Tailoring biomass composition during the optimization of the integral upgrading of biogas in microalgal-bacterial processes

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KEYWORDS

Carbohydrate accumulation, CO_2 removal, H_2S removal, L/G ratio, macromolecular composition, photobioreactor.

ABSTRACT

The influence of biogas flow rate (0, 0.3, 0.6 and 1.2 $m^3 m^{-2} h^{-1}$) on the elemental and macromolecular composition of the algal-bacterial biomass produced from biogas upgrading in a 180 L photobioreactor interconnected to a 2.5 L external bubbled absorption column was investigated using diluted anaerobically digested vinasse as cultivation medium. The influence of the external liquid recirculation/biogas ratio ($0.5 < L/G < 67$) on the removal of CO_2 and H_2S, and on the concentrations of O_2 and N_2 in the upgraded biogas was also evaluated. A L/G ratio of 10 was considered optimum to support CO_2 and H_2S removals of 80% and 100%, respectively, at all biogas flow rates tested. Biomass productivity increased at increasing biogas flow rate, with a maximum of 12±1 g $m^{-2} d^{-1}$ at 1.2 $m^3 m^{-2} h^{-1}$, while the C, N and P biomass content remained constant at 49±2%, 9±0% and 1±0%, respectively, over the 175 days of experimentation. The high carbohydrate contents (60-80%), inversely correlated to biogas flow rates, would allow the production of ≈100 L of ethanol per 1000 m^3 of biogas upgraded under a bio-refinery process approach.

INTRODUCTION

Biogas from the anaerobic digestion of residual organic matter is typically composed of CH_4 (40-75%), CO_2 (25-60%), H_2S (up to 2%) and N_2, O_2 or H_2 at trace level concentrations.[1] The primary biogas production estimated in the European Union in 2012 was 12.0 Mtoe, which corresponded to the generation of 46.3 TWh of electricity.[2] In this context, the cost-effective conversion of biogas to biomethane via CO_2 and H_2S removal is crucial to boost biogas applications (e.g use as a vehicle biofuel or injection in natural gas grids).[3] CO_2 removal from biogas reduces its costs of compression and transportation, while increasing its specific calorific value.[4] Likewise, H_2S removal is also recommended due to its toxicity and hazards associated to

the corrosion of pipelines, engines and biogas storage structures.[3,5] Physical/chemical technologies such as water/chemical absorption and cryogenic separation can reduce biogas CO_2 content, while activated carbon filtration and chemical scrubbing with metal ions can be efficiently used for H_2S removal.[1,6-8] Despite water/chemical scrubbing and membrane separation can support a simultaneous removal of CO_2 and H_2S from biogas, these technologies exhibit high environmental impacts and operating costs.[1,9] On the other hand, conventional biological technologies such as algal photobioreactors only allow for the removal of CO_2, while aerobic or anoxic biotrickling filters exclusively support H_2S removal.[6,7] Therefore, the development of innovative low-cost biotechniques for an integral upgrading of biogas via the simultaneous removal of CO_2 and H_2S is mandatory.

Algal-bacterial processes constitute a low-cost and environmentally friendly alternative to physical/chemical technologies or conventional biotechniques for an integral biogas purification.[10] Biogas upgrading in algal-bacterial processes is characterized by the photosynthetic conversion of CO_2 to microalgae biomass in the presence of light and by the oxidation of H_2S to sulfate by sulfur oxidizing bacteria using the O_2 produced from microalgal photosynthesis.[10] The economic and environmental sustainability of this novel biotechnology can be improved using wastewater as a free nutrient and water source to support the growth of microalgae and bacteria, with the environmental benefits associated to the mitigation of the eutrophication potential of wastewaters.[3,10] In addition, the microalgal-bacterial biomass produced during biogas upgrading could be used as a feedstock for the production of renewable energy (bioethanol, biogas, biodiesel and biohydrogen) or of commercial bio-products such as proteins, carbohydrates, lipids or poly-β-hydroxybutyrates (PHB).[11,12] Unfortunately, the number

of studies on the integral upgrading of biogas coupled with wastewater treatment in algal-bacterial photobioreactors is scarce, with a knowledge gap on the influence of operational conditions on biomass composition. In a preliminary study, Bahr et al.[10] recorded CO_2 and H_2S removals from simulated biogas (using N_2 instead of CH_4 due to its potential explosion hazards) of 40 and 100%, respectively, during the treatment of diluted centrates in a pilot high rate algal pond (HRAP) connected to an external biogas absorption column (AC). Despite these promising results, the potential of this novel biotechnology to simultaneously treat biogas and wastewater can be further boosted by optimizing CO_2 and H_2S removal while tailoring the composition of the algal-bacterial biomass to allow for a more cost-effective biomass valorization.

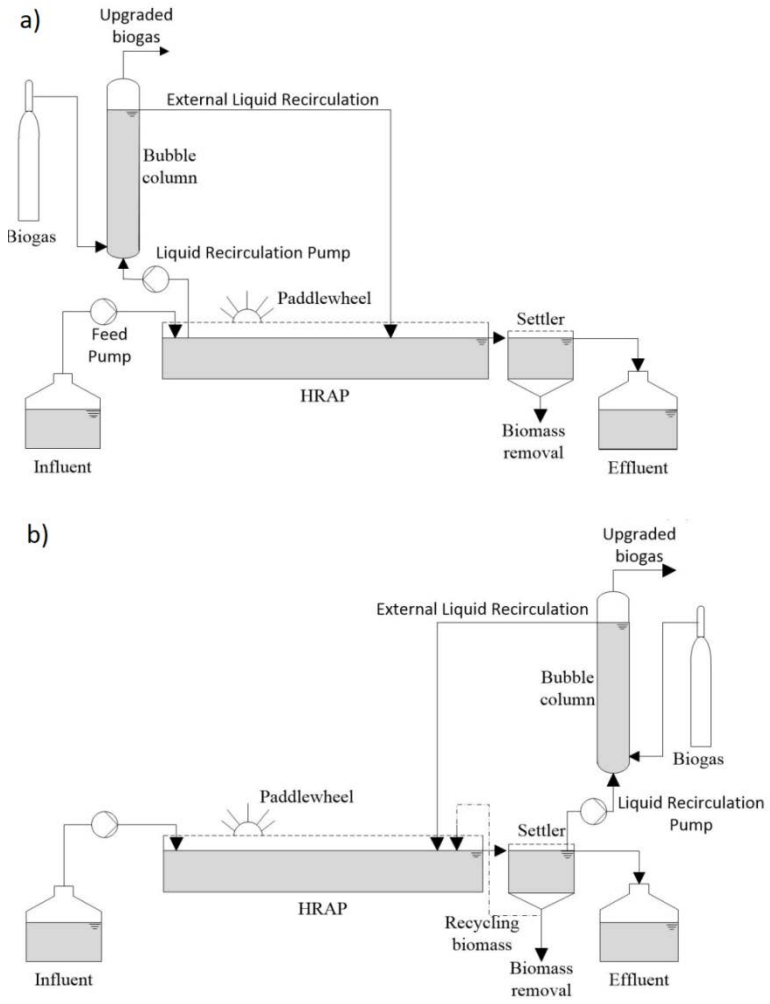
The main objective of this work was to investigate the influence of biogas flow rate on the macromolecular and elemental composition of the algal-bacterial biomass produced during biogas upgrading in a 180 L algal-bacterial HRAP treating anaerobically digested vinasse (ADV) and interconnected to an external AC. The influence of the external liquid recirculation/biogas (L/G) ratio on the removal of CO_2 and H_2S, and on the O_2 and N_2 content of the upgraded biogas was also evaluated. Furthermore, the potential carbon and nutrient removal from ADV and the dynamics of microalgae population in the HRAP were also investigated.

MATERIALS AND METHODS

Experimental set-up

The experimental set-up consisted of a 180 L HRAP with an illuminated surface of 1.2 m^2 (202 cm length × 63 cm width × 15 cm depth) and two water channels divided by a central wall, interconnected to a 2.5 L (Ø = 4.4 cm; height = 165 cm) external absorption column. The HRAP

and AC were interconnected via an external recirculation of the microalgae broth (Fig. 1), with a varying flow rate. The HRAP cultivation broth was continuously agitated using a 6-blade paddlewheel at an internal liquid recirculation velocity of ≈20 cm s^{-1}. HRAP illumination was conducted using 16:8 h 1ight:dark cycles at 104±25 μmol m^{-2} s^{-1} during the illuminated period (7:00-23:00 h) using 33 fluorescent bulbs (20 W, DUOLEC E27, Portugal) and 12 Gro-lux fluorescent lamps (Sylvania, Germany). Effluent sedimentation was carried out in an 8 L settler located at the outlet of the HRAP. The absorption unit consisted of a bubble column provided with a metallic sparger located at its bottom. The system was operated indoors at the Department of Chemical Engineering and Environmental Technology of University of Valladolid (Spain) for 175 days at 26±2 °C.



92

93 **Figure 1.** Schematic of the combined biogas upgrading and ADV treatment experimental set-up.
94 a) External liquid recirculation drawn from the cultivation broth (Stages I to III); b) External
95 liquid recirculation drawn from the supernatant of the settler (Stages IV and V).

96 **Microorganisms and culture conditions**

97 The pilot HRAP was inoculated with 10 L of a 0.6 g TSS L^{-1} *Chlorella vulgaris* culture
98 (previously acclimated to dilute ADV) and 2 L of a 6.2 g TSS L^{-1} nitrifying-denitrifying activated

sludge from Valladolid wastewater treatment plant (WWTP). The initial TSS concentration in the cultivation broth of the HRAP was of 0.08 g L^{-1}.

Chlorella vulgaris was isolated from a vinasse storage pond of a sugar and ethanol industry located in Mato Grosso do Sul (Brazil). Twelve 1.0 L e-flasks were incubated at 30 ºC and 200 r.p.m. under light:dark periods of 16:8 h at 61±6 μmol m^{-2} s^{-1} for 21 days in order to acclimate *Chlorella vulgaris* to ADV wastewater prior to HRAP inoculation.

Biogas and anaerobically digested vinasse wastewater

Two synthetic biogas mixtures were used for biogas upgrading. Biogas mixture 1 (BM1) was composed of CO_2 (30%) and N_2 (70%), while biogas mixture 2 (BM2) was composed of CO_2 (29.5%), H_2S (0.5%) and CH_4 (70%) (Abello Linde, Spain). ADV was collected from the anaerobic wastewater treatment line of a food industry located in Valladolid (Spain) and stored at 4 °C prior to use.

Influence of L/G on CO_2 and H_2S removal efficiency and O_2 biomethane concentration in the absorption column

Synthetic biogas mixtures BM1 and BM2 were sparged into the AC at 0.2 $m^3.m^{-2}.h^{-1}$ and at 0.2, 0.6 and 1.2 m^3 m^{-2} h^{-1}, respectively (flow rates referred to the AC cross sectional area) at external liquid recirculation (LR) rates of 0.6, 2.7, 4.9, 8.4 and 13.1 m^3 m^{-2} h^{-1} in order to determine the influence of LR on CO_2 and H_2S removal, and on the O_2 content of the upgraded biogas. Hence, the L/G ratios in the AC ranged from 0.5 to 67. Biogas composition (CO_2, H_2S, CH_4, N_2 and O_2) was measured by GC-TCD at the inlet and outlet of the AC at each tested L/G ratio.

Influence of biogas flow rate on biomass composition and wastewater treatment

Five operational stages using 2 different biogas mixtures and 3 different biogas flow rates were tested in order to optimize biogas upgrading coupled with ADV treatment, and to evaluate the influence of biogas flow rate on the macromolecular and elemental composition of the algal-bacterial biomass generated (Table 1). The hydraulic retention time (HRT) in the HRAP was set at a typical value of 7.4±0.3 days[13] (corresponding to a HRT in the settler of ≈12±3 h) and each operational stage was maintained for approximately 35 days (≈5×HRT). The LR was adjusted to L/G ratios of ≈10 in all stages. ADV was diluted with tap water at 10% prior to feeding the HRAP based on the results obtained in preliminary ADV biodegradability tests in algal-bacterial systems performed according to Posadas et al.[14] These assays revealed maximum removals of soluble total organic carbon (TOC), inorganic carbon (IC) and total nitrogen (TN) of 20%, 91% and 72%, respectively, in the tests provided with ADV diluted at 10% and incubated at 300 r.p.m. under 284±17 μmol m^{-2} s^{-1} with a light:dark photoperiod of 16:8 h. The average concentrations of TOC, IC, chemical oxygen demand (COD), TN, $N\text{-}NH_4^+$, soluble phosphorus (P) and TSS in the 10 times diluted ADV wastewater were 117±17 mg L^{-1}, 142±20 mg L^{-1}, 306±37 mg L^{-1}, 71±13 mg L^{-1}, 56±14 mg L^{-1}, 3.3±0.9 mg L^{-1} and 0.13±0.04 g L^{-1}, respectively, while the average pH was 7.84±0.13.

Table 1. Biogas mixtures and flow rates tested in the AC along with the average cultivation broth temperature, evaporation rate, pH and DO monitored in the HRAP during each experimental stage.

STAGE	ABSORPTION COLUMN	HIGH RATE ALGAL POND

	Biogas Mixture	Biogas flow rate ($m^3\ m^{-2}\ h^{-1}$)	HRAP Temperature (°C)	Evaporation Rate ($L\ m^{-2}\ d^{-1}$)	pH	DO ($mg\ L^{-1}$)
I	-	-	22.2±1.3	5.1±0.8	7.8±0.1	8.2±0.9
II	BM1	0.2	24.6±2.0	7.2±1.0	8.0±0.2	6.5±0.6
III	BM2	0.2	25.1±1.3	7.4±1.6	8.1±0.1	4.5±0.6
IV	BM2	0.6	24.8±1.6	6.0±1.7	7.8±0.1	4.2±0.5
V	BM2	1.2	25.1±0.7	6.1±1.5	7.9±0.1	5.9±0.7

Stage I corresponded to the start-up of the process and was carried out without biogas addition. BM1 and BM2 at 0.2 $m^3\ m^{-2}\ h^{-1}$ were continuously sparged into the AC during stages II and III, respectively (Fig. 1a). Finally, BM2 flow rate was increased to 0.6 and 1.2 $m^3\ m^{-2}\ h^{-1}$ during stages IV and V, respectively, while the external liquid recirculation in these stages was drawn from the supernatant of the settler in order to avoid biomass accumulation in the AC (Fig. 1b).

Gas samples of 100 µL were drawn twice a week at the inlet and outlet of the AC in order to monitor CO_2, H_2S, CH_4, O_2 and N_2 concentrations. The inlet and outlet biogas flow rates were also measured to accurately determine CO_2 and H_2S removals. Similarly, liquid samples of 250 mL were drawn twice a week from the influent and effluent (settler output, Fig. 1) wastewater to monitor the concentration of TOC, IC, TN, N-NH_4^+, NO_2^-, NO_3^-, P and TSS. COD concentration was only measured at steady state, which was considered reached under constant concentrations of the monitored parameters (≈4-5 times the elapsed HRT). TOC, IC, COD, TN, NH_4^+, NO_2^-, NO_3^- and P concentrations corresponded to the soluble phase, which required liquid

sample filtration through 0.20 µm nylon filters prior to analysis. Likewise, liquid samples of 250 mL were drawn from the cultivation broth twice a week to monitor TSS concentration. The sludge volume index (SVI) of the algal-bacterial broth was also determined in duplicate under steady state conditions. The ambient and cultivation broth temperatures, dissolved oxygen (DO) concentration and pH in the cultivation broth, and the influent and effluent wastewater flow rates were daily measured. Light intensity at the HRAP surface was monitored under steady state conditions. Sampling was always conducted at 9:00 a.m along the entire experimental period. Biomass harvesting was performed twice a week from stages I to III and every day in stages IV and V (due to the fact that the high external liquid recirculation drawn from the settler implied a high biomass accumulation in the settler, which also entailed the need for daily settled biomass recirculation to the HRAP in order to avoid biomass wash-out). The elemental (C, N and P) and macroscopic (lipids, proteins, carbohydrates, PHB and ash) biomass composition was determined at each steady state. Starch content analysis was performed only at stage V. Finally, the microalgae assemblage structure was also determined under steady state conditions.

The biogas CO_2 and H_2S removal efficiency (RE) in the AC was quantified as follows:

$$RE_{CO_2/H_2S}(\%) = \frac{C_{CO_2/H_2S,IN} \cdot F_{IN} - C_{CO_2/H_2S,OUT} \cdot F_{OUT}}{C_{CO_2/H_2S,IN} \cdot F_{IN}} \cdot 100 \quad (1)$$

where $C_{CO_2/H_2S,IN}$ and $C_{CO_2/H_2S,OUT}$ stand for the concentrations (%) of CO_2 and H_2S, respectively, in the raw and upgraded biogas in the AC, while F_{IN} and F_{OUT} correspond to the flow rate of the raw and upgraded biogas (L d^{-1}). Likewise, the overall TOC, IC, total carbon (TC= TOC + IC + C-CO_2), COD, TN, N-NH_4^+ and P removal efficiencies were determined according to equation (2).

$$RE_i(\%) = \frac{C_{i,IN} \cdot Q_{IN} - C_{i,OUT} \cdot Q_{OUT}}{C_{i,IN} \cdot Q_{IN}} \cdot 100 \qquad (2)$$

where $C_{i,IN}$ and $C_{i,OUT}$ are, respectively, the influent and effluent aqueous concentrations (mg L^{-1}) of the target monitored parameter i (TOC, IC, TC, COD, TN, N-NH_4^+ or P), and Q_{IN} and Q_{OUT} represent the influent and effluent wastewater flow rates in the HRAP (L d^{-1}).

The suspended solid removal efficiency of the settler (RE_{TSS}) was quantified according to equation (3):

$$RE_{TSS}(\%) = \frac{TSS_{HRAP} - TSS_{Effluent}}{TSS_{HRAP}} \cdot 100 \qquad (3)$$

where TSS_{HRAP} and $TSS_{Effluent}$ correspond to the TSS concentration (g L^{-1}) in the HRAP and in the supernatant of the settler, respectively. Biomass production (W, g $m^{-2}_{Surface\ HRAP}$ d^{-1}) was estimated according to equation (4):

$$W = \frac{TSS_{HRAP} \cdot Q_{OUT}}{S} \qquad (4)$$

where S represents the total HRAP illuminated surface (1.2 m^2).

Analytical procedures

CO_2, H_2S, CH_4, N_2 and O_2 gas concentrations were analyzed by GC-TCD according to Posadas et al.[14] Dissolved TOC, IC and TN concentrations were determined using a Shimadzu TOC-VCSH analyzer (Japan) equipped with a TNM-1 chemiluminescence module. N-NH_4^+ was measured using an ammonia electrode Orion Dual Star (Thermo Scientific, The Netherlands). N-NO_3^- and N-NO_2^- were analyzed via HPLC-IC using a Waters 515 HPLC pump coupled with

192 an ion conductivity detector (Waters 432), and equipped with an IC-PAK Anion HC column
193 (4.6×150 mm) and an IC-Pak Anion Guard-Pak (Waters). P concentration was determined
194 spectrophotometrically using the ammonium-molybdate method (Spectrophotometer U-2000,
195 Hitachi, Japan). All analyses, including COD, TSS and SVI were carried out according to
196 Standard Methods.[15] The pH was measured in a Eutech CyberScan pH510 pHmeter (Eutech
197 Instruments, The Netherlands), while DO and temperature in the HRAP were measured using an
198 OXI 330i oximeter (WTW, Germany). The photosynthetic active radiation (PAR) was recorded
199 with a LI-250A light meter (LI-COR Biosciences, Germany). The harvested biomass in the settler
200 was dried for 24 hours at 105 ºC in a P-Selecta laboratory stove (SELECTA, Spain). The
201 determination of the C and N content of the algal-bacterial biomass was performed using a LECO
202 CHNS-932, while phosphorus content analysis was carried out spectrophotometrically after acid
203 digestion in a microwave according to the internal procedure of the Instrumental Technical
204 Laboratory of the University of Valladolid. Lipid content was determined gravimetrically
205 following biomass extraction with chloroform:methanol (2:1) (v/v).[16] The biomass protein
206 content was determined using the Lowry method and the carbohydrate content was determined
207 spectrophotometrically using the Dubois method.[17,18] The starch content was quantified using the
208 996.11 AOAC enzymatic method.[19] The PHB content of the biomass was analyzed by GC-MS
209 following the analytical procedure developed by Zúñiga et al[12]. and the ash content was
210 determined according to APHA.,[15]

211 The identification, quantification and biometry measurements of microalgae were carried out by
212 microscopic examination (OLYMPUS IX70, USA) of microalgal samples (fixed with lugol acid
213 at 5% and stored at 4 ºC prior to analysis) according to Sournia.[20]

Statistical treatment

The results were evaluated using an analysis of variance (ANOVA) with a Fisher's least significant difference (LSD) test using a 95% confidence level. The data analyzed always showed variance homogeneity (Bartlett test).

RESULTS AND DISCUSSION

Influence of external liquid recirculation on CO_2 and H_2S removal efficiency and O_2 biomethane concentration in the absorption column

A complete H_2S removal (REs = 100%) was obtained within the range of L/G ratios studied (Fig. 2a). The results obtained also showed that CO_2-RE and the O_2 concentration in the upgraded biogas increased linearly at increasing the L/G ratio up to a ratio of 15 (Fig. 2b). In this context, Kasikamphaiboon et al.[21] also reported increasing CO_2-REs at increasing L/G ratios in a packed column using a Monoethanolamine solution. This increase in CO_2 removal concomitant with the higher O_2 concentrations observed in the treated biogas were likely due to the combined effects of an increase in the overall mass transfer coefficients ($klaCO_2$ and $klaO_2$) at increasing L/G ratios (higher turbulence in the AC) and of the enhanced CO_2/O_2 carry over potential of the external liquid recirculation in the AC (which avoided liquid saturation with CO_2, thus increasing CO_2 concentration gradients available for gas-liquid mass transport, and increased the amount of O_2 potentially stripped-out).[22] On the other hand, no significant differences were found on CO_2-REs (95±2%) at L/G ratios above 15, likely due to the limited increase in $klaCO_2$ when increasing external liquid recirculations over a critical flow rate and to the fact that the absorption process was always operated at a maximum CO_2 concentration gradient under these particular

conditions. Similar CO_2-REs were however recorded by Bahr et al.[10] (86±5%) in a bubble column at a L/G ratio of 1.0 in mineral salt medium at a pH of 9.4 due to significantly higher overall CO_2 solubility at high pH values. This suggests that the complete CO_2 removal in our experiments was probably limited by the relatively low cultivation broth pH (≈7.9). Despite O_2 concentrations in the treated biogas also remained stable at L/G ratios above 15 (3±1%), the rapid DO fluctuations in the algal broth of the HRAP (DO ranged from 3.4 to 7.3 mg L^{-1} along the 175 days of experimentation) used for CO_2 and H_2S absorption could eventually increase O_2 concentrations in the upgraded biogas above 5%, which constitutes the lower explosive limit (LEL) for methane/O_2 mixtures.[23] Therefore, a L/G ratio of ≈10 (corresponding to CO_2-REs of ≈80% and O_2 concentrations < 2%) was here selected.

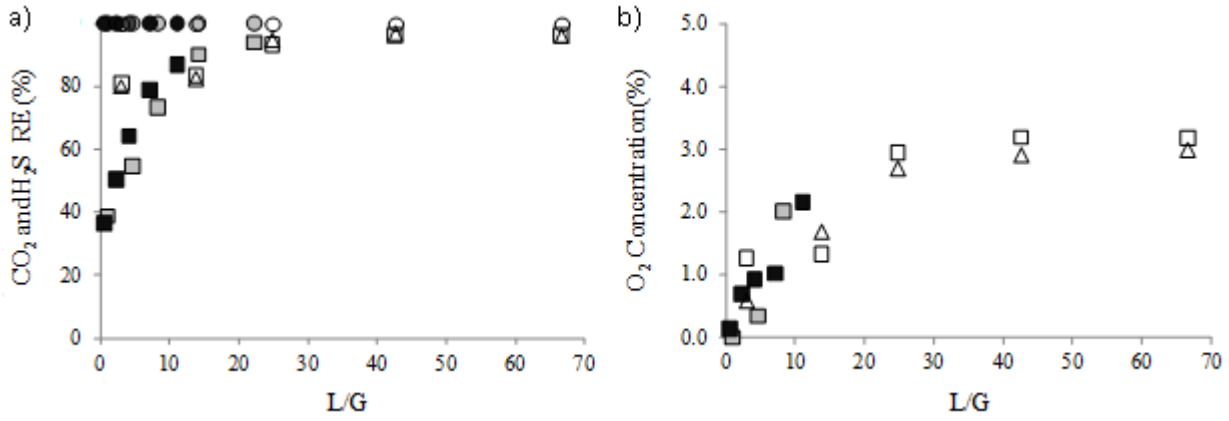


Figure 2. Influence of L/G on CO_2 and H_2S removal efficiency (a) and O_2 biomethane concentration (b) during the biogas absorption experiments in the AC conducted with BM1 (triangles) and BM2 (squares for CO_2 and O_2; circles for H_2S) at 5 mL min^{-1} (white), 15 mL min^{-1} (grey) and 30 mL min^{-1} (black).

Influence of biogas flow rate on biomass composition and wastewater treatment

The HRAP cultivation broth temperature ranged from 22.2±1.3 to 25.1±1.3 °C over the 175 days of HRAP operation (Table 1), which lied within the optimum growth temperature range for most freshwater microalgae (20-30 °C).[24] On the other hand, the high turbulence in the HRAP resulted in high evaporation rates (5.1±0.8 and 7.4±1.6 L m^{-2} d^{-1}) over the entire experimental period, similar to the rates estimated by Guieysse et al.[25] under outdoor conditions in temperate climates (1.3-6.2 L m^{-2} d^{-1}). The DO concentration recorded in the cultivation broth remained always above of 3.4 mgO_2 L^{-1}, which ruled out the absence of oxygen limitation during nitrification or the oxidation of organic matter and H_2S. In this regard, steady state TOC and COD-REs ranging from 24±6 to 57±6% and from 31±1 to 51±6%, respectively, were recorded. These removals were not correlated with the different biogas flow rates tested, but were similar to the aerobic biodegradability of the anaerobically digested wastewater (Table 2). A carbon mass balance calculation over the entire experimental period revealed that assimilation into biomass was the main C removal mechanism. Likewise, assimilation into biomass also was the principal mechanism of N and P removal in the HRAP. A complete NH_4^+ removal was recorded during the 5 operational stages, all effluent TN corresponding to the in-situ produced N-NO_3^- (45±8 mg L^{-1}). N-NO_2^- was not detected in the cultivation broth likely due to the moderate temperatures (T <28ºC) and the occurrence of high DO concentrations.

During stage I (no biogas supply), the pH remained at 7.8±0.1 due to the high ADV buffer capacity. This pH ≈7.9 is optimum for freshwater microalgae cultivation while preventing ammonia toxicity and phosphate precipitation.[3] IC and TC-REs of 71±1 and 50±2%, respectively, were obtained, while low TN-REs (as a result of the limited biomass productivity and high inlet NH_4^+ concentrations) and P-REs of 50±11% were recorded in stage I. TSS concentration

increased to 0.13±0.07 g L^{-1} with an associated biomass productivity of 2.5±0.2 g m^{-2} d^{-1}, comparable to the average productivity of 2.1±0.6 g m^{-2} d^{-1} reported by Posadas et al.[26] during the treatment of fishery wastewater in a similar 180 L HRAP under outdoor conditions.

Table 2. TOC, IC, TC, COD, TN, P, and TSS settler removal efficiencies, TSS concentration, biomass productivity and SVI under steady state in the 5 experimental stages evaluated.

STAGE	RE (%)							TSS	W	SVI
	TOC	IC	TC	COD	TN	P	TSS	(g L^{-1})	(g m^{-2} d^{-1})	(mL g^{-1})
I	24±6	71±1	50±2	31±1	1±15	50±11	93±1	0.13±0.07	2.5±0.2	61±3
II	45±8	66±3	60±4	48±4	37±7	71±11	100±0	0.35±0.02	7.1±0.8	391±77
III	50±11	76±6	66±7	51±6	35±12	86±11	99±0	0.39±0.07	6.6±1.9	358±13
IV	57±6	78±2	73±2	48±5	25±12	75±10	97±3	0.48±0.09	9.4±2.0	466±68
V	45±2	68±4	69±2	38±6	21±3	36±1	99±1	0.60±0.06	11.8±0.9	266±10

BM1 upgrading during stage II increased the overall process C/N ratio from 3.4±0.2 to 4.7±0.0. A low biogas flow rate of 0.2 m^3 m^{-2} h^{-1} (at a L/G of 10) was initially set to avoid the acidification of the HRAP cultivation broth, whose pH remained at 7.9±0.2. The IC-REs remained constant at 66±3%, while the TC-REs increased to 60±4%. Similarly, TN-REs and P-REs increased to 37±7% and 71±11%, respectively. The increase in algal-bacterial biomass concentration up to 0.35±0.02 mgTSS L^{-1} as a result of the enhanced C availability entailed a biomass productivity of 7.1±0.8 g m^{-2} d^{-1}. Finally, CO_2-REs of 80±1% concomitant with O_2 concentrations of 2±0% were recorded during stage II (Fig. 3a), which were consistent with the CO_2-REs of ≈50% reported by

Kao et al.[5] during the upgrading of a H_2S-free biogas by *Chlorella* sp. MB-9 in a 50 L outdoor photobioreactor at 0.05 vvm.

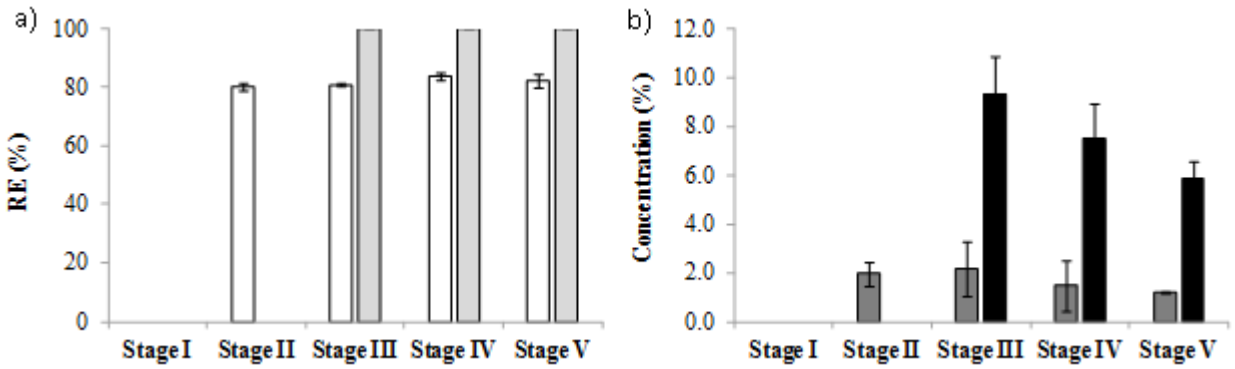


Figure 3. a) CO_2 (□) and H_2S (▫) removal efficiency, and b) O_2 (▪) and N_2 (■) concentrations in the biogas upgraded in the AC during the five experimental stages.

BM1 was replaced by BM2 in stage III in order to elucidate the influence of simulated real biogas supply on HRAP performance and biogas upgrading. The presence of H_2S at 0.5% did not influence significantly the removal of IC, TC, TN and P and biomass productivity. However, carbon supply continued being the main process limitation as a result of the low C/N ratio in the process. On the other hand, while CO_2-RE remained constant at 81±1% (Fig. 3a), a complete H_2S removal was achieved as reported by Bahr et al.[10]. The O_2 and N_2 concentrations in the upgraded biogas during stage III, stripped out from the recycling cultivation broth, averaged 2±1 and 9±2%, respectively (Fig. 3b).

Despite BM2 flow rate was increased to 0.6 $m^3\ m^{-2}\ h^{-1}$ in stage IV (with the corresponding increase in external liquid recirculation to maintain a L/G of 10), IC and TC-REs remained comparable to those recorded in stage III. TN-REs dropped to 25±12%, while P-REs remained similar to stage III (75±7%). Both TSS concentration and biomass productivity increased to

304 0.48±0.09 mg L^{-1} and 9.4±2.0 g m^{-2} d^{-1}, respectively, despite the occurrence of carbon limitation.
305 The CO_2 and H_2S-REs remained constant at 84±2% and 100%, respectively, as well as the O_2
306 (1±1%) and N_2 (8±1%) concentrations in the upgraded biogas.

307 The increase in BM2 flow rate to 1.2 m^3 m^{-2} h^{-1} in stage V (at a L/G of 10) brought about an
308 increase in the overall C/N ratio of 7.0±0.3. The IC and TC-REs slightly decreased to 68±4 and
309 69±2%, respectively, along with a decrease in TN-RE to 21±3%, and in P-RE to 36±1% (as a
310 result of the slightly higher TN and P concentrations in the influent wastewater). The TSS and W
311 slightly increased to 0.60±0.06 mg L^{-1} and 11.8±0.9 g m^{-2} d^{-1}, respectively, which were
312 comparable to the productivities of 10-35 g m^{-2} d^{-1} reported by Hoffmann[27] in outdoors HRAPs
313 treating domestic wastewater at HRTs of 2-6 d. At this point it must be stressed that biomass
314 productivity was positively correlated (R^2= 0.9622) with the C/N ratio applied to the HRAP (data
315 not shown). Finally, CO_2-REs (82±2%), H_2S REs (100%) and the O_2 (1±0%) and N_2 (6±1%)
316 concentrations in the upgraded biogas remained similar to stage IV.

317 Steady state CO_2 and H_2S removals of ≈80 and 100%, respectively, were achieved in this
318 combined AC-HRAP process by maintaining a constant L/G of 10 regardless of the biogas flow
319 rate applied, which were similar to those often supported by conventional high pressure (8-10
320 bar) water scrubbing units.[8] Lower CO_2-REs (40±6%) were reported by Bahr et al.[10] when
321 assessing biogas upgrading in a AC-HRAP using diluted centrate wastewater instead of mineral
322 salt medium (CO_2-REs ≈86±5%), while H_2S-REs remained at 100% regardless of the cultivation
323 medium in this preliminary study. Similarly, Conde et al.[28] obtained 74-93% CO_2-REs and
324 60-67% H_2S-REs in a HRAP equipped with an absorption column inside the pond. In a more
325 recent study Mann et al.[29] recorded CO_2-REs of up to 97% with a complete H_2S removal in a 1 L

enclosed tubular photobioreactor. However, in spite of these promising results, the O_2 levels in the upgraded biogas ranged from 18-23% (far above the LEL of CH_4-O_2 mixtures). Converti et al.[7] (10-24%) also observed high O_2 biomethane concentrations during biogas upgrading by *Arthrospira platensis* in a 1.0 L photobioreactor. In our particular study, the maximum O_2 concentrations still remained above the upper limit required for injection of the upgraded biogas in most natural gas networks in Europe (≈0.5-1%).[10] Finally, negligible methane losses during biogas upgrading (< 1%) were recorded regardless of the operational stages.

Microalgae population and biomass harvesting

Despite *Chlorella* sp. (100%) was initially inoculated in the HRAP, this microalga was mainly overcome by *Pseudanabaena* sp. (48%) and *Chloromonas* sp. (26%) during stage I (Fig. 4). High average TSS-REs of 93±1% were achieved at a settler HRT of 12±3 h (Table 2), which were significantly higher to those reported by Park et al.[30] in settling ponds at 1-2 d of HRT (TSS-REs of 50-80%) and comparable to the maximum TSS-REs of 90±15% observed by Posadas et al.[26] in a similar HRAP treating fishery and domestic wastewaters. A low SVI of 61±3 mL g^{-1} was also recorded during stage I, which confirmed the good compaction of the algal-bacterial sludge visually observed (SVI <100 mL g^{-1} is desired in conventional activated sludge plants).[15,31]*Pseudanabaena* sp. decreased to 25% as a result of the dominance of *Stigeoclonium* sp. (72%) during stage II. This change in the microalgae assemblage resulted higher TSS REs (≈100±0%) in the settler but in a deterioration in the SVI to 391±77 mL g^{-1}, respectively. The supplementation of biogas with H_2S in stage III entailed a further modification in the microalgae population structure, with a complete dominance of *Microspora* sp. (100%). Surprisingly, this new microalgae assemblage maintained similar SVIs and TSS-REs in the settler

348 to those recorded in stage II. The increase in biogas flow rate in stage IV was characterized by a
349 gradual shift in microalgae population to *Stigeoclonium* sp. (14%) and *Planktolyngbya* sp. (81%),
350 which also exhibited a poor sludge compaction (SVI≈466±68 mL g^{-1}) but similar TSS-REs
351 (97±3%). Finally, a decrease in SVI to 262±13 mL g^{-1} along with an efficient settler performance
352 (TSS-REs of 99±1%) were mediated by the change in the microalgae population to
353 *Stigeoclonium* sp. (16%) and *Geitlerinema* sp. (60%) during stage V. Contrary to previous
354 observations in HRAPs, the microalgae harvesting efficiency in our experimental set-up was not
355 dependent on the microalgae population structure.[30] It must be stressed that microalgae
356 population was totally composed of filamentous microalgae from stage II to V, which was
357 consistent with the high SVI recorded but surprisingly did not hinder the rapid biomass
358 sedimentation.[32]

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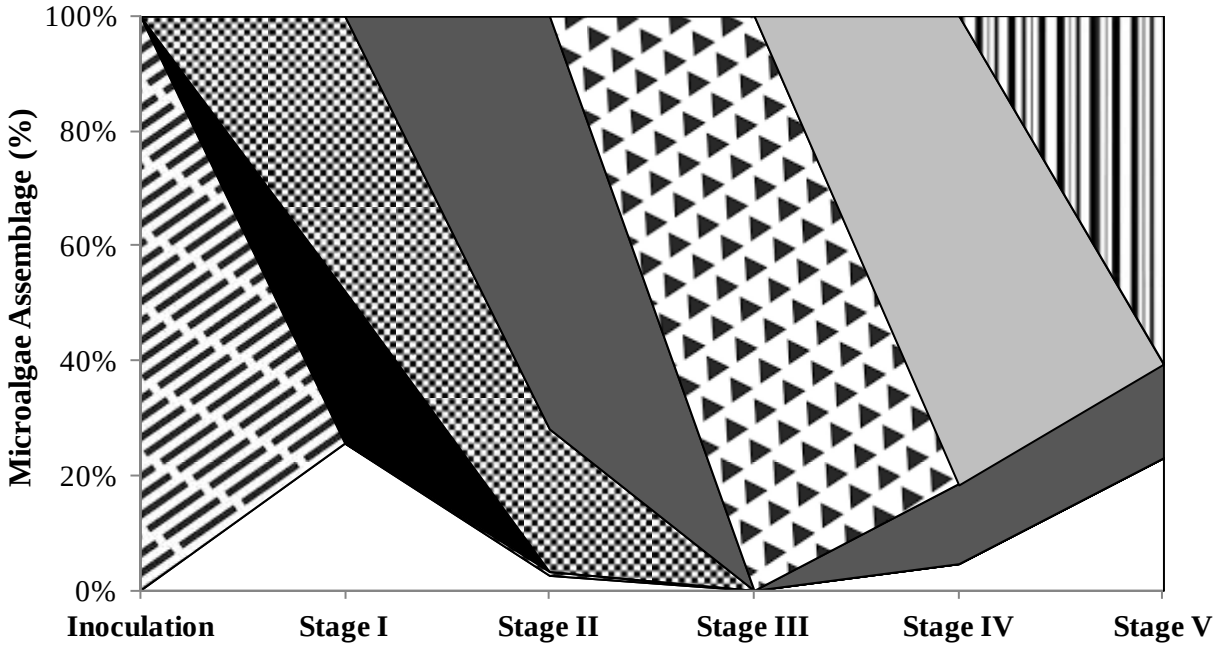

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Figure 4. Dynamics of microalgae population in percentage of number of cells during the inoculation and the five experimental stages evaluated. (▨) *Chlorella* sp., (■) *Chloromonas* sp., (▥) *Geitlerinema* sp., (▶) *Microspora* sp., (▩) *Pseudanabaena* sp., (■) *Stigeoclonium* sp., (■) *Planktolyngbya* sp., and (□) other species (<8%) (*Acutodesmus* sp., *Entosiphon* sp., *Leptolyngbya* sp., *Nitzschia* sp., *Staurosira* sp., *Synechococcus* sp., and *Ulothrix* sp.).

Macromolecular and elemental composition

The algal-bacterial C, N and P content (on a dry weight basis) remained constant at 49±2%, 9±0% and 1±0%, respectively, regardless of the operational stage. The elemental composition of the biomass here obtained (C/N biomass ratio of 5.6±0.2) was in agreement with previous literature findings (C 40-60%; N 4-9%).[33] The algal-bacterial consortium used for HRAP inoculation exhibited carbohydrate, protein, lipid and ash concentrations of 68±8%, 20±1%, 9±1% and 8±0%, respectively (Fig. 5). The carbohydrate content increased to 82±1% along with a decrease in lipid content to 1±1% during stage I. The stepwise increase in biogas flow rate from stage I to IV induced in a decrease in carbohydrate content (R^2= 0.9537) concomitant with an increase in the protein content (R^2= 0.9474). During stage V, the carbohydrate, protein, lipid and ash concentrations remained comparable to those obtained in stage IV (60±7%, 36±6%, 1±0% and 14±0%, respectively). The low recorded PHBs content of the algal-bacterial biomass during the five operational stages compared to the values of 11% reported by Panda and Mallick[34] under nutrient starvation conditions ruled out the possibility to use this biomass for biopolymer production (Fig. 5). On the other hand, overall the carbohydrate content of the algal-bacterial biomass recorded in this study was superior to that typically reported for microalgae (8-35%),[35,36] which suggests a straight forward biomass valorization in the form of bioethanol production via

carbohydrate fermentation.[24,36] In this context, the microalgae population present in the HRAP during experimentation was mainly composed by cyanobacteria, which are known to contain glycogen rather than starch as storage carbohydrates.[36] This was confirmed by the low starch content of the algal-bacterial biomass (5±1%) in stage V. Based on the results obtained in stage V and assuming both a yield of carbohydrate extraction (prior hydrolysis) into glucose of 80% and a theoretical maximum conversion of glucose into ethanol of 0.51 g ethanol per g glucose,[36] 1000 m^3 d^{-1} of biogas could eventually produce 328 $Kg_{biomass}$ d^{-1} and be converted to 102 L of ethanol. This specific production (302 $L_{ethanol}$ $ton_{biomass}^{-1}$) is superior to that reported for sugar cane (70 L ton^{-1}) and comparable to that of bagasse (280 L ton^{-1}).[37] In this context, the potential of biotechnology to produce high-added value products from biogas upgrading was recently reported by Angelidaki and co-workers during the succinic acid production using glucose as external electron donors.[38]

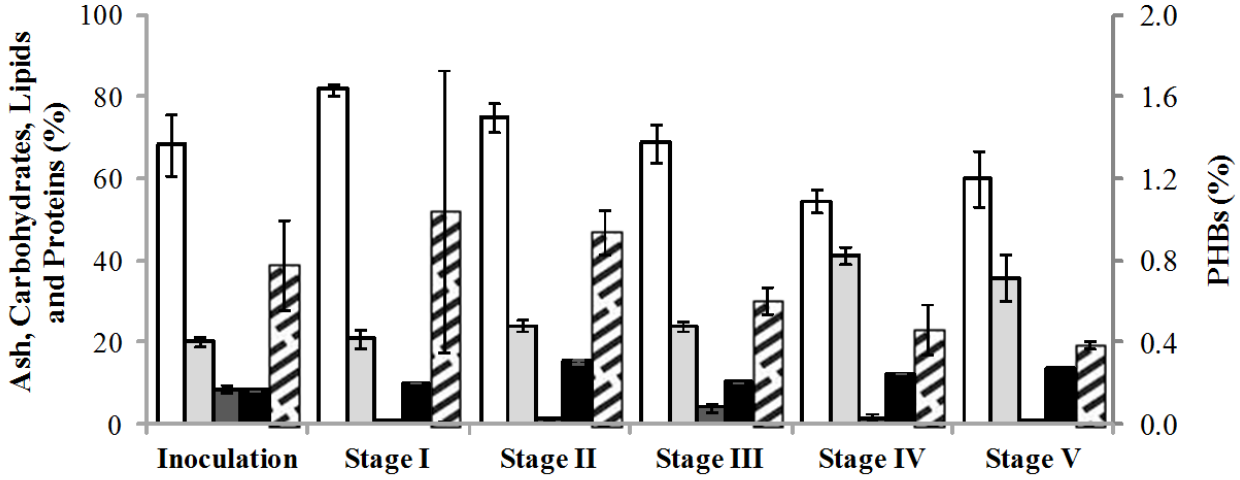

Figure 5. Carbohydrates (□), proteins (▫), lipids (■), PHBs (▨) (expressed on ash free basis) and ash (■) concentrations in the harvested biomass under steady state during the inoculation and the 5 experimental stages evaluated.

In brief, this research work confirmed the potential of algal-bacterial processes to support an integral upgrading of biogas coupled to both wastewater treatment and the production of biomass with a high plasticity in terms of macromolecular composition. The use of an external absorption column interconnected to a HRAP via the external recirculation of the microalgae broth at a L/G of 10 supported sustained CO_2 and H_2S removals of 80% and 100%, respectively. Unfortunately, O_2 concentrations in the upgraded biogas were above maximum recommended levels for biomethane injection in natural gas networks in Europe, which represents a niche for further research. Finally, the high carbohydrate content (60-80%) of the algal-bacterial biomass produced, which was inversely correlated with the biogas load, would eventually allow the production of 102 $L_{ethanol}$ per 1000 m^3_{biogas} using a bio-refinery process approach.

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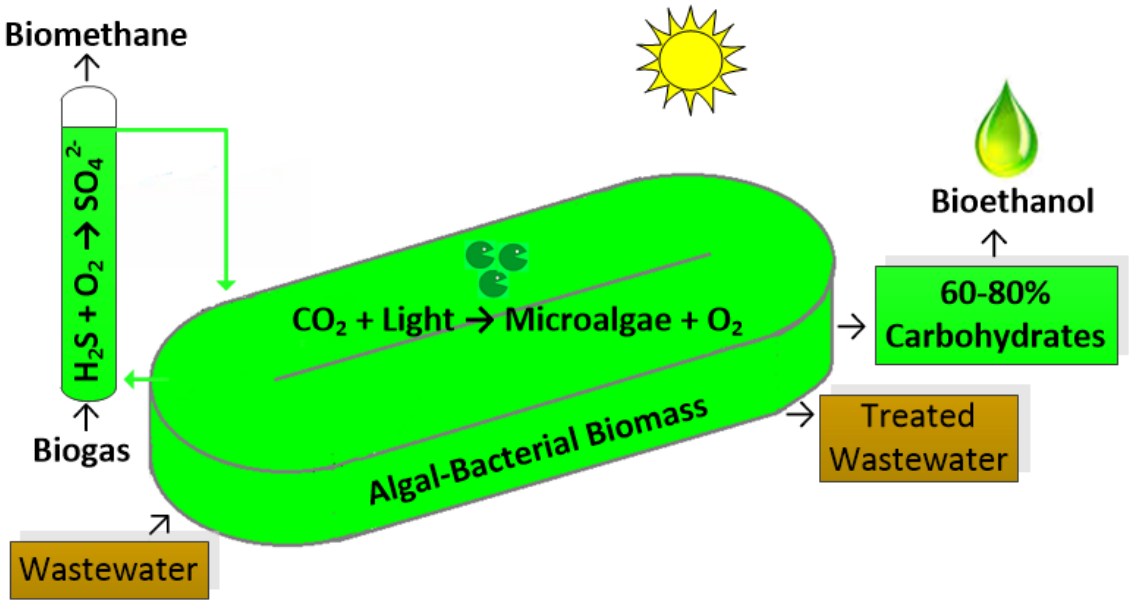

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