Title: Influence of the gas-liquid flow configuration in the absorption column on photosynthetic biogas upgrading in algal-bacterial photobioreactors

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Keywords: Algal-bacterial photobioreactor; biogas upgrading; bio-methane; nutrients recovery; digestate

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Suggested Reviewers: Leslie Meier PhD
Universidad de La Frontera, Chile
l.meier01@ufromail.cl
Expert in wastewater treatment and Biogas upgrading using microalgae cultures

Cheng Yan Chen PhD
School of Environmental Studies, China
yancheng622@126.com
Expert in digestate treatment and Biogas upgrading using microalgae cultures

Simon Murray PhD
Queen's University Belfast, UK
s.murray@qub.ac.uk
Expert in Biogas upgrading
Enrica Uggetti PhD
Universitat Politècnica de Catalunya, Spain
enrica.uggetti@upc.edu
Expert in wastewater and sludge treatment, low cost technologies, microalgae, anaerobic digestion and biogas.
Dear Editor-in-Chief

Please find enclosed our original unpublished paper “Influence of the gas-liquid flow configuration in the absorption column on photosynthetic biogas upgrading in algal-bacterial photobioreactors” co-authored by Alma Toledo-Cervantes, Cindy Madrid-Chirinos, Sara Cantera, Raquel Lebrero and Raúl Muñoz. All authors are aware of the ethics policy of Bioresource Technology Journal, declare no conflict of interest and accept responsibility for the present manuscript. The manuscript is submitted for publication in Bioresource Technology for the first time, considering that it is the best-suited journal for the research area of the present work, more specifically Biological waste treatment: Environmental bioengineering (20.100).

Photosynthetic biogas upgrading coupled with nutrient removal from digestate represents a competitive and environmentally friendly technology to conventional physical-chemical technologies for biogas upgrading. This innovative technology, here evaluated at pilot scale, consisted of a high rate algal pond (HRAP) treating digestate interconnected to a CO₂-H₂S absorption column (AC) via recirculation of the HRAP cultivation broth for biogas scrubbing. Preliminary studies in our lab have consistently showed that despite the high potential of photosynthetic biogas upgrading, N₂ and O₂ stripping from the recycling cultivation broth to the upgraded biogas often results in CH₄ concentrations < 95 % (the minimum concentration for biomethane injection into natural gas grids in most EU countries). Thus, an optimization of biogas scrubbing in the AC of this photosynthetic biogas upgrading system is needed in order to obtain a bio-methane complying with the quality standards for injection into natural gas grids.

This research assessed the influence of the gas/liquid flow configurations (co-current and counter-current) in the AC on bio-methane quality and nutrient recovery from a real digestate in the form of algal-bacterial biomass. The influence of the liquid recycling to biogas flowrate (L/G) ratio on bio-methane quality was also tested under both gas-liquid flow configurations in order to minimize both O₂ and N₂ content in the bio-methane. Additionally, an innovative process design was evaluated by interconnecting an external coagulation-flocculation tank to the HRAP, which allowed obtaining a biomass productivity of 15 g m⁻² d⁻¹ (thus maximizing the recovery of C, N, P and S in the form of algal-bacterial biomass) while minimizing the effluent to be discharged. Process operation in a co-current configuration enabled to maintain this biomass productivity, while counter-current operation decreased biomass productivity likely due to a sulphur-mediated heavy metal deprivation. A bio-methane composition complying with most international regulatory limits for injection into natural gas grids was obtained regardless of the gas/liquid flow configuration. Furthermore, an optimal L/G ratio of 0.5 under co-current flow operation in the AC allowed obtaining a bio-methane composition of 0.8 ± 0.0 % CO₂, 0.01 ± 0.0 % O₂, 0.7 ± 0.2 % N₂ and 98.5 ± 0.2 % CH₄.

We look forward to your evaluation.

Best regards,

Alma Toledo-Cervantes
Raúl Muñoz
Highlights

- EU standard bio-methane was obtained regardless of the gas-liquid flow configuration
- Optimum bio-methane composition was achieved at a L/G=0.5 under co-current operation
- Counter-current operation decreased biomass productivity and the cultivation broth pH
- High C, N, P and S recoveries were achieved by decoupling the HRT from the SRT
Influence of the gas-liquid flow configuration in the absorption column on photosynthetic biogas upgrading in algal-bacterial photobioreactors

Alma Toledo-Cervantes¹, Cindy Madrid-Chirinos¹, Sara Cantera¹, Raquel Lebrero¹,

Raúl Muñoz¹*

1.-Department of Chemical Engineering and Environmental Technology, University of Valladolid, Dr. Mergelina s/n., Valladolid 47011, Spain.

*Corresponding author: mutora@iq.uva.es
Abstract

The potential of an algal-bacterial system consisting of a high rate algal pond (HRAP) interconnected to an absorption column (AC) via recirculation of the cultivation broth for the upgrading of biogas and digestate was investigated. The influence of the gas-liquid flow configuration in the AC on the photosynthetic biogas upgrading process was assessed. AC operation in a co-current configuration enabled to maintain a biomass productivity of 15 g m$^{-2}$ d$^{-1}$, while during counter-current operation biomass productivity decreased to 8.7 ± 0.5 g m$^{-2}$ d$^{-1}$ as a result of trace metal limitation. A bio-methane composition complying with most international regulatory limits for injection into natural gas grids was obtained regardless of the gas-liquid flow configuration. Furthermore, the influence of the recycling liquid to biogas flowrate (L/G) ratio on bio-methane quality was assessed under both operational configurations obtaining the best composition at an L/G ratio of 0.5 and co-current flow operation.

Keywords: Algal-bacterial photobioreactor; biogas upgrading; bio-methane; nutrients recovery; digestate.
1. Introduction

Anaerobic digestion is a sustainable platform technology to reduce the environmental impact of biodegradable organic wastes. During anaerobic digestion, ~20-95% of this residual organic matter is biologically converted into biogas (consisting of 50-70% of CH₄, 30-50% of CO₂ and trace gases such as H₂S, H₂ and N₂ (Appels et al., 2011)) and digestate (a nutrient rich liquid effluent) (Möller and Müller, 2012). Biogas is a renewable energy source typically used in industry for heat and power generation or as natural gas substitute after upgrading. Nowadays, the high energy and chemicals consumption associated to conventional physical-chemical technologies for biogas upgrading (to a CH₄ content of at least 95% as required by most international biomethane standards) limits their environmental and economic sustainability (Muñoz et al., 2015). On the other hand, digestate is applied in agriculture as biofertilizer, although environmental problems such as ammonia emission, nitrate leaching or phosphorus soil saturation might derive from inappropriate digestate handling, storage and application (Holm-Nielsen et al., 2009).

In this context, photosynthetic biogas upgrading coupled to nutrient removal from digestate can enhance the sustainability and economic viability of biogas and digestate management (Bahr et al., 2014; Posadas et al., 2015; Serejo et al., 2015). During photosynthetic biogas upgrading, microalgae use light energy to fix the CO₂ from biogas via photosynthesis, while sulphur-oxidizing bacteria oxidize H₂S to sulphate using the O₂ photosynthetically produced. Both microalgal and bacterial growth can be supported by the N and P contained in the digestate, with the subsequent reduction of its eutrophication potential. The algal–bacterial biomass produced during photosynthetic biogas upgrading can be used as slow-release bio-fertilizer or as a feedstock for biofuel
production, thus contributing to improve the economic and environmental viability of this innovative technology (Posadas et al., 2014).

Despite the high potential of photosynthetic biogas upgrading, N$_2$ and O$_2$ stripping from the recycling cultivation broth to the upgraded biogas often results in CH$_4$ concentrations < 95 %. (Muñoz et al., 2015). N$_2$ and O$_2$ are often present in the recycling cultivation broth at concentrations of ~14 mg-N$_2$ L$^{-1}$ and > 8 mg-O$_2$ L$^{-1}$ as a result of its direct contact with the atmosphere (in open HRAPs) and the intensive microalgal photosynthetic activity in the photobioreactor, respectively (Toledo-Cervantes et al., 2016). In fact, the O$_2$ stripped out from the cultivation broth is a function of the biomass productivity, which is directly linked to the irradiation impinging into the cultivation broth. All studies evaluating the performance of this technology to date were conducted under low light intensities (75-420 μmol m$^{-2}$ s$^{-1}$), which could have partially biased the results obtained in terms of final bio-methane quality (Posadas et al., 2015; Serejo et al., 2015; Toledo-Cervantes et al., 2016). On the other hand, the liquid to biogas flow (L/G) ratio in the external absorption column (AC) has been recently identified as one of the key operational parameters determining the final composition of bio-methane. Unfortunately, the influence of the biogas/recycling liquid flow configuration in the AC (counter-current vs co-current) on bio-methane composition has not been yet systematically assessed. Meier et al. (2015) operated a counter-current flow bubble column interconnected to a stirred tank photobioreactor and reported a bio-methane O$_2$ content of ~1.2 % at a L/G of 6.3. Likewise, bio-methane O$_2$ concentrations ranging from 0.7 to 1.2 were recorded by Posadas et al. (2015) in a HRAP interconnected to a bubble column operated at co-current flow. These O$_2$ concentrations were significantly higher than the limit of 0.3 % required by most international regulations for bio-methane injection into natural gas networks, which
entails the need for a systematic optimization of biogas scrubbing in the absorption
column of photosynthetic biogas upgrading systems.

This research assessed the influence of the gas-liquid flow configuration (co-current and
counter-current) in the AC on bio-methane quality and nutrient recovery performance
from real digestate. Additionally, the influence of the L/G ratio (0.3-1) on bio-methane
quality was investigated under steady state at the two target gas-liquid flow
configurations in order to minimize both O₂ and N₂ content.

2. Materials and methods

2.1 Experimental set-up operation

The experimental set-up consisted of a HRAP interconnected to a bubble column
(referred to as absorption column, AC) and to a harvesting tank via recirculation of the
cultivation broth (Figure S1, Supplementary information). The system was operated
indoors at the Dept. of Chemical Engineering and Environmental Technology at
University of Valladolid (Spain). The HRAP dimensions were 170 cm length and 82 cm
width, with a working volume of 180 L and an illuminated area of 1.21 m². The HRAP
was continuously agitated at an internal liquid recirculation velocity of 20 cm s⁻¹ and
illuminated at 1500 ± 600 µmol m⁻² s⁻¹ by six high intensity LED PCBs (Phillips SA,
Spain) using 14:10 h light:dark cycles. The composition of the rendering digestate fed
continuously at an influent flow rate of 1 L d⁻¹ was (mg L⁻¹): ammonium (NH₄⁺) 1668 ±
249, total nitrogen (TN) 1815 ± 109, total phosphorous (TP) as P-PO₄³⁻ 48 ± 2, chemical
oxygen demand (COD) 1745 ± 413, inorganic carbon (IC) 1500 ± 168 and sulphate
(SO₄²⁻) 15 ± 2. Tap water was daily supplied to the HRAP to compensate for
evaporation losses. The AC (165 cm height and 4.4 cm diameter) was fed with a
synthetic biogas mixture (70 % of CH₄, 29.5 % of CO₂ and 0.5 % of H₂S, Abello Linde (Barcelona, Spain)) and cultivation broth from the HRAP at a similar flow rate of 1.6 m³ m⁻² h⁻¹ (flow rate referred to the AC cross sectional area). The algal-bacterial cultivation broth exiting the AC was returned to the HRAP. A fraction of the cultivation broth (26 L d⁻¹) was transferred to an external stirred tank for biomass harvesting, thus decoupling biomass productivity from the hydraulic retention time (HRT). A polyacrylamide-based flocculant solution (Chemifloc CV-300, (de Godos et al., 2011)) was dosed at 120 mg L⁻¹ to recover the algal-bacterial biomass by coagulation-flocculation. The biomass-free cultivation broth was then returned to the HRAP. This harvesting method represents a low cost alternative for algal-bacterial broths with a sludge volume index > 100 mL g⁻¹. The effluent from the system was removed at 0.5 L d⁻¹ from the harvesting tank, along with the flocculated biomass in the stirred tank, in order to minimize the effluent discharged into the environment while avoiding the accumulation of potentially toxic compounds present in the digestate.

2.2 Influence of the gas-liquid flow configuration on biogas upgrading and nutrients recovery

The HRAP was inoculated with Mychonastes homosphaera (Skuja) Kalina & Puncchárová (a taxonomic synonym of Chlorella minutissima Fott & Novákóvá) from a previous culture grown in synthetic anaerobically digested stillage (Toledo-Cervantes et al., 2016). The AC was operated under co-current flow for 94 days (stage I) and for 110 days (stage II) under a counter-current flow configuration. Samples of 100 mL from the rendering digestate and the cultivation broth were collected twice a week to measure the pH and concentration of IC, TN, NH₄⁺, TP, nitrite (NO₂⁻), nitrate (NO₃⁻), SO₄²⁻ and TSS. The inlet and outlet biogas flow rate and composition (CO₂, H₂S, O₂, N₂, and CH₄)
were also recorded twice a week. Temperature and dissolved O$_2$ concentration (DO) were *in-situ* determined in the HRAP. Algal-bacterial cultivation broth samples were drawn at each steady state to characterize the structure of the population of both microalgae and bacteria, and their elemental composition (C, N, P and S).

2.3 **Influence of the L/G ratio on bio-methane composition under co-current and counter-current operation**

Liquid to biogas flow rate ratios ranging from 0.3 to 1.0 were tested under co-current and counter-current operation. The synthetic biogas was constantly sparged into the AC at 40 mL min$^{-1}$, while the cultivation broth recycling rate was set at 12, 20, 32 and 40 mL min$^{-1}$. The system was allowed to stabilize for at least two times the liquid HRT before the analysis of bio-methane composition.

2.4 **Analytical methods**

The biogas and bio-methane CO$_2$, H$_2$S, O$_2$, N$_2$ and CH$_4$ concentrations were analysed by GC-TCD according to Posadas *et al.*, (2015). The DO and pH were monitored with an OXI 330i oximeter (WTW, Germany) and a pH meter Eutech Cyberscan pH 510 (Eutech instruments, The Netherlands), respectively. Dissolved TOC, IC and TN concentrations were analysed using a Shimadzu TOC-VCSH analyser (Japan) equipped with a TNM-1 chemiluminescence module. NO$_2^-$, NO$_3^-$, PO$_4^{3-}$ and SO$_4^{2-}$ concentrations were measured by HPLC-IC according to Serejo *et al.*, (2015), while NH$_4^+$ concentration was determined using an ammonia electrode Orion Dual Star (Thermo Scientific, The Netherlands). COD and TSS analyses were carried out according to standard methods for the examination of wastewater (Eaton *et al.*, 2005). The photosynthetic active radiation (PAR) at the HRAP surface was measured with a LI-
250A light meter (Lincoln, Nebraska, USA). The biomass C and N content was determined using a CHNS analyser (LECO CHNS-932), while P and S content was analysed by an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES, Varian 725-ES) after microwave-acid digestion (Alcántara et al., 2015). The structure of the bacterial population was determined by denaturing gradient gel electrophoresis (DGGE) according to Posadas et al., (2015), and the sequences were deposited in GenBank Data Library under accession numbers KX146512-KX146523, while the microalgae community was morphologically characterized by microscopical observations (OLYMPUS IX70, USA) after fixation with 5% of lugol acid.

3. Results and discussion

3.1 Influence of the gas-liquid flow configuration on nutrient recovery from rendering digestate

An innovative HRAP operational strategy based on decoupling the HRT from the solids (biomass) retention time (SRT) was applied in this study. This strategy allows maximizing nutrient recovery from high-strength wastewaters (i.e. digestate) in the form of algal-bacterial biomass while maintaining biomass concentration below light limiting values (Toledo-Cervantes et al., 2016). The system was operated for a period of 2 folds the SRT (25 ± 2 d) before reaching steady state. From day 50 to 94 (stage I), the algal-bacterial consortium was able to maintain a biomass productivity of 15 g m⁻² d⁻¹ with a TSS concentration in the cultivation broth of 2.6 ± 0.3 g L⁻¹ (Figure 1, table 1). The high irradiance here applied, which mimicked solar irradiance (1500 ± 600 μmol m⁻² s⁻¹), prevented excessive mutual shading and supported this dense microalgae culture. The latter was also mediated by the high nutrients concentrations of the high-strength wastewater used. The AC interconnected to the HRAP was operated under counter-
current flow configuration from day 104 onwards without a significant variation in the TSS concentration of the HRAP for 7 weeks. However, biomass concentration unexpectedly decreased from day 154 to 167 (Figure 1). In this context, an increase in the phosphorous (P) concentration in the cultivation broth to 4 mg-P L\(^{-1}\) was conducted in the HRAP by direct K\(_2\)HPO\(_4\) salt addition at day 172 in order to elucidate whether a limitation in this nutrient was responsible for the decrease in the TSS concentration recorded. Phosphorous addition to the cultivation broth stabilized the TSS concentration at 1.3 ± 0.1 g L\(^{-1}\) but did not induce the expected 800 mg-TSS L\(^{-1}\) concentration increase based on the biomass P content (0.005 g-P/g-biomass). An increment of Mg to 20 mg L\(^{-1}\) by day 181 did not entail an increase in the TSS concentration, which confirmed the absence of magnesium limitation in the cultivation broth. Finally, 180 mL of a micronutrients solution composed of (g L\(^{-1}\)) 2.86 H\(_3\)BO\(_3\), 1.81 MnCl\(_2\).4H\(_2\)O, 0.22 ZnSO\(_4\).7H\(_2\)O, 0.39 Na\(_2\)MoO\(_4\).2H\(_2\)O, 0.08 CuSO\(_4\).5H\(_2\)O, 0.05 Co(NO\(_3\))\(_2\).6H\(_2\)O was added at day 195, which led to a rapid increase in TSS concentration up to 2.1 g L\(^{-1}\) by day 200. Since no variation in digestate composition occurred over the experimental period, the results herein obtained suggested that microalgae growth was limited by trace metal availability due to their precipitation as sulphur-salts. This sulphur-induced precipitation likely occurred as a result of the O\(_2\) deprivation at the bottom of the AC under counter-current operation, since O\(_2\) was gradually consumed or stripped out on its way downwards (See section 3.2). On the other hand, the DGGE analysis revealed the presence of *Vampirovibrio chlorellavorus* (Band 5 in figure S2, Supplementary information), a non-photosynthetic cyanobacteria and obligate predator that only grows by consuming species of the green alga *Chlorella* (Coder and Goff, 1986; Soo et al., 2015). Thus, the combination of a sulphur-mediated heavy metal limitation and the presence of this *Chlorella* predator might have contributed to the decrease in TSS down
to 1.4 ± 0.3 g L\(^{-1}\), which only supported a biomass productivity of 8.7 ± 0.5 g m\(^{-2}\) d\(^{-1}\).

The high buffer capacity of the rendering digestate, together with the photosynthetic activity of microalgae mediated by the high productivity imposed during stage I, maintained the pH at 10.2 ± 0.5 and the DO concentration at 15.9 ±1.6 mg L\(^{-1}\). During stage II, a decrease in the pH and DO concentration of the cultivation broth to 9.5 ±0.1 and 13.3 ±1.1 mg L\(^{-1}\), respectively, was recorded due to the lower biomass productivity induced by the trace metal limitation (Table 1). These high DO concentrations prevented oxygen limitation during the bacterial oxidation of the organic matter contained in the digestate, and supported COD removal efficiencies (RE) of 83 ± 4.3 % and 89 ± 2.5 % in stages I and II, respectively.

Assimilatory rather than abiotic mechanisms governed C, N and P removal during the experiment. A complete removal of NH\(_4^+\) concomitant with a TN-RE of 98 ± 0.4 % were observed in stage I (Figure 2a). Nitrogen assimilation into algal-bacterial biomass during stage I accounted for 64 ± 7 % (0.065 g-N/g-Biomass), ~33% of the N input being stripped out as NH\(_3\) as a result of the high pH of the cultivation broth (10.2). The low nitrification activity observed (Figure 2b) allowed a significant N-NH\(_4^+\) removal by stripping. This nitrogen loss would be eventually overcome by either decreasing the N-load or increasing the biomass withdrawal rate from the harvesting tank (a parameter that can be controlled in the experimental set-up) since the digestate nitrogen load selected could theoretically support biomass productivities up to 23 g m\(^{-2}\) d\(^{-1}\) provided that no other nutrient limitation occurs. In spite of the slight increase in nitrification activity recorded during stage II, the decrease in biomass productivity mediated by counter-current operation resulted in a nitrogen assimilation of 45 ± 12 %, with 49 ± 2 % of the N input being stripped out to the environment. Despite the decrease in biomass
productivity, P concentration in the cultivation broth remained below the detection limit of the spectrophotometric method used at both operational configurations. The low phosphorous content measured in the biomass (0.005 g-P/g-biomass) and the ability of microalgae to accumulate energy in the form of polyphosphate suggested a total P recovery during both steady states (Alcántara et al., 2015).

The carbon mass balance conducted estimated that 88 ± 4 % of the carbon supplied (considering both the inorganic and organic carbon in the digestate and the C-CO$_2$ absorbed in the AC) was recovered as biomass during stage I. Carbon recovery decreased in stage II down to 57 ± 5 % due to the above mentioned decrease in biomass productivity. In contrast, IC-RE significantly increased (t-test, $p \leq 0.05$) (Figure 2a) from 90 ± 1.1 % to 95 ± 0.5 % mainly due to the enhanced CO$_2$-stripping (38.6 ± 5 %) mediated by the decrease in pH (Table 1). Additionally, the sulphur mass balance estimated that 38 and 24 % of the sulphur contained in the biogas was assimilated into biomass during stage I and II, respectively (0.007 g-S/g-biomass).

On the other hand, the alkaline conditions prevailing during HRAP-operation (pH >9.5), together with the high average IC (1550 ± 471 mg L$^{-1}$) and sulphate (539 ± 113 mg L$^{-1}$) concentrations, promoted the dominance of the unialgal culture of Mychonastes homosphaera (Skuja) Kalina & Puncchárová. The morphological identification of this microalga was confirmed by the DGGE analysis with observation of bands 2 and 3 (Figure S2, Supplementary information), which belonged to the genus Chlorophyta and were related to Chlorella species. The DGGE analysis also revealed 12 bands belonging to four different phyla: Cyanobacteria/Chloroplast (4 bands), Proteobacteria (5 bands), Chloroflexi (2 bands) and Bacteroidetes (1 band) (Table S1, Supplementary material). Aerobic bacteria from the genus Sphingomonas (band 11) and Sphingobacteriales order
likely supported the biodegradation of the organic matter contained in the digestate (Shokrollahzadeh et al., 2008; Ye and Zhang, 2013)

Finally, the low effluent flow rate (0.5 L d$^{-1}$), together with the low N and P effluent concentrations recorded, entailed a low environmental impact in terms of wastewater discharge to the environment. At this point it should be also stressed that the coagulation-flocculation process implemented in the interconnected tank was efficient at removing biomass from the cultivation broth to an average effluent TSS concentration of 28 ± 4 mg L$^{-1}$, which complies with the limit established by the European Union legislation (European Directive 91/271/CEE).

3.2 Influence of gas-liquid flow configuration on biogas upgrading performance

Conventional water scrubbing for biogas upgrading relies on the contact between the biogas flowing upwards through a packed absorption column and a pressurized water stream trickling down in a counter-current mode. The column is typically filled with random packing materials in order to increase the specific gas-liquid contact area and thus maximize the gas-liquid mass transfer. State of the art water scrubbers can provide a bio-methane with a CH$_4$ content of 96-98 % (Ryckebosch et al., 2011). In contrast, the absorption columns coupled to photobioreactors have been mostly operated at co-current flow with no packing materials to avoid biomass clogging (Toledo-Cervantes et al., 2016), with only one experimental study conducted using a counter-current flow configuration (Meier et al., 2015). The study here reported constitutes, to the best of our knowledge, the first systematic comparison addressing the influence of the biogas-recycling liquid flow configuration on bio-methane composition. Statistically different (t-test, p≤ 0.05) CO$_2$-REs of 98.8 ± 0.8 % (co-current) and 96.9 ± 1.6 % (counter-
current) were recorded during stages I and II, respectively, while statistically similar
REs ~100 % were obtained for H₂S. The CO₂ and H₂S REs observed under a co-current
configuration were in agreement with those reported by Toledo-Cervantes et al. (2016).
The lower CO₂-REs recorded under counter-current flow operation were attributed to
the decrease in the pH of the cultivation broth from 10.2 to 9.5 (Table 1), mediated by
the decrease in microalgal photosynthetic activity (See section 3.1). These results
confirmed that CO₂ removal highly depends on the photosynthetic activity of
microalgae in spite of the high buffer capacity of the digestate. Furthermore, the nearly
complete H₂S removal observed at both configurations highlighted the robustness of
this biological technology for the abatement of H₂S from biogas.

Table 2 shows the bio-methane composition under co-current and counter-current flow
configurations. The CO₂ and CH₄ contents of the bio-methane were statistically
different, with a higher CH₄ content under a co-current flow configuration (96.2 ± 0.7
%) (Figure 3a). The bio-methane obtained in both operational stages presented a low
oxygen content due to the active oxygen demand resulting from the oxidation of H₂S to
sulphate (Figure 3b). No significant differences in O₂ and N₂ content were observed at
both operational configurations. Toledo-Cervantes et al. (2016) reported a similar N₂
concentration (2.4 ± 0.2 %) but a lower O₂ content (0.03 ± 0.04 %) in the biogas
upgraded in a similar experimental set-up operated under co-current flow configuration
at a L/G=1. The lower O₂ content observed by these authors was likely due to the lower
irradiance (420 ± 105 μmolm⁻² s⁻¹) and biomass productivity (7.5 g m⁻² L⁻¹) used in
their experimentation, which entailed a lower DO concentration in the cultivation broth
(9.6 ± 0.4 mg O₂ L⁻¹). Moreover, since the same L/G ratio was applied at both
operational configurations, the nitrogen content in the bio-methane (stripped out from
The cultivation broth was statistically similar (Figure 3b).

The bio-methane obtained under both gas-liquid flow configurations complied with the regulatory limits of most international standards for bio-methane injection into natural gas grids regardless of the operational configuration. Nonetheless, several operational problems were observed during counter-current flow operation. First, elemental sulphur accumulation at the bottom of the AC resulted in diffuser clogging, while biomass accumulation at the top of the AC caused the obstruction of pipelines. The elemental sulphur accumulation observed under counter-current configuration was attributed to the stripping and gradual DO consumption along the AC, which resulted in a low DO concentration at the bottom of the AC where biogas was sparged. Therefore, the dissolved H$_2$S at the bottom of the column was not completely oxidized to sulphate but to elemental sulphur, which accumulated at the surface of the diffuser and the absorption column’s walls. The limited H$_2$S oxidation at the bottom of the AC was also responsible of the trace metal precipitation hypothesized in section 3.1.

3.3 Influence of the L/G ratio on bio-methane composition under co-current and counter-current operation

The recycling liquid to biogas ratio constitutes as a key operational parameter determining the final quality of bio-methane in algal-bacterial photobioreactors (Toledo-Cervantes et al., 2016). Theoretically, an increased overall concentration gradient and volumetric mass transfer coefficient were expected under counter-current flow operation. Nonetheless, the decrease in pH and biomass productivity during stage II counterbalanced the beneficial mass transfer effects of counter-current flow operation. In this context, a systematic evaluation of the influence of the L/G ratio on
bio-methane composition was carried under both gas-liquid flow configuration. This experimentation was carried out from days 95 to 98 and therefore it was not biased by the above-mentioned secondary effects of the counter-current flow operation on the cultivation broth (i.e. lower biomass productivity and pH) and allowed to minimize the O₂ and N₂ content in the bio-methane without compromising the CO₂ removal.

Table 3 shows the REs recorded under both gas-liquid flow configurations. The CO₂-REs at the L/G ratios tested were significantly different under co-current and counter-current flow operation (t-test, p ≤ 0.05), except for the REs obtained under counter-current flow operation at a L/G ratio of 1 and 0.8. The CO₂-REs observed at a L/G ratio of 1 were in agreement with those previously reported by Toledo-Cervantes et al. (2016) (98.8 ± 0.2 %) using a similar experimental fed with synthetic digestate. The results obtained also indicated that the CO₂-REs increased at increasing the L/G ratio up to 1, likely due to the higher carry over capacity when increasing the recycling liquid rate. As expected, higher CO₂-REs were observed under counter-current flow operation (Table 3) due to the enhanced overall concentration gradient and mass transfer coefficient (k_La-CO₂), the latter mediated by an extended gas-liquid contact time. In contrast, the CO₂-REs recorded at a L/G ratio of 0.3 were lower at both operational configurations (70.3 ± 1.0 % and 60.4 ± 1.9 % under co-current and counter-current flow operation, respectively). These low CO₂-REs were attributed to the decrease in pH in the recycling cultivation broth from 10 to 8.5 ± 0.1 induced by the increase in the liquid HRT in the AC. No significant differences were observed in the H₂S-REs under both operational configurations regardless of the L/G ratio, which confirmed the robustness of this technology in terms of H₂S.
Counter-current flow operation involved higher mass transfer rates, which resulted in higher O₂ and N₂ desorption rates from the cultivation broth concomitant with enhanced CO₂ removals in the AC, but slightly lower CH₄ concentrations than under co-current operation (Figure 4). However, the two gas-liquid flow configurations tested allowed obtaining a bio-methane complying with most international regulations. Under co-current flow operation at a L/G of 0.5, a bio-methane composition of 0.8 ± 0.0 % of CO₂, 0.01 ± 0.0 % of O₂, 0.7 ± 0.2 % of N₂ and 98.5 ± 0.2 % of CH₄ was obtained, which to the best of our knowledge constitutes the best composition ever reported for any stand-alone biological biogas upgrading technology.

4. Conclusions

Microalgae photosynthetic activity was identified as a key process parameter determining both the quality of bio-methane and the extent of the nutrients removal mechanisms. Process design here evaluated, allowed decoupling biomass productivity from the HRT, which overcame the light limitation problem associated with the use of high strength digestates. Despite counter-current flow operation supported a more efficient gas-liquid mass transfer, both the enhanced N₂/O₂ stripping and the lower microalgal activity observed, resulted in a lower bio-methane quality. However, the bio-methane composition achieved under both operational configurations complied with the regulatory limits required for its injection into natural gas grids.

Acknowledgments

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References


Figure caption.

**Figure 1.** Time course of the total suspended solids concentration in the HRAP. The vertical line indicates the change in the gas-liquid flow configuration in the AC.

**Figure 2.** a) Removal efficiencies of chemical oxygen demand (COD), ammonium ($\text{NH}_4^+$), total nitrogen (TN), inorganic carbon (IC), sulphate ($\text{SO}_4^{2-}$) and phosphate ($\text{PO}_4^{3-}$) in the HRAP and b) effluent concentrations of nitrate ($\text{N-NO}_3^-$), nitrite ($\text{N-NO}_2^-$) and sulfate ($\text{SO}_4^{2-}$) under co-current (black bars) and counter-current (white bars) gas-liquid flow operation. Vertical lines represent standard deviations from replicate measurements under steady state operation. All REs were significantly different (t-student test, $p<0.05$) except those of $\text{NH}_4^+$ and $\text{PO}_4^{3-}$.

**Figure 3.** Time course of the concentration of a) $\text{CO}_2$ (○) and $\text{CH}_4$ (△), and b) $\text{O}_2$ (□) and $\text{N}_2$ (◊) in the bio-methane. Vertical lines represent standard deviations from replicate measurements.

**Figure 4.** Influence of the recycling liquid to biogas ratio on the concentration of a) $\text{O}_2$, b) $\text{N}_2$, c) $\text{CH}_4$ and d) $\text{CO}_2$ in the bio-methane under co-current (□) and counter-current (○) gas-liquid flow operation. Vertical lines represent standard deviations from replicate measurements.
Figure 1.
Figure 2.

(a) RE (%)

COD NH\textsuperscript{+} TN IC SO\textsubscript{4}\textsuperscript{2-} PO\textsubscript{4}\textsuperscript{3-}

(b) Concentration (mg L\textsuperscript{-1})

N-NO\textsubscript{3} N-NO\textsubscript{2} SO\textsubscript{4}\textsuperscript{2-}
Figure 3.

(a) Changes in CO₂ and CH₄ concentrations over time. (b) Changes in O₂ and N₂ concentrations over time.
Figure 4.

(a) [Graph showing 
\( \text{O}_2 \) (%)]

(b) [Graph showing 
\( \text{N}_2 \) (%)]

(c) [Graph showing 
\( \text{CH}_4 \) (%)]

(d) [Graph showing 
\( \text{CO}_2 \) (%)]

L/G ratio vs. gas concentrations.
Table 1. Operating conditions under co-current and counter-current flow configurations in the absorption column.

<table>
<thead>
<tr>
<th></th>
<th>HRAP (°C)</th>
<th>TSS</th>
<th>pH-digestate</th>
<th>pH-HRAP</th>
<th>DO (mg L⁻¹)</th>
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</thead>
<tbody>
<tr>
<td>Co-current flow</td>
<td>23.8 ±1.7</td>
<td>2.6 ± 0.3</td>
<td>7.8 ±0.4</td>
<td>10.2 ±0.5</td>
<td>15.9 ±1.6</td>
</tr>
<tr>
<td>Counter-current flow</td>
<td>19.4 ±1.6</td>
<td>1.4 ± 0.3</td>
<td>7.6 ±0.2</td>
<td>9.5 ±0.1</td>
<td>13.3 ±1.1</td>
</tr>
<tr>
<td></td>
<td>CO₂ (%)</td>
<td>O₂ (%)</td>
<td>N₂ (%)</td>
<td>CH₄ (%)</td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
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<td></td>
</tr>
<tr>
<td>Co-current flow</td>
<td>0.4 ± 0.3</td>
<td>0.7 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.7 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>96.2 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Counter-current flow</td>
<td>0.9 ± 0.3</td>
<td>1.2 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.6 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95.1 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>

*Same letter means no significantly different (t-test, p≤ 0.05)*
Table 3. Influence of the L/G ratio on the carbon dioxide and hydrogen sulphide removal efficiencies under co-current and counter-current flow configurations in the absorption column.

<table>
<thead>
<tr>
<th>L/G ratios</th>
<th>RE at co-current flow (%)</th>
<th>RE at counter-current flow (%)</th>
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<tbody>
<tr>
<td></td>
<td>CO₂</td>
<td>H₂S</td>
</tr>
<tr>
<td>1</td>
<td>98.8 ± 0.0</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.8</td>
<td>98.3 ± 0.0</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.5</td>
<td>97.3 ± 0.1</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.3</td>
<td>70.3 ± 1.0</td>
<td>98.3 ± 2.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Same letter means no significantly different (t-test, P≤ 0.05)
Electronic Annex
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