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Influence of Biogas Flow Rate on Biomass Composition During the Optimization of Biogas Upgrading in Microalgal-Bacterial Processes

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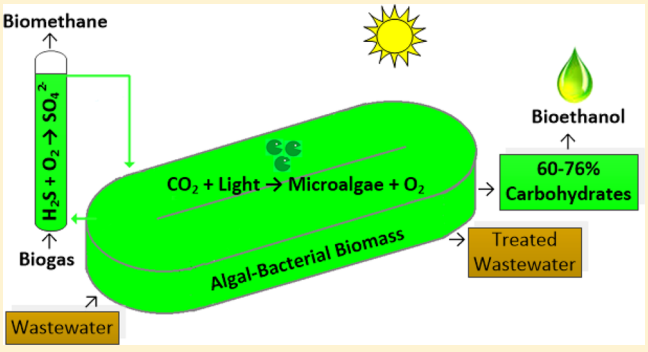
Supporting Information

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 (60−76%), inversely correlated to biogas flow rates, would allow the production of ≈100 L of ethanol per 1000 m^3 of biogas upgraded under a biorefinery 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 (0.005−2%), 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 with 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 supporting 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

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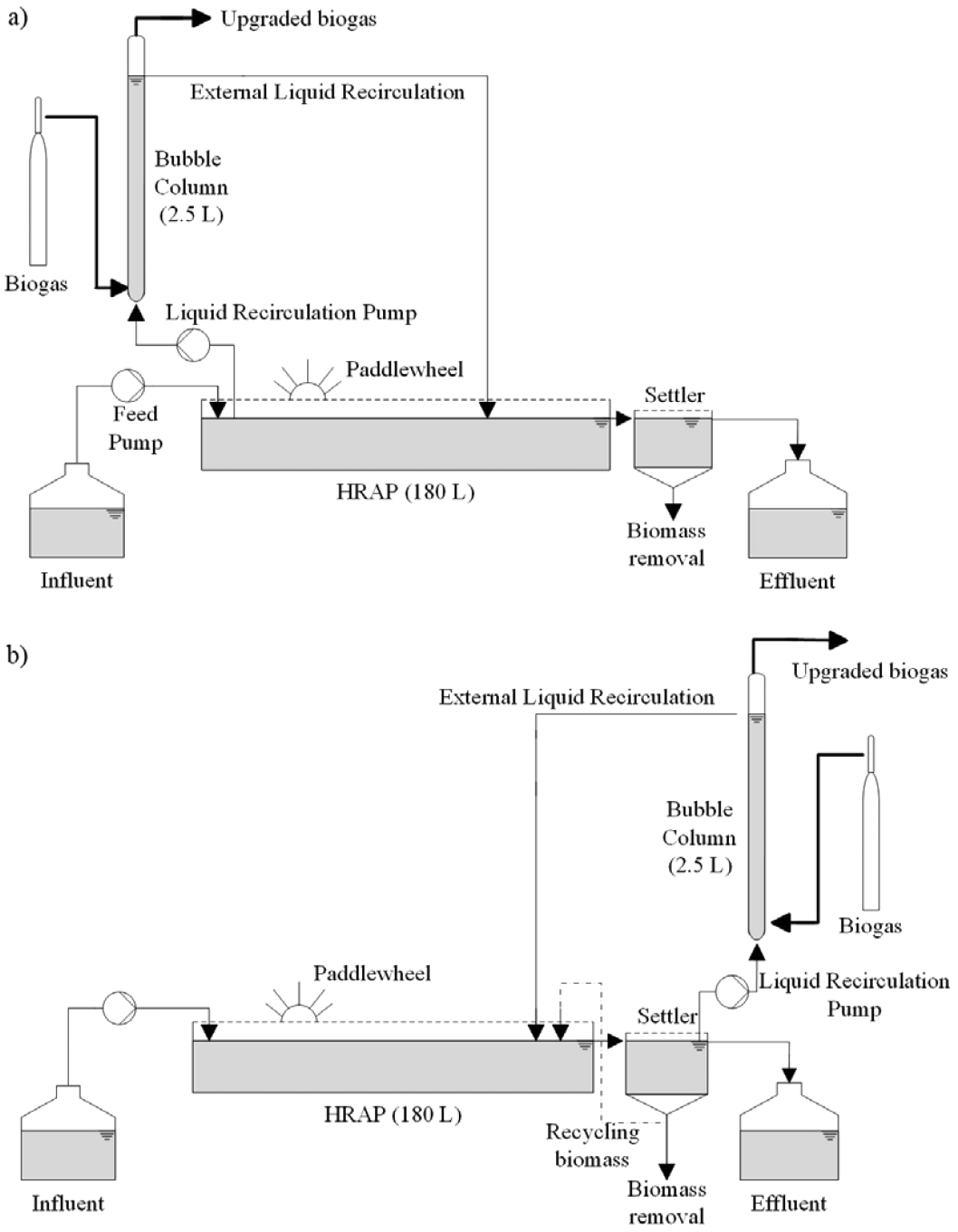

Figure 1. Schematic of the combined biogas upgrading and ADV treatment experimental setup. (a) External liquid recirculation drawn from the cultivation broth (stages I–III). (b) External liquid recirculation drawn from the supernatant of the settler (stages IV and V).

the environmental benefits associated with 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 bioproducts 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 intercon-

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Table 1. Biogas Mixtures and Flow Rates Tested in the AC with the Average Cultivation Broth Temperature, Evaporation Rate, pH, and DO Monitored in the HRAP During Each Experimental Stage

	absorption column		high rate algal pond			
stage	biogas mixture	biogas flow rate ($m^3\ m^{-2}\ h^{-1}$)	HRAP temp (°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

nected 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 on the basis of the key impact of this operational variable on both the CO_2 and H_2S mass transport from the biogas and the stripping of O_2 and N_2 from the algal-bacterial cultivation broth.[10] 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 Setup. The experimental setup 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 transparent PVC 2.5 L (Ø = 4.4 cm; height = 165 cm) external absorption column, with lateral connections at its bottom and top for the upflow recirculation of the cultivation broth. The HRAP and AC were interconnected via an external recirculation of the microalgae broth (Figure 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) 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.

Microorganisms and Culture Conditions. The pilot HRAP was inoculated with 10 L of a 0.6 g TSS L^{-1} *Chlorella vulgaris* culture (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 analyzed TSS concentration in the cultivation broth of the HRAP was of 0.08 g L^{-1}. The amount of bacteria inoculated in the HRAP was twice higher compared to that of microalgae to ensure a rapid biodegradation start-up.

Chlorella vulgaris was isolated from a vinasse storage pond of a sugar and ethanol industry located in Mato Grosso do Sul (Brazil). There were 12 1.0 L e-flasks incubated at 30 °C and 200 rpm 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). N_2 was replaced by CH_4 in BM2 in order to simulate a typical biogas composition. 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. The TSS concentration in the cultivation broth during the experimentation was 0.13 ± 0.07 g L^{-1}.

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 (7.4 ± 0.3 days) was selected on the basis of the results of the ADV biodegradability tests mentioned below, which was similar to HRTs reported for domestic wastewater treatment in HRAPs.[13,14] In addition, the HRT in the settler was ≈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 on the basis of the results obtained in preliminary ADV biodegradability tests in algal-bacterial systems performed according to methodology described by Posadas et al.[15] These preliminary assays with ADV wastewater revealed maximum removals of soluble total organic carbon (TOC), inorganic carbon (IC), and total nitrogen (TN) of 20%, 91%, and 50%, respectively, in the tests provided with ADV diluted at 10% and incubated at 300 rpm under 284 ± 17 μmol m^{-2} s^{-1} with a light:dark photoperiod of 16:8 h after 7 days. The average concentrations of soluble TOC, IC, chemical oxygen demand (COD), TN, N-NH_4^+, and 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.

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

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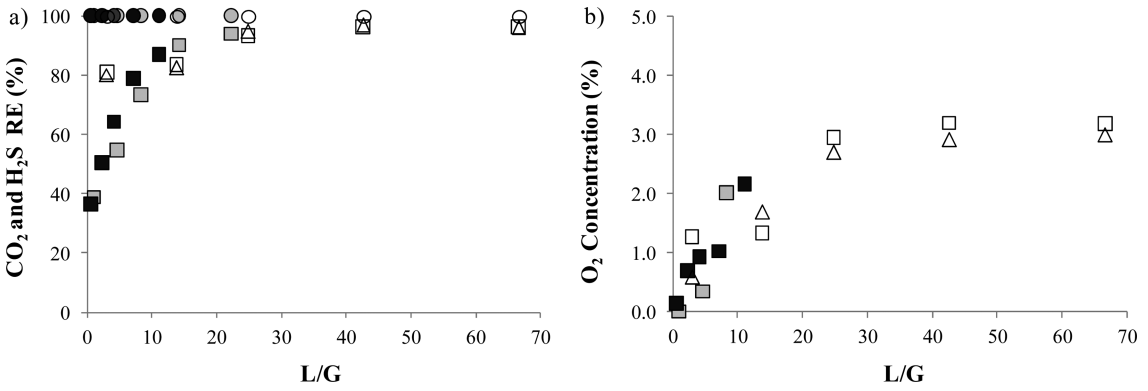

Figure 2. Influence of L/G on CO_2 and H_2S removal efficiency (a) and O_2 biomethane concentration (b) during the biogas absorption experiments in the AC conducted with BM1 (△) and BM2 (□ for CO_2 and O_2; ○ for H_2S) at 5 mL min^{-1} (white), 15 mL min^{-1} (gray), and 30 mL min^{-1} (black).

m^{-2} h^{-1} were continuously sparged into the AC during stages II and III, respectively (Figure 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 (Figure 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, Figure 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 measured daily. The latter were used to determine the daily evaporation rate from the difference between both flow rates. 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 calculation of biogas CO_2 and H_2S removal efficiency, soluble TOC, IC, total carbon, COD, TN, N-NH_4^+, and P removal efficiencies, suspended solid removal efficiency of the settler (RE_{TSS}), biomass production, and C, N, and P mass balance were described in the Supporting Information (SI) section.

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.[15] 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 an ion conductivity detector (Waters 432), and equipped with an IC-PAK Anion HC column (4.6 mm × 150 mm) and an IC-Pak Anion Guard-Pak (Waters). P concentration was determined spectrophotometrically using the ammonium-molybdate method (Spectrophotometer U-2000, Hitachi, Japan). All analyses, including COD, TSS, and SVI were carried out according to Standard Methods.[16] The pH was measured in a Eutech CyberScan pH510 pHmeter (Eutech Instruments, The Netherlands), while DO and temperature in the HRAP were measured using an OXI 330i oximeter (WTW, Germany). The photosynthetic active radiation (PAR) was recorded with a LI-250A light meter (LI-COR Biosciences, Germany). The harvested biomass in the settler was dried for 24 h at 105 °C in a P-Selecta laboratory stove (SELECTA, Spain). The determination of the C and N content of the algal-bacterial biomass was performed using a LECO CHNS-932, while phosphorus content analysis was carried out spectrophotometrically after acid digestion in a microwave according to the internal procedure of the Instrumental Technical Laboratory of the University of Valladolid.[13] Lipid content was determined gravimetrically following biomass extraction with chloroform:methanol (2:1) (v/v).[17] The biomass protein content was determined using the Lowry method, and the carbohydrate content was determined spectrophotometrically using the Dubois method.[18,19] The starch content was quantified using the 996.11 AOAC enzymatic method.[20] The PHB content of the biomass was analyzed by GC-MS following the analytical procedure developed by Zúñiga et al.,[12] and the ash content was determined according to APHA.[16]

The identification, quantification, and biometry measurements of microalgae were carried out by microscopic examination (OLYMPUS IX70) of microalgal samples (fixed with lugol acid at 5% and stored at 4 °C prior to analysis) according to Sournia.[21]

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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

		RE (%)								
stage	TOC	IC	TC	COD	TN	P	TSS	TSS (g L^{-1})	W (g m^{-2} d^{-1})	SVI (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

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 (Figure 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 (Figure 2b). In this context, Kasikamphaiboon et al.[22] 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 was likely due to two combined effects: (i) an increase in the overall mass transfer coefficients ($klaCO_2$ and $klaO_2$) at increasing L/G ratios as a result of the higher turbulence in the AC; (ii) an enhanced CO_2/O_2 transportation potential of the external liquid recirculation in the AC, which avoided liquid saturation with CO_2 and consequently supported an increase in the CO_2 concentration gradients available for biogas−liquid mass transport (but at the expenses of an increase in the amount of O_2 potentially stripped-out).[23] 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 an 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 remaining 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.[24] Therefore, a L/G ratio of ≈10 (corresponding to CO_2-REs of ≈80% and O_2 concentrations <2%) was here selected.

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).[25] 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.[26] 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 mg O_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 (see Supporting Information for a detailed 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 and the occurrence of high DO concentrations. In this context, NO_2^- oxidation kinetics are often limited at temperatures above 28 °C,[27] while the entire nitrification process becomes limited by oxygen at DO concentrations below 2 mg O_2 L^{-1}.[28]

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.[29] during the treatment of fishery wastewater in a similar 180 L HRAP under outdoor conditions. In addition, the ADV C/N/P ratio of ≈100/26/0.7 suggested a simultaneous carbon and phosphorus limitation based on the optimum ratio reported for microalgae growth (≈100/18/2).[13,15,30]

BM1 upgrading during stage II increased the overall process C/N ratio from 3.4 ± 0.2 to 4.7 ± 0.0, remaining still lower than the optimum ratio for microalgae growth of ≈5.6.[13,15,30] A low biogas flow rate of 0.2 m^3 m^{-2} h^{-1} (at an 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%.

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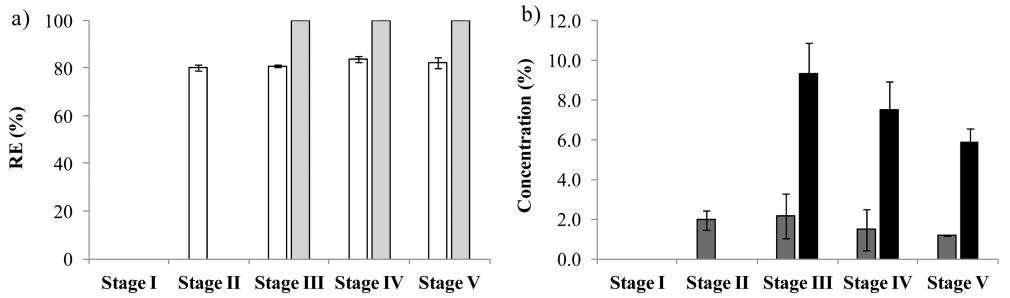

Figure 3. (a) CO_2 (white) and H_2S (gray) removal efficiency, and (b) O_2 (gray) and N_2 (black) concentrations in the biogas upgraded in the AC during the five experimental stages.

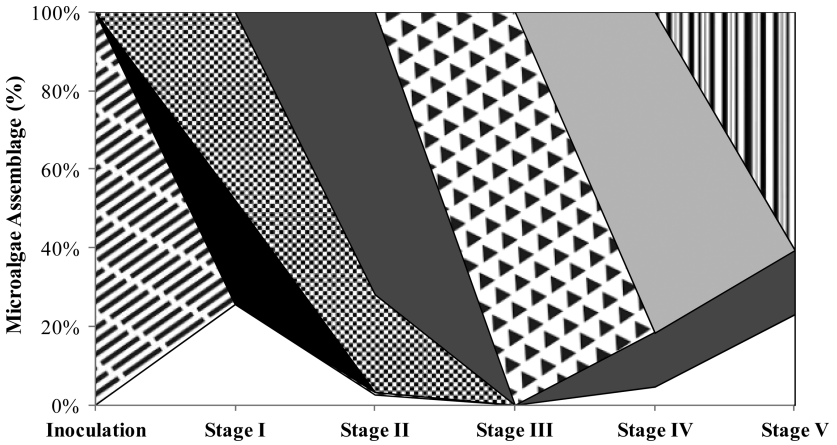

Figure 4. Dynamics of microalgae population in percentage of number of cells during the inoculation and the five experimental stages evaluated: (diagonal stripes) *Chlorella* sp., (black) *Chloromonas* sp., (vertical stripes) *Geitlerinema* sp., (triangles) *Microspora* sp., (cross-hatch) *Pseudanabaena* sp., (dark gray) *Stigeoclonium* sp., (light gray) *Planktolyngbya* sp., and (white) other species (<8%) (*Acutodesmus* sp., *Entosiphon* sp., *Leptolyngbya* sp., *Nitzschia* sp., *Staurosira* sp., *Synechococcus* sp., and *Ulothrix* sp.).

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 mg TSS 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 (Figure 3a), which were superior to 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.

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 (4.7 ± 0.4) in the process.[13,15,30] On the other hand, while CO_2-RE remained constant at 81 ± 1% (Figure 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 (Figure 3b). Similar O_2 (1.8 ± 0.2%) and N_2 (7.5 ± 1.4%) concentrations landfill were obtained by Läntelä et al.[31] during the upgrading of biogas in high pressure (20 bar) water absorption units.

Despite BM2 flow rate increasing 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 and a C/N ratio of 5.6 ± 0.1), 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 0.48 ± 0.09 mg L^{-1} and 9.4 ± 2.0 g m^{-2} d^{-1}, respectively. The CO_2 and H_2S-REs remained constant at 84 ± 2% and 100%, respectively, as well as the O_2 (1 ± 1%) and N_2 (8 ± 1%) concentrations in the upgraded biogas.

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 increase in the overall C/N ratio of 7.0 ± 0.3. The IC and TC-REs slightly decreased to 68 ± 4% and 69 ± 2%, respectively, along with a decrease in TN-RE to 21 ± 3%, and in P-RE to 36 ± 1% (as a result of the slightly higher TN and P concentrations in the influent wastewater). The TSS and W slightly increased to 0.60 ± 0.06 mg L^{-1} and 11.8 ± 0.9 g m^{-2} d^{-1}, respectively, which were comparable to the productivities of 10−35 g m^{-2} d^{-1} reported by Hoffmann[32] in outdoors HRAPs treating domestic wastewater at HRTs of 2−6 d. At this point it must be stressed that biomass productivity was positively correlated (R^2 = 0.9622) with the C/N ratio applied to the HRAP. Finally, CO_2-REs (82 ± 2%), H_2S REs (100%), and the O_2 (1 ± 0%) and N_2 (6 ± 1%) concentrations in the upgraded biogas remained similar to stage IV. Despite the decreasing trend in both the average oxygen and nitrogen concentrations from stage III to V, the N_2/O_2 ratio in the upgraded biogas during these three stages remained similar (4.8 ± 0.5), which suggest that this biogas contamination was originated from the dissolution of N_2/O_2 in the open HRAP and their further desorption in the AC. The gradual increase in the cultivation broth temperature from stage

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

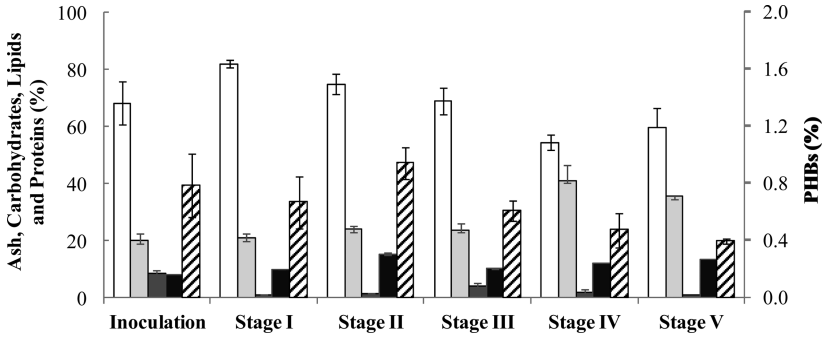

Figure 5. Carbohydrates (white), proteins (light gray), lipids (dark gray), PHBs (diagonal stripes) (expressed on ash free basis), and ash (■) concentrations in the harvested biomass under steady state during the inoculation and the 5 experimental stages evaluated.

III to V likely induced these lower N_2 and O_2 concentrations in the upgraded biogas as a result of the lower aqueous solubility of these two gases at increasing temperatures.

Steady state CO_2 and H_2S removals of ≈80% and 100%, respectively, were achieved in this combined AC-HRAP process by maintaining a constant L/G of 10 regardless of the biogas flow rate applied, which were similar to those often supported by conventional high pressure (8−10 bar) water scrubbing units.[8] Lower CO_2-REs (40 ± 6%) were reported by Bahr et al.[10] when assessing biogas upgrading in a AC-HRAP using diluted centrate wastewater instead of mineral salt medium (CO_2-REs ≈86 ± 5%), while H_2S-REs remained at 100% regardless of the cultivation medium in this preliminary study. Similarly, Conde et al.[33] obtained 74−93% CO_2-REs and 60−67% H_2S-REs in a HRAP equipped with an absorption column inside the pond. In a more recent study Mann et al.[34] 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% to 23% (far above the LEL of CH_4−O_2 mixtures). Converti et al.[7] also observed high O_2 biomethane (10−24%) 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%) being initially inoculated in the HRAP, this microalga was mainly overcome by *Pseudanabaena* sp. (48%) and *Chloromonas* sp. (26%) during stage I (Figure 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.[35] 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.[29] 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).[16,36] *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 to those recorded in stage II. The increase in biogas flow rate in stage IV was characterized by a gradual shift in microalgae population to *Stigeoclonium* sp. (14%) and *Planktolyngbya* sp. (81%), which also exhibited a poor sludge compaction (SVI ≈ 466 ± 68 mL g^{-1}) but similar TSS-REs (97 ± 3%). Finally, a decrease in SVI to 262 ± 13 mL g^{-1} along with an efficient settler performance (TSS-REs of 99 ± 1%) were mediated by the change in the microalgae population to *Stigeoclonium* sp. (16%) and *Geitlerinema* sp. (60%) during stage V. Contrary to previous observations in HRAPs, the microalgae harvesting efficiency in our experimental setup was not dependent on the microalgae population structure.[35] It must be stressed that microalgae population was totally composed of filamentous microalgae from stage II to V, which was consistent with the high SVI recorded but surprisingly did not hinder the rapid biomass sedimentation.[37]

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%).[38] 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 (Figure 5). A similar carbohydrate content (≈69%) was found by Margarites and Costa[39] when *Chlorella minutissima* was cultivated in a synthetic mineral salt medium under phosphorus deprivation conditions. The carbohydrate content increased to 76 ± 1% along with a decrease in lipid content to 1 ± 0% during stage I. High carbohydrate contents in microalgae have been associated with phosphorus limitation.[39,40] For instance, Markou et al.[40] reported a carbohydrate content up to 67 ± 3% in *A. platensis* under phosphorus starvation. Furthermore, the stepwise increase in biogas flow rate from stage I to IV (resulting in an increase in the C/N ratio from 3.4 ± 0.2 to 5.6 ± 0.1, respectively) induced a decrease in carbohydrate content (R^2 = 0.9762) concomitant with an increase in the protein content (R^2 = 0.9629). During the noncarbon-limited 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). These results suggest that the algal-bacterial biomass accumulated carbohydrates due to a potential carbon limitation below the optimum C/N ratio (5.6) concomitant with a limitation in P supply to the process.

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

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[41] under nutrient starvation conditions ruled out the possibility to use this biomass for biopolymer production (Figure 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%),[42,43] which suggests a straightforward biomass valorization in the form of bioethanol production via carbohydrate fermentation.[39,43] 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.[43] This was confirmed by the low starch content of the algal-bacterial biomass (5 ± 1%) in stage V. On the basis of the results obtained in stage V and assuming both a yield of carbohydrate extraction (prior hydrolysis) into glucose of 80% as reported by Möllers et al.[43] and a theoretical maximum conversion of glucose into ethanol of 0.51 g ethanol per g glucose,[43] 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}).[44] 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.[45]

In brief, this research work confirmed the potential of algal-bacterial processes to support an efficient 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. Similarly, studies focused on the removal of other biogas pollutants such as methyl siloxanes, halocarbons, and N_2 during biogas upgrading by microalgal-bacterial processes are also necessary to exploit the potential of this biotechnology in order to achieve an integral biogas upgrading. Finally, the high carbohydrate content (60−76%) 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 biorefinery process approach.

■ ASSOCIATED CONTENT

Supporting Information

Additional details, including calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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