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H_2 addition through a submerged membrane for in-situ biogas upgrading in the anaerobic digestion of sewage sludge

Natalia Alfaro[a], María Fdz-Polanco[a,b], Fernando Fdz-Polanco[a,b], Israel Díaz[a,b,*]

[a] Department of Chemical Engineering and Environmental Technology, Escuela de Ingenierías Industriales, Sede Dr. Mergelina, University of Valladolid, Dr. Mergelina s/n, 47011 Valladolid, Spain

[b] Institute of Sustainable Processes, University of Valladolid, Dr. Mergelina s/n, 47011 Valladolid, Spain

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ABSTRACT

In-situ upgrading of biogas in a mesophilic anaerobic digester of sewage sludge by sparging H_2 through a membrane was studied. Large gas recirculation rates were required to facilitate H_2 transfer to the bulk liquid phase; at ~200 $L\,L_{reactor}^{-1}\,d^{-1}$, H_2 utilization efficiency averaged 94% and the specific CH_4 production increased from 0.38 $L\,L_{reactor}^{-1}\,d^{-1}$, during conventional digestion, to 0.54 $L\,L_{reactor}^{-1}\,d^{-1}$. Sludge digestion was not compromised by elevated H_2 partial pressure nor by the associated rise in the pH (8.1) because of CO_2 removal. In this regard, VFA accumulation was not detected and the performance of VS removal was similar to the observed without H_2 supply. Microbial analysis revealed that homoacetogens were outcompeted by hydrogenotrophic methanogens. *Methanoculleus* sp., *Methanospirillum* sp., *Methanolinea* sp. and *Methanobacterium* sp. were the hydrogenotrophic archaea present over the experiment.

1. Introduction

Biomethane could show a substantial impact in future energy systems in Europe because of EU policies to rise the portion of renewable and low carbon fuels and contribute to the decarbonization of heat and transport (European Commission, 2017; Wall et al., 2018). High purity biomethane (CH_4) results from biogas upgrading, a process in which pollutants (CO_2, N_2, H_2O, H_2S…) are removed from biogas so that CH_4 content is enhanced. Normally, a concentration of CH_4 larger than 80–95% (v/v), depending on the legislation and set standards (Muñoz et al., 2015), is required in biomethane in order to be injected into natural gas grid or to be used as transport fuel. The removal of CO_2, a major contaminant of biogas, is mostly performed by scrubbing, membranes or pressure swing adsorption technologies at full-scale facilities. However, the recent progress in biological technologies brings the possibility to convert CO_2 into valuable products (Muñoz et al., 2015; Wall et al., 2018). In particular, power to gas strategies have gained increased attention recently (Bailera et al., 2017; Götz et al., 2016). The power to gas technology uses excess electricity produced by renewable energy sources, during off-peak production times, to convert water into H_2 and O_2 through electrolysis and subsequent biological or catalytic methanation of H_2 and CO_2 ($4\,H_2 + CO_2 \rightarrow CH_4 + 2\,H_2O$).

Biological methanation has shown higher tolerance to the impurities usually present in biogas in comparison to the catalytic pathway. However, insufficient transfer of H_2 to the liquid phase, in which the biological reaction occurs, limits the biomethanation and results in large bioreactor size (Götz et al., 2016). Several approaches have been employed to enhance H_2 transfer in bioreactors for ex-situ upgrading, such as stirred reactors (Luo and Angelidaki, 2012), packed-columns (Jee et al., 1988), bubble columns (Alfaro et al., 2018) and biotrickling filters (Dupnock and Deshusses, 2017). Nonetheless, H_2 could be supplied directly to the anaerobic digester so that *archaea* can consume H_2 and CO_2 (in-situ upgrading), hence additional units for upgrading may be avoided (Rittmann, 2015; Zabranska and Pokorna, 2018).

The methane evolution rate (MER), which expresses the increase in the specific CH_4 production rate ($L\,L_{reactor}^{-1}\,d^{-1}$) under H_2 supply with respect to the lack thereof, reported in in-situ studies ranged from 0.08 to 0.39 $L\,L_{reactor}^{-1}\,d^{-1}$, while the concentration of CH_4 in upgraded biogas was between 58 and 99% (v/v) (Lecker et al., 2017). The feasibility of in-situ upgrading does not only require efficient H_2 and CO_2 conversion into CH_4 but also preserved organic matter removal and convenient integration in the available facilities (Agneessens et al., 2017). In this regard, the rise of pH resulting from CO_2 removal is a challenge, pH values higher than 8 (up to 9.2) have been reported with uneven effects on organic matter removal efficiency (Bassani et al.,

* Corresponding author at: Department of Chemical Engineering and Environmental Technology, Escuela de Ingenierías Industriales, Sede Dr. Mergelina, University of Valladolid, Dr. Mergelina s/n, 47011 Valladolid, Spain.
E-mail address: israel.diaz@iq.uva.es (I. Díaz).

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2016; Garcia-Robledo et al., 2016; Luo et al., 2012). Additionally, the endergonic character of syntrophic fermentations is emphasised by an increase in H_2 partial pressure (Rittmann and McCarty, 2001; Speece, 2008), thus reducing available energy for acetogenic bacteria and resulting in VFA accumulation (Mulat et al., 2017).

From a different point of view, mechanical stirring has been applied in completely mixed reactors (Agneessens et al., 2017, 2018; Luo and Angelidaki, 2013a,b) to ease organic matter removal and H_2 conversion while biogas recirculation or liquid recirculation have been evaluated only for pulse H_2 additions (Agneessens et al., 2017; Jensen et al., 2018). In this respect, the application of biogas recirculation through membranes for gas sparging has shown to increase H_2 and CO_2 conversion efficiency up to 95% (by increasing k_La values for H_2) in ex-situ hydrogenotrophic reactors (Alfaro et al., 2018; Díaz et al., 2015). Therefore, in-situ upgrading during the anaerobic digestion of sludge could benefit from biogas recirculation, commonly employed for mixing in full-scale digesters (Appels et al., 2011), to increase the conversion efficiency of H_2 and CO_2 as in the aforementioned ex-situ experiments. Nevertheless, such a reactor configuration (gas recirculation and H_2 supply through a submerged membrane) has never been evaluated for in-situ upgrading.

The aim of this work is to evaluate the feasibility of supplying H_2 to an anaerobic digester of sewage sludge through a submerged membrane module for in-situ upgrading of biogas. The effect of biogas recirculation rate on upgrading efficiency and the performance of the organic matter removal were assessed. Dynamics of the microbial community were studied using molecular biology tools.

2. Materials and methods

2.1. Sludge digesters setup

Two cylindrical bioreactors with an internal diameter of 188 mm and a height of 1000 mm were filled to a working volume of 20 L. One reactor (R1) was used for upgrading with H_2, while the other R2 was a control (conventional digestion). A hollow-fibre membrane module (ZeeWeed®-1, General Electric, Spain) was submerged in R1.

Membrane consisted of polymeric fibres (0.4 μm pore size) and an area of 0.093 m^2 for gas sparging. Peristaltic pumps (Watson-Marlow) were used for feeding and mixing of R1 and R2. A feeding tank, equipped with a magnetic stirrer, was installed for both reactors. H_2 was fed from a gas cylinder using a mass-flow controller (Aalborg, USA). The gas mixture composed by H_2 feeding and biogas recirculation lines was injected in R1 with a peristaltic pump through the membrane (Fig. 1a). A pH probe was installed in R1 for continuously monitoring of pH. R2 was fed only with thickened mixed sludge without biogas recirculation and H_2 supply (Fig. 1b).

2.2. Operating conditions

Inoculum was obtained from mesophilic sludge digesters in municipal WWTP of Valladolid (Spain). The content of total and volatile solids in the inoculum was 22.0 g kg^{-1} and 12.8 g kg^{-1}, respectively. Temperature was controlled at 35 ± 1 °C in R1 and R2 and both were fed at HRT of 20 days with sewage sludge (thickened mixed primary and secondary sludge) collected periodically from the aforementioned WWTP. VS content in sewage sludge was variable, then, OLR to the digesters was allowed to change with the aim of replicating the conditions in the WWTP (Table 1). After a set-up period of 60 d, H_2 was added to R1 (stage 1). H_2 addition at a rate of 0.87 L $L_{reactor}^{-1}$ d^{-1} was maintained during the whole experiment in all stages, to achieve a ratio 4:1 to the average gaseous CO_2 production during the set-up period. Gas recirculation rates applied to R1 ranged between 50 and 202 L $L_{reactor}^{-1}$ d^{-1} in the different experimental stages (Table 1). Peristaltic pumps for sludge recirculation were operated at 72 L $L_{reactor}^{-1}$ d^{-1}. Volumes of gases and volumetric gas rates are reported at 273 K and 1 atm.

2.3. Monitoring and analysis

Gas production was measured by liquid displacement (Alfaro et al., 2018) and the composition of biogas was determined by GC-TCD as in Díaz et al. (2010). pH, total solids (TS), volatile solids (VS) and NH_4^+ concentration for raw and digested sludge were analysed weekly according to Standard Methods (APHA, 2005). VFA concentration was analysed by GC-FID (Díaz et al., 2010).

2.4. Calculations

H_2 gas-liquid mass transfer rate (r_t, L $L_{reactor}^{-1}$ d^{-1}) and efficiency of H_2 utilization (η_{H2}, %) were calculated according to Eq. (1) and Eq. (2), respectively:

$$r_t = H_2 \text{ flow rate} - H_2 \text{ in output gas} \quad (1)$$

$$\eta_{H_2} = \frac{H_2 \text{ flow rate} - H_2 \text{ in output gas}}{H_2 \text{ flow rate}} \cdot 100 \quad (2)$$

where H_2 flow rate is 0.87 L $L_{reactor}^{-1}$ d^{-1} and H_2 in output gas is the H_2 rate (L $L_{reactor}^{-1}$ d^{-1}) in the produced biogas. It was assumed that all the H_2 transferred to the liquid phase was ultimately converted to CH_4 or employed for microbial growth as in Díaz et al. (2015). Specific gas transfer coefficient (k_La_{H2}) (h^{-1}) was calculated by mass balances according to Bassani et al. (2016) and Alfaro et al. (2018) H_2 rate converted to methane (L $L_{reactor}^{-1}$ d^{-1}) was calculated according to Eq. (3):

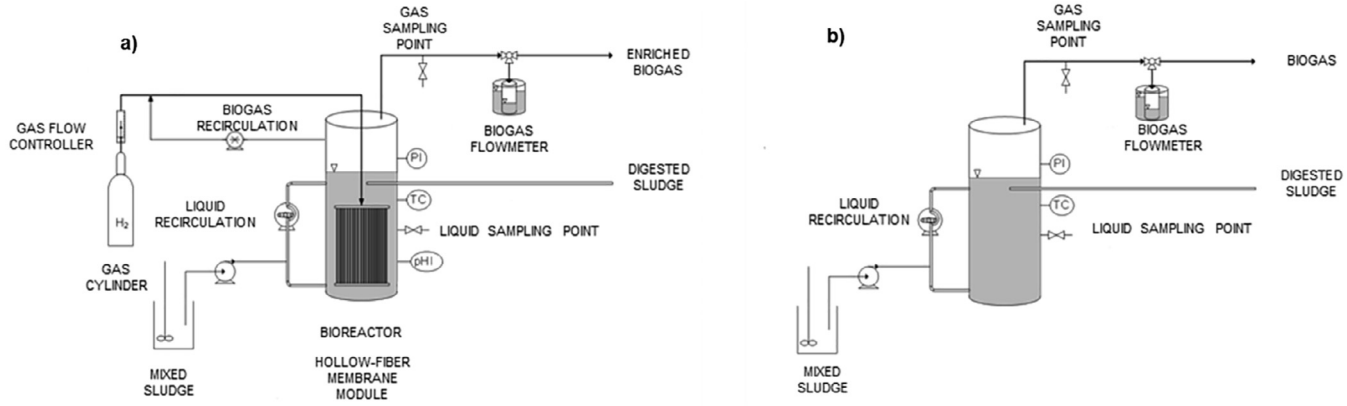

Fig. 1. Diagram of the pilot plants. a) Upgrading reactor (R1); b) Control reactor (R2).

Table 1
Operating conditions and characteristics of sewage sludge utilized during the experiment.

	Set-up	Stage 1	Stage 2	Stage 3
Operating conditions				
t (d)	0	62	120	184
H_2 flow rate ($L\,L_{reactor}^{-1}\,d^{-1}$)	0	0.87	0.87	0.87
Gas recirculation rate ($L\,L_{reactor}^{-1}\,d^{-1}$)	0	50	101	202
HRT (d)	20	20	20	20
OLR ($g\,VS\,L^{-1}\,d^{-1}$)	1.3 ± 0.1	1.3 ± 0.2	1.5 ± 0.2	1.8 ± 0.5
Raw sludge composition				
TS ($g\,kg^{-1}$)	31.7 ± 0.5	42.5 ± 1.7	38.3 ± 8.8	58.8 ± 2.3
VS/TS ratio	0.78 ± 0.13	0.75 ± 0.13	0.69 ± 0.11	0.69 ± 0.12
Acetate ($mg\,L^{-1}$)	350 ± 38	291 ± 42	489 ± 49	555 ± 48
NH_4^+-N ($mg\,L^{-1}$)	102 ± 13	108 ± 16	125 ± 10	166 ± 36

$$H_2\ rate\ to\ biomethane = 4(CH_4\ in\ output\ gas R1 - CH_4\ in\ output\ gas\ R2) \quad (3)$$

where 4 is the stoichiometric coefficient according to stoichiometric equation, CH_4 in output gas R1 ($L\,L_{reactor}^{-1}\,d^{-1}$) is the rate of CH_4 produced in R1 and CH_4 in output gas R2 ($L\,L_{reactor}^{-1}\,d^{-1}$) is the rate of CH_4 produced in R2.

2.5. Microbial analysis

Liquid samples from R1, R2 and inoculum, were frozen at −20 °C in order to evaluate the evolution of the microbial population during the experiment in both reactors. DNA isolation, PCR, DGGE analysis were performed according to Alfaro et al. (2018). The V6-V8 regions of the bacterial 16S rRNA and the V2-V3 regions of the archaeal 16S rRNA were amplified for DGGE employing the universal primers previously reported in Rodríguez et al. (2012). DGGE profiles were compared using the GelCompar IITM software (Applied Maths BVBA, Sint-Martens-Latem, Belgium) and similarity indices were calculated as in Lebrero et al. (2013). The taxonomic position of the sequenced DGGE bands was obtained using the RDP classifier tool (50% confidence level) (Wang et al., 2007). The closest cultured and uncultured relatives to each band were obtained using the BLAST search tool at the NCBI (McGinnis and Madden, 2004). Sequences were deposited in GenBank Data Library under accession numbers MG383910- MG383931 (*archaea*) and MG664852- MG664869 (bacteria).

3. Results and discussion

3.1. Efficiency of H_2 and CO_2 conversion

During the set-up period, both reactors showed analogous biogas production rates (Fig. 2). The average concentration of CH_4 in the biogas from R1 and R2 was around 66% (Table 2), which agrees with typical CH_4 concentration in biogas from sludge digestion as reported in literature (Metcalf & Eddy et al., 2002). Therefore, R2 was validated as control reactor to establish comparisons. After the set-up period (on day 62), the first stage started injecting $0.87\,L\,L_{reactor}^{-1}\,d^{-1}$ of H_2 into R1. In addition, a gas recirculation rate of $50\,L\,L_{reactor}^{-1}\,d^{-1}$ was applied in R1. The conversion took place at a low rate at the beginning of stage 1; the efficiency of H_2 utilization (η_{H2}) and the H_2 flow rate converted to methane showed an increasing trend during this period (Fig. 3). On average, only 55% of the H_2 supplied was consumed (Table 2). CH_4 production rate experienced an increase of 23% in R1 in comparison with R2 (Fig. 2, Table 2) and MER reached $0.10\,L\,L_{reactor}^{-1}\,d^{-1}$. CO_2 flow rate in output gas in R1 was 43% lower than in R2 because of the reaction of H_2 with the in-situ produced CO_2 (Table 2). Gas recirculation rate was increased to $101\,L\,L_{reactor}^{-1}\,d^{-1}$ on day 120, marking the beginning of stage 2 of the experiment. Consequently, a significant improvement of the H_2 mass transfer in R1 was observed. 87% of the H_2 injected was transferred (Fig. 3 and Table 2) thus reducing the unused concentration of H_2 in upgrading gas while CH_4 concentration rose to 71%. In R1, CH_4 production rate experienced an increase of 47% in comparison to R2 while CO_2 flow rate in output gas was 47% lower. MER also reached a larger value ($0.15\,L\,L_{reactor}^{-1}\,d^{-1}$) than in stage 1. On day 181, gas recirculation rate was doubled to $202\,L\,L_{reactor}^{-1}\,d^{-1}$ with the purpose of raising η_{H2} (stage 3). In this stage, η_{H2} increased to an average of 94%, thus showing larger H_2 utilization compared to stage 2 and 1 (Fig. 3 and Table 2). Therefore, this stage showed an important improvement as almost all H_2 was transferred. CH_4 concentration increased to 73% and H_2 content dropped to 7%. However, the CO_2 content increased (Table 2), probably because of the higher OLR applied during stage 3. CH_4 production rate of R1 was on average 42% higher compared to R2 during the same period. From another point of view, CH_4 production rate was similar in stages 1 and 3 for R1 ($0.54\,L\,L_{reactor}^{-1}\,d^{-1}$) in spite of the larger H_2 transfer rate observed during stage 3. This could be attributed to a lower CH_4 production from organic matter removal in stage 3 in comparison to stage 1. In fact, R2, without H_2 addition, produced $0.38\,L\,L_{reactor}^{-1}\,d^{-1}$ during stage 3 versus $0.44\,L\,L_{reactor}^{-1}\,d^{-1}$ in stage 1. Conversely, the contribution of the added H_2 to the observed CH4 production rate was significantly higher in stage 3 ($0.16\,L\,L_{reactor}^{-1}\,d^{-1}$) than in the first stage ($0.10\,L\,L_{reactor}^{-1}\,d^{-1}$).

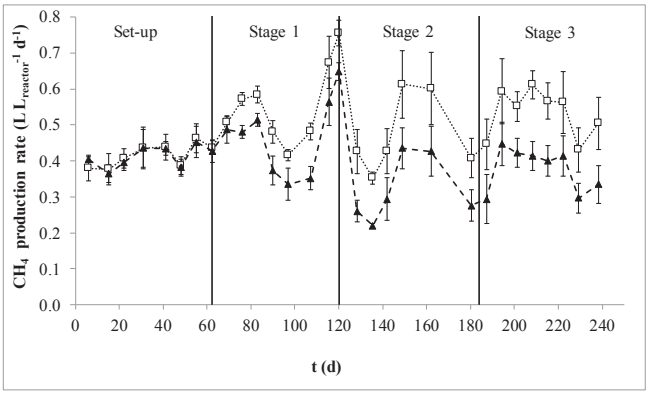

Fig. 2. Methane production rates in R1 (□) and R2 (▲) during the experiment.

H_2 converted to CH_4 can be calculated from a mass balance to H_2 according to Eq. (3) (Fig. 4). The rate of converting H_2 to methane showed an increasing trend over the experiment, from an average H_2 conversion to methane of 46% during stage 1, to 72 and 76% in stages 2 and 3, respectively. This fact emphasizes the positive correlation between gas recirculation rate and the conversion of H_2 and CO_2 into CH_4. The portion H_2 employed for microbial growth was estimated as the gap between H_2 supply rate (input) and the sum of H_2 rate and CH_4 as equivalent H_2 in the biogas (output). The portion of H_2 dedicated to microbial growth represented approximately 9, 14 and 18% of total H_2 supply and 16, 16 and 19% of transferred H_2 in stages 1, 2 and 3,

Table 2
Upgrading (R1) and control (R2) reactor performances.

	Set- up		Stage 1		Stage 2		Stage 3	
	R1	R2	R1	R2	R1	R2	R1	R2
Biogas Production rate ($L\,L_{reactor}^{-1}\,d^{-1}$)	0.64 ± 0.08	0.63 ± 0.08	1.06 ± 0.18	0.67 ± 0.23	0.67 ± 0.22	0.47 ± 0.16	0.74 ± 0.16	0.56 ± 0.13
Biogas Composition (%)								
H_2	/	/	36.5 ± 7.1	/	17.7 ± 3.9	/	7.2 ± 2.4	/
CO_2	34.4 ± 1.4	34.4 ± 1.0	12.4 ± 1.9	34.1 ± 1.0	11.4 ± 4.8	32.0 ± 1.3	19.7 ± 3.0	32.9 ± 1.1
CH_4	65.6 ± 1.4	66.0 ± 1.0	51.1 ± 6.5	65.8 ± 1.0	70.9 ± 3.6	68.0 ± 1.3	73.1 ± 3.4	67.1 ± 1.1
CH_4 production rate ($L\,L_{reactor}^{-1}\,d^{-1}$)	0.42 ± 0.05	0.41 ± 0.05	0.54 ± 0.10	0.44 ± 0.10	0.47 ± 0.14	0.32 ± 0.11	0.54 ± 0.11	0.38 ± 0.08
CO_2 in output gas ($L\,L_{reactor}^{-1}\,d^{-1}$)	0.22 ± 0.03	0.22 ± 0.03	0.13 ± 0.06	0.23 ± 0.05	0.08 ± 0.08	0.15 ± 0.05	0.15 ± 0.04	0.18 ± 0.04
η_{H2} (%)	/	/	54.6 ± 9.4	/	86.2 ± 3.0	/	93.9 ± 2.9	/
H_2 transfer rate ($L\,L_{reactor}^{-1}\,d^{-1}$)	/	/	0.48 ± 0.09	/	0.75 ± 0.03	/	0.82 ± 0.03	/
k_La_{H2} (h^{-1})	/	/	2.7 ± 0.8	/	8.9 ± 1.9	/	24.9 ± 6.8	/
Acetate ($mg\,L^{-1}$)	35.6 ± 30.9	35.8 ± 42.0	45.0 ± 28.8	25.5 ± 15.2	25.3 ± 13.2	25.2 ± 22.2	31.1 ± 14.8	12.5 ± 11.4
pH	7.23 ± 0.12	7.45 ± 0.18	7.28 ± 0.14	7.41 ± 0.10	7.80 ± 0.23	7.42 ± 0.23	8.09 ± 0.23	7.41 ± 0.27
TS ($g\,kg^{-1}$)	21.5 ± 1.0	21.4 ± 1.5	21.1 ± 1.0	20.6 ± 1.8	22.3 ± 2.0	23.0 ± 3.5	27.6 ± 5.5	27.8 ± 3.9
VS/TS ratio	0.66 ± 0.05	0.66 ± 0.07	0.63 ± 0.06	0.63 ± 0.08	0.59 ± 0.07	0.60 ± 0.08	0.56 ± 0.05	0.57 ± 0.06
VS removal (%)	47.1 ± 4.9	47.4 ± 6.4	48.4 ± 7.7	49.4 ± 9.2	48.5 ± 15.0	48.5 ± 13.3	55.8 ± 9.3	55.7 ± 10.6
NH_4^+-N ($mg\,L^{-1}$)	729 ± 106	780 ± 172	670 ± 117	692 ± 83	721 ± 99	702 ± 102	794 ± 105	756 ± 123

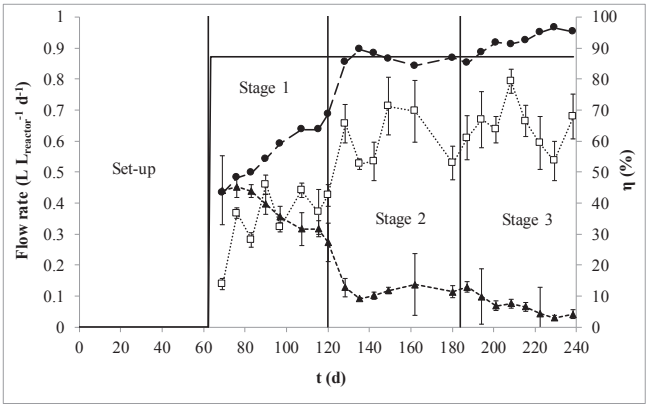

Fig. 3. Gas flow rates and efficiency of H_2 utlization in R1 throughout the experiment. H_2 supplied rate (—), H_2 rate in biogas (▲), CH_4 as H_2 equivalent rate in biogas (□), H_2 conversion efficiency (●).

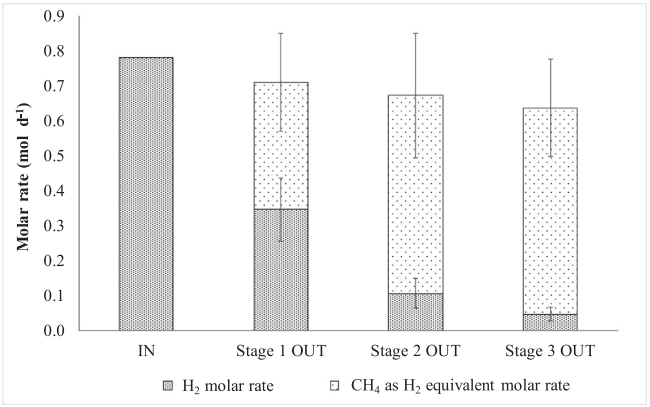

Fig. 4. Balance of H_2.

respectively. These values are within the range of previous studies performed in ex-situ bioreactors with similar configurations (Alfaro et al., 2018). In contrast, ex-situ studies reached an asymptote of around 8–10% (of transferred H_2) after long time operation at low dilution rates (Díaz et al., 2015), while microbial growth seemed to remain stable or slightly increase in this study. This can be the consequence of continuous biomass washout, as the amount of substrate used for growth has been reported to be larger at the beginning of the experiments, where the consumers of H_2 are in small proportion (Díaz et al., 2015). The amount of acetate as H_2 equivalent can be neglected because of the low acetate concentrations observed during the study (Table 2).

In brief, the application of increasing gas recirculation rates successfully increased MER, the efficiency of H_2 utilization and the concentration of CH_4 in upgraded biogas up to $0.16\,L\,L_{reactor}^{-1}\,d^{-1}$, 94% and 73% respectively. Gas recirculation is frequently applied to mix anaerobic digesters of sludge and in-situ upgrading could benefit from that to avoid high-speed stirring to achieve efficient H_2 transfer and biogas upgrading (Agneessens et al., 2017, 2018). However, gas recirculation rates typically used for mixing in full-scale digesters are around 7.2 and $10\,L\,L_{reactor}^{-1}\,d^{-1}$ (Appels et al., 2008), 5 to 20 times lower than those applied in this study. In this regard, the energy required for gas recirculation has been identified as the largest component in energy expenses for ex-situ upgrading in membrane bioreactors (Alfaro et al., 2018), then, the viability of the system in economic terms could be compromised.

From another point of view, the CH_4 production rate achieved in stage 3 was like that found in Luo and Angelidaki (2013a) under similar operating conditions but with a 24 times lower membrane area to reactor volume ratio in this study, showing an alternative to biofilm formation over the membrane and pressure drop reported. On the contrary, H_2 diffusion achieved a larger concentration of CH_4 in the upgraded biogas (up to 96.1%) (Luo and Angelidaki, 2013a).

Gas recirculation rate increased the transfer of H_2 to the liquid, thus improving the efficiency of the upgrading. However; CH_4 content in the output gas was not higher than 73%. This is the result of the excess of H_2 feed flow rate supplied in stages 2 and 3 (as it was maintained constant during the whole experiment at $0.87\,L\,L_{reactor}^{-1}\,d^{-1}$), leading to extra fed H_2 which could not couple with the real total amount of CO_2 produced in-situ during that stages. Thus, unutilized H_2 went out from the process producing a dilution effect on final CH_4 content in the produced biogas. Further studies should be conducted with regulated H_2 supply rates to fit the variable CO_2 production because of seasonal changes in OLR in order to maximize the CH_4 concentration.

3.2. MBR mass transfer capacity

k_La_{H2} value showed an increasing trend during the experiment, in accordance with the positive correlation observed with gas recirculation rate. The average k_La_{H2} values (h^{-1}) observed in the upgrading reactor are shown in Table 2. Literature on in-situ biogas upgrading reactors shows scarce k_La_{H2} values; 6.6 and $11.8\,h^{-1}$ with a column diffuser and $16.0\,h^{-1}$ with a ceramic diffuser (Luo and Angelidaki,

2013b). In the present study, k_La_{H2} values are several orders of magnitude lower than reported in ex-situ experiments with a similar configuration (Alfaro et al., 2018). Despite everything, in-situ upgrading digesters with high HRT do not require specific mass transfer coefficients as high as the ex-situ process because of the lower specific CO_2 rates ($L\,L_{reactor}^{-1}\,d^{-1}$) to convert. In fact, the H_2 concentration of 7.2% with a k_La_{H2} value of 25 h^{-1} (stage 3) agrees with a modification of ADM1, to account for H_2 injection, that showed that k_La_{H2} values around 21 h^{-1} should be achieved in in-situ digesters to reduce H_2 concentration to below 5% and around 35 h^{-1} to meet gas grid injection requirements (Bensmann et al., 2014).

3.3. Anaerobic digestion performance

3.3.1. VFA evolution

During the set-up period, the two reactors showed similarly low VFA content with acetate concentration of 36 mg L^{-1} (Table 2), which agrees with literature of biogas production from sewage sludge (Metcalf & Eddy et al., 2002). During the first HRT of stage 1, acetate concentration increased from 15 mg L^{-1} to 95 mg L^{-1} to decrease suddenly afterwards. This finding agrees with the statement of (Agneessens et al., 2018) about the likelihood of acetate accumulation during the initial phase of a continuous in-situ biogas upgrading reactor with the later stabilisation after 1 HRT.

During the rest of the experiment, VFA accumulation was not observed (Table 2). In contrast, acetate accumulation was reported in some studies (Agneessens et al., 2018, 2017; Luo and Angelidaki, 2013a; Mulat et al., 2017). Agneessens et al. (2018, 2017) showed that acetate accumulation was more probable during high H_2 (1.3–1.7 $L\,L_{reactor}^{-1}\,d^{-1}$), low CO_2 (< 7%) and high pH (> 8.33) levels as H_2 was introduced in the headspace of the reactors in intermittent pulses. In these experiments, homoacetogenesis was stimulated by those conditions of H_2, CO_2 and pH, decreasing the activity of acetoclastic methanogens contributing to acetate accumulation and outcompeting methane production from H_2 and CO_2 by hydrogenotrophic methanogens. High acetate accumulation (2070 mg L^{-1}) was also observed in Luo and Angelidaki (2013a) with a H_2 flow rate of 1.76 $L\,L_{reactor}^{-1}\,d^{-1}$, high pH (8.31) and low CO_2 content in the output gas (4%). This accumulation was in accordance with the parameters affecting acetate concentrations during in-situ biogas upgrading described by Agneessens et al. (2018). The present study was carried out with continuous H_2 injection (instead of sporadic pulses and lower H_2 load) in which CO_2 content ≥11% and pH reached lower values (≤8.1), thus avoiding the possible stimulation of homoacetogens, being outcompeted by hydrogenotrophic methanogens. Agneessens et al. (2018) reported as well that more frequent H_2 injection rate reduces the possibility of acetate accumulation which can be linked to the lack of VFA accumulation obtained in the present experiment. Contrary to previous studies (Liu and Whitman, 2008; Speece, 2008), there was no accumulation of propionic acid despite the elevated H_2 partial pressure. Thus, the injection of H_2 through the hollow-fibre membrane module did not inhibit propionate degradation as in (Luo and Angelidaki, 2013a).

3.3.2. OLR

OLR is a critical parameter for anaerobic digestion reactor performance and it was recently shown to be an important parameter for in-situ biomethanation (Agneessens et al., 2017, 2018). OLR had a slightly increasing trend during the experiment, ranging from 1.3 to 1.8 g VS $L^{-1}\,d^{-1}$ (Table 1). Increasing OLR (0.5–2 g VS $L^{-1}\,d^{-1}$) has shown to stimulate acetate accumulation by homoacetogenesis, an incapability of acetoclastic methanogenesis to consume acetate or both (Agneessens et al., 2018). Additionally, the abundance of homoacetogenic species augmented when the OLR was increased (Ju et al., 2017; Li et al., 2016, 2015). At a large OLR, acidogenesis was faster than methanogenesis, which can contribute to acetate accumulation (Goux et al., 2015). In the study of Agneessens et al. (2018), performed with pulse H_2 injections to the reactors, it was observed the influence of the increasing OLR and uneven distribution of H_2 in favour of homoacetogens and acetate generation. In addition, hydrogenotrophic methanogens, instead of homoacetogens, were benefited from repeated H_2 injections with an OLR of 2 g VS $L^{-1}\,d^{-1}$ (Agneessens et al., 2018).

Acetate accumulation was not observed in the present study, presumably because of homogeneous H_2 distribution in the sludge obtained with the hollow-fibre membrane module with continuous H_2 injection, indicating hydrogenotrophic methanogens outcompeted homoacetogens. Under the studied conditions, the increasing OLR had no effect on the biomethanation process. Conversely, in a previous experiment (Luo and Angelidaki, 2013a), at equivalent H_2 flow rates and similar OLR using a hollow-fibre membrane as H_2 diffusion system, acetate accumulation was observed. This may be explained as gas recirculation was not applied in the reactor thus, H_2 distribution was less homogeneous than in the present study leading to an important homoacetogen activity. Thus, gas recirculation rate seems to have a positive effect on the in-situ biomethanation when OLR is increasing.

3.3.3. pH, NH_4^+ and solids removal

One of the main technical challenges of in-situ biogas upgrading technology is pH increase over 8.5, thus inhibiting methanogenesis (Angelidaki et al., 2018; Weiland, 2010). During the set-up period, the two reactors showed similar pH values (~7.4) which are in accordance with literature reported on biogas production from sewage sludge (Metcalf & Eddy et al., 2002). The pH for R2 remained relatively unchanged throughout the experiment, while a gradual pH increase to 8.1 was recorded in R1 (Table 2). No inhibition was observed as previously reported in Agneessens et al. (2017, 2018) at pH 8.3. Then, the direct H_2 addition to the anaerobic reactor had no effect on methanogenesis performance. In contrast, previous experiments on in-situ biogas upgrading reactors (Luo et al., 2012; Luo and Angelidaki, 2013a,b) showed slight inhibition when pH was more than 8.3.

During the experiment, the two reactors showed similar NH_4^+ concentrations (Table 2), in harmony with literature of biogas production from sewage sludge (Metcalf & Eddy et al., 2002; Speece, 2008). Thus, the H_2 addition to the anaerobic digestion of sewage sludge had no effect on NH_4^+ levels.

TS concentration, VS/TS ratio and %VS removal in feeding raw sludge, R1 and R2 are reported in Table 2. The two reactors showed similar performance of organic matter removal (Table 2) during the set-up period. This similarity of solids removal yield was maintained during the whole experiment regardless the injection of H_2 and the increase in the gas recirculation rate in R1. According to the results shown in Table 2, the removal of VS was not affected by the introduction of H_2 in any stage in R1 considering its high similarity with the VS removal results obtained in R2 with no significant differences. In addition, all these solids removal yields were inside the normal range for the digestion of sewage sludge (Metcalf & Eddy et al., 2002; Speece, 2008).

3.4. Microbial community

From the archaeal DGGE gel (Fig. 5a), 22 bands were sequenced. They belonged to the *Euryarchaeota* and *Pacearchaeota* phyla. In the case of *Euryarchaeota* phyla, the bands were ascribed to two classes, almost all to *Methanomicrobia* (band 1–15) and only one band to *Methanobacteria* (band 16). The *Pacearchaeota* phyla was found in bands 17–22. The BLAST search tool provided consistent results with those given by the RDP classifier. Five families were present in which *Methanotrix, Methanospirillum, Methanoculleus* and *Methanolinea* were the four genus assigned to *Methanomicrobia* class and *Methanobacterium* genus to *Methanobacteria* class. The *Pacearchaeota* phyla ascribed the genus *Pacearchaeota Incertae Sedis AR13*. During the experiment, some *archaea* disappeared corresponding only with the *Euryarchaeota* phyla. On the one hand, they disappeared completely in R1 (band 1, 3, 4, 5, 6

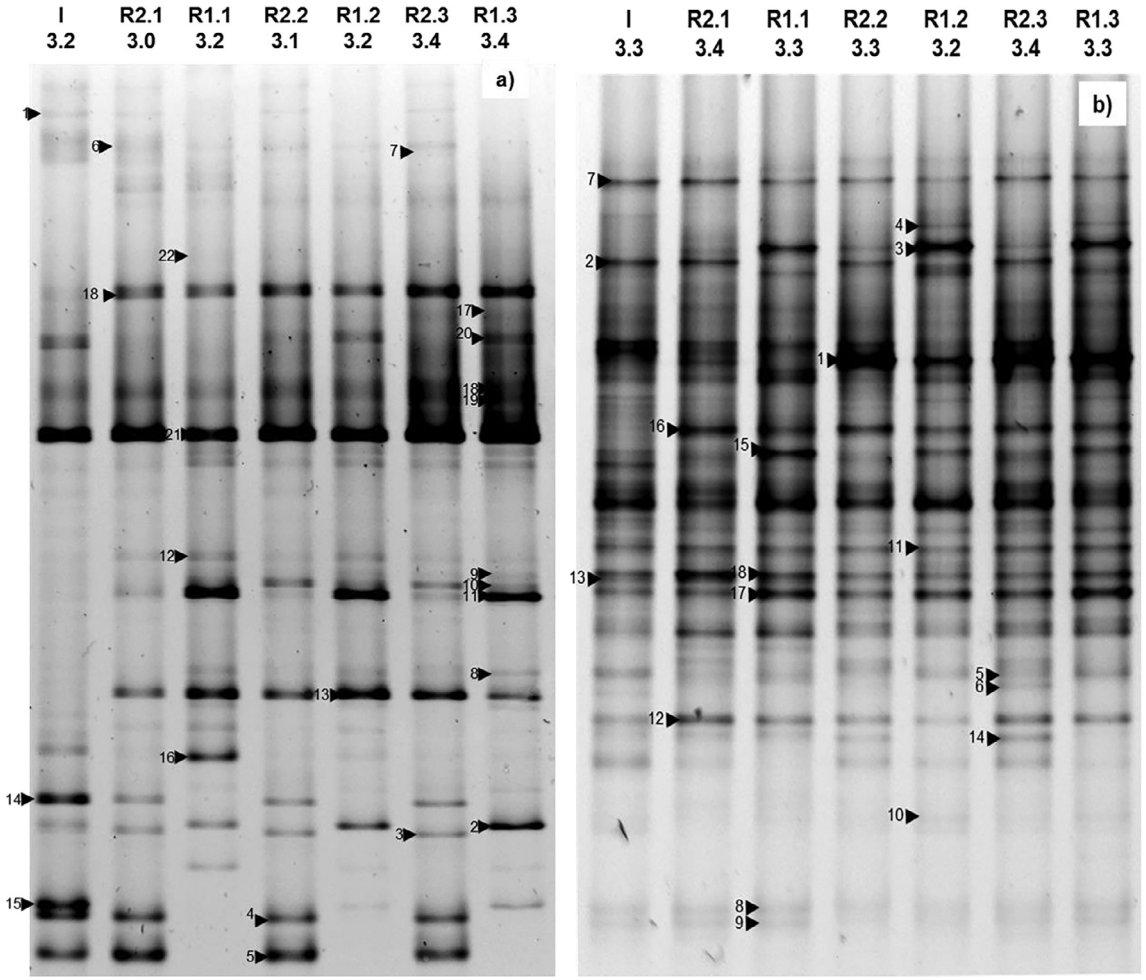

Fig. 5. a) Archaeal DGGE profiles and b) Bacterial DGGE profiles of the 16S rRNA amplicons of the samples with their respective diversity indices. Samples: Inoculum (I), upgrading reactor (R1) and control reactor (R2) in the three stages (1–3) of the experiment.

and 14) although they were present in the inoculum and R2. On the other hand, the missing *archaea* in R2 (band 2, 8 and 16) were present in R1 with an increasing trend. This trend might be the result of favourable environmental conditions for those species when larger H_2 transfer rates were observed at larger gas recirculation rates. A new *archaeon* appeared after the injection of H_2 in R1 and it was present since then (band 9), corresponding with an uncultured specie (*Methamicrobiales* CU916161.1 or archaeon KJ402292.1) with an identity of 99%. *Methanobacterium* sp. was an *archaeon* present in inoculum and R1 during the experiment but not in R2. Therefore, it was revealed the selection-effect of H_2 on archaeal community composition over time. Although *Methanoculleus sp*. was no present initially in the inoculum, it was highly present in R1 and R2 during the experiment. This fact could be explained as it was not detected in the inoculum sample due to its low abundance or it could be introduced with the feed sludge (not analysed). With high values of abundance, both reactors had some common *archaea* (band 10, 11 and 12) belonging to *Euryarchaeota* phyla. *Pacearchaeota* phyla population was maintained during the experiment in R1 and R2 in terms of high abundance. According to the genus obtained in the archaeal DGGE analysis, some hydrogenotrophic *archaea* were present over the experiment in R1 as *Methanoculleus* sp., *Methanospirillum* sp., *Methanolinea* sp. and *Methanobacterium* sp. with *Pacearchaeota Incertae Sedis AR13* as a potential hydrogenotrophic methanogen as well. R1 had the possible presence of an acetoclastic methanogen, *Methanotrix* sp. present in R2 too. From the bacterial DGGE gel (Fig. 5b) and according to the RDP classifier, 18 bands belonging to seven different phyla were sequenced: *Proteobacteria* (band 1–4), *Firmicutes* (band 5–7), *Verrucomicrobia* (band 8–9), *Lentisphaerae* (band 10–11), *Actinobacteria* (band 12–13), *Acidobacteria* (band 14) and *Cloacimonetes* (band 15) while three bands remained unclassified (band 16–18). In general, the BLAST search tool provided consistent results with those given by the RDP classifier. No homoacetogens were found in R1, which links with the previous discussion of VFA and OLR results. Homoacetogens were potentially outcompeted by hydrogenotrophic methanogens because of the reactor configuration and operation (homogeneous H_2 distribution by hollow-fibre membrane module, use of gas recirculation rate, continuous H_2 injection, obtained pH and CO_2 levels).

High *archaea* richness and evenness was found with Shannon-Wiener diversity index (between 3.2 and 3.4), close to the upper range value of 3.5 (MacDonald, 2003), in R1, with the maximum value in the last stage of the experiment with the highest biogas recirculation rate (Fig. 5a). For *archaea*, H index of R1 was similar, but always slightly higher than the diversity index obtained in the inoculum and in R2 during the experiment. The diversity indices for bacteria were between 3.2 and 3.3 in R1, showing a high bacterial richness and evenness (Fig. 5b). For bacteria, the diversity index of R1 was similar but always slightly lower to the H index obtained in the inoculum and in R2 during the experiment.

Regarding the similarity between samples, those from the control reactor showed high similarity indices of bacteria during the experiment (79.8–92.0) in comparison to the inoculum, with the same being observed in the upgrading reactor (73.8–76.7). The samples from the control reactor (R2) presented high similarity indices of *archaea* in

comparison with the inoculum (values between 78.3 and 87.8) as expected due to the same operating conditions in both cases. During the different stages of the experiment in R2 the similarity indices were high and not so different (92.4–96.2), indicating the maintenance of *archaea* population in the reactor over the time. In the upgrading reactor, similar high similarity indices were found between stage 1 and 2 (83.6) and stage 2 and 3 (81.5) although the biogas recirculation rate was increased, in contrast to stage 1 and 3 for which the similarity index was significantly lower (51.4). This evidence revealed a gradual change in archaeal population over the time as gas recirculation increased, instead of an abrupt change from stage to stage. Comparing stage-by-stage similarity index of *archaea* found in R1 and R2 samples, it can be observed not only the highest difference in stage 1 as a result of the injection of H_2 in the upgrading reactor (similarity value of 57.9) but also an increasing trend in stages 2 (82.2) and 3 (93.5). This increasing *archaea* similarity trend between R1 and R2 and the decreasing similarity values in R1 cannot link with the increasing H_2 utilization efficiency obtained during the operation of the reactors described previously. These values might be explained by the appearance of a biofilm (not analysed), around the hollow-fibre membrane module, which was likely to be created after stage 1, where some hydrogenotrophic *archaea* population potentially could be accumulated near the H_2 source and were responsible for the high process bioconversion. Kougias et al. (2016) reported the dominance of a hydrogenotrophic *archaea* in the biofilm formed on top of the H_2 diffuser surface for an ex-situ biogas upgrading experiment. However, the biofilm formed on the hollow-fibre membrane module employed by Luo and Angelidaki (2013a) increased the resistance of H_2 diffusion to the liquid. In their study, it was also informed that less than 25% of H_2 conversion took place in the biofilm, while the rest was consumed in the bulk liquid phase. Thus, to ensure the contribution of the biofilm to H_2 consumption and CH_4 production, further research on in-situ biogas upgrading membrane bioreactors should focus on the biofilm microbial analysis.

4. Conclusions

In-situ biological biogas upgrading by coupling CO_2 with external H_2 was feasible in a mesophilic anaerobic digester of sludge. CH_4 production rate ($0.54\ L\ L_{reactor}^{-1}\ d^{-1}$) was 42% larger than the conventional digestion. Organic matter removal in the upgrading reactor was not compromised by H_2 supply or by the high pH level (8.1), indicating adaptation of microbial population. Increasing gas recirculation rates improved the H_2 gas-liquid mass transfer significantly and seemed to have a positive effect on biomethanation when OLR increased. VFA accumulation was not observed and hydrogenotrophic methanogens outcompeted homoacetogens.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2019.01.135.

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