

1 **Photosynthetic biogas upgrading to bio-methane: boosting nutrient**
2 **recovery via biomass productivity control.**

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15

16 **Abstract.**

17 A pilot high rate algal pond (HRAP) interconnected to an external CO₂-H₂S absorption
18 column via settled broth recirculation was used to simultaneously treat a synthetic
19 digestate and to upgrade biogas to a bio-methane with sufficient quality to be injected
20 into natural gas grids. An innovative HRAP operational strategy with biomass
21 recirculation based on the control of algal-bacterial biomass productivity (2.2, 4.4 and
22 7.5 g m⁻² d⁻¹) via settled biomass wastage was evaluated in order to enhance nutrient
23 recovery from digestate at a constant hydraulic retention time. The influence of the
24 recycling liquid to biogas (L/G) ratio on the quality of the upgraded biogas was
25 assessed. The bio-methane composition under a L/G ratio of 1 (0.4 ± 0.1% CO₂, 0.03 ±
26 0.04% O₂, 2.4 ± 0.2% N₂ and 97.2 ± 0.2% CH₄) complied with the technical
27 specifications of most European bio-methane legislations regardless of the biomass
28 productivity established. The HRAP operational strategy applied allowed increasing the
29 N and P recovery from 19 and 22% to 83 and 100%, respectively, when the biomass
30 productivity was increased from 2.2 to 7.5 g m⁻² d⁻¹. Finally, the dynamics of
31 microalgae and bacteria population structure were characterized by morphological
32 identification and DGGE analysis.

33

34 **Keywords:** Biogas upgrading; bio-methane; microalgae-based processes; nutrients
35 recovery; wastewater treatment.

36

37 **Highlights:**

- 38 • A removal of CO₂ and H₂S from biogas higher than 99% was achieved.
- 39 • A low L/G ratio prevented O₂ and N₂ contamination of the upgraded biogas.
- 40 • The bio-methane complied with EU legislation for injection into natural gas
41 grids.
- 42 • A novel HRAP operation based on biomass productivity control was developed.
- 43 • This operation strategy allowed maximizing nutrient recovery from digestate.
- 44

45 **Introduction.**

46 Anaerobic digestion offers a cost-effective and environmentally feasible solution for
47 organic waste management while contributing to satisfy the global demand for
48 renewable energy via biogas production. In this context, the annual biogas production in
49 the European Union accounted for ~13.4 Mtoe in 2013 [1]. Biogas is composed mainly
50 of methane (CH₄) (40-75%), carbon dioxide (CO₂) (25-50%), hydrogen sulfide (H₂S)
51 (0.005-2%) and ammonia (NH₃) (<1%). Other gases such as hydrogen (H₂), nitrogen
52 (N₂), oxygen (O₂) and halogenated hydrocarbons are also present in raw biogas at lower
53 concentrations [2]. The concentration of these biogas pollutants depends on the
54 composition of the initial organic substrate and the type of anaerobic digestion process.
55 The H₂S present in biogas corrodes metal parts, reduces the durability of the motors and
56 generates hazardous sulfur dioxide when biogas is combusted for the generation of heat
57 and electricity. Likewise, CO₂ reduces the specific calorific value of biogas and
58 increases carbon monoxide and hydrocarbon emissions during combustion. Therefore,
59 these biogas pollutants must be previously removed in order to comply with the
60 technical specifications for biogas to be used as a transport fuel or injected into natural
61 gas grids. Most international legislations for bio-methane, which is the most common
62 term to refer to the upgraded biogas, require concentrations of CH₄ ≥95%, CO₂ ≤2%, O₂
63 ≤0.3 % and negligible amounts of H₂S [3].

64

65 Conventional physical-chemical technologies such as water scrubbing, chemical
66 scrubbing and membrane separation are commonly applied for CO₂ removal from
67 biogas. However, these technologies often require a previous H₂S cleaning step such as
68 activated carbon adsorption or chemical scrubbing [4]. On the contrary, biological H₂S
69 removal technologies such as anoxic and aerobic biotrickling filters are not able to

70 remove CO₂ and present operational problems such as elemental sulfur accumulation
71 (and subsequent clogging of the packed-bed) and biogas contamination with O₂ and N₂
72 [5, 6]. In addition, the physical-chemical technologies capable of simultaneously
73 removing CO₂ and H₂S (for example chemical scrubbing with alkali aqueous solutions)
74 exhibit high operating costs and a significant environmental impact [7].

75

76 In this regard, microalgae-based processes have emerged as a competitive and
77 environmentally sustainable alternative for the simultaneous removal of CO₂ and H₂S
78 from biogas [8]. These processes are based on the fixation of CO₂ via photosynthesis by
79 microalgae and the oxidation of H₂S to sulfate by sulfur oxidizing bacteria using the
80 oxygen photosynthetically produced. Moreover, the anaerobic effluents produced on-
81 site can eventually support microalgae growth, thus reducing their associated treatment
82 costs and eutrophication potential [9]. In addition, the algal biomass generated during
83 the photosynthetic biogas upgrading process can be used as a feedstock for bio-fuel or
84 bio-fertilizer production [10, 11], provided that biomass production has been properly
85 maximized. However, the increase in pH and modification of metal ion speciation (e.g.
86 Ca²⁺, Mg²⁺ and Fe²⁺) in the cultivation broth induced by microalgae growth can
87 promote the abiotic removal of N and P by volatilization and precipitation, respectively
88 [12]. This abiotic nutrient removal mechanism contributes to a detrimental loss of
89 nutrients and causes a severe environmental impact derived from the indirect N₂O
90 emissions associated to NH₄⁺ stripping [13].

91

92 Several proof of concept studies of this innovative photosynthetic biogas upgrading
93 process coupled with nutrient removal from digestate have been recently conducted by
94 Bahr *et al.* [8], Serejo *et al.* [14] and Posadas *et al.*, [15] in a HRAP interconnected to an

95 external CO₂-H₂S absorption column (AC). However, while a complete H₂S removal
96 was always observed, CO₂ removal was low (<80%) and the upgraded biogas was
97 contaminated with N₂ and O₂ (stripped out from the cultivation broth), the latter
98 decreasing the CH₄ content in the upgraded biogas to ~80%. Therefore, the O₂ and N₂
99 content in the upgraded biogas represents nowadays the main limitation of this
100 technology to achieve a high quality bio-methane, which entails the need to explore new
101 operational strategies to minimize the desorption of these bio-methane pollutants from
102 the algal-bacterial broth. In addition, little attention has been also paid to the
103 optimization of nutrient recovery from digestates, which would enhance the
104 environmental sustainability of the photosynthetic biogas upgrading process.

105

106 This research aimed at optimizing both the photosynthetic biogas upgrading process and
107 nutrient recovery from digestate in an algal-bacterial HRAP interconnected to a biogas
108 absorption column via recirculation of the settled broth. A preliminary optimization of
109 the recycling liquid to biogas ratio was conducted in order to obtain a bio-methane with
110 sufficient quality to be injected into natural grids. Then, an innovative HRAP
111 operational strategy based on the control of algal-bacterial biomass productivity via
112 settled biomass wastage was evaluated in order to enhance nutrient recovery from a
113 synthetic digestate while producing a high quality bio-methane.

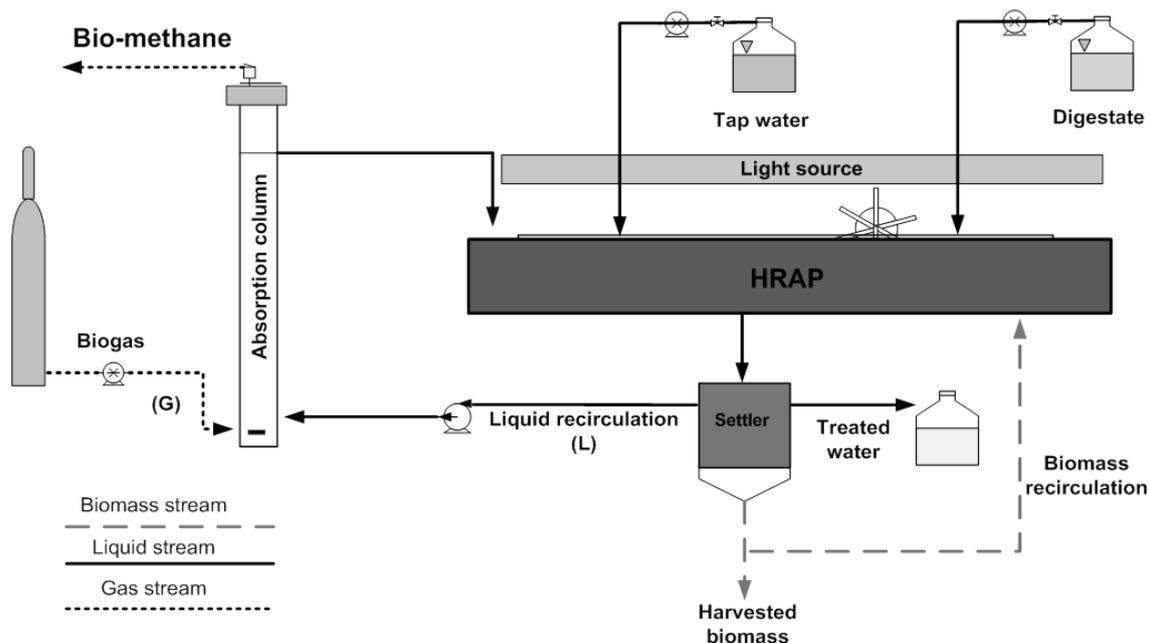
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115 **2. Materials and methods.**

116 **2.1 Experimental setup and operational conditions.**

117 The experimental setup, located at the Dept. of Chemical Engineering and
118 Environmental Technology at Valladolid University (Spain), consisted of a 180 L high
119 rate algal pond (170 cm length × 82 cm width × 15 cm depth) with an illuminated area

120 of 1.21 m², interconnected to a 8 L conical settler and to a 2.2 L absorption column (4.4
121 cm diameter, 165 cm height) via recirculation of the settled algal cultivation broth
122 (Figure 1). The HRAP was fed with a synthetic digestate at an influent flow rate of 1.3
123 ± 0.2 L m⁻² d⁻¹, continuously agitated at an internal liquid recirculation velocity of ≈20
124 cm s⁻¹, and illuminated with fluorescent lamps at 420 ± 105 μmol m⁻² s⁻¹ using 16:8 h
125 light:dark cycles. Tap water was supplied to compensate evaporation losses. The
126 composition of the synthetic digestate was (mg L⁻¹): ammonium (NH₄⁺) = 526 ± 132,
127 total nitrogen (TN) = 646 ± 61, total phosphorous (TP) as P-PO₄³⁻ = 53 ± 11, inorganic
128 carbon (IC) = 4458 ± 106 and sulfate (SO₄²⁻) = 317 ± 83. Digestates are characterized
129 by a high alkalinity and nutrient concentrations [16]. The effluent from the HRAP was
130 collected in the settler and the clarified effluent was then pumped to the bottom of the
131 AC at 1.6 m³ m⁻² h⁻¹ (flow rates referred to the AC cross sectional area) co-currently
132 with the biogas sparged (70% CH₄, 29.5% CO₂, 0.5% H₂S, Abello Linde (Barcelona,
133 Spain)) through a metallic diffuser at 1.6 m³ m⁻² h⁻¹. The liquid phase exiting the AC
134 was returned to the HRAP, while the excess of effluent from the system was removed
135 by overflow from the settling tank. This innovative photobioreactor configuration
136 allowed decoupling the hydraulic retention time from the algal bacterial biomass
137 productivity by controlling the rate of settled biomass wasted and returned to the HRAP.
138



139

140 **Figure 1.** Schematic diagram of the experimental setup used for the continuous
 141 upgrading of biogas coupled to digestate treatment.

142

143 **2.2 Influence of the recycling liquid to biogas ratio on the quality of the upgraded**
 144 **bio-methane.**

145 L/G ratios ranging from 0.5 to 60 were tested in order to maximize CO₂ and H₂S
 146 removal while minimizing O₂ and N₂ desorption from the recycling liquid to the
 147 upgraded biogas. The synthetic biogas was sparged into the AC at 5.3, 16.0, and 31.5
 148 mL min⁻¹, while the external liquid recirculation rate was set at 15, 60, 120, 203, and
 149 315 mL min⁻¹ for each biogas flow rate tested. The AC was constantly fed with the
 150 algal-bacterial broth at a pH of 10 ± 0.3. The absorption system was allowed to stabilize
 151 for two times the AC hydraulic retention time (HRT) prior to the monitoring of the
 152 upgraded biogas composition by GC-TCD.

153

154 **2.3 Influence of biomass productivity on biogas upgrading and nutrient recovery.**

155 The HRAP was inoculated with a consortium of cyanobacteria/microalgae composed of
156 *Geitlerinema* sp. (61.5%), *Staurosira* sp. (1.5%) and *Stigeoclonium tenue* (37%) from a
157 previous culture grown in diluted centrate wastewater. The consortium was then
158 acclimated to the digestate for 40 days prior to the experiment start-up. A biomass
159 productivity of $2.2 \text{ g m}^{-2} \text{ d}^{-1}$ was set during stage I (days 0-77) by controlling the rate of
160 withdrawal of settled biomass based on the total suspended solids (TSS) concentration
161 in the settler. The biomass productivity was increased to $4.4 \text{ g m}^{-2} \text{ d}^{-1}$ during stage II
162 (days 78-159) and to $7.5 \text{ g m}^{-2} \text{ d}^{-1}$ during stage III (days 160-202). The latter
163 productivity was selected based on the maximum biomass productivity expected from
164 the TP daily fed into the HRAP (assuming a P biomass content of 1 % according to
165 Alcántara *et al.*, [17]). The experimental system was operated indoors for 202 days.
166 Liquid samples (100 mL) were collected twice a week from the digestate influent, the
167 treated digestate and the cultivation broth of the HRAP to monitor the pH and
168 concentration of IC, TN, NH_4^+ , nitrite (NO_2^-), nitrate (NO_3^-), phosphate (PO_4^{3-}), SO_4^{2-}
169 and TSS. The TSS concentration of the settled biomass was also determined twice a
170 week to control biomass productivity. The temperature and dissolved O_2 concentration
171 (DO) were monitored in-situ. Gas samples from the inlet and outlet of the biogas
172 absorption column were periodically drawn to monitor the concentrations of CO_2 , H_2S ,
173 O_2 , N_2 , and CH_4 . The inlet and outlet gas flow rates in the AC were also measured. An
174 aliquot of 50 ml of algal-bacterial biomass was taken in each steady state to characterize
175 the populations of microalgae and bacteria.

176

177 **2.4 Analytical procedures.**

178 Dissolved IC and TN concentrations were determined using a Shimadzu TOC-VCSH
179 analyzer (Japan) equipped with a TNM-1 chemiluminescence module. NH_4^+ was

180 measured using an ammonia electrode Orion Dual Star (Thermo Scientific, The
181 Netherlands). NO_3^- , NO_2^- , PO_4^{3-} and SO_4^{2-} concentrations were analyzed by HPLC-IC
182 according to Serejo *et al.*, [14]. TSS analyses were carried out according to Standard
183 Methods [18]. The pH in the cultivation broth was monitored with a pH meter Eutech
184 Cyberscan pH 510 (Eutech instruments, The Netherlands). The light intensity at the
185 HRAP surface was measured with a LI-250A light meter (LI-COR Biosciences,
186 Germany). The C and N contents of the algal-bacterial biomass were determined using a
187 CHNS analyser (LECO CHNS-932), while P and S contents were determined using an
188 Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES, Varian 725-ES)
189 after microwave-acid digestion [19]. The biogas CO_2 , H_2S , O_2 , N_2 , and CH_4
190 concentrations were analyzed by GC-TCD according to Posadas *et al.*, [15]. The
191 morphological identification of microalgae was carried out by microscopic observations
192 (OLYMPUS IX70, USA) after sample fixation with 5% of lugol acid. The bacterial
193 community determination was conducted by DGGE-sequencing according to Posadas *et*
194 *al.*, [15] and the sequences were deposited in GenBank Data Library under accession
195 numbers KU605583-KU605606.

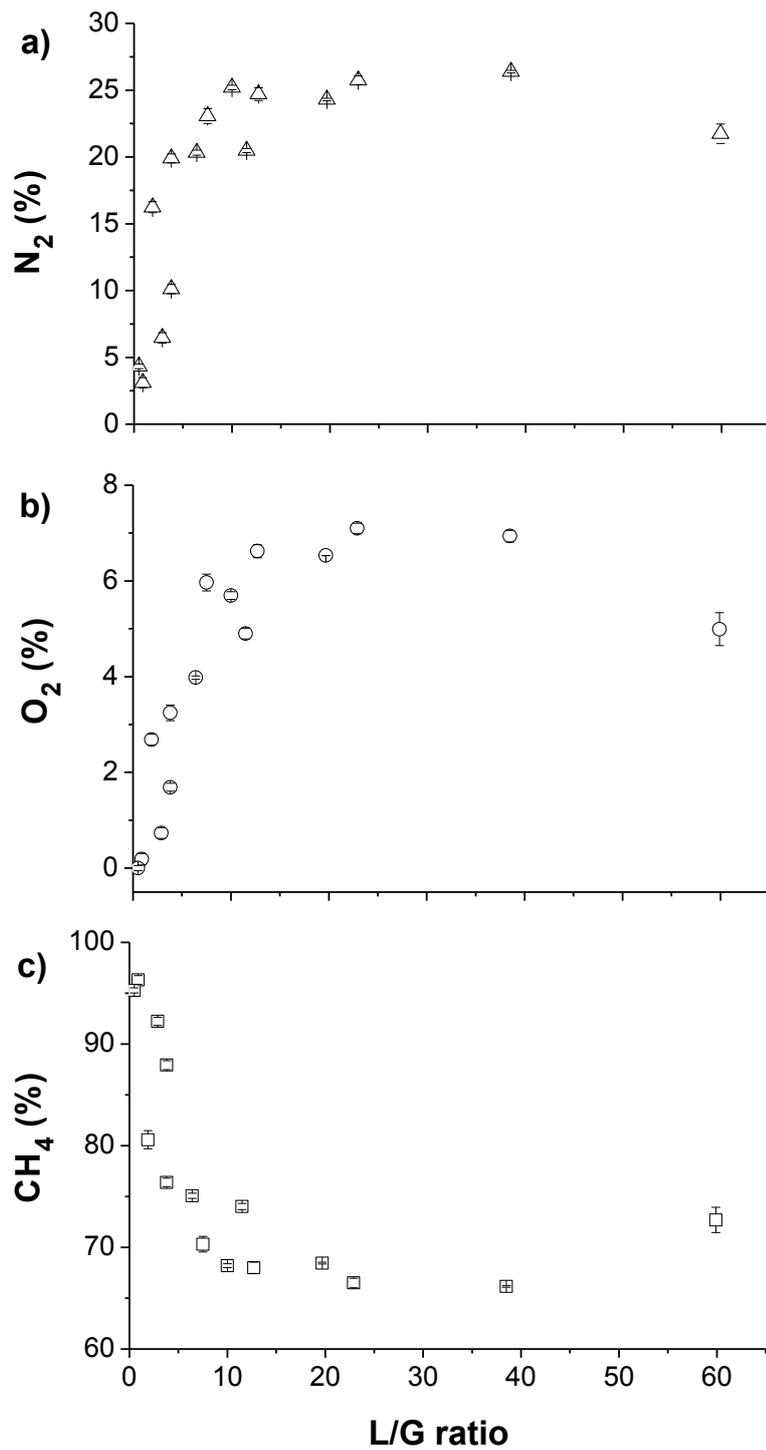
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197 **3. Results and discussion.**

198 **3.1 Influence of the recycling liquid to biogas ratio on the quality of the upgraded** 199 **biogas.**

200 The performance of the photosynthetic biogas upgrading process can be optimized by
201 determining the optimum L/G ratio in order to prevent O_2 and N_2 desorption while
202 boosting the absorption of CO_2 and H_2S . CO_2 mass transfer from the biogas is a
203 function of pH, CO_2 concentration, temperature, pressure and ionic strength of the
204 recycling algal-bacterial broth. Since H_2S and CO_2 are acidic gases, a more efficient

205 absorption of these biogas pollutants would be expected at a high pH. In our particular
206 study, the pH of the algal-bacterial broth was 10, which supported CO₂ and H₂S
207 removal efficiencies (REs) of 98.8 ± 0.19% and 97.1 ± 1.4%, respectively, regardless of
208 the L/G ratio tested. However, the N₂ and O₂ stripped from the cultivation broth
209 increased linearly at increasing the L/G ratio, to finally stabilize at 25% and 7%,
210 respectively (Figures 2a and 2b). In contrast to the results here obtained, Serejo *et al.*,
211 [14] reported a stabilization in the CO₂-REs at 95 ± 2% at L/G ratios above 15 (likely
212 due to the relatively low pH of the cultivation broth ≈7.9), with a maximum O₂
213 concentration in the upgraded biogas of 3 ± 1%. The higher N₂ and O₂ concentrations
214 here observed were likely due to the increase in the overall mass transfer coefficients in
215 the AC as a result of the higher ionic strength of the cultivation broth (IC =2300 mg L⁻¹),
216 which prevented the coalescence of the fine bubbles produced by the diffuser. This
217 increased contamination of the upgraded biogas at increasing L/G ratios resulted in a
218 concomitant decrease in CH₄ concentration from 95% at a L/G of 1 down to 68% at L/G
219 >15. Similar results were reported by Posadas *et al.* [15], who despite the high CO₂ and
220 H₂S REs obtained, observed a decrease in the final CH₄ concentration down to 81 ± 2%
221 as a result of a high N₂ content in the upgraded biogas. Hence, a L/G ≤1 resulted in CH₄
222 concentrations over 95% (Figure 2c), and in H₂S, CO₂, O₂ and N₂ concentrations lower
223 than 0.007%, 0.4%, 0.2% and 3%, respectively, which complied with most European
224 bio-methane legislations. Therefore, the recycling liquid to biogas ratio was identified
225 as a key operating factor determining the final quality of the upgraded biogas.



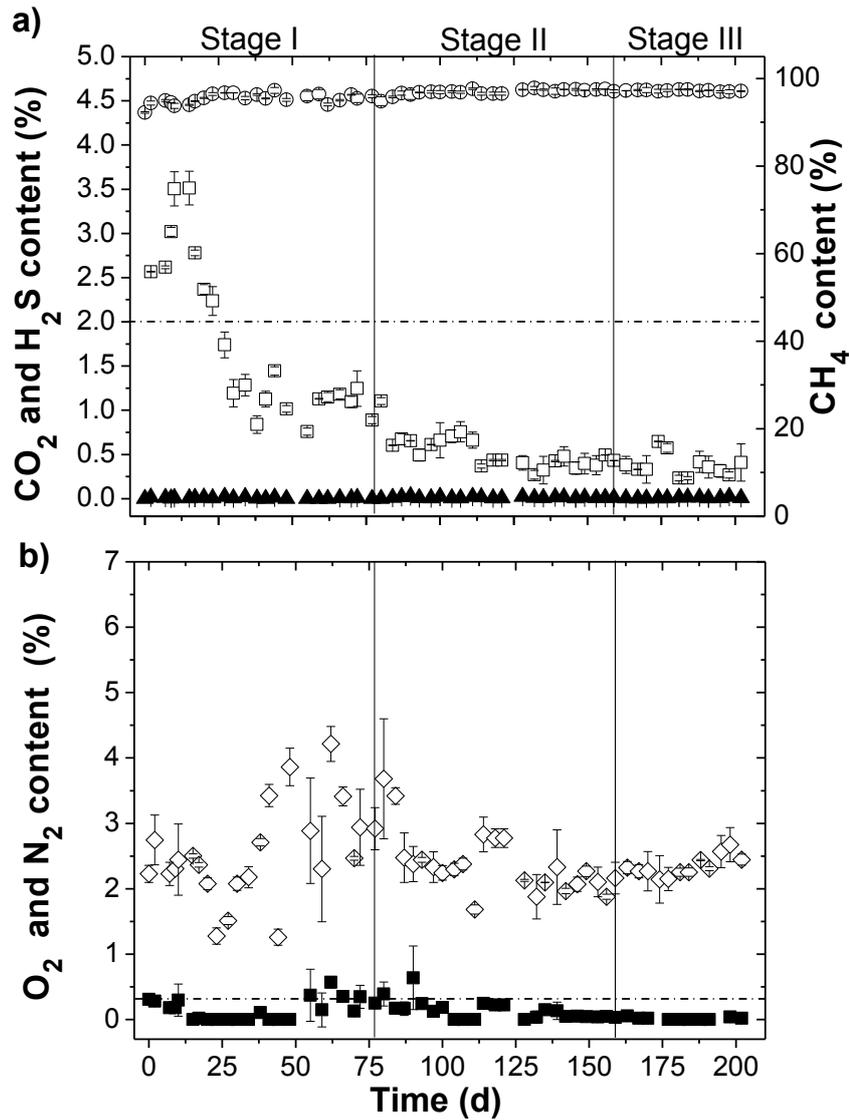
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227 **Figure 2.** Influence of the recycling liquid to biogas ratio on the concentrations of (a)
 228 N₂, (b) O₂ and (c) CH₄ in the upgraded biogas. Vertical bars represent the standard
 229 deviation from replicate measurements.

230

231 **3.2 Influence of biomass productivity on biogas upgrading to bio-methane.**

232 The CO₂ content in the bio-methane gradually decreased during stage I from 3.5% to
233 1.2% (Figure 3a), concomitantly with the increase in the pH of the cultivation broth in
234 the HRAP up to 9.1 ± 0.1 due to microalgal photosynthetic activity (Table 1). These
235 results confirmed that the high CO₂-REs here recorded significantly depended on the pH
236 of the cultivation broth. In spite of the high CO₂ absorption recorded in the AC, only a
237 slight decrease in the pH (0.1-0.3 gradient) from the bottom to the top of the AC was
238 observed. These results were not in agreement with those reported by Meier *et al.*, [20]
239 using a similar two-stage system, who observed a pH gradient of ~1-2 along the column
240 depending on the cultivation broth recycling rate. This difference was attributed to the
241 high buffer capacity of the digestate used in this study for microalgae growth. The
242 average steady CO₂-REs obtained in stages I, II and III were $96.6 \pm 1.2\%$, $98.4 \pm 0.8\%$
243 and $99 \pm 0.3\%$, respectively (Figure 4). The increase in the pH of the cultivation broth
244 from 9.1 to 10.6, likely mediated by the increase in the overall photosynthetic activity
245 (which itself was induced by the increase in biomass productivity), supported the higher
246 CO₂-REs recorded. These values were higher than those recorded by Bahr *et al.* [8] (RE
247 = $86 \pm 5\%$) using a similar experimental setup operated with a highly carbonated
248 mineral salt medium at a pH of 9.4 and at a L/G ratio of 1. On the other hand, despite
249 similar CO₂-REs (97%) from a synthetic biogas containing 41% of CO₂ were reported
250 by Mann *et al.* [21], contamination of the upgraded biogas with up to 23.4% of O₂ was
251 also observed in their study.
252

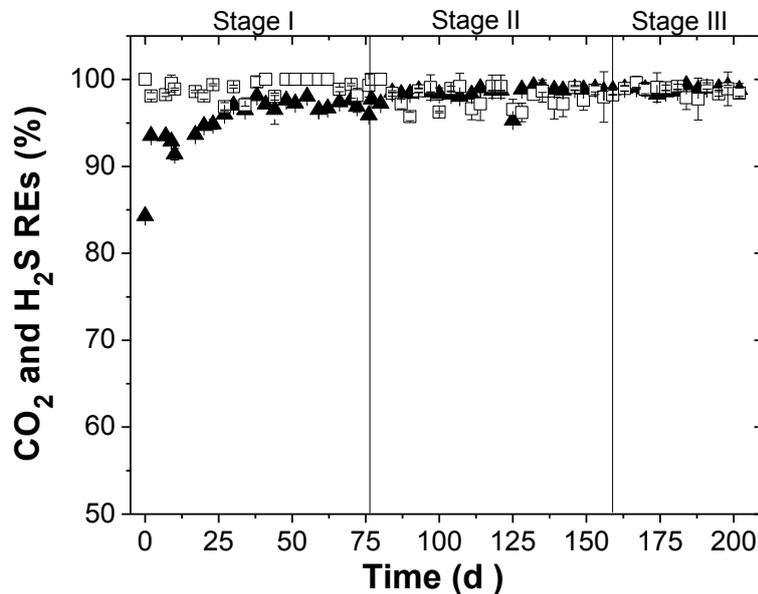


253 **Figure 3.** Time course of the concentration of (a) CO₂, (□) H₂S (▲) and CH₄ (○), and
 254 b) oxygen (■) and nitrogen (◇) in the upgraded biogas. The horizontal dashed lines
 255 indicate the maximum CO₂ and O₂ concentrations required for bio-methane injection
 256 into natural gas grids. Vertical bars represent standard deviation from replicate
 257 measurements.

258

259 An almost complete H₂S removal was recorded regardless of the biomass productivity
 260 set: $99.0 \pm 1.0\%$, $98.0 \pm 1.2\%$ and $98.5 \pm 1.0\%$ in stages I, II and III, respectively
 261 (Figure 4), which was in accordance with those reported by Serejo *et al.* [14] and
 262 Posadas *et al.* [15]. Sulfate formation was observed as a result of the biological H₂S

263 oxidation. At this point it must be highlighted that no oxygen limitation occurred
 264 throughout the entire experimentation. Dissolved oxygen concentration increased from
 265 $5.4 \pm 0.8 \text{ mg O}_2 \text{ L}^{-1}$ in stage I to $9.6 \pm 0.4 \text{ mg O}_2 \text{ L}^{-1}$ in stage III as a result of the
 266 increase in microalgae productivity. The sulfate concentrations during stages I, II and III
 267 were $388 \pm 43 \text{ mg-SO}_4^{2-} \text{ L}^{-1}$, $483 \pm 24 \text{ mg-SO}_4^{2-} \text{ L}^{-1}$ and $386 \pm 52 \text{ mg-SO}_4^{2-} \text{ L}^{-1}$,
 268 respectively. The decrease observed during stage III was attributed to the increase in
 269 biomass productivity (sulfate assimilation into biomass). The sulfur mass balance
 270 revealed that only 40% of the sulfur removed was oxidized to sulfate, the remaining
 271 60% being likely present (dissolved or in suspension) as S-intermediates such as S^0 ,
 272 thiosulfate or sulfite. Partial oxidation of the H_2S transferred from biogas has been
 273 previously reported [22], however a further analysis of the sulfur compounds present in
 274 the cultivation broth is necessary in order to elucidate the fate of the H_2S removed.
 275



276
 277 **Figure 4.** Time course of the removal efficiencies of CO_2 (▲) and H_2S (□). Vertical
 278 bars represent standard deviation from replicate measurements.
 279

280 The biological oxidation of CH₄ resulted in average CH₄ losses of $4.9 \pm 2.4\%$ (on a
281 mass basis) during stage I, no methane losses being recorded afterwards. The CH₄
282 content in the upgraded biogas was $95.8 \pm 0.8\%$, $96.9 \pm 0.7\%$ and $97.2 \pm 0.2\%$ in stages
283 I, II and III, respectively (Figure 3a). These values are comparable to those achieved by
284 water scrubbing technologies, where CH₄ losses by dissolution in the pressurized water
285 of 3-5% result in CH₄ purities of 80-99%, depending on the N₂ and O₂ content of the
286 upgraded biogas [23].

287

288 The O₂ demand in the absorption column resulting from the biological oxidation of H₂S
289 caused an oxygen content in the upgraded biogas of $0.1 \pm 0.2\%$ in stages I and II and
290 $0.03 \pm 0.04\%$ in stage III (Figure 3b). These O₂ concentrations recorded in the bio-
291 methane were lower than the values obtained by Meier *et al.* [20] (1.2%) and Posadas *et*
292 *al.*, [15] (0.7-1.2%), and remained significantly below those reported in literature during
293 biogas upgrading in algal photobioreactors (10-24%) [21, 24]. Finally, the N₂ stripped
294 out from the recycling cultivation broth resulted in average concentrations of $2.6 \pm$
295 0.9% , $2.4 \pm 0.5\%$ and $2.4 \pm 0.2\%$ during stages I, II and III, respectively (Figure 3b),
296 due to the low L/G ratio applied in this study (which limited the amount of N₂
297 potentially desorbed). Higher N₂ concentrations in the upgraded biogas of 6-8% were
298 recorded by Posadas *et al.*, [15] and Serejo *et al.*, [14] during photosynthetic biogas
299 upgrading at a L/G of 10. In this context, the optimum bio-methane composition was
300 obtained at the highest microalgae productivity evaluated ($0.4 \pm 0.1\%$ CO₂, $0.03 \pm$
301 0.04% O₂, $2.4 \pm 0.2\%$ N₂ and $97.2 \pm 0.2\%$ CH₄), which complied with the regulatory
302 limits of most European legislations for bio-methane injection in natural gas grids
303 (Figures 3a and 3b). For instance, the injection of bio-methane into the Spanish network

304 allows up to 0.3% of O₂ provided that CO₂ concentration does not exceed 2% and CH₄
305 concentration remains over 95% [25].

306

307 **3.3 Influence of biomass productivity on nutrient removal and nutrient recovery.**

308 Most recent life cycle analyses have shown that the use of wastewater as a low-cost
309 nutrients and water source can reduce the overall energy requirements and improve the
310 environmental sustainability of microalgae mass production [26, 27]. In our particular
311 case, microalgae production using the N and P present in anaerobically digested
312 wastewaters can significantly decrease the operating costs of the biogas upgrading
313 process, while preserving fresh water resources and recovering these nutrients in the
314 form of a microalgae biomass that can be further valorized as a bio-fertilizer. Despite
315 the potential of microalgal biotechnology to fix nutrients from digestates, abiotic
316 removal still represents an important mechanism for nutrient removal from wastewater
317 in algal-bacterial processes. Thus, N removal by stripping can account for up to 82%
318 [28] and P removal by precipitation for up to 63% [29] of the total nutrients supplied.
319 Nonetheless, the monitoring of this abiotic nutrient removal in HRAPs is often
320 disregarded [12].

321

Table 1. Average dissolved oxygen concentration, pH, temperature, total suspended solid concentration and biomass productivity recorded during the three operational stages.

Stage	T _{HRAP} (°C)	PH _{HRAP}	DO (mg O ₂ L ⁻¹)	TSS HRAP (g L ⁻¹)	Productivity (g m ⁻² d ⁻¹)
I	22 ± 3	9.1 ± 0.1	5.4 ± 0.8	1.6 ± 0.1	2.2 ± 1.4
II	25 ± 2	9.6 ± 0.3	7.5 ± 1.4	1.2 ± 0.4	4.4 ± 1.5
III	28 ± 1	10.6 ± 0.1	9.6 ± 0.4	0.9 ± 0.1	7.5 ± 0.1

322

323 The high buffer capacity of the cultivation broth as a result of the high IC
324 concentrations present in the digestate and the high water evaporation losses, together
325 with the high photosynthetic activity in the system, maintained high pH values during
326 the three operational stages without an automatic pH control (Table 1). The temperature
327 of the algal–bacterial broth slightly increased concomitantly with the seasonal variation
328 of the ambient temperature, but remained close to optimum values for microalgae and
329 bacteria cultivation. Apart from the impinging radiation, other variables such as the
330 nutrients load (determined by the flow rate and nutrients concentration of the target
331 wastewater) and biomass concentration in the cultivation broth (determining light
332 penetration) influence microalgae productivity in HRAPs devoted to wastewater
333 treatment. For instance, low biomass concentrations ($\sim 0.5 \text{ g L}^{-1}$) are typically
334 encountered in open ponds treating domestic wastewaters at HRTs of 5-10 days.
335 Biomass productivity can be thus boosted by increasing the nutrients load into the
336 HRAPs, provided that light supply does not limit the process. However, while an
337 increase in wastewater flow rate might induce microalgae washout, the use of
338 wastewaters with high nutrient concentrations (such as digestates) would entail very
339 dense microalgae cultures, which would ultimately limit microalgae productivity as a
340 result of an excessive mutual shading. In this context, the decoupling between the
341 hydraulic retention and biomass retention time (inversely related to microalgae
342 productivity) represents an innovative strategy for maximizing biomass productivity
343 during microalgae cultivation in high-strength wastewaters. The control of biomass
344 productivity via regulation of the settled biomass wastage rate would allow maximizing
345 nutrient recovery from wastewaters. A TSS concentration of $1.6 \pm 0.1 \text{ g L}^{-1}$ was
346 recorded in the HRAP when operating at an average productivity of $2.2 \text{ g m}^{-2} \text{ d}^{-1}$ in
347 stage I. This TSS concentration decreased to $1.2 \pm 0.4 \text{ g L}^{-1}$ and $0.9 \pm 0.1 \text{ g L}^{-1}$ under

348 operation at $4.4 \text{ g m}^{-2} \text{ d}^{-1}$ and $7.5 \text{ g m}^{-2} \text{ d}^{-1}$, respectively. The results clearly showed that
 349 an increase in the rate of biomass wastage from the settler resulted in lower TSS
 350 concentrations, which likely improved the overall photosynthetic efficiency as a result
 351 of an enhanced light penetration. In addition, the control of biomass productivity was
 352 supported by the good settling properties of the algal-bacterial biomass present in the
 353 HRAP. However, a decrease in the TSS removal efficiency of the settler from $95 \pm 3\%$
 354 in stage I to $84 \pm 4\%$ in stage III was recorded, which was attributed to the shift in
 355 microalgae population observed in stage II (see section 3.4). Unfortunately, the effluent
 356 TSS concentrations ($70 \pm 50 \text{ mg L}^{-1}$) remained always over the maximum discharge
 357 limit in European Union legislation (35 mg L^{-1}) [30].
 358

Table 2. Average removal efficiencies of total nitrogen, ammonium, phosphorus, inorganic carbon and total suspended solids recorded during the three operational stages.

Stage	Removal efficiencies (%)				
	TN	N-NH ₄ ⁺	P-PO ₄ ³⁻	IC	TSS
I	91 ± 4	100	77 ± 16	86 ± 6	95 ± 3
II	92 ± 4	100	63 ± 18	78 ± 10	91 ± 10
III	98 ± 2	100	73 ± 19	70 ± 9	84 ± 4

359
 360 A complete removal of ammonium was observed during all stages, while TN-REs
 361 increased from $91 \pm 4\%$ up to $98 \pm 2\%$ when biomass productivity increased from 2.2 to
 362 $7.5 \text{ g m}^{-2} \text{ d}^{-1}$ (Table 2). Despite the slight influence of biomass productivity on TN-REs,
 363 the share of the inlet TN assimilated into biomass varied from $19 \pm 13\%$ at the lowest
 364 microalgae productivity to $83 \pm 9\%$ at the highest productivity (Table 3). In this context,
 365 the low nitrification activity recorded along with the high pH value supported a
 366 significant N-NH₄⁺ removal by stripping, which decreased from $75 \pm 12\%$ in stage I to
 367 $13 \pm 9\%$ in stage III. On the other hand, phosphorus removal remained stable regardless
 368 of the biomass productivity set, with REs of $77 \pm 16\%$, $63 \pm 18\%$ and $73 \pm 19\%$ in

369 stages I, II and III, respectively. Serejo *et al.* [14] recorded similar phosphorous REs (71
 370 $\pm 3\%$) at a comparable biomass productivity ($7.1 \pm 0.8 \text{ g m}^{-2} \text{ d}^{-1}$) during the treatment of
 371 anaerobically digested vinasse coupled to biogas upgrading. Similar to the share of TN
 372 assimilated, the increase in biomass productivity resulted in an increase in the
 373 contribution of P assimilation to the TP removal from $22 \pm 12\%$ to 100%. The absence
 374 of PO_4^{3-} volatilization, together with the high pH prevailing in the cultivation broth
 375 throughout the entire experimental period, suggested that precipitation was the main
 376 phosphorous removal mechanism under low biomass productivities. Therefore, the
 377 control of biomass productivity via regulation of the biomass wastage rates allowed
 378 maximizing nutrient recovery in the form of algal biomass in detriment of the abiotic
 379 nutrients removal mechanisms.

380

Table 3. Nutrient recovery via biomass assimilation estimated from the nutrient removed and the elemental composition and mass flow rate of the biomass harvested during the three operational stages.

Stage	Nutrient recovery as biomass (%)			Biomass elemental composition (%)		
	C	P	N	C	P	N
I	6 ± 3	22 ± 12	19 ± 13	43.6	0.7	6.5
II	16 ± 5	50 ± 19	36 ± 18	46.5	0.8	7.2
III	30 ± 1	100	83 ± 9	48.0	0.9	6.7

381

382 **3.4 Consortia of cyanobacteria/microalgae and bacteria.**

383 The microalgae and cyanobacteria species initially present in the inoculum were
 384 gradually replaced along the three operational stages. The cyanobacterium prevailing in
 385 the inoculum (*Geitlerinema* sp.) was not observed under steady state conditions in
 386 stages I, II and III. Thus, the cyanobacteria/microalgae consortium was mainly
 387 composed of *Limnithrix planktonica* (32.9%), *Acutodesmus obliquus* (2.6%), *Chlorella*
 388 *vulgaris* (2.6%), *Mychonastes homosphaera* (5.9%), *Navicula* sp. (0.7%), *Phormidium*
 389 sp. (19.7%) and *Stigeoclonium tenue* (35.5%) during stage I. This high diversity was

390 similar to that reported in indoor HRAP treating digestates [14, 15]. Surprisingly, this
391 high microalgae diversity disappeared in stage II with the establishment of an unialgal
392 culture of the Chlorophyta *Mychonastes homosphaera*. This unialgal culture remained
393 dominant throughout stage III likely due to the extreme environmental conditions
394 prevailing in this study (high pH and salinity as a result of the high water evaporation
395 losses). In addition, the control of biomass productivity via regulation of the settled
396 biomass wastage rate applied might have also influenced the dominant species and
397 algal/bacterial ratio since the increase in biomass productivity likely induced the
398 development of fast growing microorganisms. *Mychonastes homosphaera* (Skuja)
399 Kalina & Puncochárová is currently regarded as a taxonomic synonym of *Chlorella*
400 *minutissima* Fott & Nováková. The potential of this microalga for wastewater treatment
401 [31], heavy metal removal [32] and biodiesel production has been consistently
402 demonstrated, *Mychonastes homosphaera* being capable of storing a desirable fatty acid
403 profile under nitrogen starvation [33]. The valorization of this microalga into high-
404 added value chemicals or biofuels such as syngas, bioethanol or bio-oil using a
405 biorefinery approach will certainly enhance the sustainability and economic viability of
406 microalgae-based biogas upgrading [34].

407 The high diversity revealed by microscopic observation was confirmed by the Shannon-
408 Wiener diversity indexes obtained, which ranged from 1.5 to 3.5 (Figure 1,
409 supplementary material). The slight decrease of this index from 3.2 in stage I to 2.9 in
410 stage II also confirmed the shift in algae diversity microscopically observed. Likewise,
411 the analysis of the Pearson similarity coefficients showed a high similarity between the
412 microbial communities present in stages II and III (99%), which was in agreement with
413 the above mentioned establishment of a dominant microalga specie. The DGGE analysis
414 (Figure 1, supplementary material) showed 24 bands, which were sequenced. Six

415 different phyla were retrieved from the RDP database: Cyanobacteria/Chloroplast (10
416 bands), *Acidobacteria* (4 bands), *Proteobacteria* (4 bands), *Deinococcus-thermus* (1
417 band), *Chloroflexi* (1 band), *Actinobacteria* (1 band) (Table 1, supplementary material).
418 The morphological identification of *Mychonastes homosphaera* was confirmed by
419 bands 8, 9 and 10, which belonged to the genus Chlorophyta and were related to
420 *Chlorella* species. The phyla *Acidobacteria* (bands 12 and 13), *Proteobacteria* (bands
421 15 and 16) and *Actinobacteria* (band 21) were found in the three operational stages,
422 while the phylum *Chloroflexi* was detected in the inoculum and stages II and III.
423 Bacteria from the genus *Blastocatella* (band 11) and the *Gammaproteobacteria* class
424 (band 15), which have been identified in activated sludge [35] and HRAPs treating
425 piggery wastewater [36], respectively, likely supported the aerobic biodegradation of
426 the organic matter and ammonia contained in the digestate. Finally, the identification of
427 the genus *Thioalbus* (band 16) confirmed the biological nature of H₂S oxidation [37].
428 To the best of our knowledge, this is the first time that sulfur-oxidizing bacteria
429 (facultative microorganisms that can use O₂ or NO₃⁻ as electron acceptors) have been
430 found in these photosynthetic biogas upgrading processes.

431

432 **3.5 Conclusions.**

433 This study confirmed the potential of photosynthetic biogas upgrading to support a cost-
434 efficient bio-methane production coupled to nutrient recovery from digestate. To the
435 best of our knowledge, this is the first experimental study reporting biological biogas
436 upgrading to a bio-methane complying with most European legislations for biogas
437 injection into natural gas grids. An almost complete removal of H₂S and CO₂, and
438 concentrations of O₂ and CH₄ in the upgraded biogas <0.1% and >95%, respectively,
439 were achieved regardless of the biomass productivity set. The innovative HRAP

440 operational strategy here developed allowed enhancing nutrient recovery by shifting
441 from an abiotic-based nutrients removal to an assimilatory-based removal. Furthermore,
442 the extreme cultivation conditions established in the HRAP expedited the dominance of
443 the microalga *Mychonastes homosphaera*, and supported the growth of sulfur-oxidizing
444 bacteria. The presence of sulfur-oxidizing bacteria from the genus *Thioalbus* confirmed,
445 for the first time, the biological nature of H₂S oxidation during biogas upgrading in
446 algal-bacterial photobioreactors.

447

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