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1 **In-situ biogas upgrading in thermophilic granular UASB**
2 **reactor: key factors affecting the hydrogen mass transfer rate**

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10 **Highlights**

- 11 • Biogas upgrading to 82% CH₄ is feasible in a thermophilic granular UASB reactor.
- 12 • H₂ is introduced in a separate chamber having a volume of 25% the reactor.
- 13 • H₂ low gas-liquid mass transfer rate limits the availability of H₂ for methanogens.
- 14 • H₂ distribution can be improved using porous inert devices, like ceramic sponge.
- 15 • Gas recirculation and chamber configuration help to maximize CO₂ conversion to
- 16 CH₄.

17

18 **Abstract**

19 Biological biogas upgrading coupling CO₂ with external H₂ to form biomethane opens
20 new avenues for sustainable biofuel production. For developing this technology
21 efficient H₂ to liquid transfer is fundamental. This study proposes an innovative setup
22 for in-situ biogas upgrading converting the CO₂ in the biogas into CH₄, via
23 hydrogenotrophic methanogenesis. The setup consisted of a granular reactor connected
24 to a separate chamber, where H₂ was injected. Different packing materials (rashig rings
25 and alumina ceramic sponge) were tested to increase gas-liquid mass transfer. This
26 aspect was optimized by liquid and gas recirculation and chamber configuration. It was
27 shown that by distributing H₂ through a metallic diffuser followed by ceramic sponge in
28 a separate chamber, having a volume of 25% of the reactor, and by applying a mild gas
29 recirculation, CO₂ content in the biogas dropped from 42 to 10% and the final biogas
30 was upgraded from 58 to 82% CH₄ content.

31

32 **Keywords**

33 In-situ biogas upgrading; Hydrogen; Gas-liquid mass transfer rate; UASB; Granules;
34 Anaerobic digestion

35

36 **1. Introduction**

37 Anaerobic Digestion (AD) of organic waste is a promising technology for sustainable
38 energy production (Weiland, 2010). The potato-starch processing industry produces, as
39 byproduct, up to 1 m³ of potato juice per ton of potatoes (Abeling and Seyfried, 1993).
40 Potato-starch wastewater contains high concentration of biodegradable compounds,
41 such as starch and proteins, suitable for biogas production via AD (Barampouti et al.,
42 2005). Biogas typically contains ~50-70% CH₄ and 30-50% CO₂. Biogas upgrading to
43 CH₄ content higher than 90% increases its heating value and its potential applications as
44 alternative to natural gas (Deng and Hägg, 2010).

45 Methods currently available for biogas upgrading are mainly based on
46 physicochemical CO₂ removal. Nevertheless, these technologies require use of
47 additional materials and chemicals considerably increasing the cost of the process and
48 energy input. Alternatively, biogas can be upgraded by biologically coupling H₂,
49 derived from water electrolysis, with CO₂ present in the biogas to convert them to CH₄.
50 H₂ can be produced using the electricity generated by the surplus of energy from wind
51 mills or photovoltaic facilities, which may result from variable weather conditions. This
52 reaction is carried out by a group of microorganisms known as hydrogenotrophic
53 methanogenic archaea that utilize CO₂, as carbon source, and H₂, as electron donor, to
54 produce CH₄ via hydrogenotrophic methanogenesis (Muñoz et al., 2015). Previous
55 studies demonstrated that the addition of H₂ to a conventional biogas reactor can lead to

56 20 to 40% increase in CH₄ production rate, as result of the conversion of the CO₂
57 present in the biogas to additional CH₄ (Luo and Angelidaki, 2013; Luo et al., 2012).

58 Although biological biogas upgrading offers economical and technical advantages
59 compared to traditional methods (Nordberg et al., 2012), H₂ mediated biogas upgrading
60 is still challenging. One of the main limitations is the low H₂ gas-liquid mass transfer
61 rate (Bassani et al., 2015; Luo and Angelidaki, 2012; Luo et al., 2012).

62 H₂ gas-liquid mass transfer rate can be described by the following equation (1):

$$r_t = 22.4k_L a(H_{2gTh} - H_{2l})$$

63 where r_t (L/(L-day)) is the H₂ gas-liquid mass transfer rate, 22.4 (L/mol) is the gas
64 volume to mole ratio (1 mol gas corresponds to 22.4 L at STP), $k_L a$ (day⁻¹) is the gas
65 transfer coefficient, H_{2gTh} (mol/L) represent the H₂ concentration in the gas phase while
66 H_{2l} (mol/L) the H₂ dissolved in the liquid phase. One way to increase H₂ gas-liquid
67 mass transfer rate is by increasing $k_L a$. This coefficient is specific for given reactor
68 configuration and operating conditions (Pauss et al., 1990). Therefore, $k_L a$ can be
69 modulated by changing parameters such as mixing speed (Bhattacharyya and Singh,
70 2010; Luo and Angelidaki, 2012), gas recirculation (Guiot et al., 2011) and H₂ diffusion
71 device (Luo and Angelidaki, 2013; Díaz et al., 2015).

72 Besides, high-rate anaerobic treatment using up-flow anaerobic sludge blanket
73 (UASB) reactors is commonly applied in industrial wastewater treatment plants
74 (Gomec, 2010; Sevilla-Espinosa et al., 2010). Moreover, typically a UASB process is
75 expected to provide higher methane content in the biogas than a CSTR process (Nizami
76 et al., 2012).

77 UASB reactors' technology is based on the presence of granular sludge comprised of
78 microorganisms responsible for catalyzing the biological conversion of organic matter

79 to biogas. High recirculation flow rates and consequent high up-flow velocities have an
80 in important role for the hydraulic mixing improving the wastewater to granules contact
81 (Powar et al., 2013; Zheng et al., 2012). It has been previously reported that
82 carbohydrate degraders and hydrogenotrophic methanogens are predominant in starch-
83 grown granules, likely due to their role in the interspecies H₂ transfer with syntrophic
84 bacteria (Lu et al., 2015). Moreover, previous studies on H₂ mediated biogas upgrading
85 demonstrated that H₂ affected the microbial community composition enhancing the
86 hydrogenotrophic methanogenic pathway and the syntrophic relationship between
87 bacteria and hydrogenotrophic methanogens (Bassani et al., 2015).

88 In this study an innovative setup consisting of a UASB granular reactor connected to
89 a separate chamber, where the H₂ was injected, was designed to mediate efficient H₂
90 transfer to liquid phase for biological conversion of H₂ and CO₂ to CH₄. Key factors
91 affecting the H₂ gas-liquid mass transfer rate were evaluated. More specifically, the
92 effect of different operating conditions aiming in increasing $k_L a$ of H₂ to gas, and
93 thereby increase the gas to liquid transfer, were studied to elucidate their role in
94 improving CO₂ and H₂ conversion to CH₄. Parameters examined were liquid and gas
95 recirculation and configuration of diffusion devices. Moreover, the addition of packing
96 materials as a mean to minimize the gas bubble size and thus increase the gas
97 dissolution in the liquid was tested. Finally, the effect of gas retention time was
98 evaluated using single or serial chamber configurations with different working volumes.

99

100 **2. Materials And Methods**

101 **2.1 Substrate characteristics and feedstock preparation**

102 Potato-starch wastewater substrate was obtained from Karup Kartoffelmelfabrik
103 potato-starch processing factory, Denmark. Because potato-starch processing involves
104 an up-concentration step, the provided substrate was diluted 10 times with water and
105 Basal Anaerobic (BA) medium, to adjust the volatile solids (VS) content to the required
106 operation conditions. Successively, the substrate was stored at -20°C, in 5 L bottles and
107 thawed at 4°C for 2-3 days, before usage. BA medium was prepared as described in
108 Supplementary Information (SI). The diluted substrate had a pH of 6.05, chemical
109 oxygen demand (COD) of 21.76±0.15 g/L, total solids (TS) and VS content of
110 26.14±0.17 and 18.73±0.12 g/L, respectively. The concentration of total volatile fatty
111 acids (VFA) was 49.29±4.94 mg/L. Total Kjeldahl Nitrogen (TKN) and ammonium
112 nitrogen NH⁴⁺ (NH₄-N) were 1.24 ± 0.01 and 0.30 ± 0.01 g-N/L, respectively.

113

114 **2.2 Setup and operation of the reactors**

115 Each setup was composed of a UASB reactor with a working volume of 1.4 L,
116 connected to a separate H₂-injection chamber with a working volume of 0.2 L. The
117 feeding was introduced from the bottom of the UASB. The reactors were inoculated
118 with 550 g of mesophilic granules, obtained from Colsen wastewater treatment plant
119 treating potato starch wastewater (The Netherlands) and BA medium. The granules
120 were adapted to thermophilic conditions for 25 days by feeding the reactors with diluted
121 potato starch wastewater at hydraulic retention time (HRT) of 7 days and organic
122 loading rate (OLR) of 2.79 gVS/L.day. A double net-separator was located in the upper
123 part of each UASB to prevent the wash out of granules. One setup (R1) was used as
124 upgrading reactor, while the other (R2) was utilized as control reactor operated
125 throughout the experiment without H₂ injection. Both reactors were maintained at

126 thermophilic conditions (55 ± 1 °C) by circulating hot water through a water jacket
 127 around the UASB reactors glass walls.

128 After the startup phase, the whole experiment was divided in 8 periods. During period I
 129 the OLR was increased to 3.73 gVS/L day shortening the HRT to 5 days (Pre H₂ phase).
 130 The recirculation flow rate was set to 4 L/h. From period II, H₂ was continuously
 131 injected to R1 through a diffuser placed at the bottom of the H₂-injection chamber (In-
 132 situ phase). Rashig rings (5 mm internal diameter) were inserted into the separate
 133 chamber of both reactors to maximize the H₂ gas-liquid mass transfer rate in case of R1.
 134 The volumetric H₂ flow rate was set to 4 times the CO₂ production rate (in the gas
 135 phase) recorded before the H₂ addition, according to Luo and Angelidaki (2013b), i.e.
 136 3.5 L/L.day, and then reduced to improve the H₂ consumption. In period III, the
 137 recirculation flow rate of both reactors was increased to 7 L/h. Successively, in period
 138 IV, rashig rings were replaced by an inert alumina ceramic sponge, while in periods V
 139 and VI different gas recirculation flow were applied. In order to evaluate the effect of
 140 the gas retention time, the H₂-injection chamber volume was doubled to 400 mL by
 141 connecting two chambers in series (Period VII) or by assembling them as a single
 142 chamber with extended length (Period VIII).

143 The percentage of H₂ utilized was calculated according to the following equation (2):

$$\text{H}_2 \text{ utilization efficiency} = \frac{\text{H}_2 \text{ injected} \left(\frac{\text{L}}{\text{L} - \text{day}} \right) - \text{H}_2 \text{ in biogas} \left(\frac{\text{L}}{\text{L} - \text{day}} \right)}{\text{H}_2 \text{ injected} \left(\frac{\text{L}}{\text{L} - \text{day}} \right)} * 100$$

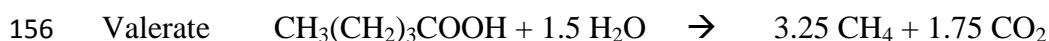
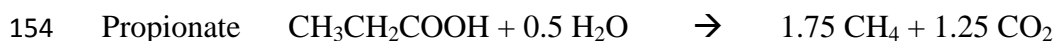
144 The percentage of CH₄ derived from the conversion of CO₂ and H₂ was calculated
 145 according to the equation 3:

146 CH₄ from CO₂ and H₂ conversion (%) =

147
$$\left(\frac{(\text{CH}_4 \text{ production rate in R1 } (\frac{\text{L}}{\text{L.day}}) - \text{CH}_4 \text{ production rate in R2 } (\frac{\text{L}}{\text{L.day}}))}{\text{CH}_4 \text{ production rate in R2 } (\frac{\text{L}}{\text{L.day}}) + \text{CH}_4 \text{ production rate equivalent to VFA in R2 } (\frac{\text{L}}{\text{L.day}})} \right) +$$

148
$$\frac{(\text{CH}_4 \text{ production rate equivalent to VFA in R1 } (\frac{\text{L}}{\text{L.day}}) - \text{CH}_4 \text{ production rate equivalent to VFA in R2 } (\frac{\text{L}}{\text{L.day}}))}{\text{CH}_4 \text{ production rate in R2 } (\frac{\text{L}}{\text{L.day}}) + \text{CH}_4 \text{ production rate equivalent to VFA in R2 } (\frac{\text{L}}{\text{L.day}})} * 100$$

149 Where CH₄ production rate represents the volume of CH₄ produced per liter of
150 reactor, per day, measured at the outflow of the reactor. While CH₄ production rate
151 equivalent to VFA was calculated converting VFA concentrations, in the reactors, to
152 CH₄ production equivalent according the following conversion reactions:



157 This was done to take into account the biomethanation inhibition caused by the injection
158 of H₂ in the upgrading reactor and provide a more accurate estimation of the CH₄
159 produced from the conversion of CO₂ and H₂.

160

161 **2.3 Analytical methods**

162 The biogas production was recorded in daily basis. TS, VS, NH₄-N and TKN were
163 measured according to the Standard Methods for Examination of Water and Wastewater
164 (APHA, 2005). Liquid samples from the reactors were collected for pH and VFA
165 analysis every second day. VFA and pH were measured according to Kougiyas et al.,
166 (2015) as described in SI. Detailed description of chromatographs utilized to measure
167 biogas composition and CH₄ production (for batch assays) are given in SI. Detection
168 limits for the measurement of CH₄, CO₂ and H₂ by GC were defined by the calibration
169 curve (5–100%), while the detection limits for VFA were 5–1500 mg/L.

170

171 **2.4 Specific methanogenic activity test**

172 Specific methanogenic activity (SMA) assays were conducted during reactors' steady
173 state operation. 1 g of granules and 9 mL of liquid sample obtained from the reactors
174 were immediately transferred to 36 ml serum bottles under anaerobic conditions. The
175 bottles were supplemented with acetate (20 mM) or H₂/CO₂ (80:20, 1 atm). Bottles with
176 glucose (10 mM) or water as substrate were prepared as control and blank, respectively.
177 All the tests were prepared in triplicates, flushed with N₂, sealed with rubber stoppers
178 and aluminum caps and incubated at 55 °C and 155 rpm.

179

180 **3. Results And Discussion**

181 **3.1 Process performances and biogas upgrade**

182 Operational data from upgrading (R1) and control (R2) reactor under steady state
183 conditions are reported in Table 1 and 2.

184

185 **3.1.1 Period I: the pre H₂ phase**

186 In the pre H₂ phase (Period I), the two reactors showed similar performance in terms of
187 biogas production rate (on average 2147 mL/L-reactor.day) and CH₄ yield (335
188 mL/gVS, corresponding to ~70% of the theoretical) (Table 1). This result is in
189 accordance with previous studies on biogas production from starch biomasses (Frigon
190 and Guiot, 2010). The average CH₄ content of the reactors was ~59% (Table 1 and Fig.
191 1), the pH was ~7.5 and the total VFA content >1 g/L (Table 1 and Fig. 2).

192

193 **3.1.2 Period II: effect of rashig rings as H₂ distribution device on biogas upgrading**
194 **performance**

195 To increase the $k_L a$ and thereby enhance gas-liquid transfer, rashig rings were placed in
196 the H₂-injection chamber to break H₂ bubbles and thus increase contact surface area
197 between gas and liquid phases (Kramer and Bailey, 1991). Once steady state conditions
198 were achieved, H₂ was continuously injected (3.5 L/L.day), through a metallic diffuser,
199 in the H₂-injection chamber (In-situ phase). By comparing reactors' performance, in R1,
200 45% higher CH₄ production rate was observed (Table 1 and Fig. 3). Additionally, a pH
201 increase to 7.9 was recorded in R1, as a result of the CO₂ removal (Table 1 and Fig. 2a).
202 Nevertheless, because of the low H₂ gas-liquid mass transfer rate, only 51% of the H₂
203 injected was utilized leading to a high amount of unutilized H₂ in the output gas (45%)
204 (Table 1 and Fig 1a). Additionally, a remarkable increase in VFA levels, reaching 3.4
205 g/L, was recorded in the upgrading reactor, while VFA concentration in the control
206 reactor remained stable (Table 1 and Fig. 2b). This is likely due to the high H₂ partial
207 pressure that affected negatively acidogenic VFA conversion resulting in their
208 accumulation. Moreover, the continuous H₂ injection led to a progressive higher H₂
209 partial pressure, which shifted the metabolic pathway towards homoacetogenesis
210 inhibiting methanogenesis (Cord-Ruwisch et al., 1997). This argument was supported
211 by the predominance and accumulation of acetate over other VFA in R1 accounting for
212 55% of total VFA (Table 1). Moreover, this level was 4 % higher than the
213 correspondent level in R2, which, together with higher total VFA concentrations,
214 demonstrates the instability caused by the excessive H₂ flow rate provided in R1.
215 Therefore, to provide a more accurate estimation of the increment of the CH₄ production
216 rate due to CO₂ and H₂ conversion, the total VFA concentrations in the two systems

217 were converted in equivalent CH_4 production, as described in section 2.2. The difference
218 in the VFA concentration between the two reactors was taken into account to estimate
219 the inhibition of liquid substrate degradation occurring in the upgrading reactor and
220 allow the reactors' performances to be comparable. Thus, the CH_4 derived from CO_2 and
221 H_2 conversion was calculated (equation 3) based on the difference between the CH_4
222 production rates of the two systems after normalization of VFA.

223 To overcome the negative effect of the H_2 on the biomethanation process and improve
224 the H_2 consumption, in the last part of this period the H_2 flow rate was reduced to 2.6
225 L/L.day reducing the unutilized H_2 to 34% of the output gas and increasing the CH_4
226 content to 47%.

227

228 **3.1.3 Period III: effect of liquid recirculation on upgrading performance**

229 Good mixing is known to be crucial to make substrates available for microorganisms
230 (Bhattacharyya and Singh, 2010; Luo and Angelidaki, 2012). Moreover good mixing
231 increases the $k_L a$ for gasses, which is function of the surface area per unit volume,
232 thereby increasing gas-liquid contact (Kramer and Bailey, 1991). Therefore, to improve
233 H_2 -liquid contact, the liquid recirculation flow was increased from 4 to 7 L/h, while the
234 H_2 flow rate was maintained to 2.6 L/L.day leading to a slight increase of the utilized H_2
235 (53%) (Table1). The unutilized H_2 and the CH_4 content in the output gas stabilized to
236 37% and 45%, respectively (Table 1 and Fig. 1a). Similarly, in this period in R1 36%
237 higher CH_4 production rate was recorded, compared to R2 (Table 1 and Fig. 3). As these
238 results did not markedly differ from the last part of period I (i.e. H_2 flow rate was
239 reduced to 2.6 L/L.day), it can be concluded that the improved upgrading efficiency was
240 mainly attributed to the lower H_2 flow rate applied, rather than to the higher liquid

241 recirculation flow. In fact, upon H₂ addition, the granular bed appeared less expanded,
242 probably due to reduced dissolved CO₂ concentration in the liquid, due to the
243 hydrogenotrophic consumption of CO₂ to CH₄ (Ohsumi et al., 1992; Song et al., 2005).
244 Therefore, the positive effect of the higher liquid recirculation on biogas production and
245 upgrading was not achieved.

246

247 **3.1.4 Period IV: effect of alumina ceramic sponge as H₂ distribution device on** 248 **upgrading performance**

249 An alternative method to reduce H₂ bubbles size and thus increase gas-liquid contact
250 is by increasing the surface area of the material over which the bubbles travelled and
251 thereby breaking them to a smaller size. Based on that, the rashig rings in the H₂-
252 injection chamber were replaced with alumina ceramic sponge. Alumina ceramic
253 sponge introduced in the chamber had 16 m² (0.3 m²/g) surface area which is
254 significantly higher compared to the surface area in rashig rings (0.1 m², corresponding
255 to 0.002 m²/g). Interestingly, in this period, the H₂ utilization and the CH₄ production
256 rate derived from CO₂ and H₂ conversion increased (Table 1 and Fig. 3). On average,
257 67% of the H₂ injected was utilized reducing the H₂ content in the output gas to 31%
258 and increasing the CH₄ content to 52% (Table 1 and Fig. 1a). These results clearly show
259 the influence of the H₂ distribution on the upgrading performances indicating the
260 importance of porosity and pore size of the H₂ distribution device for an effective H₂
261 utilization by microorganisms.

262 In this period lower biogas and CH₄ production rates were observed in particular in
263 R2 (Table 1 and Fig. 3). Previous studies have demonstrated that aluminum oxide does
264 not cause any toxic effects on microorganisms' growth (Ingham et al., 2012).

265 Additionally, state indicators of the biomethanation process, such as VFA and pH, did
266 not demonstrate any imbalance. More specifically, the VFA levels recorded in this
267 period and particularly for R1 were at the lowest levels compared to the other periods
268 (Table 1 and Fig. 2b). Therefore, we assume that ceramic sponge pores could have
269 retained undigested biomass particles with consequent decrease of CH₄ production.

270 In the last part of this period, in order to reduce the unutilized H₂, the H₂ flow rate was
271 further decreased to 2 L/L.day resulting in reduced H₂ and increased CH₄ content in the
272 output gas to 20% and 57%, respectively.

273

274 **3.1.5 Period V and VI: effect of gas recirculation on upgrading performance**

275 As previously described, gas recirculation would have a positive effect on $k_L a$
276 coefficient, increasing H₂ gas-liquid mass transfer rate (Equation 1) (Guiot et al., 2011).

277 Therefore, in period V, 4 mL/min gas recirculation (then increased to 6 mL/min, in
278 period VI) were applied to R1 improving the H₂ dissolution and thus significantly
279 increasing the CO₂ conversion. In fact, in these periods on average 87% of the H₂
280 injected was utilized leading to 37% higher CH₄ production rate (Table 2 and Fig. 3).
281 Nevertheless, an increase in the pH value to 8.2 was recorded as a result of the CO₂
282 removal (Table 2 and Fig. 2a). The CH₄ content in the biogas markedly increased to
283 66% and the unutilized H₂ decreased to 14% (Table 2 and Fig. 1a). To further decrease
284 the unutilized H₂, at the end of the period the H₂ flow rate was reduced to 1.8 L/L.day
285 (corresponding to ~2.5 times the CO₂ production rate recorded in R2). Nevertheless, no
286 substantial difference in biogas composition and upgrading performances was recorded.
287 In previous studies, H₂ distribution in the reactor's liquid phase was optimized by the
288 application of gas recirculation flow rates ~4-folds higher than the input gas flow rate

289 (Díaz et al., 2015). Unfortunately, in this experiment, beside the positive effect on
290 upgrading performances, the application of such a high gas recirculation flow rate led to
291 an excessive pressure through the diffuser and to turbulent movements causing granules
292 disintegration. The subsequent reduction of reactor's active biomass can explain the
293 lower CH₄ production rate and VFA levels higher than 5 g/L observed in R1 from
294 period V (Table 2, Fig. 2b and Fig. 3).

295

296 **3.1.6 Period VII and VIII: Effect of gas retention time using H₂-injection chamber** 297 **configuration on upgrading process performance**

298 To increase the contact area between H₂ bubbles and liquid, and therefore increase H₂
299 transfer coefficient (Equation 1), the ceramic sponge surface area was doubled. This
300 was done by doubling H₂-injection chamber volume, either by connecting two chambers
301 in series (Period VII), or by assembling them in a single longer chamber (Period VIII).
302 The connection of two chambers in series did not lead to a substantial improvement of
303 upgrading performances, indicating that chamber's volume itself has not a direct
304 correlation with H₂ distribution. Nevertheless, by assembling two chambers in a single
305 longer one, a higher H₂ percentage was utilized (94%) resulting in only 8% H₂
306 unutilized (Table 2 and Fig. 1a). Therefore, CO₂ and CH₄ contents in the output biogas
307 dropped to 10% and increased to 81% (with a maximum of 82%) respectively (Table 2
308 and Fig. 1a). However, in this period the pH raised to 8.4 as a consequence of the high
309 CO₂ conversion (Table 2 and Fig. 2a). The results clearly demonstrate the importance of
310 a proper reactor configuration design that increases the gas retention time leading to
311 more efficient H₂ distribution and CO₂ conversion to CH₄.

312 Moreover, from the comparison of reactors CH₄ production rate, it was shown that, in
313 the upgrading reactor, on average the CH₄ produced from the conversion of CO₂
314 represented ~37% of the total recorded CH₄ production rate (Table 1 and 2 and Fig. 3).

315 Finally, it should be mentioned that the lower CH₄ production and higher VFA levels
316 of control reactor observed in period VII were due to the disassembly of the separate
317 chamber in order to be mounted in the upgrading reactor (Table 2 and Fig. 2b and 3).

318 The CH₄ productivity and the VFA concentration of the control reactor were recovered
319 in period VIII.

320

321 **3.2 Specific methanogenic activity test**

322 H₂ addition is known to promote the hydrogenotrophic methanogenic pathway (Bassani
323 et al., 2015; Luo and Angelidaki, 2013a, 2013b). Therefore, in this experiment, SMA
324 tests were performed to validate the effect of the H₂ addition on methanogenesis
325 pathways. Granules and liquid samples were taken from the reactors at steady state of
326 periods IV (introduction of ceramic sponge as H₂ distribution device) and V
327 (application of gas recirculation). It was shown that the preferable methanogenic
328 pathway in both reactors (i.e. R1 and R2) was hydrogenotrophic (Table 3). This result
329 was expected because hydrogenotrophic methanogens are known to be predominant in
330 starch-grown granules (Lu et al., 2015).

331 In period IV, CH₄ production rate achieved by batches fed with H₂/CO₂ did not show
332 markedly difference between the two reactors. Conversely, in period V, higher
333 hydrogenotrophic activity was observed in R1 compared to the control reactor, likely
334 due to the gas recirculation enhancing the effect of H₂ addition on microbial community
335 composition and thus stimulating hydrogenotrophic methanogenic pathway.

336 Both tests showed low acetoclastic activity which can be explained by the high acetate
337 levels detected in the reactors before the tests which further increased in period V (~3.3
338 g/L in R1 and ~1.5 g/L in R2; Table 2). Moreover, by comparing the concentration of
339 unutilized acetate at the end of SMA tests and in the UASB reactors, it was shown that
340 acetate levels markedly decreased in all batches (from 3 to 2.5 g/L in the upgrading
341 system and from 1.4 to 1.3 g/L in the control treatment), apart from batches fed with
342 acetate, where acetate levels increased to 3.3 and 1.8 g/L in R1 and R2, respectively.
343 These results indicate that high acetate levels in the inoculum obtained from the reactor
344 probably inhibited the process not allowing the further degradation of the supplemental
345 amount of acetate that was added in the batch bottles (Gorris et al., 1989).
346 Finally, it was found that the specific microbial activity for the degradation of glucose
347 was lower in period V compared to period IV. This could be possibly due to the
348 negative effect of gas recirculation on the granules as previously discussed in the
349 continuous reactor operation (Tables 1, 2 and 3).

350

351 **4. Conclusions**

352 The current research demonstrated the feasibility of in-situ biogas upgrading using an
353 external chamber with 25% of the conventional biogas reactor volume. Key factors
354 affecting the H₂ gas-liquid mass transfer rate were tested to improve the efficiency of
355 the overall process. It was shown that the use of porous devices benefit the H₂ uptake as
356 the active contact area is increasing and the gas retention time is extended. Moreover,
357 the gas recirculation flow rate and the chamber design are fundamental elements that
358 must be considered to maximize the gas retention time and thus the H₂ dissolution to the
359 liquid media.

360

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366

367 **Appendix A. Supplementary data**

368 Supplementary data associated with this article can be found, in the online version, at

369

370

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458 **Table captions:**

459 **Table 1:** Upgrading (R1) and control (R2) reactor performances under steady state
460 conditions (Periods I-IV).

461 **Table 2:** Upgrading (R1) and control (R2) reactor performances under steady state
462 conditions (Periods V-VIII).

463 **Table 3:** Specific methanogenic activity (SMA) results, expressed as CH₄ production
464 rate (mL/L.day), under steady state conditions.

465 **Figure captions:**

466 **Fig. 1:** Biogas composition (CH_4 (●), CO_2 (○) and H_2 (■) %) of (a) upgrading and (b)
467 control reactor.

468 **Fig. 2:** pH (a) and total VFA (b) of upgrading (◆) and control (○) reactor.

469 **Fig. 3:** CH_4 production rate of upgrading (◆) and control (○) reactor.

Table 1

Phase	Pre H ₂				In-situ			
Period	I		II		III		IV	
H ₂ distribution device	-		rashig rings		rashig rings		ceramic sponge	
Reactor	R1	R2	R1	R2	R1	R2	R1	R2
Liquid recirculation flow (L/h)	4	4	4	4	7	7	7	7
Gas recirculation flow (mL/min)	NA*	/	NA*	/	NA*	/	NA*	/
Biogas production rate (mL/L.day)	2167±180	2127±180	2093±232	2229±129	2072±102	2015±75	1953±97	1787±57
Biogas composition (%):								
CH ₄	58.2±3.4	60.3±3.0	40.4±4.3	60.6±1.8	44.9±2.3	60.9±1.0	52.0±1.9	62.5±0.3
CO ₂	41.8±3.4	39.7±3.0	14.9±3.2	39.4±1.8	18.5±3.2	39.1±1.0	17.0±0.7	37.5±0.3
H ₂	NA*	/	44.6±6.7	/	36.6±1.9	/	31.0±1.9	/
CH ₄ production rate (mL/L.day)	1255±54	1277±61	1528±147	1350±74	1497±73	1227±53	1471±72	1117±39
CO ₂ in output gas (mL/L.day)	912±148	850±134	565±115	878±73	618±55	789±33	482±34	670±19

H ₂ flow rate (mL/L.day)	NA*	/	3477±594	/	2636±89	/	2629±93	/
H ₂ consumption rate (mL/L.day)	NA*	/	1769±330	/	1412±212	/	1756±121	/
pH	7.46±0.03	7.49±0.06	7.92±0.11	7.59±0.09	7.90±0.06	7.60±0.05	7.93±0.12	7.56±0.09
Total VFA (g/L)	1.69±0.37	1.21±0.25	3.40±0.31	1.41±0.28	3.60±0.23	2.26±0.11	2.81±0.46	2.37±0.32
Acetate content in VFA (%)	41.3±4.3	49.0±3.9	55.3±4.0	51.5±3.8	51.8±2.3	47.3±3.7	49.7±3.8	47.2±4.2

*NA: not applicable to this period

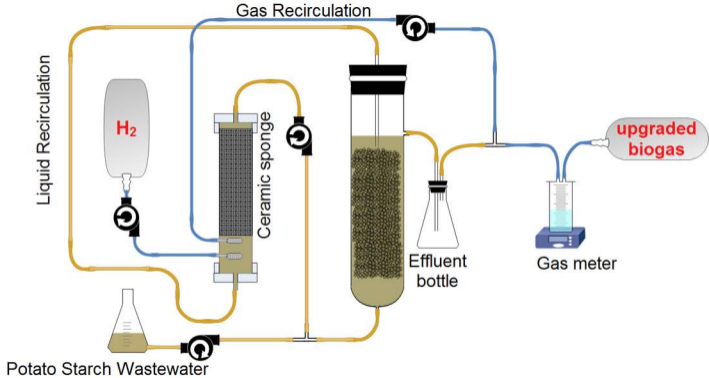
Table 2

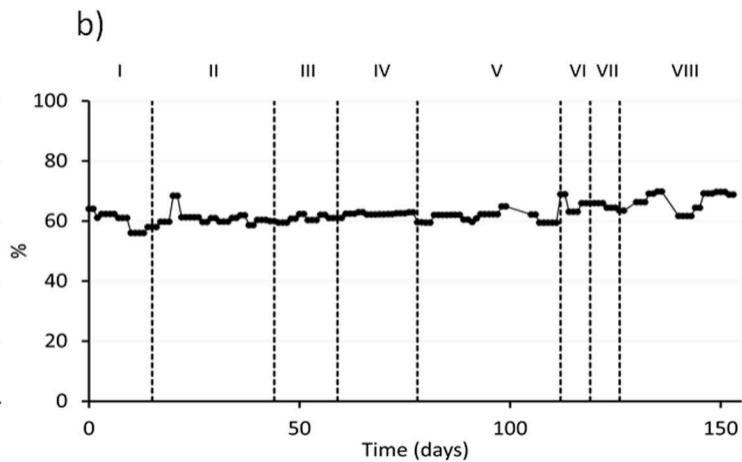
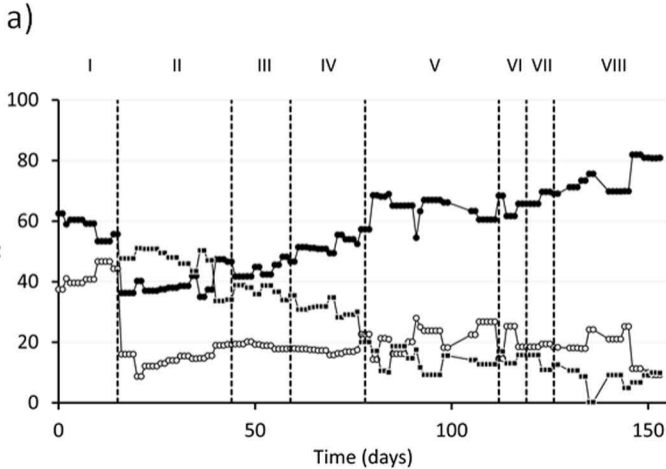
Phase	In-situ							
Period	V		VI		VII		VIII	
H ₂ distribution device	ceramic sponge		ceramic sponge		serial chambers		single chamber with extended length	
Reactor	R1	R2	R1	R2	R1	R2	R1	R2
Liquid recirculation flow (L/h)	7	7	7	7	7	7	7	7
Gas recirculation flow (mL/min)	4	/	6	/	6	/	6	/
Biogas production rate (mL/L.day)	1786±68	1900±85	1521±98	2018±275	1337±72	1175±138	1261±157	1558±188
Biogas composition (%):								
CH ₄	66.4±1.9	61.1±1.2	66.0±2.5	65.0±2.4	67.6±2.0	65.0±1.0	81.3±0.6	66.7±2.8
CO ₂	20.5±4.0	38.9±1.2	18.35±3.9	35.0±2,4	18.8±0.5	35.0±1.0	10.2±1.0	33.2±2.8
H ₂	13.0±4.3	/	15.7±1.4	/	13.5±2.4	/	8.5±1.5	/
CH ₄ production rate (mL/L.day)	1365±52	1161±55	1188±55	1308±149	1046±57	763±92	1145±134	1039±121

CO ₂ in output gas (mL/L.day)	421±65	740±47	333±82	710±134	291±16	412±48	121±21	615±83
H ₂ flow rate (mL/L.day)	2144±312	/	1834±30	/	1768±55	/	1828±14	/
H ₂ consumption rate (mL/L.day)	1873±234	/	1551±44	/	1536±80	/	1717±23	/
pH	7.83±0.10	7.64±0.07	8.24±0.20	7.85±0.12	8.18±0.08	7.92±0.07	8.38±0.07	7.99±0.09
Total VFA (g/L)	5.11±0.06	3.24±0.48	3.66±0.97	2.37±0.27	4.34±0.40	3.21±0.39	3.87±0.40	2.36±0.15
Acetate content in VFA (%)	64.6±3.4	46.0±4.7	39.9±2.6	39.4±4.3	37.0±2.2	36.5±2.9	30.3±1.4	34.5±6.5

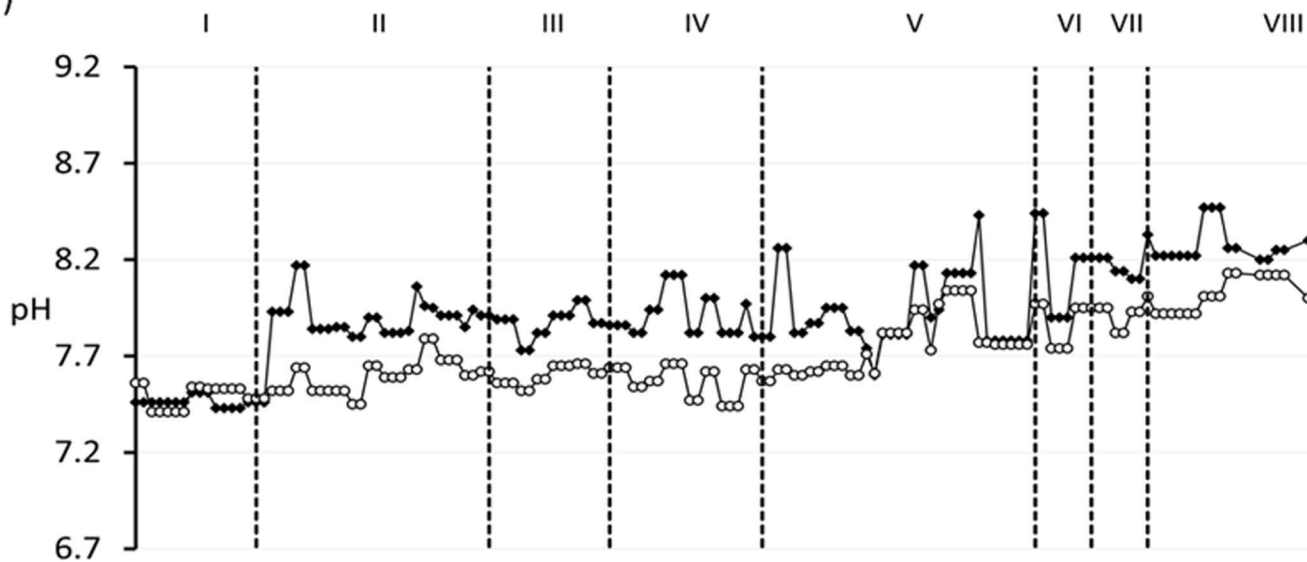
Table 3

Period	IV		V	
Reactor	R1	R2	R1	R2
Blank	36±2	11±2	6±1	7±1
Glucose	589±67	219±6	73±22	23±12
Acetate	159±4	4±1	4±1	3±2
H ₂ /CO ₂	1270±20	1296±29	986±212	520±65





a)



b)

