

1 **Influence of the seasonal variation of environmental conditions on**
2 **biogas upgrading in an outdoors pilot scale high rate algal pond**

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11
12 **ABSTRACT**

13 The influence of the daily and seasonal variations of environmental conditions on the
14 quality of the upgraded biogas was evaluated in an outdoors pilot scale high rate algal
15 pond (HRAP) interconnected to an external absorption column (AC) via a conical
16 settler. The high alkalinity in the cultivation broth resulted in a constant biomethane
17 composition during the day regardless of the monitored month, while the high algal-
18 bacterial activity during spring and summer boosted a superior biomethane quality. CO₂
19 concentrations in the upgraded biogas ranged from 0.1% in May to 11.6% in December,
20 while a complete H₂S removal was always achieved regardless of the month. A limited
21 N₂ and O₂ stripping from the scrubbing cultivation broth was recorded in the upgraded
22 biogas at a recycling liquid/biogas ratio in the AC of 1. Finally, CH₄ concentration in
23 the upgraded biogas ranged from 85.6% in December to 99.6% in August.

24 **Keywords:** Algal-bacterial photobioreactor; Biogas upgrading; Biomethane; Outdoors
25 operation; Yearly evaluation.

26

27 **1. Introduction**

28 Biogas from the anaerobic digestion of wastewaters and organic waste constitutes a
29 renewable source of energy to generate electricity or heat (Muñoz et al., 2015).
30 However, the use of biogas as a substitute of natural gas or fuel in transportation
31 requires an effective purification to levels set by national regulations. For instance,
32 biogas injection into natural gas grids typically requires concentrations of $\text{CH}_4 \geq 95\%$,
33 $\text{CO}_2 \leq 2\%$, $\text{O}_2 \leq 0.3\%$ and trace levels of H_2S (Muñoz et al., 2015; Toledo-Cervantes et
34 al., 2017).

35

36 Algal-bacterial processes have emerged as a platform technology capable of
37 simultaneously removing CO_2 and H_2S in a single stage, and constitute a cost-effective
38 and environmentally friendly alternative to conventional biogas upgrading technologies
39 (Bahr et al., 2014; Muñoz et al., 2015). Biogas upgrading in algal-bacterial
40 photobioreactors is based on the oxidation of H_2S to SO_4^{2-} by sulfur oxidizing bacteria
41 promoted by the high dissolved oxygen (DO) concentrations in the scrubbing
42 cultivation broth, and on the photosynthetic fixation of the absorbed CO_2 by microalgae.
43 The economic and environmental sustainability of this biotechnology can be boosted via
44 digestate supplementation as a nutrient and water source, which will support an
45 effective recovery of nutrients in the form of algal-bacterial biomass (Posadas et al.,
46 2017; Toledo-Cervantes et al., 2016).

47

48 Biogas upgrading coupled to digestate treatment has been typically evaluated indoors in
49 high rate algal ponds (HRAPs) interconnected to biogas absorption columns (AC) under
50 artificial illumination (Alcántara et al., 2015; Bahr et al., 2014; Meier et al., 2015;

51 Posadas et al., 2016, 2015; Serejo et al., 2015; Toledo-cervantes et al., 2017; Toledo-
52 Cervantes et al., 2017, 2016). The optimization of this process has reached promising
53 results in terms of biomethane quality (CH₄ concentrations of 96.2±0.7 %), nutrient
54 removal (total nitrogen (TN)-removal efficiencies (REs) of 98.0±1.0 % and P-PO₄⁻³-
55 REs of 100±0.5 %) and biomass productivities (15.0 g m⁻² d⁻¹) (Toledo-Cervantes et al.,
56 2017). Comparable results were also obtained by Posadas et al. (2017) in a similar
57 biogas upgrading photobioreactor configuration operated outdoors during summer in
58 Spain, when solar irradiation, temperature and the number of sun hours were most
59 favorable to support algal-bacterial activity. In this context, a systematic year-round
60 evaluation of the influence of the daily and seasonal variations of environmental
61 conditions on biogas upgrading and nutrient recovery from digestate is needed to
62 validate this technology under outdoor conditions.

63

64 This study investigated for the first time the year-round performance of biogas
65 upgrading in an outdoors pilot HRAP interconnected to an external AC by monthly
66 monitoring the daily variations of biogas quality and cultivation broth parameters under
67 continental climate conditions.

68

69 **2. Materials and methods**

70 **2.1. Biogas and centrate**

71 A synthetic biogas mixture composed of CO₂ (29.5%), H₂S (0.5%) and CH₄ (70%) was
72 used as a raw biogas in the present study (Abello Linde; Spain). Centrate was monthly
73 obtained from the centrifuges dehydrating the anaerobically digested mixed sludge of
74 Valladolid wastewater treatment plant (WWTP) and stored at 4 °C. The composition of

75 centrate varied along the experimental period as a result of the seasonal operational
76 variations of the WWTP: total organic carbon (TOC) = 16-523 mg L⁻¹, inorganic carbon
77 (IC) = 450-600 mg L⁻¹, TN = 374-718 mg L⁻¹, P-PO₄³⁻ = 26-135 mg L⁻¹ and SO₄²⁻ = 0-
78 38 mg L⁻¹. The IC concentration in the centrate was adjusted to 1999 ± 26 mg L⁻¹ via
79 addition of NaHCO₃ and Na₂CO₃ in order to maintain the required high alkalinity and
80 pHs (≥9) in the cultivation broth to support an effective CO₂ and H₂S absorption in the
81 AC (Posadas et al., 2017).

82

83 **2.2. Experimental set-up**

84 The experimental set-up, constructed according to Posadas et al. (2017), was located
85 outdoors at the Department of Chemical Engineering and Environmental Technology of
86 Valladolid University (41.39° N, 4.44° W). The pilot plant consisted of a 180 L HRAP
87 with an illuminated area of 1.20 m² (width = 82 cm; length = 170 cm; depth = 15 cm)
88 and two water channels divided by a central wall and baffles in each side of the
89 curvature. The internal recirculation velocity of the cultivation broth in the HRAP was ≈
90 20 cm s⁻¹, which was supported by the continuous rotation of a 6-blade paddlewheel.
91 The HRAP was interconnected to an external 2.5 L bubble AC (height = 165 cm;
92 internal diameter = 4.4 cm) provided with a metallic biogas diffuser of 2 μm pore size
93 located at the bottom of the column. The HRAP and the AC were interconnected via an
94 external liquid recirculation of the algal-bacterial cultivation broth from an 8 L conical
95 settler (Fig. 1). **The efficiency of the settler in terms of biomass removal was almost**
96 **complete.**

97

<Figure 1>

98 **2.3. Operational conditions and sampling procedures**

99 Process operation was carried out from November the 1st 2016 to October the 30st 2017.
100 The HRAP was inoculated to an initial concentration of 210 mg TSS L⁻¹ with a
101 microalgae inoculum composed of *Leptolyngbya lagerheimii* (54%), *Chlorella vulgaris*
102 (28%), *Parachlorella kessleri* (9%), *Tetrademus obliquus* (5%) and *Chlorella*
103 *minutissima* (2%) from an indoor HRAP treating biogas and centrate at the Department
104 of Chemical Engineering and Environmental Technology of Valladolid University
105 (Spain). Five different operational stages (namely I, II, III, IV and V) were defined as a
106 function of the temperature, photosynthetic active radiation (PAR), number of sun hours
107 and biomass productivity imposed (Table 1). The synthetic biogas was sparged into the
108 AC under co-current flow operation at 74.9 L d⁻¹ under a recycling liquid to biogas ratio
109 (L/G) of 1.0 according to Posadas et al. (2017), which resulted in gas and liquid
110 retention time of 48 min and. The liquid velocity accounted for 2 m h⁻¹. The HRAP was
111 fed with IC-supplemented centrate as a nutrient source at a flow rate of 3.5 L d⁻¹, which
112 entailed a hydraulic retention time of 50 d. Tap water was supplied in order to
113 compensate water evaporation losses and allow process operation without effluent
114 (Table 1).

115 <Table 1>

116 The pH, temperature and DO concentration in the cultivation broth of the HRAP, AC
117 and settler, along with PAR, were monitored every thirty minutes during the daytime of
118 one day every month where the environmental conditions were representative of the
119 conditions in the entire month. Gas samples of 100 µL from the upgraded biogas were
120 drawn every hour to monitor the gas concentrations of CH₄, CO₂, H₂S, O₂ and N₂.
121 Liquid samples of 100 mL from the cultivation broth of the HRAP, AC and settler were
122 drawn every two hours to monitor the concentrations of dissolved TOC, IC, TN.

123

124 **2.4. Analytical procedures**

125 PAR was measured using a LI-250A light meter (LI-COR Biosciences, Germany),
126 while pH was determined with an Eutech Cyberscan pH 510 (Eutech instruments, The
127 Netherlands). Temperature and DO were measured using an OXI 330i oximeter (WTW,
128 Germany). Gas concentrations of CH₄, CO₂, H₂S, O₂ and N₂ were determined using a
129 Varian CP-3800 GC-TCD according to Posadas et al. (2015) (Palo Alto, USA).
130 Dissolved TOC, IC and TN concentrations were measured using a Shimadzu TOC-
131 VCSH analyzer (Japan) coupled with a TNM-1 chemiluminescence module.

132

133 **3. Results and discussion**

134 **3.1. Biogas Upgrading**

135

<Figure 2>

136 **3.1.1 CO₂ biomethane concentration**

137 Negligible variations in CO₂ concentration in the biomethane were recorded throughout
138 the daytime regardless of the operational month likely due to the high alkalinity of the
139 cultivation broth (Fig. 2; Fig. S6). These results were in agreement with Posadas et al.
140 (2017), who observed a constant CO₂ concentration in the upgraded biogas during the
141 daytime in a similar set-up operated with a high ionic strength cultivation broth (IC
142 concentration $\approx 2660 \pm 48$ mg L⁻¹). This study also suggested that the influence of the
143 cultivation broth temperature on CO₂ absorption (Henry's law constant ranged from
144 $H_{CO_2} \approx 1.27$ at 8.3 °C in November to $H_{CO_2} \approx 0.59$ at 40.3 °C in July) was lower than that
145 of the IC concentration (Sander, 2015). Hence, the biomethane CO₂ concentration in
146 stage I ranged from 1.4% in January to 11.6% in December. This concentration varied
147 from 0.1% in March to 3.9% in May during stage II, and from 0.6% in June to 2.2% in
148 July in stage III. CO₂ concentrations in stage IV and V ranged from 0.4% to 1.8% and

149 from 0.8% to 1.2%, respectively (Fig. 2). Thus, the concentration of CO₂ in the
150 biomethane produced in the algal-bacterial photobioreactor complied during most of the
151 year with European regulations, which require CO₂ concentrations ≤2% prior injection
152 into natural gas grids or use as a vehicle fuel (Muñoz et al., 2015). The high CO₂ REs
153 here obtained (estimated from ≈60.7% in December to 99.7% in May) were promoted
154 by the optimum L/G ratio reported by Posadas et al. (2017) and the high pHs/alkalinity
155 of the cultivation broth in the AC, which enhanced CO₂ absorption (Lebrero et al.,
156 2016; Posadas et al., 2015; Toledo-Cervantes et al., 2016). These results were in
157 accordance with Rodero et al. (2017), who reported an increase in the CO₂-RE from
158 30.8% to 99.3% when alkalinity increased from 102±7 mg IC L⁻¹ to 1581±135 mg IC L⁻¹
159 at 35.0°C in a similar photobioreactor configuration under indoor conditions.

160

161 This year-round evaluation of the performance of the algal-bacterial photobioreactor
162 confirmed the key role of biotic mechanisms on this biogas upgrading technology (Fig.
163 2). Hence, despite the low temperatures of the cultivation broth during winter increased
164 CO₂ aqueous solubility, the lower pHs of the cultivation broth supported by the low
165 photosynthetic activity (from 8.1 to 9.0) resulted in higher CO₂ concentrations in the
166 upgraded biogas. The higher photosynthetic activity mediated by the favorable
167 environmental conditions prevailing during spring and summer, along with the
168 accumulation of IC in the cultivation broth from 1785 mg L⁻¹ to 4599 mg L⁻¹ from stage
169 II to V, increased the pH from 8.8 to 9.8, which resulted in biomethane CO₂
170 concentrations complying with most international regulations. In this context, although
171 a 60% decrease in CO₂ solubility is expected when the cultivation broth temperature
172 increases from 10 to 40°C, the high CO₂ concentration gradient supported by the high

173 alkalinity/pH of the cultivation broth during stages II - V compensated this decrease in
174 CO₂ solubility.

175

176 **3.1.2 H₂S biomethane concentration**

177 H₂S was completely removed in the system regardless of the environmental parameters
178 and alkalinity. This higher elimination compared to the removal of CO₂ was attributed
179 to the higher H₂S aqueous solubility (Henry's law constant ranging from $H_{H_2S} \approx 3.58$ at
180 8.3 °C to $H_{H_2S} \approx 1.80$ at 40.3 °C) (Sander, 2015). The high pHs also promoted the
181 complete removal of this acidic gas in the AC (Bahr et al., 2014). These results were in
182 accordance to Posadas et al. (2017), who reported a complete removal of H₂S during the
183 simultaneous treatment of centrate and biogas in a similar outdoors experimental set-up,
184 and to Toledo-Cervantes et al. (2016) who also observed a complete depletion of H₂S
185 during the optimization of photosynthetic biogas upgrading under laboratory conditions.
186 In brief, the H₂S concentration in the biomethane herein obtained complied with most
187 European regulations for biomethane injection into natural gas grids or use as a vehicle
188 fuel, which requires H₂S levels $\leq 5 \text{ mg m}^{-3}$ (Muñoz et al., 2015).

189

190 **3.1.3 N₂ and O₂ concentrations in the biomethane**

191 Despite no clear trend in the evolution of biomethane N₂ concentration along the
192 daytime was recorded, the highest O₂ concentrations in the upgraded biogas were
193 recorded around midday, concomitantly with the highest DO concentrations in the
194 cultivation broth (Fig. S3; Fig. S8). Biomethane N₂ and O₂ concentrations during stage I
195 ranged from 0.0% in November to 5.5% and 1.8%, respectively, in January. During
196 stage II, N₂ and O₂ concentrations varied from 1.2% (April) and 0.3% (March),
197 respectively, to 5.9% (March) and 2.4% (May), respectively. In stage III, these

198 concentrations ranged from 0.1% and 0.0% (July), respectively, to 3.3% (June) and
199 1.5% (July), respectively. During stage IV, N₂ and O₂ concentrations fluctuated from
200 0.0% (August) to 5.2% and 1.9% (September), respectively. Finally, N₂ and O₂
201 concentrations during stage V ranged from 1.9% and 0.4%, respectively, to 3.2% and
202 1.2%, respectively (Fig. S8). Overall, the highest N₂ and O₂ concentrations in the
203 upgraded biogas were recorded during stages I and II (and during September in stage
204 III) likely due to the lower ambient temperatures, which increased the solubility of these
205 gases in the HRAP and their further desorption in the AC.

206

207 The previous optimization of the L/G ratio in the AC entailed a low N₂ and O₂
208 desorption (Posadas et al., 2017). Thus, the O₂ concentrations here recorded in the
209 biomethane were in accordance to Posadas et al. (2017) and Serejo et al. (2015), who
210 reported values ranging from 0% to 2% and from 0% to 4%, respectively, in a similar
211 experimental set-up (under outdoors and laboratory conditions, respectively) at a L/G of
212 0.5. The O₂ concentration in the upgraded biogas only complied with international
213 regulations during the periods of low PAR ($\leq 1\%$), which requires a further optimization.

214

215 **3.1.4 CH₄ biomethane concentration**

216 Negligible variations in the CH₄ concentration of the upgraded biogas were recorded
217 throughout the daytime regardless of the operational month (Fig. 2). Hence, CH₄
218 concentration in the biomethane in stage I ranged from 85.6% in December to 94.8% in
219 January. During stage II, CH₄ concentration varied from 90.4% in March to 97.2% in
220 May, and from 94.5% to 99.0% in stage III (July). Finally, the range of CH₄
221 concentrations in stage IV and V were 93.0%-99.6% and 94.5%-96.0%, respectively
222 (Fig. 2). Therefore, the CH₄ concentration in the biomethane here produced during

223 stages II-V complied with most European regulation for injection into natural gas grids
224 or use as a vehicle fuel (Muñoz et al., 2015). The higher CH₄ concentrations from stage
225 II onwards were mainly due to the higher CO₂ removals and lower N₂ and O₂
226 desorptions recorded (Fig. 2). These concentrations were in accordance to Posadas et al.
227 (2017) and Toledo-Cervantes et al. (2017), who reported CH₄ concentrations of 92.0%
228 and 96.2%, respectively, in the upgraded biogas using the same photobioreactor
229 configuration. Finally, negligible CH₄ losses by absorption in the AC were measured
230 regardless of the operational month as a result of the low CH₄ aqueous solubility
231 (Henry's law constant of CH₄ ranged from $H_{CH_4} \approx 0.044$ at 8.3 °C to $H_{CH_4} \approx 0.028$ at
232 40.3°C) (Sander, 2015). **Finally, it should be noted that the CH₄ content in the upgraded
233 biogas remained constant during the night period as a result of the high buffer capacity
234 and pH of the cultivation broth.**

235

236 **4. Conclusions**

237 This work constitutes the first year-round evaluation of biogas upgrading in a pilot scale
238 outdoors HRAP. The high alkalinity and pHs in the cultivation broth were identified as
239 key parameters to maintain a constant biomethane composition during the daytime.
240 Environmental conditions significantly influenced the quality of biomethane. CO₂, H₂S
241 and CH₄ concentrations in the upgraded biogas complied with most international
242 regulations for biomethane injection into natural gas grids or use as a vehicle fuel. This
243 study confirmed the year-round feasibility of outdoors algal-bacterial processes for the
244 simultaneous removal of CO₂ and H₂S from biogas coupled to nutrient removal from
245 digestates.

246

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252

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302

303 **FIGURE CAPTIONS**

304 **Figure 1.** Schematic diagram of the outdoors experimental set-up used for the
305 continuous photosynthetic upgrading of biogas.

306 **Figure 2.** Time course of the concentration of CO₂ (■) and CH₄ (▲) in the upgraded
307 biogas during one diurnal cycle under steady state as a function of the operational
308 months.

Figure 1
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Figure 1. Schematic diagram of the outdoors experimental set-up used for the continuous photosynthetic upgrading of biogas.

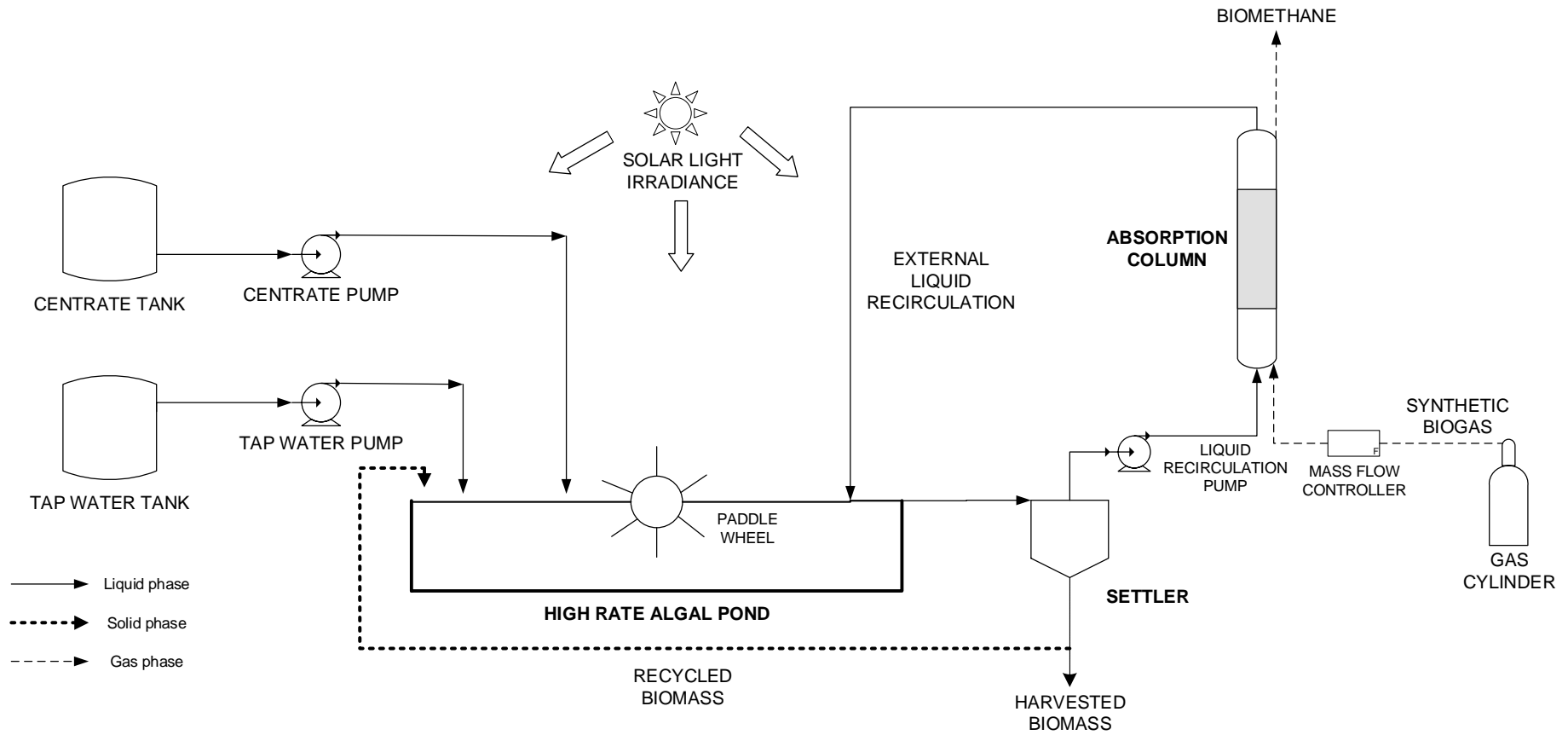


Figure 2
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Figure 2. Time course of the concentration of CO₂ (■) and CH₄ (▲) in the upgraded biogas during one diurnal cycle under steady state as a function of the operational months.

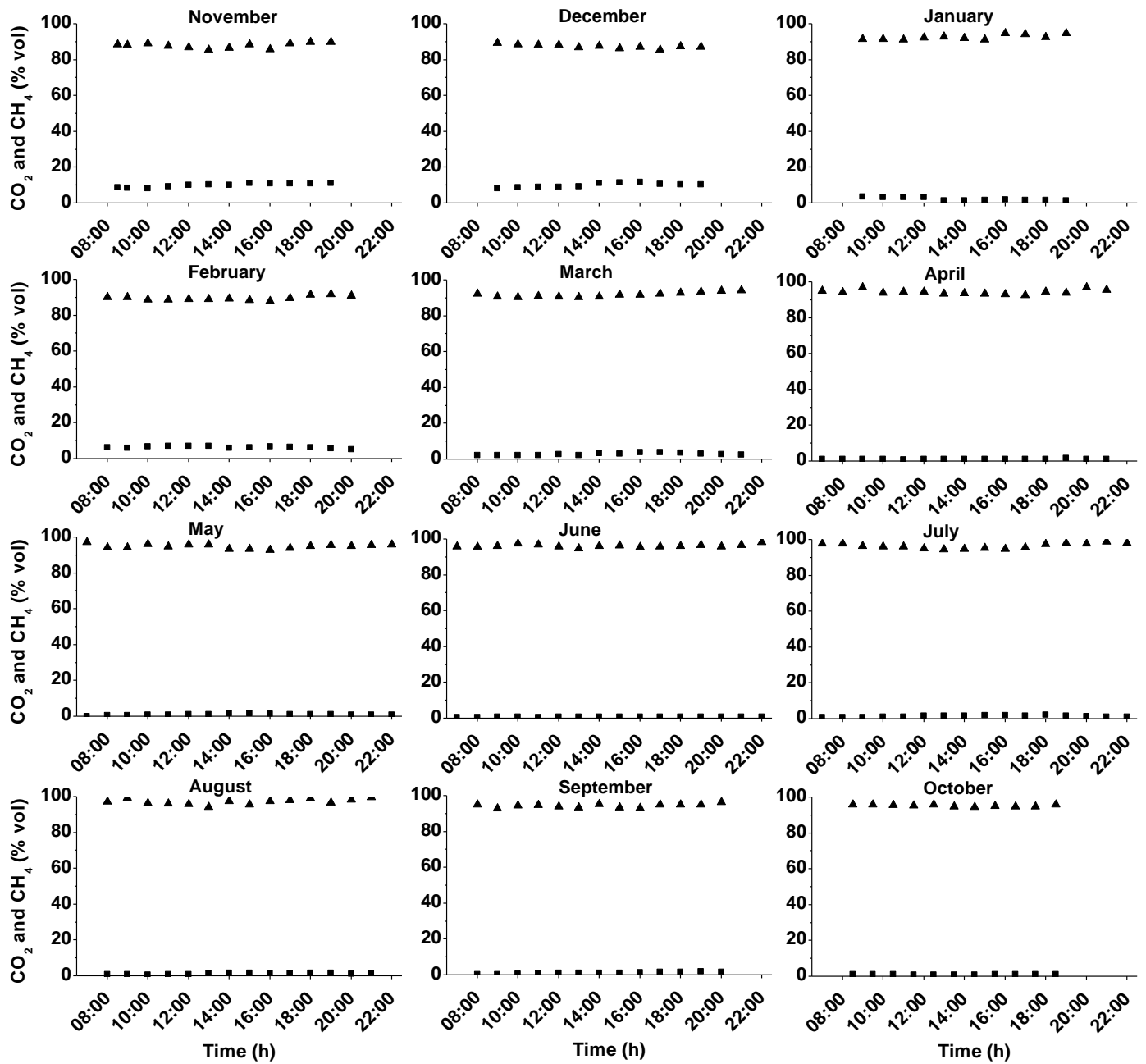


Table 1

Table 1. Environmental and operational parameters during the five operational stages.					
		Parameter			
Stage	Month	Average ambient temperature (°C)	Average photosynthetic active radiation ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	N° of sun hours (h)	Biomass Productivity ($\text{g m}^{-2} \text{d}^{-1}$)
I	November 30, 2016	4.4 ± 1.6	170 ± 33	10 ± 1	0.0
	December 28, 2016	7.5 ± 4.9	349 ± 119	10 ± 1	
	January 31, 2017	10.2 ± 3.9	339 ± 174	10 ± 1	
	February 28, 2017	14.1 ± 6.6	921 ± 237	12 ± 1	
II	March 29, 2017	14.2 ± 6.2	1213 ± 191	13 ± 1	7.5
	April 26, 2017	8.6 ± 1.5	301 ± 138	14 ± 1	
	May 31, 2017	23.1 ± 5.8	1399 ± 183	15 ± 1	
III	June 28, 2017	20.3 ± 2.7	297 ± 105	15 ± 1	15.0
	July 27, 2017	28.5 ± 6.5	1411 ± 155	15 ± 1	
IV	August 25, 2017	26.0 ± 6.3	1070 ± 199	13 ± 1	22.5
	September 27, 2017	20.7 ± 7.2	1009 ± 237	12 ± 1	
V	October 26, 2017	18.4 ± 7.0	113 ± 83	10 ± 1	15.0

Electronic Annex

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