mainly of methane (about 60 %) and carbon dioxide (about 40 %). This is today a widespread way of producing green energies and that may simultaneously allow the recovery of organic wastes. To increase the rate of methane in the biogas, one possibility is the injection of hydrogen into the digesters. In fact, the natural production of hydrogen transformed into methane by hydrogenotrophic methanogenic *Archae* ($4H_2 + CO_2 \longrightarrow CH_4 + 2H_2O$) is limiting. Thus, by injecting exogenous hydrogen, the hydrogenotrophic methanogenic *Archae* could consume more $CO_2$ naturally produced in the reactor, and thus increase the rate of methane in biogas.

Injection of gaseous hydrogen to improve methanation reaction in digesters has been the subject of publications which demonstrated that several parameters may affect the efficiency of hydrogen injection, including the operating temperature and the mode of process performance. The main problem identified by Luo et al. (2012), Luo and Angelidaki (2012), Luo and Angelidaki (2013a) and Bassani et al. (2015) is indeed a problem of efficiency of hydrogen mass transfer in the reaction medium, leading to the presence of hydrogen in the biogas. These studies using different substrates (sludge STEP for Luo and Angelidaki (2012), bovine manure for Luo et al. (2012) and Luo and Angelidaki (2013a)) demonstrated the impact of some operating conditions on the effectiveness of the injection of hydrogen. These were the pressure of the $H_2/CO_2$ mixture injected in the headpsace of the reactor (Luo et al., 2012), the agitation rate (Luo et al., 2012; Luo and Angelidaki, 2012, 2013a) and the design of the gas supplier (Luo and Angelidaki, 2012, 2013a).

Luo and Angelidaki (2012) also showed that the amount of dissolved hydrogen

was very small regarding the amount of hydrogen that was expected by applying a mass balance on the gaseous hydrogen. With an injection of $H_2$ to 12 L/(L d) and an agitation rate of 500 rpm, the dissolved concentration of $H_2$ was indeed about 8 µmol/L while the expected one was 45 µmol/L. The difference between these two values highlighted a limiting step of transfer from gas to liquid phase due to non-adapted hydrodynamic conditions.

To improve the absorption of hydrogen in the liquid phase, two strategies were considered: the biogas recirculation and injection of hydrogen through membranes, in order to avoid the formation of bubbles and the loss of $H_2$ in the outlet gas phase. Biological methanation by recirculation of the biogas in the solution was proposed by Alfaro et al. (2019) and Kougias et al. (2017). Three configurations of digesters and different recirculation rates were compared by these last authors. Studied reactors were two columns in series, a Continuous Stirred Tank Reactor and a bubble column. The best results were achieved in the column reactors with the highest recirculation and flowrate reaching a consumption of up to 100 % $H_2$ and in the bubble column, with a rate of methane reaching 98 % in the biogas.

In order to address the problem of hydrogen absorption, in several studies, pure hydrogen or hydrogen mixed with CO or $CO_2$ was injected by using permeation hollow fiber membranes. The membranes used were non-porous polyurethane membranes (Luo and Angelidaki, 2013b; Wang et al., 2013) or PVDF (polyvinylidene fluoride) (Díaz et al., 2015). Studies using non-porous membranes agreed on the fact that hydrogen was entirely consumed, since there was no hydrogen in the biogas. Methane rates above 96 % in biogas were reported (Ju et al., 2008; Luo and Angelidaki, 2013b) which illustrated the effectiveness of an injection by permeation. The study of Díaz et al. (2015) also showed a very good efficiency in the consumption of

hydrogen, with more than 95 % of the $H_2$ injected effectively consumed. However, it was found in this last study that, on the first days of digestion, a large part of the injected hydrogen was used for biomass growth, and not for the production of methane. Moreover, the development of a biofilm on the surface of the membrane was observed (Luo and Angelidaki, 2013b) and could increase the resistance of hydrogen mass transfer. Apart from the study of Kim et al. (2013), carried out in a 100 L reactor and considering an *ex-situ* culture of hydrogenotrophic *Archae*, the studies were conducted in low volumes laboratory-scale reactors.

In this study, biomethanation reaction was carried-out in a 100 L bioreactor, using a silicone permeation membrane, to assess the robustness of *in-situ* biomethanation at the pilot-scale. First, the permeation characteristics of the membrane and the gas-liquid mass transfer performance of this system were determined. Next, the impact of the shear rate and hydrogen flow rate on the biogas production and composition were studied. Finally, the performance of methanation were determined using a co-injection of hydrogen and $CO_2$.

## 2. Materials and methods

### *2.1. Pilot-scale digester*

The total volume of the tank was 142 L, with a diameter of $D_{vessel}$ = 500 mm and a height $H$ =760 mm. The sketch of the bioreactor is reported on Figure 1. As demonstrated in a previous study (Lebranchu et al., 2017), the stirrer preferred was a double helical ribbon as it offered better mixing performance and allowed enhanced biogas productivity. This stirrer was combined here with a central Archimede's screw wrapped around the axis of agitation. Both systems were connected to an ATEX motor. The ribbon was sized in geometric similarity with the one previously used in

a 2 L digester as reported by Lebranchu et al. (2017). The pitch of the ribbon was 500 mm, its width was 48 mm while its internal and external diameters were 384 and 80 mm respectively. For the screw, the dimensions were a pitch of 298 mm, a blade width of 54 mm and an internal radius of 12 mm. The wrapping direction was opposite to that of the external ribbon. The agitator was also equipped with a Teflon scraper with a height of 35 mm to prevent the formation of crust at the bottom of the reactor. The control of the temperature at 40 $^{o}$C was ensured by a double-heated jacket. The agitation rate varied, depending on the operating conditions used, as detailed in the results section. The digester operated in continuous-mode with a mean residence time of 28 days, which implied the supply of 3.5 L of cattle manure and removal of 3.5 L of digestate each day. Due to the viscosity of the liquid phase, two Archimede's screw pumps (Air et Eau systèmes, Ludres, France) were used for supply and outlet. The supply was preliminary filtered using a grid with 6 mm diameter holes to prevent pipe plugging. Biogas composition and production were determined using dedicated on-line gas chromatography and gasmeter as previously described in Lebranchu et al. (2017).

### *2.2. Dense membrane properties and characterization*

To avoid the presence of hydrogen in the biogas at the outlet of the reactor, which would require the introduction of a recirculation loop of the biogas, the hydrogen injection was not carried out by a conventional sparger but by permeation into a silicone tube pressurized by closing it at one end and wrapped around a cylindrical support. The silicone tube used was a membrane with 0.3 mm thickness and an internal diameter of 2 mm (Witeg, Wertheim, Germany). The permeability $\mathcal{P}$ of the hydrogen in the silicone was obtained from equation 1 by applying various pressure gradients and measuring the related gas flow rate. The experiments were conducted

in air, water and digestate.

$$\mathcal{P} = \frac{Q \cdot e}{A \cdot \Delta P} \tag{1}$$

with $\mathcal{P}$ ($m^3$ m/(s $m^2$ Pa)) the membrane permeability, $Q$ the volumetric flow rate ($m^3$/s), $e$ the membrane thickness (m), $A$ the membrane surface ($m^2$) and $\Delta P$ (Pa) the pressure difference. In order to enhance liquid circulation in the core of the vessel, the diameter of the membrane support was increased to a maximal value of 324 mm. A gap of 65 mm was left between the top of the scraper placed on the stirrer and the bottom of the wrapping of the membrane tube to allow the circulation of the fluid. For the same reason, a gap of 65 mm was applied between the top of the membrane tube and the surface of the liquid. Using these conditions, a maximum of 100 m of membrane could be wrapped. According to the equation 1, the expected hydrogen flow rate should be 35 mL/min approximately.

The hydrogen flow rates and the volumetric gas-liquid mass transfer coefficients $k_La$ of the set-up were also determined. Gas-liquid mass transfer measurements were carried-out by setting the permeation rate at the inlet of the membrane and measuring the pressure inside the membrane tube. Dissolved $H_2$ was obtained from the mass balance between the inlet and the outlet. The experiment was carried-out as follows : The reactor was filled with 100 L of water heated gradually to 40 °C for 12 hours. Before the start of the measurements, a purge of the membrane tube was done at 20 mL / min of $H_2$ for 20 min in order to remove the gases that could be introduced inside the tube. The end of the tube was then closed and the flow rate studied was fixed to the flow meter. Continuous hydrogen injections were made in water at 10, 30 and 50 mL / min and, by the plot of equation 2, $k_La$ ($s^{-1}$) value was then obtained.

$$\ln\left(\frac{[H_2]^*}{[H_2]^* - [H_2]}\right) = k_L a \cdot t \quad (2)$$

with $[H_2]^*$ the concentration of $H_2$ at saturation, $[H_2]$ the concentration of $H_2$ in the solution and $t$ the time (s).

## 3. Results and discussion

### *3.1. Gas-liquid mass transfer*

The permeability of hydrogen in the silicone was determined at 40°C using several injection rates (30, 50 and 70 mL / min) and considering permeation in air to neglect the resistance of mass transfer in the fluid phase (Figure 2). A value of $\mathcal{P}$ = 7.33×10$^{-14}$ (m$^3$ m) / (s m$^2$ Pa) was obtained. As expected, the increase of the resistance of mass transfer entailed a slower permeation of hydrogen in water and in digestate due to the significant increase of the resistance to mass transfer when air is replaced by these two fluids (Figure 2). In parallel, the determination of $k_L a$ values, carried-out at a agitation rate of 10 rpm (namely for a constant power dissipation per unit of volume) provided values of approximately 1 h$^{-1}$ (Figure 2). No study determining the value of $k_L a$ of a dense membrane for hydrogen injection in digesters have been carried out at the pilot-scale making hard the comparison of the present values with literature data. An increase of the agitation rate to 40 rpm did not promote an increase in the hydrogen absorption rate as $H_2$ microbubbles, formed and attached to the membrane, detached then due to higher local shear stress, causing a sudden release of gaseous $H_2$ and then a decrease in the amount of dissolved hydrogen.

### *3.2. Validation of pilot-scale digester*

The biogas flowrate profile showed that, after 17 days of production, it reached an asymptotic value of about 5.2 NL/h (or 1 NL/(h kg$_{OM}$)), which corresponded to

a production of 125 L of biogas per day. Considering the daily addition of 3.5 L of liquid cattle manure, at a rate of about 11 % of organic matter, biogas production was approximately 324 $L/kg_{OM}$. This result was thus in agreement with the expected values of biogas production using cattle manure (Teixeira Franco et al., 2017). The initial pH was 7.9, corresponding to the pH of the digestate used for initial reactor filling. During the first 7 days of digestion, a slight pH decrease was observed before a stabilization to 7.5 after 20 days of digestion, which corresponded to range of values of 7.5 to 8, typically encountered in digesters (Bassani et al., 2015).

The analysis of biogas composition revealed a maximal methane content of 63.6 % and a minimal $CO_2$ rate of 36.3 % after 3 days of digestion. This initial process sequence, rather favourable to methane production was already noticed in different metagenomic studies such as the works of Montero et al. (2008) on a synthetic medium or of Chachkhiani et al. (2004) using liquid cattle manure. This suggested thus an initial production of $CH_4$ by the hydrogenotrophic *Archae* rather than by the acetotrophic populations leading to the reduction of $CO_2$. Biogas composition progressively stabilized after 7 days of digestion at rate values of 57.7 % for $CH_4$ and 42 % for $CO_2$. The results obtained without hydrogen injection thus validated the pilot-scale digester as a scale-up of the equipment used by Lebranchu et al. (2017) for cattle manure methanization and could thus be used for further study of biomethanation process.

### *3.3. In-situ biomethanation*

#### *3.3.1. Impact of $H_2$ flowrate on biogas flowrate*

After each steady-state, $H_2$ flow rate was progressively increased from 12 to 31 mL/min. Biogas flow rate per kg of organic matter was determined and given in Figure 3A. Hydrogen injection, whatever the flowrate used, did not seem to significantly

modify biogas flowrate. This was previously observed by Luo et al. (2012) by increasing partial pressure of $H_2$ in a digester headpsace. This result was also consistent with the bioreaction stoichiometry as one molecule of $CO_2$ should be transformed into one molecule of methane. This also meant that all injected hydrogen was effectively consumed. As the consumption of exogenous $H_2$ led to the consumption of a fraction of the dissolved $CO_2$, there was also a concomitant slight increase in the pH value after each increase of hydrogen flowrate (Figure 3B), from 7.5 to almost 7.7 for $H_2$ flow rate of 31 mL/min. However, this increase, also observed in other studies of biological methanation (Szuhaj et al., 2016; Luo et al., 2012; Luo and Angelidaki, 2013a), remained weak and did not seem to significantly affect the production process as the flow rate of biogas remained overall constant (Figure 3A).

### *3.3.2. Impact of $H_2$ flowrate on biogas composition*

On the contrary to biogas flow rate, $CH_4$ and $CO_2$ biogas contents significantly depended on the $H_2$ flowrate as indicated by the measurements reported in Figure 3A. A progressive decrease in $CO_2$ content and a corresponding increase in $CH_4$ content were indeed observed when $H_2$ flowrate increased. Figure 3A also shows that, in the mean time, the time interval between change in $H_2$ flow rate and biogas content stabilization was reduced. Whereas five days were needed to achieve the stabilization after switching the $H_2$ flowrate from 0 to 12 mL/min, three days were indeed sufficient to achieve stability after the switch from 20 to 31 mL/min. This suggested a possible adaptation of microbial populations to the presence of $H_2$, including the increase in the hydrogenotrophic bacteria population, promoting a reduction of the necessary stabilization time. Studies of Agneessens et al. (2017), Treu et al. (2018), and Alfaro et al. (2018, 2019) demonstrated by microbial community characterization that microbial populations were able to adapt to a $H_2$ injection but also that this

adaptation was strongly linked to the process operation parameters (modes of injection of $H_2$, flow rates, substrates, reactor scale). The relationships between $CH_4$ and $CO_2$ contents and injected $H_2$ flowrates were reported in Figure 4. Interestingly, the experimental measurements could be fitted using a linear model which was expected regarding reaction stoichiometry. Considering that the molar volume remained indeed the same whatever the gas, a mole of $CH_4$ should be produced for each mole of $CO_2$ consumed. Knowing that the flow of biogas is 5.2 L/h, the slope obtained of 0.35 corresponded to 0.3 mL/min of additional $CH_4$ for 1 mL/min $H_2$ added. However, theoretically, with the addition of 1 mL/min of $H_2$, the $CH_4$ flowrate should be of 0.25 mL/min. This clearly confirmed the efficiency of conversion of hydrogen into methane and that the injected hydrogen was completely consumed. This also suggested that consumed hydrogen seemed not significantly used for biomass growth, as Díaz et al. (2015) already showed. The total consumption of hydrogen was confirmed by the analysis of biogas with $H_2$ rates lower than 0.05 % (Figure 3A) despite weak amounts of $H_2$ of approximately 0.04 % at an hydrogen flowrate of 31 mL/min were determined. The simultaneous presence of $CO_2$ at high volume fractions also seemed to indicate the occurrence of local $H_2$ saturations in the bioreactor inducing a release of gaseous hydrogen. This may be explained either (i) by a physical limitation, namely the injected hydrogen was not dispersed quickly enough in the reactor in comparison with the inlet flowrate of hydrogen, leading to local saturation and the appearance of a gas phase hydrogen or (ii) by a biological limitation, namely *Archae* were not locally able to consume all the amount of injected hydrogen or (iii) by a local limitation of $CO_2$ that stopped the $H_2$ consumption. Even if biogas recirculation is an interesting option to limit $H_2$ loss, further developments regarding efficiency of 'one-pass' hydrogen injection, namely without biogas recirculation, should be intensified. Jensen et al. (2018) indeed demonstrated that

it should remain the optimal solution from a mass transfer performance point of view.

### *3.4. Impact of shear stress on biogas production during biomethanation*

As noted previously, when the hydrogen flow rate and the agitation rate were respectively 31 mL/min and 10 rpm, traces of $H_2$ were measured at the outlet of the reactor. Thus, the frequency of agitation was modified in order to improve the distribution of hydrogen in the reactor and potentially to reduce this loss and to increase the $CH_4$ rate. The study of the impact of agitation rate was therefore performed here in the presence of an injection of $H_2$ to 31 mL/min.

#### *3.4.1. Impact on biogas flowrate*

The temporal evolution of the flowrate of biogas for different conditions of agitation is presented in Figure 5. The increase in the agitation rate from 10 to 20 rpm did not have a significant impact on the flowrate of biogas. However, the reduction of agitation from 20 to 5 rpm led to an increase in the average flow rate from 0.89 to 1.14 L/(h $kg_{OM}$), but also to temporal fluctuations of this flow rate. This could be related to the results of previous paper dealing with the impact of agitation on the performance on biogas production in a 2 L digester (Lebranchu et al., 2017). It was indeed shown that the increase in agitation from 10 to 50 and 90 rpm, led to a reduction of the amount of biogas, probably due to the increase in the maximum in shear stress the reactor and the resulting damage of microbial aggregates. As shown by Lebranchu et al. (2017), this shear stress could be expressed by equation 3:

$$\sigma = K \cdot \dot{\gamma}^n \tag{3}$$

with $K$ (Pa $s^n$) the consistency index and $n$ () the flow index for the cattle manure digestate. Then the following equation may also be obtained:

$$\frac{\sigma_{max_{100L}}}{\sigma_{max_{2L}}} \propto \left( \frac{\dot{\gamma}_{max_{100L}}}{\dot{\gamma}_{max_{2L}}} \right)^n \tag{4}$$

with $\sigma_{max_{100L}}$ and $\sigma_{max_{2L}}$ (Pa) and $\dot{\gamma}_{max_{100L}}$ et $\dot{\gamma}_{max_{2L}}$ the maximal shear stresses and maximal shear rates in both systems respectively. Assuming that maximal shear rate $\dot{\gamma}_{max}$ was encountered in the gap between the ribbon and the vessel wall, it could be estimated by equation 5.

$$\dot{\gamma}_{max} = \frac{\pi \cdot N \cdot D}{\frac{D_{vessel} - D}{2}} \tag{5}$$

with $D_{vessel}$ the vessel diameter, $D$ the external ribbon diameter and $N$ the agitation rate. The width of the gap in the 100 L reactor was also decreased relatively to its length obtained theoretically by geometric similarity of the 2L reactor in order to increase the heat transfer and limit the formation of crust close to the outer wall. Thus, the maximal shear rate reached 12.6 $s^{-1}$ in 100 L reactor at 5 rpm, which is 2 times higher than the one obtained in the 2 L reactor at 10 rpm (6.1 $s^{-1}$). The maximal shear stress obtained at 10 rpm in 2 L reactor was about 30 Pa (Lebranchu et al., 2017), so according to equation 4, the maximal shear stress obtained at 5 rpm in the pilot reactor was 37.2 Pa. Using the same method of calculation, the maximal shear stress at the pilot-scale and at an agitation rate of 10 rpm was then estimated at $\sigma_{max}$ =47 Pa. Thus, the maxim shear stress in 2 L at $N$ =10 rpm was similar to the one expected in the 100 L digester for $N$ = 5 rpm, and matched the critical shear stress that should not be exceeded, as determined in Lebranchu et al. (2017). During the experiment at $N$ =10 rpm in the 100 L reactor, the maximal stress was

thus high enough to lead to a reduction of the biogas flowrate.

Moreover, the variability of the flow rate was more important at an agitation rate of 5 rpm in comparison with 10 or 20 rpm agitations. This phenomenon found no explanation by the measurements and no high frequency fluctuations could be put into evidence. The change in the frequency of agitation did not significantly change the pH of the digestate (data not shown). The relative stability of the pH during this period indicated that the balance between the production and consumption of volatile fatty acids was globally not impacted by the agitation rate. The biogas flow variations observed at $N = 5$ rpm could therefore not be explained by pH fluctuations.

### *3.4.2. Impact on biogas composition*

The evolution of the composition in $CH_4$ and $CO_2$ flowrates for the various agitation rates (10, 20 and 5 rpm) was reported in Figure 5. The increase in agitation from 10 to 20 rpm allowed an increase in $CH_4$ rate from 68.0 to 68.8 %, a decrease in $CO_2$ rate from 31.7 to 30.9 % and a residual $H_2$ rate reduction from 0.041 to 0.034 %. This suggested that a stronger agitation allowed a better homogeneity of the $H_2$ and thus a more efficient consumption. The change from $N$ =20 to 5 rpm led also to biogas composition fluctuations. This destabilization was accompanied by a decrease in the average $CH_4$ rate to 66.7 %, an increase in the $CO_2$ rate to 33.1 % and an increase in the $H_2$ rate to 0.08 %. Considering the respective flow rates of each gas component, a slight increase in hydrogen flow rate of 1 mL/h at 20 rpm versus 3 mL/h to 5 rpm, which seemed to indicate to a slight decline in methane flow rate, but $CH_4$ flow rate increased from 3.03 to 3.5 L/h. Thus, the hydrogen mass balance was not in total agreement with the one in methane, with a simultaneous increase in the flow of methane and hydrogen. These results suggested that the additional

methane produced was not related to the injection of hydrogen but could be the consequence of better synthrophic relationships between bacteria as it was already suggested in the study of Lebranchu et al. (2017). Thus, it was concluded that the increase in agitation allowed a better distribution of hydrogen in the reactor, promoted a reduction in $H_2$ rate in biogas, but also caused a decrease in overall biogas production flow. The agitation was then set back to 10 rpm for the rest of the study.

### *3.5. Addition of exogenous $CO_2$*

To further validate the hypothesis of local $CO_2$ limitation, $CO_2$ was added in the injected gas, by applying the ratio of $H_2$ and total C carbon rates $H_2/C = 8.8$. This addition was carried-out in 2 steps. First, the addition of $CO_2$ was made at a flow rate of 3.5 mL/min, while maintaining the flow of $H_2$ at 31 mL/min. In a second step, the flow rate of $CO_2$ was maintained constant at 3.5 mL/min while $H_2$ flow rate was increased till total consumption of injected $CO_2$. The Figure 6 reports the total flow rate of biogas at the outlet of the digester. A slight increase in this flow rate was observed throughout the period of $CO_2$ injection, from 5.0 L/h to 5.6 L/h. The observed increase of the overall flow rate was consistent with what was expected, although this increase was three times higher than the expected value for a $CO_2$ inlet flow rate of 0.21 L/h. Besides, no decrease of pH during the addition of $CO_2$ was observed meaning there was no accumulation of $CO_2$ in the reactor. This suggested that dissolved $CO_2$ remained close to the permeation membrane, and that it was either consumed, or accumulated before its stripping. This would suggest a local decrease of the pH, close to the membrane, as previously demonstrated by Garcia-Robledo et al. (2016). Lastly, the increase of $H_2$ flow rate led to a slight increase in the pH measured, from 7.7 to 7.8, which was consistent with the consumption of $CO_2$ naturally formed by the digestion process.

The injection of $CO_2$ at 3.5 mL/min with a $H_2$ flow rate of hydrogen set at 31 mL/min led to an increase in the rate of $CO_2$ in biogas and, consequently, a decrease in $CH_4$ rate (Figure 6). Combined with the overall increase in the flow of biogas, this increase in $CO_2$ rate led to the increase in $CO_2$ outflow from 1.66 to 1.94 L/h, while $CO_2$ injected was 0.21 L/h, indicating an overproduction of $CO_2$ in these conditions. Figure 6 shows that the amount of $CO_2$ consumed is not zero because the present rate of $H_2$ in the biogas decreased in the presence of $CO_2$. Concerning $H_2$, its rate fell from 0.16 to 0.008 %, meaning its flow rate decreased from 0.13 to 0.0083 mL/min. At the same time, methane rate was reduced from 66.7 to 65.1 %, but the increase in overall throughput of biogas led to an increase in the overall rate of methane from 3.34 to 3.66 L/h. The increased flow rate of methane is qualitatively consistent with the decrease in the rate of hydrogen. Quantitatively, the simultaneous overproduction of $CO_2$ and $CH_4$ could be explained by the acetotrophic *Archae* that produce both of $CH_4$ and $CO_2$. The local decrease in pH mentioned earlier would be the consequence of the activity of the acetotrophic *Archae*. This phenomenon was demonstrated by the study of Hao et al. (2013) who observed changes in bacterial populations during changes of pH conditions. It has been highlighted that the increase in the pH above 7.5 led to an inhibition of the activity of the acetotrophic methanogenic *Archae* . Thus, considering the initial pH of 7.8 in the whole of the reactor, the addition of $CO_2$ could have led to a local decrease in pH below 7.5, which would cause the overproduction of methane and $CO_2$. Thus, it seems that, qualitatively, the depletion of $H_2$ in the outlet gas was explained by consumption of a fraction of $CO_2$. However, it remained difficult to quantify it more precisely due to the increased production of $CO_2$ by acetotrophic *Archae* .

The addition of extra $CO_2$ mixed with the $H_2$ allowed the total consumption of

the $H_2$ (Figure 6). This could explain why hydrogen was detected in outlet biogas for flow injection of 31 mL/min of pure hydrogen, even if $CO_2$ was macroscopically available. The study of Garcia-Robledo et al. (2016) showed that injected $H_2$ and $CO_2$ consumption was carried out in a millimetric layer near the membrane. This suggested that, in the absence of $CO_2$ injected, the existence of residual hydrogen was due to a $CO_2$ limitation near the membrane. A realistic assumption is that the injected $H_2$ locally reacted with $CO_2$ which, once the latter fully consumed, caused a local accumulation of hydrogen beyond its solubility, entailing its stripping. So, there was probably a limitation of hydrogen transport from the membrane towards the rest of the reactor and a limitation on the transport of $CO_2$ from bulk to the membrane permeation vicinity. This assumption was also previously proposed by Garcia-Robledo et al. (2016) and Agneessens et al. (2018) in 1.4 L lab-scale digesters.

In order to consume the injected $CO_2$, $H_2$ flow rate was progressively increased while keeping constant the $CO_2$ flow rate to 3.5 mL/min. It should be noted that it was necessary to increase the $H_2$ flow rate up to 42 mL/min (Figure 6) to achieve a $CO_2$ rate in the biogas similar to the rate of $CO_2$ with pure $H_2$ injection, i.e. to consume all of the exogenous $CO_2$. Even if the increase in total production of biogas was explained by overproduction by the acetotrophic *Archae*, this consumption of additional hydrogen showed that the hydrogenotrophic *Archae* were always present and active. Flow rate of biogas was stable at 5.6 L/h over the period, but the flow rate of methane increased up to 3.8 L/h and that of $CO_2$ decreased to 1.8 L/h. The difference was thus + 0.14 L/h in $CH_4$ and - 0.14 L/h in $CO_2$. The surplus of added hydrogen was thus 0.66 L/h, while the theoretical value was 0.17 L/h of additional methane and 0.17 L/h for $CO_2$ consumption. The results obtained in the digester were therefore relatively close to the theoretical values. Throughout this period,

the injected $H_2$ was completely consumed, which confirmed the effectiveness of the permeation membrane for the intensification of production as long as it remained some $CO_2$ to consume in the membrane vicinity.

Lastly, the injection of $CO_2$ was shut-off, which led to the decrease in the flow of biogas from 5.6 to 5.0 L/h (Figure 6), to the increase in $CH_4$ rate to 70.5 %, to the decrease in $CO_2$ rate at 28.9 % and the measurement of about 0.4 % $H_2$ in the biogas. The presence of hydrogen in the biogas while keeping a $CO_2$ rate of nearly 30 % indicated the appearance of a local saturation, likely located in the environment of the membrane.

## 4. Conclusion

Biomethanation of cattle manure was validated in a 100 L digester using a dense membrane for $H_2$ injection. This injection was shown to strongly limit the undissolved $H_2$ rate in the biogas produced. A linear relationship was found between the $CH_4$ content in the biogas and the $H_2$ flowrate. The co-injection of exogenous $CO_2$ with $H_2$ revealed that biogas production was probably limited by the dissolved $CO_2$ transport within the liquid phase volume, which addresses the issue of the definition of robust scale-up rules for biomethanation processes and for designs of gas injection systems.

## Acknowledgements

This work was supported by ADEME and Programme d'Investissements d'Avenir (research program VALORCO).

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## Figure captions

**Figure** 1. Sketch of the bioreactor used in the present study.

**Figure** 2. Impact of transmembrane pressure on hydrogen permeation flow rate in the case of permeation in air (■), in water (▲) and in digestate (●) and on the volumetric gas-liquid mass transfer coefficient $k_L a$ in digestate (×).

**Figure** 3. (A) Temporal profile of biogas flowrate (□) and composition in $CO_2$ (◇), $H_2$ (*) and $CH_4$ (○) and (B) pH.

**Figure** 4. Impact of $H_2$ flowrate on the rates of methane (○) and $CO_2$ (◇).

**Figure** 5. Impact of agitation rate on the total (□), $CO_2$ (◇), $H_2$ (∗) and $CH_4$ (○) volumetric flowrates.

**Figure** 6. Impact of $CO_2$+$H_2$ injection on the $CO_2$ (◇), $H_2$ (∗) and $CH_4$ (○) biogas composition and total biogas volumetric flowrate (□) .

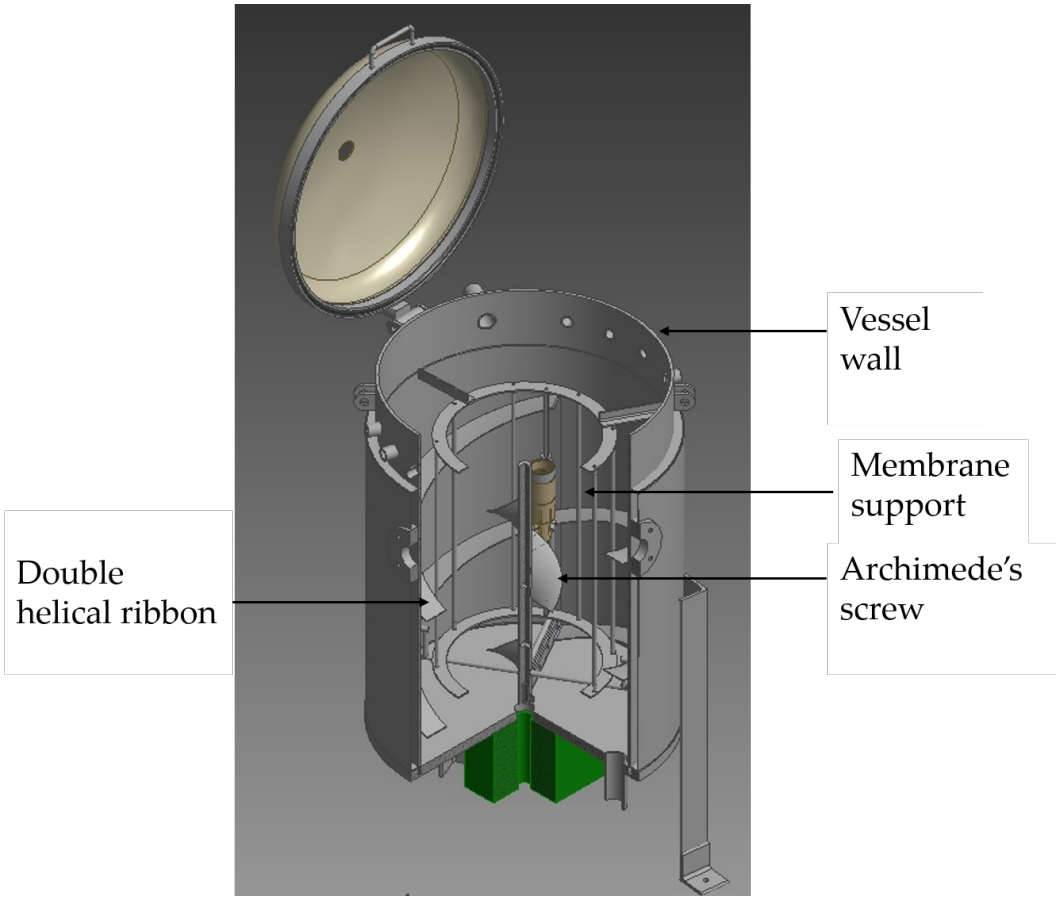



Figure 1: Sketch of the bioreactor used in the present study.

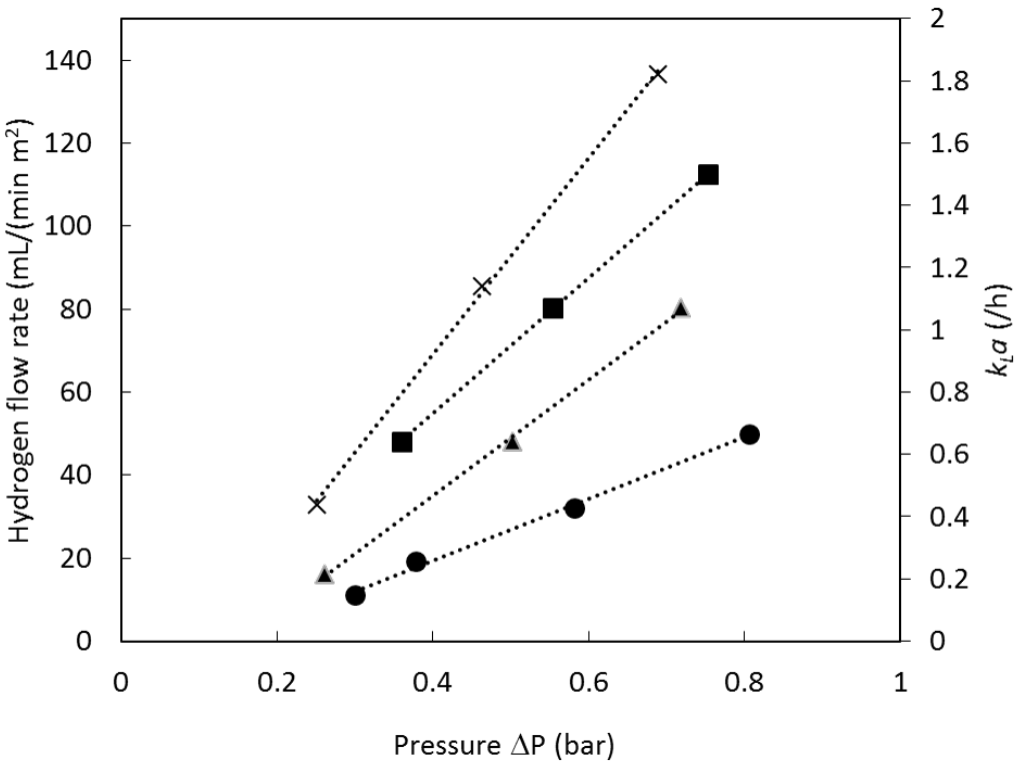
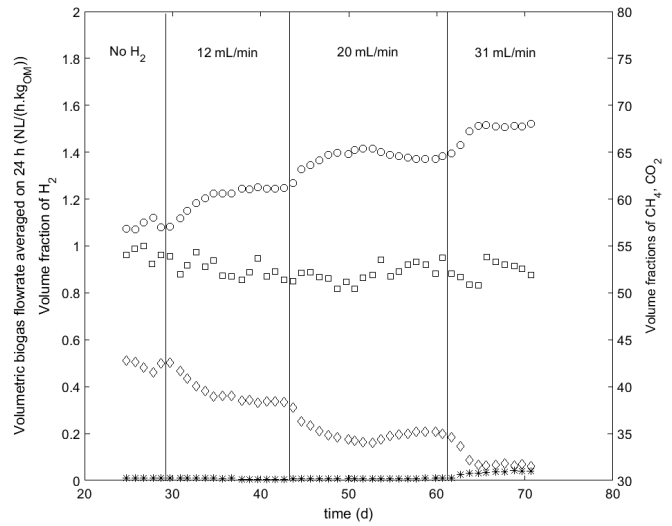
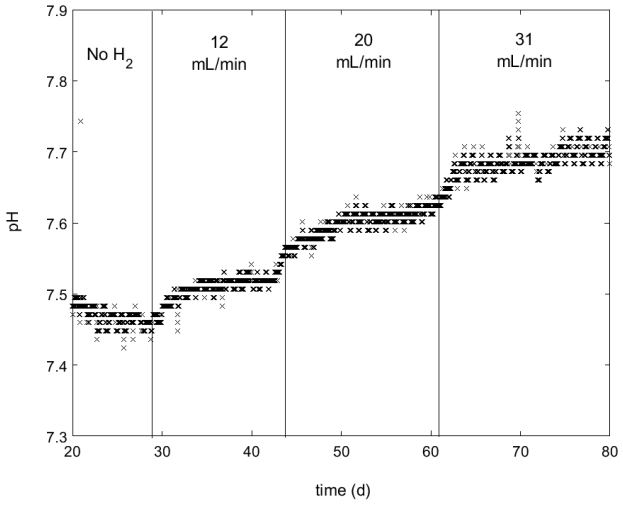



Figure 2: Impact of transmembrane pressure on hydrogen permeation flow rate in the case of permeation in air (■), in water (▲) and in digestate (●) and on the volumetric gas-liquid mass transfer coefficient $k_La$ in digestate (×.)




(A)




(B)

Figure 3: (A) Temporal profile of biogas flowrate (□) and composition in $CO_2$ (◇), $H_2$ (*) and $CH_4$ (○) and (B) pH.

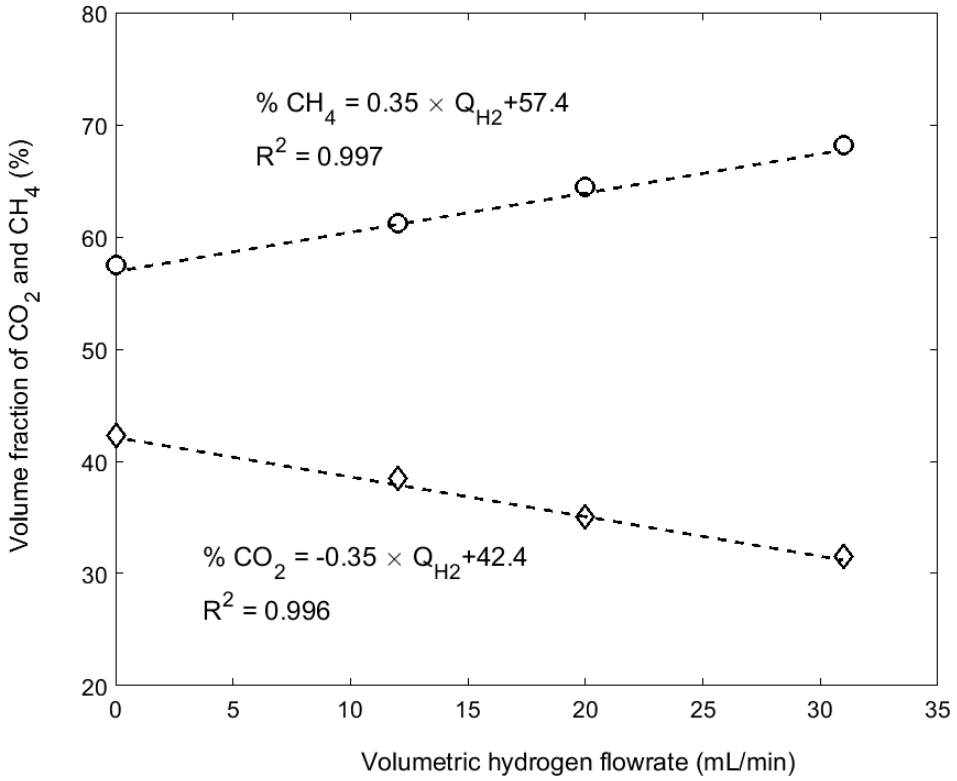



Figure 4: Impact of $H_2$ flowrate on the rates of methane in $CO_2$ ($\diamond$) and $CH_4$ ($\circ$) .

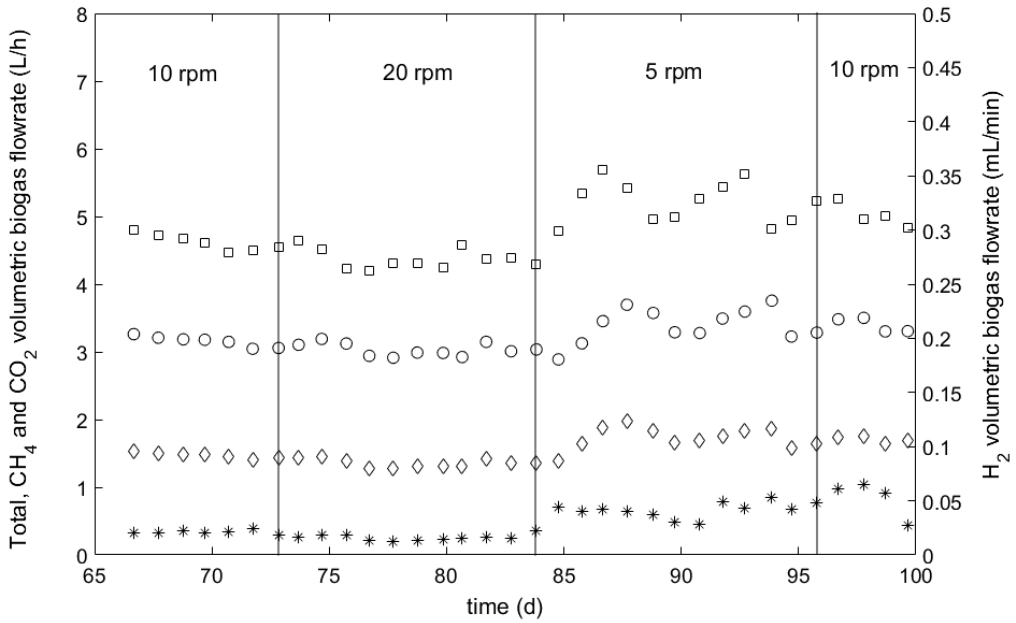



Figure 5: Impact of agitation rate on the total ($\square$), $CO_2$ ($\diamond$), $H_2$ ($*$) and $CH_4$ ($\circ$) volumetric flowrates.

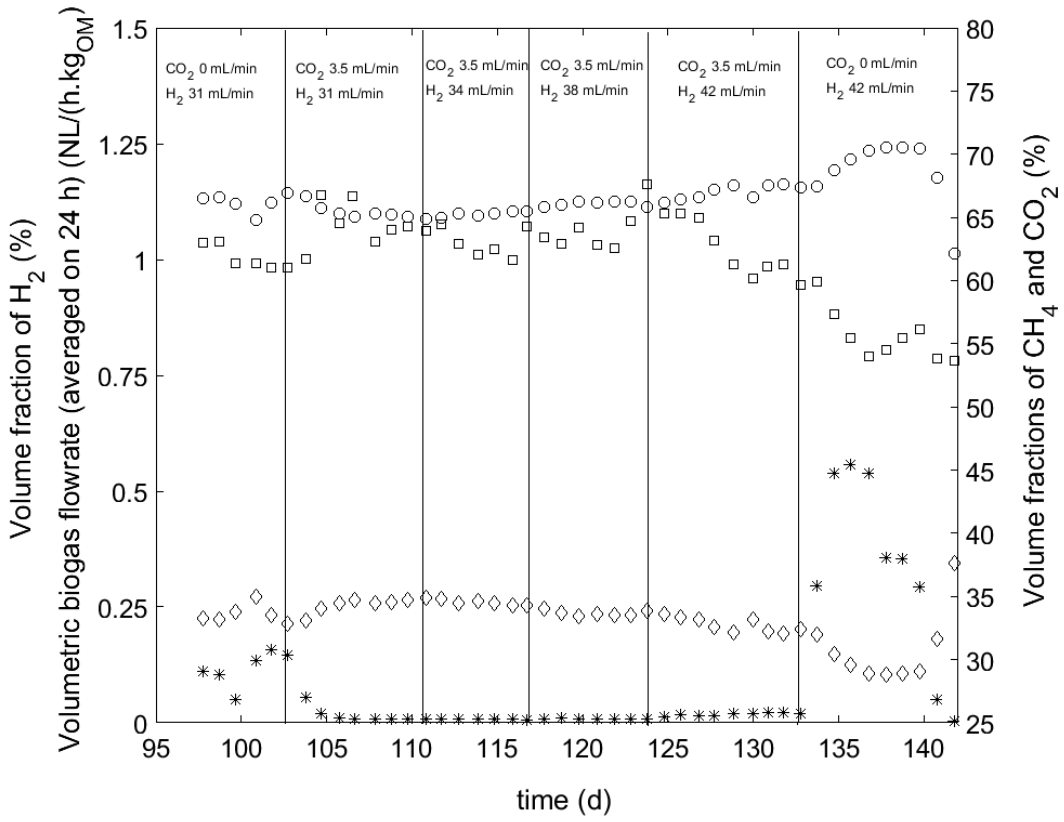



Figure 6: Impact of $CO_2$+$H_2$ gas on the $CO_2$ ($\diamond$), $H_2$ ($*$) and $CH_4$ ($\circ$) biogas composition and total biogas volumetric flowrate ($\square$).