Technology validation of photosynthetic biogas upgrading in a semi-industrial scale algal-bacterial photobioreactor

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ABSTRACT

In this work, the performance of photosynthetic biogas upgrading coupled to wastewater treatment was evaluated in an outdoors high rate algal pond (HRAP) interconnected to an absorption column at semi-industrial scale. The influence of biogas flowrate (274, 370 and 459 L h^{-1}), liquid to biogas ratio (L/G = 1.2, 2.1 and 3.5), type of wastewater (domestic *versus* centrate) and hydraulic retention time in the HRAP (HRT) on the quality of the biomethane produced was assessed. The highest CO_2 and H_2S removal efficiencies (REs) were recorded at the largest L/G due to the higher biogas-liquid mass transfer at increasing liquid flowrates. No significant influence of the biogas flowrate on process performance was observed, while the type of wastewater was identified as a key operational parameter. CO_2 and H_2S-REs of 99% and 100% at a L/G_{max}=3.5 were recorded using centrate. The maximum CH_4 content in the biomethane (90%) was limited by N_2 and O_2 desorption.

Keywords: algal-bacterial photobioreactor; biogas upgrading; microalgae; semi-industrial scale HRAP; wastewater treatment.

1. Introduction

Biogas from the anaerobic digestion of organic waste, such as sludge from wastewater treatment plants (WWTPs), constitutes a valuable bioenergy vector able to reduce our current dependence on fossil fuels. Biogas from WWTPs is typically composed of CH_4 (60-75%), CO_2 (30-40%) and other pollutants at trace level concentrations such as H_2S (0.02-2%), O_2 (0-1%), N_2 (0-2%), NH_3 (<1%) and siloxanes (0-0.2%) (Ryckebosch et al., 2011). The high concentration of CO_2 increases hydrocarbon and carbon monoxide emissions during biogas combustion, reduces its specific calorific value and increases its transportation cost. On the other hand, H_2S is a malodorous and toxic gas contaminant that generates corrosion and mechanical wear in pipelines and internal combustion engines (Lebrero et al., 2016).

Several technologies are nowadays commercially available to remove these contaminants from biogas in order to generate a high quality biomethane similar to natural gas. Physical-chemical technologies for CO_2 separation such as pressure swing adsorption, membrane separation and water/organic/chemical scrubbing often need a previous H_2S cleaning step (i.e. adsorption on activated carbon or metal ions-based *in situ* precipitation) and a high energy input (0.2-0.7 kWh/m^3_{biogas}), with the associated increase in operational costs. Thus, the high energy and chemical requirements of conventional biogas upgrading processes, among other factors such as the cost of acquisition of the organic matter and the type of process, limit the cost-effective use of biomethane as a renewable substitute of natural gas (Rodero et al., 2018a). On the other hand, biological technologies such as biofiltration or *in situ* microaerobic anaerobic digestion for H_2S removal followed by hydrogenotrophic biogas upgrading (*power to gas*) for CO_2 bioconversion into CH_4 entail the need of a two-stage process and can be only applied in locations with a sustained surplus of renewable electricity (Angelidaki et al., 2018; Muñoz et al., 2015a).

In this context, biogas upgrading using algal-bacterial processes has emerged as a cost-competitive and environmentally friendly platform capable of removing CO_2 and H_2S in a single step process (Bahr et al., 2014). Photosynthetic biogas upgrading is based on the concomitant CO_2 fixation by microalgae using solar energy and oxidation of H_2S to S^0/SO_4^{2-} by sulfur-oxidizing bacteria using the oxygen photosynthetically produced (Sun et al., 2016). Moreover, this biotechnology simultaneously supports wastewater treatment since residual nutrients can sustain algal-bacterial growth, which contributes to improve its environmental and economic sustainability (Posadas et al., 2015a; Zhang et al., 2017). Biogas upgrading combined with wastewater treatment in algal-bacterial photobioreactors has been successfully validated indoors at lab-pilot scale (Bahr et al., 2014; Meier et al., 2017; Ouyang et al., 2015; Posadas et al., 2016; Rodero et al., 2018b; Serejo et al., 2015; Toledo-Cervantes et al., 2017a, 2016; Yan et al., 2016). Likewise, promising results in terms of biogas upgrading (CH_4 contents of 85.2-97.9%) and centrate treatment (total nitrogen removal efficiencies (REs) of 80-87% and P-PO_4^{3-} REs of 85-92%) were obtained in an outdoors 180 L high rate algal pond (HRAP) interconnected to an absorption column (Marín et al., 2018; Posadas et al., 2017a). However, this innovative biogas upgrading technology has not been yet validated at semi-industrial scale, which is a must in order to foster its acceptance by the industrial sector.

This work investigated for the first time the influence of biogas flow rate and the liquid to biogas ratio (L/G) on biomethane quality in an outdoors algal-bacterial photobioreactor treating real biogas at semi-industrial scale. Moreover, the influence of the type of wastewater (domestic *versus* centrate) and the hydraulic retention time (HRT) in the HRAP on biogas upgrading and nutrient recovery efficiency was also assessed.

2. Materials and methods

2.1. Biogas and wastewaters

Biogas was produced in a semi-industrial 20 m^3 anaerobic digester treating sewage sludge at Chiclana de la Frontera WWTP (Spain). Biogas composition averaged 69.2±5.7% CH_4, 32.7±2.8% CO_2 and 1183±1006 ppm H_2S. Fresh domestic wastewater was pumped into the HRAP directly after screening and degreasing of the influent raw wastewater. The average composition of the domestic wastewater was (mg L^{-1}): chemical oxygen demand (COD) = 496±145, inorganic carbon (IC) = 46±11, total nitrogen (TN) = 41±11, ammonium ($N\text{-}NH_4^+$) = 44±9, phosphate ($P\text{-}PO_4^{3-}$) = 6±2 and total suspended solids (TSS) = 140±40. Urea, H_3PO_4, $NaHCO_3$ and Na_2CO_3 were added to the raw domestic wastewater to achieve a final IC, TN and $P\text{-}PO_4^{3-}$ concentration of 500, 500 and 75 mg L^{-1}, respectively, in order to simulate a medium-strength centrate composition.

2.2. Experimental set-up

The experimental set-up was located outdoors at Chiclana de la Frontera WWTP (36.42 N; 6.15 W) (Spain). The set-up consisted of a 9.6 m^3 HRAP made of concrete blocks with an illuminated surface of 32 m^2, 0.3 m of depth, two water channels divided by a central wall and two flow rectifiers in each side of the curvature. The cultivation broth in the HRAP was continuously agitated by a 6-blade paddlewheel operated at 7 rpm, resulting in an internal liquid velocity of 0.30 m s^{-1}. The HRAP was interconnected to a 150 L absorption column provided with a polypropylene fine bubble biogas diffuser (Ecotec AFD 270) via an external liquid recirculation of the supernatant from a 7 m^3 conical settler

(Figure 1). The algal-bacterial biomass accumulated at the bottom of the settler was continuously recirculated to the HRAP to avoid an excessive biomass accumulation in the settler. The algal-bacterial biomass was wasted from an overflow located in the HRAP in order to maintain the depth of the photobioreactor at 0.3 m.

<Figure 1>

2.3. Operational conditions and sampling procedures

The HRAP was inoculated with a consortium of cyanobacteria/microalgae and bacteria from an outdoors HRAP treating domestic wastewater at Chiclana de la Frontera WWTP prior to the experiment start-up. Three different operational conditions were tested to assess the influence of the HRT and the type of wastewater used as a nutrient source (domestic wastewater *vs* centrate) in the HRAP on biogas upgrading efficiency. During stages I and II, the HRAP was fed with domestic wastewater at a HRT of 3.5 and 8 days, respectively, which correspond to typical values used during wastewater treatment in HRAPs (Arbib et al., 2013; Posadas et al., 2015b). In stage III, simulated centrate was used as a nutrient source at a high HRT (≈73 days) in order to avoid inhibition of microalgae growth by its high NH_4^+ concentration. The high nutrient content of centrate entailed lower wastewater flowrates to satisfy nutrient requirements. L/G ratios of 1.2 and 2.1 were tested under counter-current flow operation at different biogas flowrates (274±12, 370±7 and 459±36 L h^{-1}) under steady state in the three operational stages. Moreover, a L/G ratio of 3.5 was tested only at the lowest biogas flow rate of 274 L h^{-1} since the maximum flow rate of the recycling liquid pump was 1000 L h^{-1}.

The temperature, dissolved oxygen concentration (DO) and pH in the cultivation broth of the HRAP were monitored every five minutes. Liquid samples of 1 L from the influent wastewater (obtained along 24 hours) and 500 mL from the clarified effluent were withdrawn twice a week to monitor the concentration of COD, N-NH_4^+, P-PO_4^{3-}, N-NO_2^-, N-NO_3^-, IC and TN. Liquid samples were also drawn from the cultivation broth of the HRAP to monitor algal-bacterial TSS and volatile suspended solids (VSS) concentration. The algal-bacterial biomass was dried for 24 h at 105 °C to determine its elemental composition (C, N and S) under steady state in each operational stage.

2.4. Analytical procedures

The pH, DO concentration and temperature were monitored and recorded using Crison pH 4603 and DO 6050 probes coupled to a Crison Multimeter 44 display (Spain). CH_4, CO_2, H_2S and O_2 were measured using a COMBIMASS® Portable Gas-analyzer GA-m5. The concentrations of dissolved TN and IC were determined by means of a Shimadzu TOC-VCSH analyzer (Japan) equipped with a TNM-1 chemiluminescence module. NH_4^+ was analyzed using a selective electrode (Thermo Scientific Orion, USA). COD, P-PO_4^{3-}, N-NO_2^-, N-NO_3^-, TSS and VSS were measured using Standard Methods (Eaton et al., 2005). The elemental composition of the algal-bacterial biomass (C, N and S content) was determined using a LECO CHNS-932 analyzer (LECO, Italy).

2.5. Statistical analysis

The results here presented were provided as the average values along with their standard deviation from replicate measurements. An analysis of variance (ANOVA) was performed to determine the influence of the biogas flowrate, HRT and L/G ratio on the quality of biomethane.

3. Results and discussion

3.1. Environmental parameters

The ambient temperature and the diurnal solar radiation cycle seasonally varied along the three experimental stages, with the subsequent variations in the cultivation broth temperatures (23.5±2.5, 12.4±2.3 and 18.8±3.0 ºC during stages I, II and III, respectively) (Table 1). These variations in environmental conditions are inherent to any outdoors experimentation. In this context, Rodero et al. (2018b) found a negligible impact of the temperature on biogas upgrading performance when using a moderate alkalinity cultivation broth (i.e. centrate), while at low alkalinity (i.e. domestic wastewater) the CH_4 content of the biomethane increased by 3.3% when the temperature decreased from 35 ºC to 12 ºC. The average pH of the cultivation broth under steady state during stages I, II and III was 7.3±0.2, 7.1±0.5 and 8.9±0.3, respectively. The higher pH recorded in the latter stage was attributed to the higher pH and alkalinity of the centrate fed to the HRAP in comparison with the domestic wastewater used during stages I and II. The maximum DO concentrations in the cultivation broth (8.3±2.8, 6.6±1.3 and 9.4±1.4 mg L^{-1} in stages I, II and III, respectively) (Table 1) were recorded during the daytime, and never exceeded inhibitory levels for microalgae activity (<25 mg O_2 L^{-1}) (Jiménez et al., 2003). On the other hand, minimum daily DO concentrations of 0.3±0.2, 2.8±1.4 and 4.3±0.7 were recorded in stages I, II and III, respectively, during the nighttime due to absence of photosynthetic activity and the occurrence of an active organic matter oxidation and NH_4^+ nitrification (Posadas et al., 2013). It is worth noticing that the lowest DO concentration was observed during the treatment of domestic wastewater at a HRT of 3.5 days due to the higher biological oxygen consumption resulting from the higher organic loading rates mediated by the shorter HRT (Arbib et al., 2017).

Finally, the average water losses by evaporation during stages I, II and III accounted for 14.7±18.7, 4.3±3.2 and -0.1±0.6 L m^{-2} d^{-1} (Table 1). The highest evaporation rate herein recorded was ~ 2.2 times higher than the maximum values reported by Marín et al. (2018) in a 180 L outdoors HRAP located at Valladolid (Spain) during one year operation. This high value was attributed to the higher temperatures of the cultivation broth and the high turbulence at the HRAP surface caused by the wind in Chiclana de la Frontera. On the other hand, the negative value obtained during stage III was caused by the higher average rain recorded (4.4 L m^{-2} d^{-1}) during steady state in this period compared to 1.0 L m^{-2} d^{-1} recorded during state II and the absence of rain during stage I. This value agreed with the observations of Posadas et al. (2014), who reported negative evaporation rates in an outdoors HRAP.

3.2. Biogas upgrading performance

3.2.1. CO_2 removal

CO_2 removal efficiency was a function of the gas-liquid mass transfer in the absorption column, which itself was influenced by CO_2 consumption by microalgae in the HRAP. During stage I, CO_2-REs of 59.2±3.2, 76.6±1.8 and 88.9±1.5%, which corresponded to CO_2 concentrations of 17.3±2.2, 11.8±1.4 and 5.8±1.0% in the upgraded biogas, were recorded at L/G ratios of 1.2, 2.1 and 3.5, respectively, at a biogas flowrate of 274 L h^{-1}. CO_2-REs increased with the L/G ratio due to the increase in the overall gas-liquid mass transfer coefficient and the lower CO_2 transferred per volume of recirculating medium, which prevented the acidification of the recycling cultivation broth along the absorption column as a result of the acidic nature of biogas (Anbalagan et al., 2017; Posadas et al., 2017a). Indeed, a lower decrease in pH between the top and the bottom of the absorption column was observed with the increase in the L/G ratio (ΔpH of 1.7, 1.5 and 1.2 at a L/G ratio of 1.2, 2.1 and 3.5, respectively) during stage I. Similarly, CO_2-REs varied from 59.6±2.5 to 74.2±0.5% and from 64.4±2.2 to 81.0±0.3% when the L/G increased from 1.2 to 2.1 at a biogas flowrate of 370 and 459 L/h, respectively (Figure 2a). In this context, a slight increase in CO_2-RE was recorded at the highest biogas flowrate as a result of the higher turbulence in the absorption column, which enhanced the gas-liquid mass transfer coefficient in this unit.

<Figure 2>

During stage II, CO_2-REs of 56.4±2.5, 77.2±1.5 and 90.4±0.4% were recorded at a L/G ratio of 1.2, 2.1 and 3.5, respectively, and a biogas flowrate of 274 L h^{-1} (Figure 2b). No significant differences ($p > 0.05$) were observed in CO_2-RE values compared to stage I, which revealed a negligible influence of the HRT on CO_2 removal efficiency when domestic wastewater was used to support algal-bacterial growth. In fact, although higher pH values were expected at longer HRTs based on the lower acidification caused by the reduction in CO_2 production due to the lower organic matter load, a similar pH of the cultivation broth was recorded in the HRAP in both stages as a result of the higher nitrifying activity during stage II (as discussed in section 3.3) (de Godos et al., 2016; Posadas et al., 2017b). The decrease in pH along the absorption column in stage II was similar to that recorded in stage I (ΔpH of 2.1, 1.7 and 1.5 at a L/G ratio of 1.2, 2.1 and 3.5, respectively), which was attributed to the similar IC concentration of the cultivation broth in both stages (25.6±5.5 and 29.5±9.4 mg L^{-1} during stage I and II, respectively, under steady state conditions). Similarly, CO_2-REs varied from 64.3±4.7 to 84.0±1.4% and from 63.6±0.4 to 80.1±0.4% when the L/G increased from 1.2 to 2.1 at biogas flowrates of 370 and 459 L h^{-1}, respectively (Figure 2b). These results were in accordance to Anbalagan et al. (2017), who observed an increase in CO_2-RE from 45 to 79% when increasing the L/G ratio from 1 to 15 regardless the HRT.

Similarly, the lowest CO_2-REs during stage III were obtained at a L/G ratio of 1.2 (78.0±12.1, 85.3±1.3 and 77.6±1.0%, which corresponded to CO_2 concentrations of 10.1±4.4, 7.2±1.0 and 11.1±1.1 % in the upgraded biogas at 274, 370 and 459 L h^{-1}, respectively) (Figure 2c). An increase in CO_2-REs up to 97.8±0.8, 98.4±1.4 and 97.3±0.5% at 274, 370 and 459 L h^{-1}, respectively, was obtained at a L/G ratio of 2.1. Finally, the highest CO_2-REs (99.1±0.3%) were recorded at a L/G ratio of 3.5 (Figure 2c). The superior CO_2-REs obtained during this stage compared to stages I and II was likely due to the higher pH and alkalinity of the cultivation broth, which ultimately increased CO_2 and H_2S mass transfer in the absorption column as a result of the lower decreases in pH (ΔpH of 1.9, 1.3 and 0.8 at a L/G ratio of 1.2, 2.1 and 3.5, respectively, in the assays conducted at a biogas flowrate of 274 L h^{-1} of biogas flowrate).

3.2.2. H_2S removal

H_2S-REs of 90.9±0.7, 97.9±0.1 and 98.2±0.2% were achieved during photosynthetic biogas upgrading at a L/G ratio of 1.2, 2.1 and 3.5, respectively, when operating at a biogas flowrate of 274 L h^{-1} during stage I (Figure 3a). Similarly, H_2S-REs increased from 86.4±1.3 to 94.0±2.8% and from 87.6±2.9 to 95.2±1.2% when the L/G increased from 1.2 to 2.1 at biogas flowrates of 370 and 459 L h^{-1}, respectively, under process operation with domestic wastewater at 3.5 days of HRT. The highest H_2S removals were achieved at the highest L/G ratio as a result of the higher volumetric mass transfer coefficients and higher concentrations gradients (the latter supported by the higher pH in the absorption column mediated by the increased fresh recycling liquid flowrate). In addition, the significantly higher H_2S-REs compared to the elimination of CO_2 were attributed to the higher aqueous solubility of H_2S (dimensionless Henry's Law constant = C_L/C_G three times higher than that of CO_2) (Sander, 1999).

<Figure 3>

During stage II, H_2S-REs of 90.3±4.9, 95.9±5.4 and 98.5±0.4% were recorded at a L/G ratio of 1.2, 2.1 and 3.5, respectively, at a biogas flowrate of 274 L h^{-1} (Figure 3b). No significant influence of the HRT ($p > 0.05$) on H_2S-RE was observed when feeding the HRAP with domestic wastewater. On the other hand, H_2S-REs increased from 93.7±1.4 to 97.3±0.1% and from 92.9±1.0 to 96.1±0.8% when the L/G increased from 1.2 to 2.1 at a biogas flowrate of 370 and 459 L h^{-1}, respectively, under process operation with domestic wastewater at a HRT of 8 days.

Finally, H_2S-REs of 96.4±5.1, 97.8±0.3 and 99.1±1.3% were recorded at a L/G ratio of 1.2 and biogas flowrates of 274, 370 and 459 L h^{-1}, respectively, during stage III, while a complete removal was obtained when the L/G ratio was increased to 2.1 and 3.5 (Figure 3c). The increase in H_2S-REs observed during this stage, when centrate was used as a water and nutrient source, in comparison with stages I and II, was attributed to the higher pH and buffer capacity of the recirculating cultivation broth which increased H_2S mass transfer due to its acidic nature. These results agreed with the observations of Rodero et al. (2018b), who recorded an increase in H_2S removal from 80.3 to 94.7% when the IC concentration of the cultivation broth increased from 100 to 500 mg L^{-1} at 12ºC and L/G ratio of 0.5 in a 180 L HRAP operated indoors.

3.2.3. Enhancement in the CH_4 content of the upgraded biogas

The CH_4 enhancement factor, defined as the ratio between the increase in CH_4 content (%CH_4 in biomethane - %CH_4 in raw biogas) and the CH_4 content (%) in raw biogas, was used to comparatively assess the influence of the L/G, biogas flow rate, type of wastewater and HRT. CH_4 enhancement factors of 19.9±8.4, 25.3±8.8 and 28.8±8.7%, which corresponded to CH_4 concentrations of 79.3±2.8, 83.7±1.8 and 86.8±1.8% in the upgraded biogas, were recorded at L/G ratios of 1.2, 2.1 and 3.5, respectively, at a biogas flowrate of 274 L h^{-1} during stage I. Similarly, CH_4 concentration in the upgraded biogas increased from 81.2±0.1 to 84.7±0.6% (CH_4 enhancement factors of 17.8±1.6 and 22.8±0.9%) and from 81.6±0.6 to 85.6±0.2% (CH_4 enhancement factors of 18.6±0.1 and 24.3±0.6%) when L/G increased from 1.2 to 2.1 at biogas flowrates of 370 and 459 L h^{-1}, respectively (Figure 4a). The increase in L/G ratio played a key role on the CH_4 enhancement factor mediated by CO_2 and H_2S removals, while a negligible influence ($p>0.05$) of the biogas flowrate was recorded on CH_4 concentration in the upgraded biogas. However, the increase in L/G ratio also induced a higher desorption of the N_2 and O_2 dissolved in the cultivation broth to the biogas in the absorption column, thus decreasing the CH_4 concentration in the upgraded biogas (Posadas et al., 2017a). Indeed, the O_2 + N_2 concentration in the upgraded biogas increased up to 7.4±0.4% at a L/G ratio of 3.5 under process operation with domestic wastewater at a HRT = 3.5 days. The higher stripping of N_2 and O_2 at higher L/G ratios was due to the higher turbulence in the absorption column, which increase the overall mass transfer coefficients (Serejo et al., 2015). In this context, O_2 and N_2 stripping could be controlled operating under low L/G ratios and conditions that enhance CO_2 and H_2S gas-liquid mass transfer.

<Figure 4>

During stage II, CH_4 enhancement factors of 13.8±0, 13.2±0.6 and 15.0±1.3%, which corresponded to final CH_4 concentrations of 85.4±0.3, 85.1±0.7 and 87.0±0.9 were recorded at a L/G ratio of 1.2 and biogas flowrates of 274, 370 and 459 L h^{-1}, respectively (Figure 4b). An increase in CH_4 concentration up to ~89% was recorded at a L/G ratio of 2.1 regardless of the biogas flowrate and only a slight increase in CH_4 concentration up to 90.4±0.6% was obtained when the L/G ratio was increased to 3.5 (Table 2). Despite higher CH_4 concentrations in the upgraded biogas were recorded when the HRT of the domestic wastewater in the HRAP was increased from 3.5 to 8 days, lower CH_4 enhancement factors were achieved as a result of the higher CH_4 concentrations in the raw biogas in this stage (75.3±0.3 % in stage II *vs* 68.4±1.7 % in stage I).

<Table 2>

During stage III, CH_4 enhancement factors of 29.4±5.0, 40.3±1.3 and 37.4±0%, which corresponded to CH_4 concentrations of 83.3±2.0, 90.3±2.2 and 88.2±2.2 in the upgraded biogas, were recorded at L/G ratios of 1.2, 2.1 and 3.5, respectively, at a biogas flowrate of 274 L h^{-1} (Figure 4c). The increase in L/G ratio from 2.1 to 3.5 under process operation with centrate also resulted in lower final CH_4 concentrations due to the higher N_2 and O_2 desorption from the recycling liquid to the biomethane. Interestingly, higher N_2 + O_2 concentrations in the upgraded biogas (up to 11.4±2.0%) were recorded as a result of the increase in the overall mass transfer coefficients mediated by the higher ionic strength of the recycling liquid in stage III, which prevented the coalescence of the fine bubbles produced by the biogas diffuser (Sovechles and Waters, 2015). In our particular study, the maximum CH_4 content on the upgraded biogas (90.3%) remained below the minimum limit required for biogas injection in natural gas grid (95%) or the limit imposed for some car manufactures. Nevertheless, an increase of the alkalinity will improve CO_2 and H_2S absorption that will allow operating at lower L/G ratios with the consequent decrease in O_2.

3.3. Wastewater treatment performance

The COD-REs recorded in the HRAP accounted for 86.9±1.8, 90.7±4.1 and 73.6±0 %, which resulted in effluent COD concentrations of 85.8±10.3, 49.6±16.2 and 123.8±0 mg O_2 L^{-1} during stages I, II and III, respectively (Figure 5). The higher effluent COD concentrations in stage III compared to the previous stages were likely mediated by the higher HRT (process operation without effluent), which supported a higher biomass decay. However, effluent COD concentrations always complied with the Directive 98/15/CEE (125 mg O_2 L^{-1} maximum COD concentration for wastewater discharge into the environment) regardless of the type of wastewater or HRT ("Directive 98_15_CEE," 1998).

<Figure 5>

High N-NH_4^+ REs were achieved during the three stages (93.6±3.5, 98.1±2.1 and 100±0% in stages I, II and III, respectively). However, the removals of TN under steady state were lower and averaged 85.6±1.6, 76.4±5.7 and 86.2±3.4% during stages I, II and III respectively (Figure 5). This mismatch between TN and N-NH_4^+ eliminations was caused by the active nitrification of a fraction of the inlet nitrogen to NO_2^- and NO_3^-. In this context, N-NO_3^- was the dominant form of oxidized nitrogen since N-NO_3^- effluent concentrations averaged 2.0±1.2, 9.6±0.5 and 38.1±7.4 mg L^{-1}, while N-NO_2^- effluent concentrations averaged 0.8±0.5, 0.4±0.2 and 13.3±11.7 mg L^{-1} in stages I, II and III, respectively. The maximum fraction of the inlet nitrogen converted into N-NO_2^-+N-NO_3^- was recorded during stage II (18.5%). These results agreed with Arcila and Buitrón (2016), who recorded an incomplete nitrification or no nitrification when the HRT decreased from 10 to 6 days as a result of a nitrifying biomass wash-out. On the other hand, the lower share of nitrification during stage III compared to stage II was attributed to a high NH_4^+ volatilization mediated by the high pH (~9) under operation with centrate.

Finally, $P\text{-}PO_4^{3-}$-REs of 86.7±6.3, 80.6±3.5 and 67.6±5.4%, which entailed $P\text{-}PO_4^{3-}$ effluent concentrations of 1.0±0.5, 1.3±0.3 and 19.9±5.4 mg L^{-1} during stages I, II and III, respectively, were recoded (Figure 5). In this regard, these $P\text{-}PO_4^{3-}$-REs agreed with values previously reported in literature and highlighted the high bioremediation efficiency of HRAPs devoted to biogas upgrading (García et al., 2017; Toledo-Cervantes et al., 2016).

3.4. Concentration and elemental composition of the algal-bacterial biomass

TSS concentrations in the HRAP cultivation broth of 0.33±0.10, 0.37±0.08 and 0.56±0.05 g L^{-1} were recorded during stages I, II and III, respectively, with a similar VSS/TSS ratio of ~ 0.74. These TSS values were similar to those reported by Posadas et al. (2015b) (321-494 mg L^{-1}) in three outdoors HRAP treating domestic wastewater at 2.7-6 days of HRT under different pHs. The higher TSS concentration in the HRAP during stage III was attributed to the higher nutrient concentrations of the centrate compared to domestic wastewater.

The C and N content of the harvested biomass (on a dry weight basis) remained constant at 32.1±1.7 and 5.6±0.6%, respectively, regardless the operational stage. Despite this C content was lower compared to the typical range reported in literature for different microalgae strains (40-60 wt.%)(Teles et al., 2013), this value was in agreement with Muñoz et al. (2015b) who recorded a C content of 32.2% and 30.4% in the biomass of the strains *Botryococcus Braunii* and *Nannochloropsis gaditana*, respectively. Similarly, Harman-ware et al. (2013) reported a C content of 32.1% in *Scenedesmus sp.* biomass. The N content and the C/N ratio (5.7) in the harvested biomass remained within the range of previously reported data (Ward et al., 2014). The main differences were recorded in S content, which varied from 0.68±0.08% during stages I and II to 0.30±0.05% during stage III. These results agreed with those reported by Posadas et al. (2017a), who observed a decrease in S content in the biomass from 0.4% to 0.2% concomitantly with the increase in the IC concentration of the cultivation broth. However, this decrease in S requires further investigation.

3.5 Biogas upgrading technology costs

Despite the fact the investment cost of photosynthetic biogas upgrading is ~1.5-2.2 times higher than that of conventional-physical chemical technologies, and the needed of higher extensions of land (a total HRAP surface of ~13.4 ha to treat 300 Nm3 h^{-1} of biogas considering a water depth of 0.2 m) (Toledo-Cervantes et al., 2017b), the environmental sustainability (CO_2 trapped in form of algal bacterial biomass and wastewater treatment), the simultaneous H_2S removal and the lower energy requirements, make this technology an attractive alternative for biogas upgrading (Table 3). Moreover, algal-bacterial biomass valorization as bio-fertilizer outbalanced the high investment costs of this process.

<Table 3>

4. Conclusions

This work constitutes, to the best of our knowledge, the first demo-scale validation of the simultaneous photosynthetic biogas upgrading and wastewater treatment under outdoor conditions. The type of wastewater played a key role on biogas upgrading (with higher CO_2 and H_2S removals using centrate due to its higher pH and alkalinity), while the influence of the HRT and biogas flowrate on biogas upgrading performance was negligible. Despite higher L/G ratios supported higher CO_2 and H_2S removals, the associated N_2 and O_2 stripping resulted in a lower biomethane quality. Finally, an efficient wastewater treatment was achieved regardless of the operational conditions.

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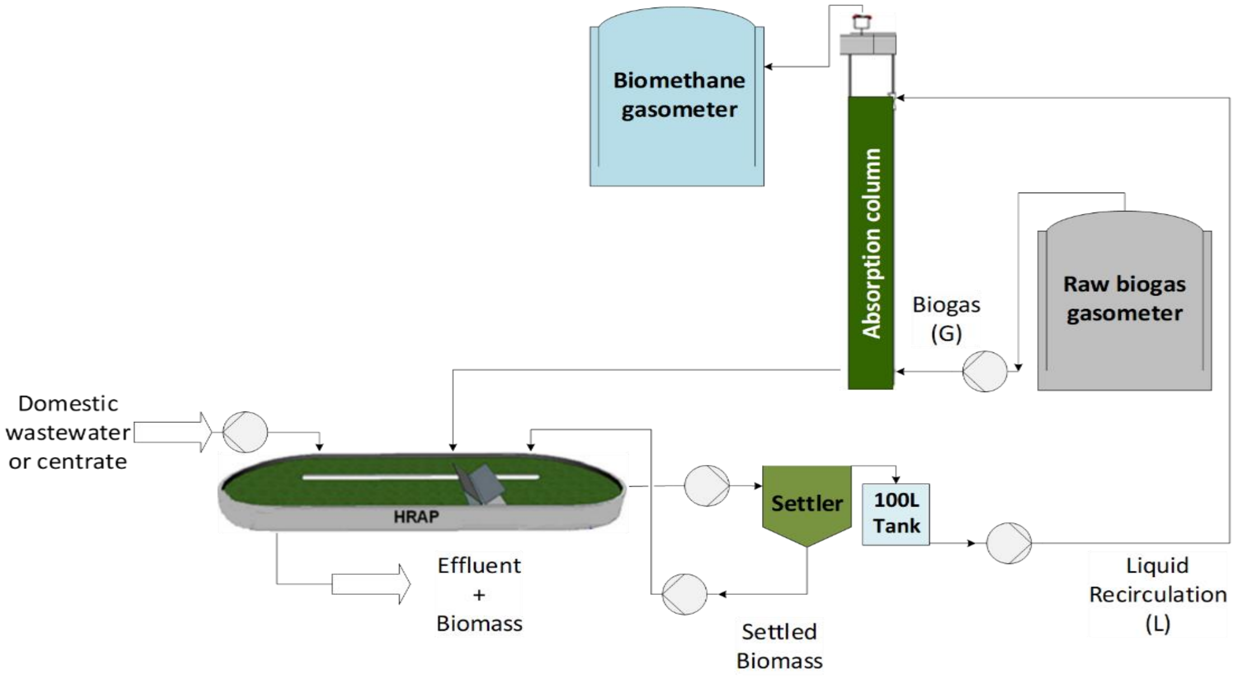

Figure 1. Schematic diagram of the experimental set-up.

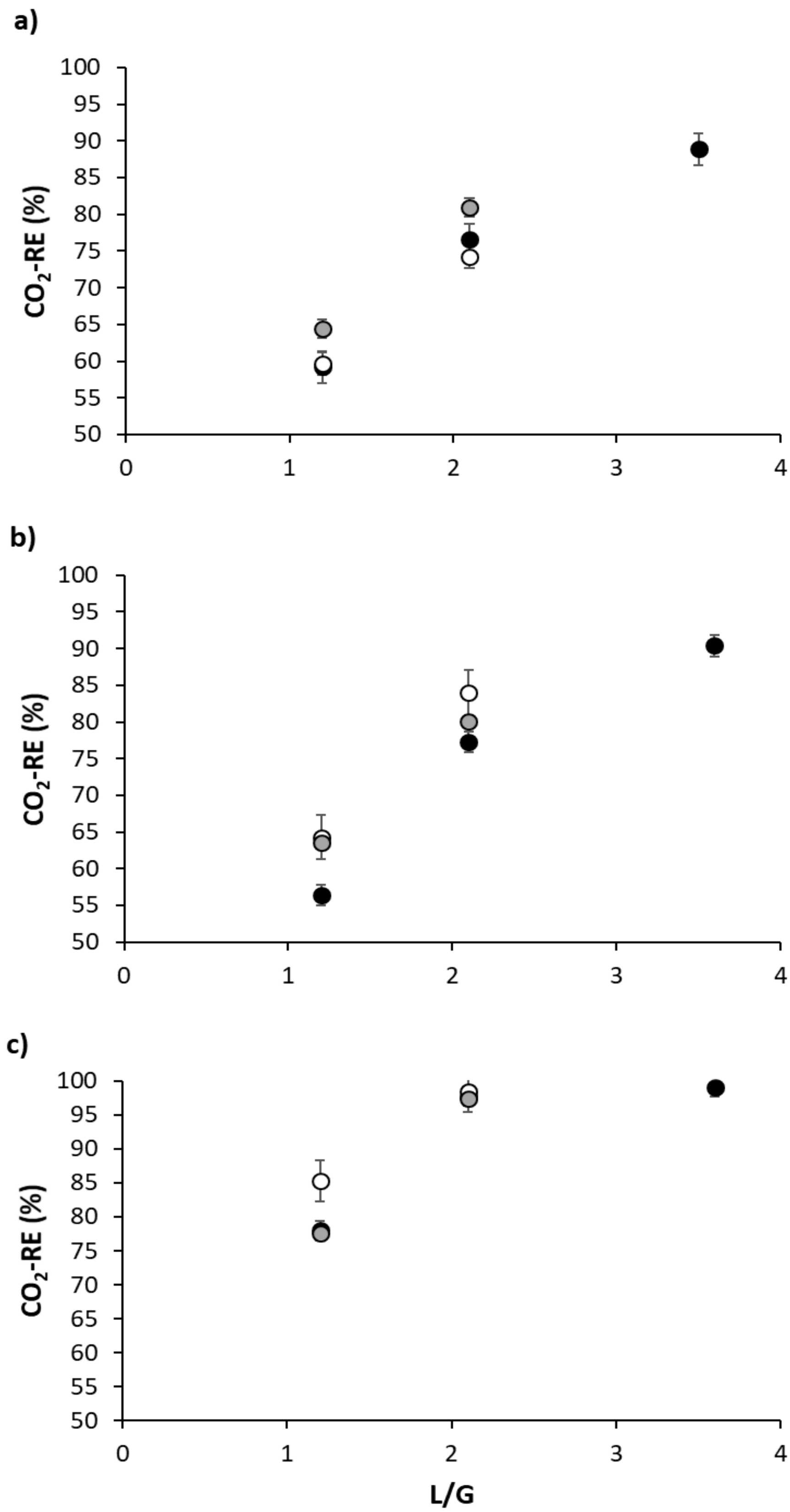

Figure 2. Influence of the L/G ratio on the removal efficiency of CO_2 at a biogas flowrate of 274 (black), 370 (white) and 459 (grey) L h^{-1} during stage I (a), stage II (b) and stage III (c).

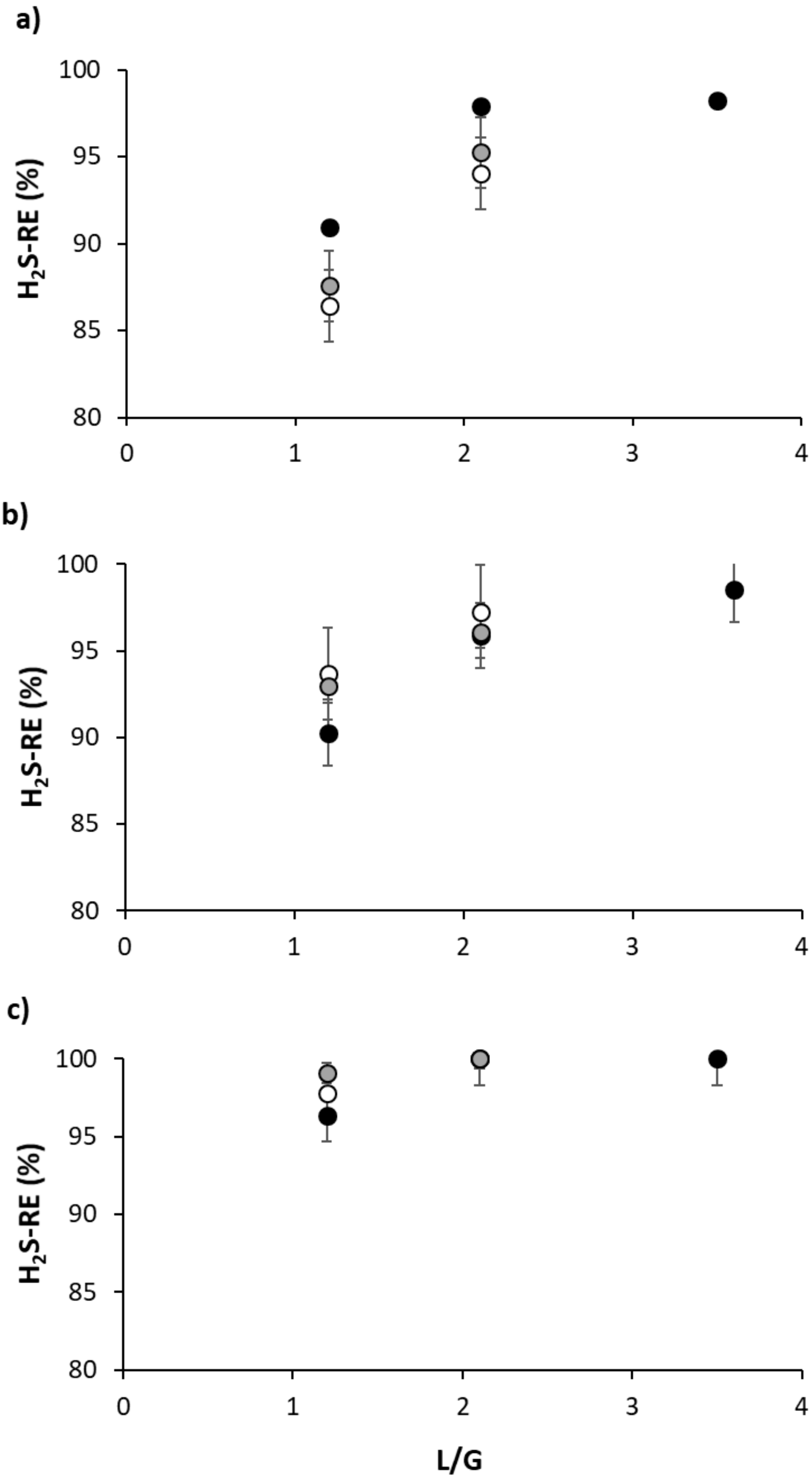

Figure 3. Influence of the L/G ratio on the removal efficiency of H_2S at a biogas flowrate of 274 (black), 370 (white) and 459 (grey) L h^{-1} during stage I (a), stage II (b) and stage III (c).

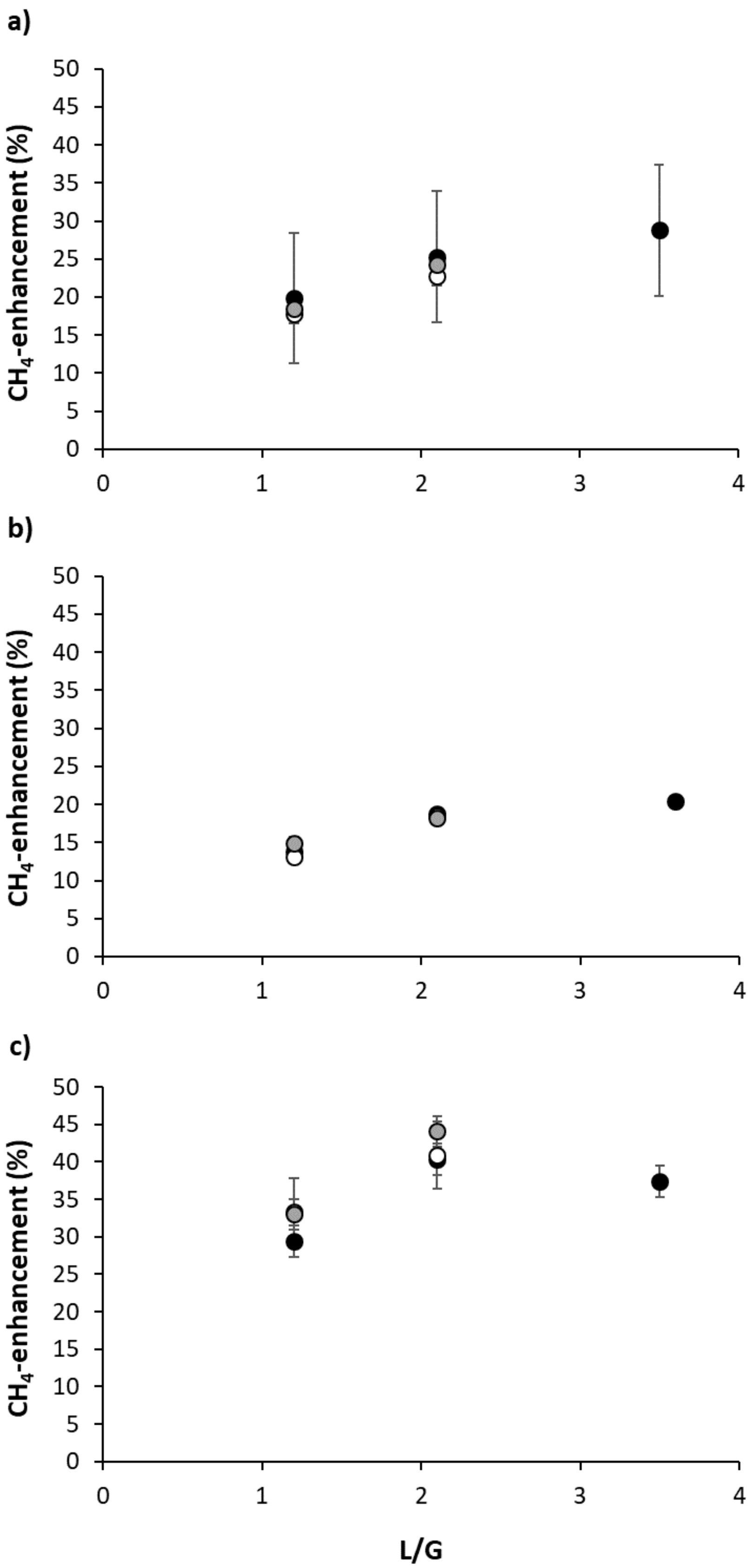

Figure 4. Influence of the L/G ratio on the CH_4 enhancement factor at a biogas flowrate of 274 (black), 370 (white) and 459 (grey) L h^{-1} during stage I (a), stage II (b) and stage III (c).

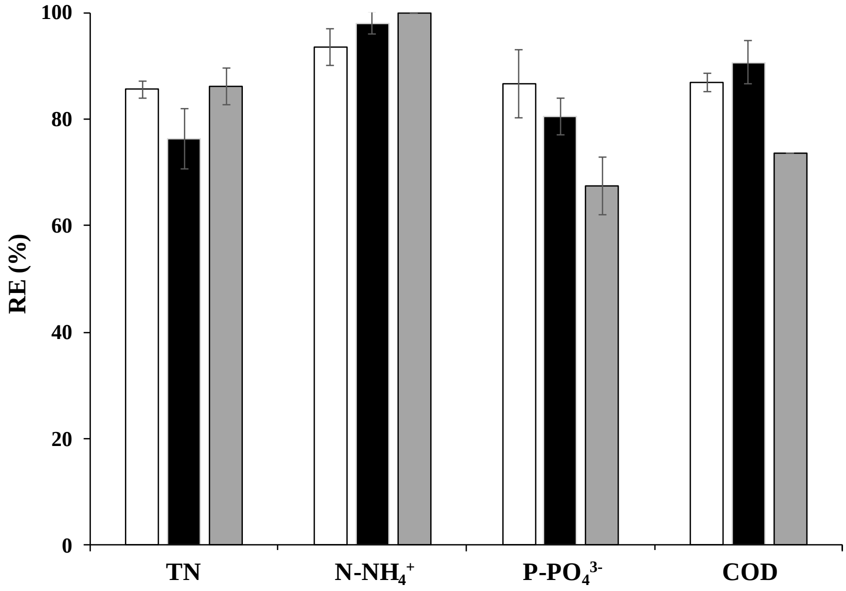

Figure 5. Steady state removal efficiencies of total nitrogen (TN), ammonium (N-NH_4^+), phosphate (P-PO_4^{3-}) and chemical oxygen demand (COD) during stage I (white), II (black) and III (grey).

Table 1. Average environmental parameters in the HRAP during the three operational stages tested under steady state conditions.

Parameter	**Stage**		
	I	**II**	**III**
Average ambient temperature (°C)	25.3±1.3	12.3±2.0	15.3±2.0
Average cultivation broth temperature (°C)	23.5±2.5	12.4±2.3	18.8±3.0
Average pH	7.3±0.2	7.1±0.5	8.9±0.3
Average maximum daily DO (mg O_2 L^{-1})	8.3±2.8	6.6±1.3	9.4±1.4
Average minimum daily DO (mg O_2 L^{-1})	0.3±0.2	2.8±1.4	4.3±0.7
Average evaporation rate (L m^{-2} d^{-1})	14.7±18.7	4.3±3.2	-0.1±0.6

Table 2. Average composition of the upgraded biogas in the different operational stages

Stage	G (L h^{-1})	L/G	Upgraded biogas			
			CH_4 (%)	CO_2 (%)	H_2S (ppm)	N_2+O_2 (%)
I	274	1.2	79.3±2.8	17.3±2.2	167±119	3.3±1.5
	274	2.1	83.7±1.8	11.8±1.4	65±49	4.5±0.4
	274	3.5	86.8±1.4	5.8±1.0	40±42	7.4±0.4
	370	1.2	81.2±0.1	17.1±0.1	442±25	1.7±0.2
	370	2.1	84.7±0.6	11.6±1.1	205±92	3.7±0.5
	459	1.2	81.6±0.6	16.6±1.1	440±63	1.7±0.6
	459	2.1	85.6±0.6	10.0±0.9	190±42	4.5±0.7
II	274	1.2	85.4±0.3	15.8±0.8	18±12	-1.2±1.1
	274	2.1	89.2±0.2	9.0±0.4	8±3	1.9±0.3
	274	3.5	90.4±0.6	4.3±0.2	3±0	5.3±0.8
	370	1.2	85.1±0.7	13.6±0.6	10±1	1.3±0.2
	370	2.1	89.1±0.4	7.0±0.1	5±0	3.9±0.3
	459	1.2	87.0±0.9	12.8±0.1	11±1	0.2±0.8
	459	2.1	89.5±0.0	7.3±0.2	6±0	3.2±0.2
III	274	1.2	83.3±2.0	10.1±4.4	65±92	6.6±2.5
	274	2.1	90.3±2.2	1.2±0.6	0±0	8.5±1.6
	274	3.5	88.2±2.2	0.5±0.2	0±0	11.4±2.0
	370	1.2	87.2±2.2	7.2±1.0	43±11	5.7±1.2
	370	2.1	90.6±0.7	0.9±0.8	0±0	8.6±0.1
	459	1.2	82.5±0.3	11.1±1.1	15±21	6.5±0.8
	459	2.1	89.3±0.7	1.8±0.3	0±0	8.9±0.5

Table 3. Biogas upgrading technology costs (Angelidaki et al. 2018, Marín et al. 2018; Muñoz et al. 2015, Toledo-Cervantes et al. 2017b)

	Water scrubbing	**Chemical scrubbing**	**Organic scrubbing**	**PSA**	**Membrane separation**	**Cryogenic separation**	**HRAP-AC**
Investment costs (€ ($Nm^3\ h^{-1}$) $^{-1}$)	3500	3200	4000	2700	2800	-	6000
Energy requirements (kW-h Nm^{-3})	0.25-0.3	0.67-0.7	0.4-0.51	0.24–0.6	0.2-0.38	0.42-1	0.08-0.14
CH_4 content (%)	>96	96-99	96–98.5	96-98	96-98	>97	90
H_2S pretreatment	Recommended	Yes	Recommended	Yes	No	Yes	No