1 **A STUDY OF PHOTOSYNTHETIC BIOGAS UPGRADING BASED ON A HIGH**

2 **RATE ALGAL POND UNDER ALKALINE CONDITIONS**

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12 **ABSTRACT**

13 Microalgal-bacterial processes have emerged as environmental friendly systems for the

14 cost-effective treatment of anaerobic effluents such as biogas and nutrients-laden

15 digestates. Environmental parameters such as temperature, irradiation, nutrient

16 concentration and pH effect the performance of the systems. In this paper, the potential of a

17 microalgal-bacterial photobioreactor operated under high pH ($\approx$9.5) and high alkalinity to

18 convert biogas into biomethane was evaluated. The influence of the illumination regime

19 (continuous light supply *vs* 12 h/12 h light/dark cycles) on the synthetic biogas upgrading

20 efficiency, biomass productivity and nutrient removal efficiency was assessed in a High-

21 Rate Algal Pond interconnected to a biogas absorption bubble column. No significant

22 differences in the removal efficiency of CO_2 and H_2S (91.5 $\pm$ 2 % and 99.5% $\pm$ 0.5,

23 respectively) were recorded regardless of the illumination regime. The high fluctuations of

24 the dissolved oxygen concentration during operation under light/dark cycles allowed to

25 evaluate the specific growth rate and the specific partial degradation rate of the microalgae

26 biomass by photosynthesis and respiration, respectively. The respiration reduced the net

27 microalgae biomass productivity under light/dark cycles compared with process operation

28 under the continuous light supply.

29 **Keywords**: alkaliphilic algal-bacterial consortium, biogas desulfurization, illumination

30 regime

INTRODUCTION

Biogas and digestate are the main byproducts from anaerobic digestion of organic waste. The composition of raw biogas varies depending on the type of residue and conditions of anaerobic digestion. A typical raw biogas composition accounts for CH_4 40-70% vol, CO_2 15-60% vol, H_2S 0.005-2% vol, and trace contaminants like water, siloxanes and volatile organic compounds (Muñoz et al., 2015) . Raw biogas can be used directly to generate heat and/or power or injected into natural gas grids when its quality is comparable with that of natural gas. The high content of CO_2 in raw biogas reduces its specific heating value and increases its transportation costs. Another undesirable biogas component is H_2S, which is a highly corrosive, toxic and malodorous gas (Noyola et al., 2006; Redondo et al., 2008). On the other hand, anaerobic digestates are characterized by a high concentration of nutrients, such as nitrogen (950 mg $N-NH_4$ L^{-1}) and phosphorus (415 mg $P-PO_4^{3-}$ L^{-1}) which are representative values from anaerobic sludge digestion made in municipal wastewater treatment plants (Uggetti et al., 2014). These aqueous effluents must be treated to avoid eutrophication of inland waterbodies and coastal areas (Batten et al., 2013). In this context, there is crucial need to develop cost-effective and environmentally friendly technologies for biogas upgrading and nutrient removal, based on both the versatile potential of biogas as a renewable energy source and the high eutrophication impact of digestates.

Algal-bacterial processes have emerged as the only technology capable of simultaneously upgrading biogas while recovering nutrients from digestates (Muñoz and Guieysse, 2006) . On one hand, microalgae can photosynthetically use the CO_2 present in the biogas as a carbon source while supporting the bacterial oxidation of H_2S via an in-situ O_2 supply (González-Sánchez et al,. 2008; de Godos et al., 2009; Bahr et al., 2014; Posadas et al., 2013; Hernández et al., 2013) On the other, the use of digestate as a nutrient source for

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microalgae and bacteria growth represents an opportunity to mitigate its eutrophication potential (Batten et al., 2013; Franchino et al., 2013; Uggetti et al., 2014). Despite the promising results obtained so far using algal-bacterial photobioreactors, biogas bioconversion to biomethane is still limited by the low CO_2 mass transfer rates of this technology (Yan et al., 2016). For instance, CO_2 removals ranging from 55 to 62 % were recorded by Alcántara et al., (2015) and Lebrero et al., (2016) using High Rate Algal Pond (HRAP) or bubble column algal-bacterial photobioreactors, which hindered the direct injection of the upgraded biogas into natural gas grids.

In this context, pH has been identified as a critical parameter determining the mass transfer rate of acidic gases such as H_2S and CO_2 in conventional gas-liquid contactors (Bahr et al., 2014; González-Sánchez et al., 2008). Process operation at pH>9 mediate an enhancement in the absorption rates of these biogas contaminants induced by chemical reactions (González-Sánchez and Revah, 2007; Markou et al., 2014). The use of carbonated alkaline cultivation media in algal-bacterial photobioreactors could support a long term efficient biogas upgrading. In these systems, medium acidification induced by CO_2 and H_2S absorption is counterbalanced by microalgae CO_2 uptake in the presence of light and CO_2 stripping under dark conditions. In addition, the use of extreme pH values promotes the stability of microalgae populations by preventing contamination with predators or other photosynthetic microorganisms (Lee, 2001). Despite the above mentioned advantages, the understanding of biogas upgrading using alkaliphilic algal-bacterial consortia is very limited.

This work aimed at evaluating the performance of a novel alkaliphilic microalgae-based process deployed for synthetic biogas upgrading and nutrient removal under different

78 illumination regimes, assessing both the fate of carbon, nitrogen, phosphorous and sulphur

79 and biomass productivity through mass balances under steady state.

80

81 **MATERIAL AND METHODS**

82 **Microorganisms and culture conditions**

83 An alkali-tolerant microalgae culture (AMC) was used as a model photosynthetic

84 consortium throughout the entire experiment. The AMC was enriched from Texcoco soda

85 lake in Mexico City according to De los Cobos-Vasconcelos et al. (2015). A culture of

86 alkaliphilic sulphur oxidizing bacteria (ASB) was used as a model H_2S degrading

87 consortium (González-Sánchez and Revah, 2007). The mixed AMC/ASB culture was

88 grown in a mineral salt medium (MSM) composed of (g/L): Na_2CO_3 (4.03); $NaHCO_3$

89 (13.61); $NaCl$ (1); K_2HPO_4 (1); K_2SO_4 (1); $CaCl·H_2O$ (0.04); KNO_3 (2.52); $MgCl_2·6H_2O$

90 (0.2) and 2 mL of a micronutrient solution (Sorokin et al., 2001). The final pH of the MSM

91 medium was 9.3.

92

93 **Experimental set-up**

94 The experimental system consisted of a 25 L HRAP interconnected with a 0.35 L

95 Absorption Bubble Column (ABC) (Figure 1). The HRAP dimensions were 1.25 m long $\times$

96 0.25 m width $\times$ 0.14 cm deep, which entailed an effective illuminated area of 0.28 m^2. The

97 HRAP was illuminated at a photosynthetically active radiation (PAR) of 500 $\mu molm^{-2}s^{-1}$

98 (model LI250A, LI-COR, USA) provided by LED white-cool light lamps (model 511919,

99 Ecosmart, USA). The cultivation broth in the HRAP was continuously mixed using a six

100 bladed paddle wheel inducing a laminar flow regime at 15 cm s^{-1}. The ABC (0.80 m height

101 × 0.019 m of internal diameter) was interconnected to the HRAP via culture broth

102 recirculation.

103

<div style="border:1px solid">Here Figure 1</div>

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Operational conditions

106 The HRAP was initially filled with 21 L of MSM and inoculated with four liters of AMC

107 containing a biomass concentration of 0.12 g TSS L^{-1}. The system was operated as fed-

108 batch under continuous illumination for 39 days. Four liters of algal cultivation broth were

109 drawn by day 20 and replaced with fresh ASB (0.2 g TSS L^{-1}) grown in MSM containing

110 10 g L^{-1} of sodium thiosulfate ($Na_2S_2O_3$) as energy source. The mixed culture was then

111 cultivated in batch mode for 19 days (stage I). From day 40 to 166 (stage II), the HRAP

112 was continuously fed at a hydraulic retention time of 9.5 days with a 1.8 times diluted

113 MSM, mimicking the composition of a digestate, supplemented with 0.35 g $Na_2S_2O_3$ L^{-1}.

114 From day 167 to 269 (stage III), a gas mixture composed of CO_2 (30%vol), H_2S (0.5%vol)

115 and balanced with N_2 was considered as synthetic biogas, it was continuously bubbled at 22

116 L d^{-1} through a porous stone located at the bottom of the ABC. The column was operated

117 co-currently at a liquid to synthetic biogas volumetric ratio (L/G) of 5 using the algal-

118 bacterial cultivation broth from the HRAP as scrubbing solution. From day 270 to 399

119 (stage IV), the illumination regime was set at a 12h:12 h light/dark photoperiod. The last

120 period (stage V) was operated for 117 days under similar operational conditions than those

121 imposed in stage II. Water evaporation rates were quantified and compensated by addition

122 of distilled water before sampling of the HRAP-ABC system.

123

Here table 1

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125   Aliquots of 70 mL of HRAP cultivation broth and influent mineral salt medium were drawn

126   three times per week in order to monitor the concentration of TSS, total organic carbon

127   (TOC), inorganic carbon (IC), total nitrogen (TN), nitrite (NO_2^-), nitrate (NO_3^-) , sulphate

128   (SO_4^{2-}), tiosulphate ($S_2O_3^{2-}$) and phosphate (PO_4^{3-}). The pH and dissolved oxygen (DO)

129   concentration of the cultivation broth were on-line recorded every 3 min. The composition

130   of synthetic biogas at the inlet and outlet of the ABC was daily monitored by non-

131   dispersive infrared sensor analysis. Finally, the elemental composition of the algal-bacterial

132   biomass was determined in stage III and IV.

133

134   **Analytical Procedures**

135   The concentration of TSS was measured according to Standard Methods (APHA, 2012).

136   Liquid samples were filtered through 0.45 μm Whatman filters prior determination of IC,

137   TOC, TN, PO_4^{3-}, SO_4^{2-} and $S_2O_3^{2-}$ concentrations. The concentration of IC, TOC and TN

138   was determined by a TOC-L CSH analyzer coupled to a TNM-L chemiluminescence

139   module (Shimadzu, Japan). The concentration of $N-NO_3^-$ and $N-NO_2^-$ were measured

140   spectrophotometrically with commercial Nitraver and Nitriver kits, respectively (HACH,

141   USA). $S-S_2O_3^{2-}$ concentration was measured by titration according to (Rodier J, 1988). The

142   concentration of $S-SO_4^{2-}$ was analyzed according to the Standard Methods (WEF, 2012). P-

143   PO_4^{3-} concentration was analyzed using a Dionex ICS 2000 Ion Chromatograph (USA)

144   equipped with a IonPacAS23 column (250 × 4 mm) eluted at 1 mL min^{-1} with a mobile

145   phase containing CO_3^{2-} and HCO_3^- at 4.5 y 0.8 mM, respectively. The elemental biomass

146   composition (C, N and S content) was determined from the biomass contained in aliquots

147   of 50 mL of cultivation broth centrifuged at 10,000 rpm (Avanti 123, Beckman Coulter,

148   USA) and washed 3 times with Milli-Q water. The salt-free pellets were dried at 105°C for

149   24h and grinded prior analysis in a Flash 2000 Elemental Analyzer CHNS-O Analyzer

150   (Thermo Scientific, USA). The gas concentration of CO_2, O_2 and H_2S at the inlet and outlet

151   of the ABC were analyzed with continuous nondispersive infrared sensor gas analyzer

152   (Ultramat 23, Siemens, Germany). The dissolved oxygen concentration and pH were online

153   determined using Applisens Z10023525 (Applikon, The Netherlands), Orion 9107BNMD

154   (Thermo Scientific,USA) and temperature probes.

155

156   **Calculations**

157   A series of elemental mass balances under steady state, showed in equations 1, 2, 3 and 4

158   and expressed as loading rates (Q_i = mass flow rate of component i/total liquid volume),

159   allowed to assess the fate of carbon, nitrogen, phosphorus and sulphur under steady state in

160   the HRAP-ABC.

161    $$Q_{C_{G_{in}}} + Q_{C_{L_{in}}} = Q_{C_{G_{out}}} + Q_{C_{L_{out}}} + Q_{C_{-X_{out}}} + Q_{C_{stripping}} \qquad\qquad (1)$$

162    $$Q_{N_{L_{in}}} = Q_{N_{L_{out}}} + Q_{N_{-X_{out}}} \qquad\qquad (2)$$

163    $$Q_{P_{L_{in}}} = Q_{P_{L_{out}}} + Q_{P_{-X_{out}}} \qquad\qquad (3)$$

164    $$Q_{S_{G_{in}}} + Q_{S_{L_{in}}} = Q_{S_{G_{out}}} + Q_{S_{L_{out}}} + Q_{S_{-X_{out}}} \qquad\qquad (4)$$

165

166   Where $Q_{i,\,in}$ and $Q_{i,\,out}$ represent the inlet and outlet mass loading rates, respectively, of all

167   species (i= carbon-C, nitrogen-N, phosphorous-P and sulphur-S) in liquid (L) and gas (G)

168   phases. Q_{i_X} represents the loading rate of each i contained in the biomass present in the

169   effluent. $Q_{c,\,stripping}$ represents the C as CO_2 lost by stripping from the HRAP. The CO_2

170   stripping from the HRAP was evaluated from equation 5 including the discrete IC

171   measurements together with the corresponding pH. The dissolved CO_2 (DCO_2) was

172   calculated as a function of pH, according to equation 6.

173   CO_2 stripping rate $= \frac{V_H}{V_T} k_L a_H \left(\frac{CO_{2air}}{H_{CO_2}} - DCO_2 \right)$ (5)

174     $[DCO_2] = \frac{IC}{1 + \frac{K_1}{[H^+]} + \frac{K_1 * K_2}{[H^+]^2}}$ (6)

175   Where V_H and V_T represent the volume of the aqueous phase in the HRAP and total

176   aqueous volume in the HRAP+ABC. H_{CO_2} represents the CO_2 Henry constant (1.1

177   dimensionless at 20 °C). $K_L a_H$ represents the global volumetric gas-liquid mass transfer

178   coefficient (20 d^{-1}) in the HRAP, which was experimentally determined . CO_{2air} is the

179   carbon dioxide concentration in air (350 $ppm_v = 1.1 \times 10^{-5}$ mol L^{-1}). K_1 and K_2 are the

180   dissociation constants for the system CO_2-H_2O (4.66×10^{-7} mol L^{-1} and 4.67×10^{-11} mol L^{-1},

181   respectively) and [H^+] is the proton concentration in the cultivation broth (mol L^{-1}).

182

183   **Assessment of the specific growth and partial degradation rates of microalgae under**

184   **light/dark cycles**

185   The fluctuations of the DO measurements recorded during stage IV under steady state were

186   described using a dynamic oxygen mass-balance applied to the liquid phase (Eq. 7). This

187   mass balance was used to estimate either the volumetric oxygen production or consumption

188   (rO_2) associated to microalgae biomass metabolism, accounting for the variation on the

189   temperature.

190     $\frac{dDO}{dt} = \frac{F}{V_T}(DO_{in} - DO) + \frac{V_C}{V_T} k_L a_C \left(\frac{O_{2g}}{H_{O_2}} - DO \right) + \frac{V_H}{V_T} k_L a_H \left(\frac{O_{2air}}{H_{O_2}} - DO \right) +$

191     $\frac{V_H}{V_T} r_{O_2} - R_{O_2-S}$ (7)

192   Where V_C represents the volume of the aqueous phase in the ABC (0.3 L), r_{O_2} represents

193   the microalgal oxygen consumption or production rate (mol L^{-1} d^{-1}), H_{O_2} represents the

194   oxygen Henry constant (32 dimensionless at 20 °C). $K_L a_C$ stands the global volumetric gas-

195   liquid mass transfer coefficient (141 d^{-1}) in the ABC, which was experimentally determined

196   using the gassing-out method. R_{O_2-S} was estimated to 0.0007 mol O$_2$ L^{-1} d^{-1} based on the

197   sulphur mass balance under steady state conditions, assuming complete oxidation to

198   sulphate.

199   The evaluation of the specific growth rate (μ_{light}) and the specific partial degradation rate

200   (μ_{dark}) of the microalgae biomass by photosynthesis and by respiration, respectively, was

201   based on the assumption of a 15 % w/w biomass loss by respiration during the 12 h dark

202   period (Grobbelaar and Soeder, 1985). This entails a μ_{light}/μ_{dark} ratio of -4.2. μ_{light} can be

203   expressed in terms of the photosynthetic oxygen/biomass ($y^p_{O_2/X}$) yield (de los Cobos-

204   Vasconcelos et al., 2015) and the oxygen production rate ($r_{O_2}{}^p$). Similarly, μ_{dark} can be

205   defined in terms of an endogenous respiration oxygen/biomass ($y^r_{O_2/X}$) yield and its

206   corresponding oxygen respiration rate ($r_{O_2}{}^r$). Therefore, the overall $y^r_{O_2/X}$ was evaluated

207   according to equation (8) for several light/dark cycles exposed to constant temperature

208   fluctuations. $r_{O_2}{}^p$ and $r_{O_2}{}^r$ represent averaged values for a set of continuous days reaching

209   uniform temperature fluctuations.

210      $$y^r_{O_2/X} = 4.2 \frac{(r_{O_2}{}^r)(y^p_{O_2/X})}{r_{O_2}{}^p} \qquad (8)$$

211   The net volumetric biomass productivity (*Px*) was evaluated from the condition of steady

212   state for a continuous cultivation (see equation 9).

213      $$Px = D \cdot X = (\mu_{light} - \mu_{dark}) \cdot X = \frac{r_{O_2}{}^p}{y^p_{O_2/X}} - \frac{r_{O_2}{}^r}{y^r_{O_2/X}} \qquad (9)$$

214 Where D is the dilution rate (d^{-1}) and X the experimental biomass concentration (g TSS L^{-}

215 ^{1}).

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220 **RESULTS AND DISCUSSION**

221 **Synthetic biogas upgrading performance**

222 The removal efficiencies of CO_2 and H_2S during stage III accounted for 89 ± 4 % and 99.5

223 $\pm 0.5\%$, respectively, and $94 \pm 1\%$ and $99.5 \pm 0.5\%$, respectively, during stage IV. The

224 similar pH values recorded during both stages explain the comparable synthetic biogas

225 upgrading performance regardless of the illumination regime. Thus, in spite of the

226 continuous feeding of CO_2 and H_2S during stage III the pH remained constant at ≈9.5 likely

227 due to high buffer capacity (alkalinity) of cultivation medium and the high photosynthetic

228 activity of the AMC under continuous illumination. However, pH fluctuated from 9.3 to 9.7

229 during stage IV as a result of the periodic medium acidification during the dark period due

230 to CO_2 and H_2S absorption, and the microalgae-based regeneration of alkalinity during the

231 light period. In this context, the increase in pH mediated by microalgae growth during the

232 illuminated period is expected to support a high biogas upgrading performance during

233 outdoors operation. These high CO_2 and H_2S removal efficiencies were also supported by

234 the high L/G ratio of 5), which prevented a deterioration in the CO_2 /H_2S mass transfer

235 induced by a decrease in the pH of the cultivation medium in the ABC (Serejo et al. 2015).

236 However, high L/G ratios often promote a high O_2 stripping from the recirculation medium

237 to the biomethane, with the concomitant contamination of the upgraded biogas. In this

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238   study, the oxygen concentration in upgraded synthetic biogas averaged 2.6% v/v during

239   stage III and IV. According to the Mexican regulation (NOM-001-SECRE 2010) oxygen

240   concentration in biomethane must be lower than 0.20% v/v. This requires an optimization

241   of the L/G ratio or the implementation of operational strategies devoted to decrease the

242   oxygen liquid concentration prior recirculation to the ABC (Toledo-Cervantes et al., 2016).

243   The complete removal of H_2S recorded in stage III and IV promoted the accumulation of

244   SO_4^{2-} in the cultivation broth concomitantly with synthetic biogas upgrading. The high DO

245   concentration prevailing in the HRAP during the entire experimental period (even during

246   dark periods DO remained > 2 mg O_2 L^{-1}) supported a complete oxidation of H_2S to

247   sulphate (González-Sánchez and Revah, 2007) (Table 2). The concentration of $S-SO_4^{2-}$

248   achieved during steady state in stages III and IV were 0.32 ± 0.02 g L^{-1} and 0.34 ± 0.05 g L^{-1}

249   , respectively.

250

Photobioreactor performance

252   The batch cultivation during stage I supported an increase in biomass concentration in the

253   HRAP-ABC system from 0.12 g TSS L^{-1} to 0.77 g TSS L^{-1} by day 39, concomitant with a

254   decrease in IC concentration from 1.25 g C L^{-1} to 0.21 g C L^{-1} (Figure 2). Process operation

255   under continuous illumination at a HRT of 9.5 days entailed steady state biomass

256   concentration of 0.72 ± 0.04 g TSS L^{-1} during stage II. The continuous supply of MSM

257   mediated an increase of the IC concentration in the HRAP, which stabilized at 1.13 ± 0.09

258   g C L^{-1} from day 120 till 166. Synthetic biogas supply during stage III under continuous

259   illumination resulted in an increase in biomass concentration up to 1.23 ± 0.05 g TSS L^{-1},

260   which suggested that microalgae growth was limited by CO_2 concentration during stage II

261   at the high pH value prevailing in the cultivation broth (pH=10.03 ± 0.16). The 50%

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262 reduction in light energy supply mediated by process operation under 12h/12h light /dark

263 cycles induced a decrease in biomass concentration from 1.23 ± 0.05 g TSS L^{-1} to steady

264 state values of 0.23 ± 0.05 g TSS L^{-1} by the end of stage IV. This decrease in TSS

265 concentration entailed a reduction in the volumetric biomass productivity from 0.129 to

266 0.023 g TSS L^{-1} d^{-1}, but did not impact on the concentration of IC in the HRAP, which

267 remained constant at 1.02 ± 0.06 g C L^{-1} (Table 2 and Figure 2) probably due to the CO_2

268 stripping as it is explained below. A detailed explanation of the mechanisms underlying the

269 unexpectedly low biomass productivity is provided below. Process operation under

270 continuous illumination during stage V supported an increase in biomass concentration and

271 productivities up to steady state values of 0.94 ± 0.09 g TSS L^{-1} and 0.098 g TSS L^{-1} d^{-1}

272 (Table 2). The absence of synthetic biogas supply during stage V resulted in an increase in

273 the pH up to 10.23 ± 0.05 and in the expected decrease in IC concentration to steady state

274 values of 0.86 ± 0.12 g C L^{-1} via biomass assimilation (Figure 2).

275

> Here figure 2

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277 The average removal efficiencies of nitrogen and phosphorous during stages III, IV and V

278 are shown in Table 2. Similar nutrient removal efficiencies were achieved during stage III

279 and V, while the decrease in biomass productivity recorded in stage IV resulted in a

280 significant decrease in the removal efficiency of phosphate. An experimental error in the

281 determination of nitrate concentrations might explain the unexpectedly high nitrate

282 removals recorded during stage IV (comparable to stage III and IV), since no noteworthy

283 differences were observed in the elemental composition of the biomass along the three last

284 operational stages and $N\text{-}NO_2^-$ concentrations were very low (≈ 0.0004 mg $N\text{-}NO_2^-$.L^{-1}). A

285   potential N_2O production in the system was also ruled out due to the relatively high DO

286   present in the HRAP during stage IV (> 2mg O_2 L^{-1}), which prevented nitrate

287   denitrification (Wang et al., 2008).

288

Here table 2

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291   **C, N, P and S mass balances**

292   Mass balance calculations for the main elemental components were conducted in the

293   experimental system under steady state in stages III, IV and V.

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Here figure 3

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296   The carbon present in the synthetic biogas as CO_2 accounted for approx. 40 % of the

297   inorganic carbon inlet load in stage III and IV (Figure 3 a). Dissolved inorganic carbon

298   accounted for 58 % of the output carbon from the system. On the other, the carbon present

299   as biomass in the liquid effluent represented 30 % of the output carbon during continuous

300   illumination, while this share decreased to 7 % under light/dark cycles as a result of the

301   limited microalgal productivity. This limited microalgae growth during stage IV resulted in

302   an enhanced carbon stripping, which increased from 5 to 12 %. The restoration of

303   continuous illumination in the absence of synthetic biogas in stage V increased the share of

304   inorganic carbon assimilated into biomass, which accounted for 37 % of the total output

305   carbon, thereby minimizing carbon stripping. The nitrogen assimilated as biomass

306   accounted for 54, 14 and 50 % of the output nitrogen in stages III, IV and V, respectively

307 (Figure 3b). Similarly, the phosphorous fixed in the biomass represented 17, 3 and 14 % of

308 the output phosphorous in stages III, IV and V, respectively. For both nutrients, the share in

309 the output streams was correlated with biomass productivity since no difference in the

310 biomass N and P content was recorded. Finally, the H_2S present in the synthetic biogas

311 accounted for 12 and 10% of the inlet Sulphur in stages III and IV, respectively. This share

312 was significantly lower than the sulphur present as thiosulphate in the feed. Both reduced

313 forms of sulphur were completely oxidized to sulphate, which represented 99.5% of the

314 output sulphur in stage III, IV and V.

315

316 **Effect of light/dark cycles on the biomass productivity**

317 During the illuminated period in stage IV, microalgal photosynthesis was responsible of a

318 net oxygen production in the HRAP. However, in the dark period, the microalgae and

319 sulfide oxidizing bacteria consumed by respiration the dissolved oxygen present in the

320 cultivation broth, with a concomitant production of CO_2 (Bahr et al., 2014; Masojídek and

321 Koblízek, 2004). Hence, the dynamic balance between microalgae growth and endogenous

322 biomass consumption entailed a steady state biomass concentration 0.2 g TSS L^{-1}, which

323 were significantly lower than the expected value derived from a 50% reduction in light

324 supply. In the absence of light supply, the microalgae consumed their intracellular organic

325 carbon (i.e. carbohydrates) for cell maintenance, which was likely responsible of the lower

326 biomass concentrations recorded. Figure 4, based on the estimated volumetric oxygen

327 production and consumption (rO_2) rates from equation 7, shows that endogenous

328 respiration activity was similar to the photosynthetic activity responsible of microalgae

329 growth.

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Two different temperature fluctuation trends can be observed in Figure 4, since the HRAP-ABC was exposed to the daily outdoors temperature fluctuations. These variations in the temperature of the cultivation broth influenced the extent of the oxygen production and endogenous respiration rates of the algal-bacterial consortium. From day 370 to 377, the temperature fluctuated from 13 to 24°C, while for days 387 to 396 the range was 16 to 28°C. This difference of 4 °C in the temperature fluctuation range induced a 60 % increase in both μ_{light}, μ_{dark} and biomass productivity (Table 3). Therefore, the microalgae productivities estimated were highly affected by the lower temperatures, which correlated with the decrease in the photosynthetic growth rates. The endogenous respiration yield, $y^r_{O_2/X}$, was up to three times higher than the photosynthetic oxygen production yield, $y^p_{O_2/X}$, and showed an inverse correlation with temperature. The $y^r_{O_2/X}$ here estimated were in agreement with the respiration yields typically reported in microalgal cultures (0.0034-0.124 mol O_2/gTSS) (Le Borgne and Pruvost, 2013; Ruiz-Martinez et al., 2016). The novel methodology for the calculation of microalgae biomass productivity here developed based on continuous DO measurements was validated by the empirical biomass productivity estimated on the empirical measurements of the dilution rate and effluent biomass concentration (Table 3).

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Conclusions

Alkaliphic algal-bacterial consortia were shown as an effective platform to bioconvert biogas into biomethane. Microalgal photosynthesis was able to effectively regenerate the alkalinity consumed during CO_2 and H_2S absorption even during light/dark illumination regimes similar to those prevailing under outdoors conditions. Carbon and nutrient recovery was a function of the biomass productivity, which itself depended on the photobioreactor illumination regime and temperature. The endogenous microalgae respiration during the dark period was likely responsible of the higher decrease in biomass compared to that expected from a 50% reduction in light supply.

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469

470 **Legends of figures**

471

472 **Figure 1**. Experimental set-up used for the alkaliphilic synthetic biogas upgrading. Dashed

473 lines represent synthetic biogas streams and continuous lines represent liquid streams.

474

475 **Figure 2.** Time course of concentration of biomass ($\triangle$) and inorganic carbon ($\bullet$) during the

476 operation of the HRAP-ABC.

477

478 **Figure 3**. Carbon (a), nitrogen (b), phosphorous (c) and sulphur (d) mass balances for

479 stages III, IV and V under steady state in the experimental HRAP-ABC. Numbers above

480 bars represent the respective input and output loading rates ($g \, L^{-1} d^{-1}$).

481

482 **Figure 4.** Time course of the estimated oxygen production and consumption rates (a) and

483 temperature (b) in the cultivation broth during the light and dark periods. Shade areas

484 represent dark periods.

Table 1. Operational conditions deployed in the experimental system HRAP-ABC.

Stage	Period (d)	MSM condition	Operation mode	Illumination	Synthetic biogas supply	
I	0-39	(a)	Fed-batch	24 h	No	
II	40-166	(b)	Continuous HRT = 9.5 d	24 h	No	
III	167-269	(b)	Continuous HRT = 9.5 d	24 h	Yes	GRT= 23 min
IV	270-399	(b)	Continuous HRT = 9.5 d	12 h/12 h light/dark	Yes	
V	400-517	(b)	Continuous HRT = 9.5 d	24 h	No	

(a) Cultivation in MSM
(b) Cultivation in 1.8 diluted MSM containing 0.35 g $Na_2S_2O_3 L^{-1}$
GRT-Gas residence time; HRT-Hydraulic residence time

Table 2. Summary of the environmental and operational parameters in the HRAP-ABC system under steady state during Stages III, IV and V.

Parameter	Stage III	Stage IV	Stage V
Px (g L^{-1} d^{-1})	0.129 ± 0.005	0.023 ± 0.001	0.100 ± 0.012
X (g TSS L^{-1})	1.23 ± 0	0.23 ± 0.05	0.94 ± 0.05
DO (mg L^{-1})	10.8 ± 0.6	Light/Dark 11.4 ± 0.5 / 2.8 ± 0.1	10.8 ± 1.3
pH	9.53 ± 0.05	Light/Dark 9.71 ± 0.00 /9.39 ± 0.00	10.23 ± 0.05
T (ºC)	19.2 ± 2.6	22.8 ± 4.0	23.9 ± 1.7
$N-NO_3^-$ removal (%)	52 ± 9	41 ± 5	55 ± 21
$P-PO_4^{3-}$ removal (%)	24 ± 8	12 ± 7	29 ± 11
C-biomass (%w)	44.7	44.5	50.0
N-biomass (%w)	8.7	7.7	8.0
S-biomass (%w)	0.8	0.6	0.1
P-biomass (%w)*	1.0	1.0	1.0

* (Grobbelaar, 2004)

Table 3. Influence of photosynthesis and respiration on the kinetic parameters and biomass productivity during light/dark cycles, based on the assumption of a 15 % w/w biomass lost by respiration during a 12 h dark period.

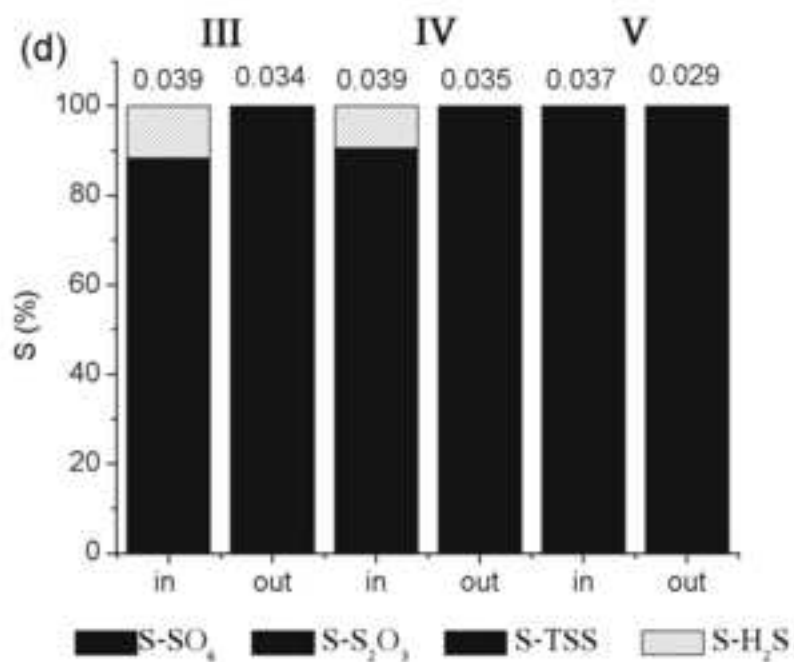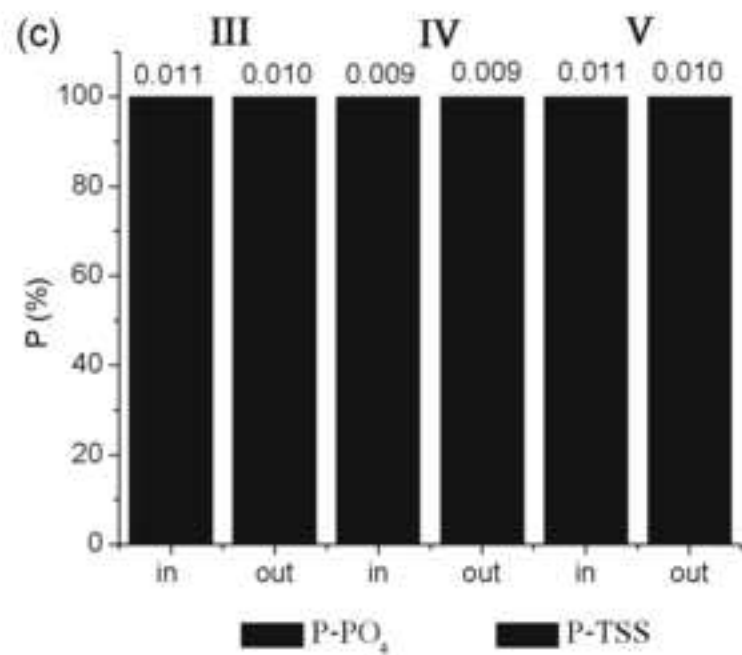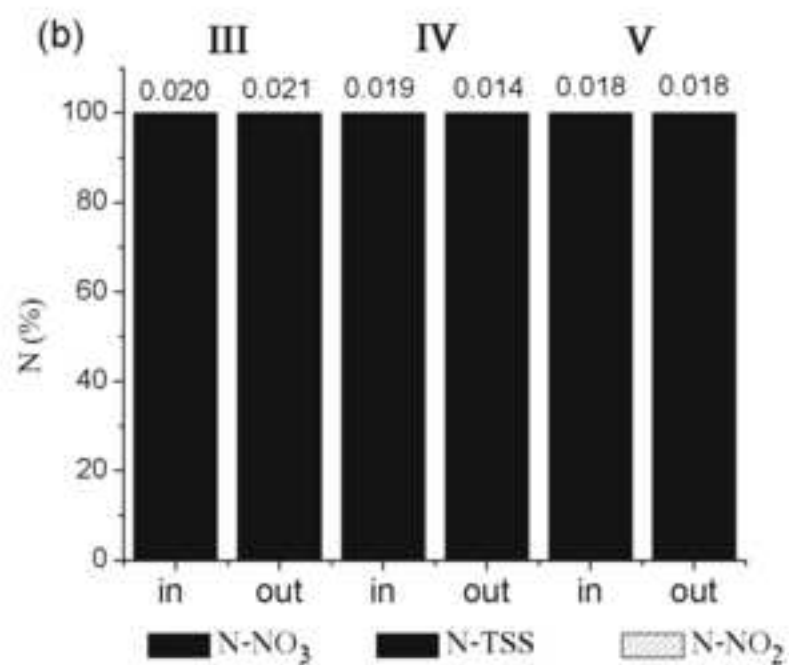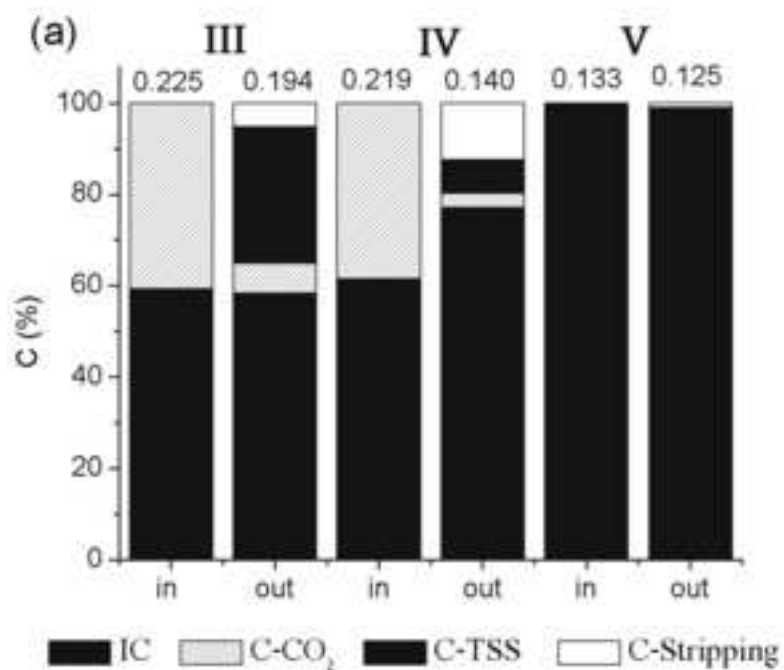| Temp. Range (°C)	Kinetic parameters					Biomass productivity (*Px*)	
	$r_{O_2}{}^p$	$r_{O_2}{}^r$	$y^r_{O_2/X}$	μ_{light}	μ_{dark}	$\dfrac{r_{O_2}{}^p}{y^p_{O_2/X}} - \dfrac{r_{O_2}{}^r}{y^r_{O_2/X}}$	$D \cdot X$
	(mol L^{-1} d^{-1})		(mol g^{-1})	d^{-1}		g L^{-1} d^{-1}	
13 to 24	0.0010	0.0009	0.2441	0.0786	-0.0184	0.0120	0.0208
16 to 28	0.0016	0.0006	0.1017	0.1258	-0.0295	0.0193	0.0208

Figure 1
Click here to download high resolution image

Clean biogas

Absorption bubble column

Light source

High Rate Algae Pond

Liquid outlet

Synthetic biogas

Liquid recirculation

Mineral salt medium

Figure 4