Photosynthetic biogas upgrading to bio-methane: boosting nutrient recovery via biomass productivity control.

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Abstract.

A pilot high rate algal pond (HRAP) interconnected to an external CO$_2$–H$_2$S absorption column via settled broth recirculation was used to simultaneously treat a synthetic digestate and to upgrade biogas to a bio-methane with sufficient quality to be injected into natural gas grids. An innovative HRAP operational strategy with biomass recirculation based on the control of algal-bacterial biomass productivity (2.2, 4.4 and 7.5 g m$^{-2}$ d$^{-1}$) via settled biomass wastage was evaluated in order to enhance nutrient recovery from digestate at a constant hydraulic retention time. The influence of the recycling liquid to biogas (L/G) ratio on the quality of the upgraded biogas was assessed. The bio-methane composition under a L/G ratio of 1 (0.4 ± 0.1% CO$_2$, 0.03 ± 0.04% O$_2$, 2.4 ± 0.2% N$_2$ and 97.2 ± 0.2% CH$_4$) complied with the technical specifications of most European bio-methane legislations regardless of the biomass productivity established. The HRAP operational strategy applied allowed increasing the N and P recovery from 19 and 22% to 83 and 100%, respectively, when the biomass productivity was increased from 2.2 to 7.5 g m$^{-2}$ d$^{-1}$. Finally, the dynamics of microalgae and bacteria population structure were characterized by morphological identification and DGGE analysis.

Keywords: Biogas upgrading; bio-methane; microalgae-based processes; nutrients recovery; wastewater treatment.
Highlights:

- A removal of CO₂ and H₂S from biogas higher than 99% was achieved.
- A low L/G ratio prevented O₂ and N₂ contamination of the upgraded biogas.
- The bio-methane complied with EU legislation for injection into natural gas grids.
- A novel HRAP operation based on biomass productivity control was developed.
- This operation strategy allowed maximizing nutrient recovery from digestate.
**Introduction.**

Anaerobic digestion offers a cost-effective and environmentally feasible solution for organic waste management while contributing to satisfy the global demand for renewable energy via biogas production. In this context, the annual biogas production in the European Union accounted for ~13.4 Mtoe in 2013 [1]. Biogas is composed mainly of methane (CH$_4$) (40-75%), carbon dioxide (CO$_2$) (25-50%), hydrogen sulfide (H$_2$S) (0.005-2%) and ammonia (NH$_3$) (<1%). Other gases such as hydrogen (H$_2$), nitrogen (N$_2$), oxygen (O$_2$) and halogenated hydrocarbons are also present in raw biogas at lower concentrations [2]. The concentration of these biogas pollutants depends on the composition of the initial organic substrate and the type of anaerobic digestion process. The H$_2$S present in biogas corrodes metal parts, reduces the durability of the motors and generates hazardous sulfur dioxide when biogas is combusted for the generation of heat and electricity. Likewise, CO$_2$ reduces the specific calorific value of biogas and increases carbon monoxide and hydrocarbon emissions during combustion. Therefore, these biogas pollutants must be previously removed in order to comply with the technical specifications for biogas to be used as a transport fuel or injected into natural gas grids. Most international legislations for bio-methane, which is the most common term to refer to the upgraded biogas, require concentrations of CH$_4$ ≥95%, CO$_2$ ≤2%, O$_2$ ≤0.3 % and negligible amounts of H$_2$S [3].

Conventional physical-chemical technologies such as water scrubbing, chemical scrubbing and membrane separation are commonly applied for CO$_2$ removal from biogas. However, these technologies often require a previous H$_2$S cleaning step such as activated carbon adsorption or chemical scrubbing [4]. On the contrary, biological H$_2$S removal technologies such as anoxic and aerobic biotrickling filters are not able to
remove CO$_2$ and present operational problems such as elemental sulfur accumulation (and subsequent clogging of the packed-bed) and biogas contamination with O$_2$ and N$_2$ [5, 6]. In addition, the physical-chemical technologies capable of simultaneously removing CO$_2$ and H$_2$S (for example chemical scrubbing with alkali aqueous solutions) exhibit high operating costs and a significant environmental impact [7].

In this regard, microalgae-based processes have emerged as a competitive and environmentally sustainable alternative for the simultaneous removal of CO$_2$ and H$_2$S from biogas [8]. These processes are based on the fixation of CO$_2$ via photosynthesis by microalgae and the oxidation of H$_2$S to sulfate by sulfur oxidizing bacteria using the oxygen photosynthetically produced. Moreover, the anaerobic effluents produced on-site can eventually support microalgae growth, thus reducing their associated treatment costs and eutrophication potential [9]. In addition, the algal biomass generated during the photosynthetic biogas upgrading process can be used as a feedstock for bio-fuel or bio-fertilizer production [10, 11], provided that biomass production has been properly maximized. However, the increase in pH and modification of metal ion speciation (e.g. Ca$^{2+}$, Mg$^{2+}$ and Fe$^{2+}$) in the cultivation broth induced by microalgae growth can promote the abiotic removal of N and P by volatilization and precipitation, respectively [12]. This abiotic nutrient removal mechanism contributes to a detrimental loss of nutrients and causes a severe environmental impact derived from the indirect N$_2$O emissions associated to NH$_4^+$ stripping [13].

Several proof of concept studies of this innovative photosynthetic biogas upgrading process coupled with nutrient removal from digestate have been recently conducted by Bahr et al. [8], Serejo et al. [14] and Posadas et al., [15] in a HRAP interconnected to an
external CO$_2$–H$_2$S absorption column (AC). However, while a complete H$_2$S removal was always observed, CO$_2$ removal was low (<80%) and the upgraded biogas was contaminated with N$_2$ and O$_2$ (stripped out from the cultivation broth), the latter decreasing the CH$_4$ content in the upgraded biogas to ~80%. Therefore, the O$_2$ and N$_2$ content in the upgraded biogas represents nowadays the main limitation of this technology to achieve a high quality bio-methane, which entails the need to explore new operational strategies to minimize the desorption of these bio-methane pollutants from the algal-bacterial broth. In addition, little attention has been also paid to the optimization of nutrient recovery from digestates, which would enhance the environmental sustainability of the photosynthetic biogas upgrading process.

This research aimed at optimizing both the photosynthetic biogas upgrading process and nutrient recovery from digestate in an algal-bacterial HRAP interconnected to a biogas absorption column via recirculation of the settled broth. A preliminary optimization of the recycling liquid to biogas ratio was conducted in order to obtain a bio-methane with sufficient quality to be injected into natural grids. Then, an innovative HRAP operational strategy based on the control of algal-bacterial biomass productivity via settled biomass wastage was evaluated in order to enhance nutrient recovery from a synthetic digestate while producing a high quality bio-methane.

2. Materials and methods.

2.1 Experimental setup and operational conditions.

The experimental setup, located at the Dept. of Chemical Engineering and Environmental Technology at Valladolid University (Spain), consisted of a 180 L high rate algal pond (170 cm length × 82 cm width × 15 cm depth) with an illuminated area
of 1.21 m², interconnected to a 8 L conical settler and to a 2.2 L absorption column (4.4 cm diameter, 165 cm height) via recirculation of the settled algal cultivation broth (Figure 1). The HRAP was fed with a synthetic digestate at an influent flow rate of 1.3 ± 0.2 L m⁻² d⁻¹, continuously agitated at an internal liquid recirculation velocity of ≈20 cm s⁻¹, and illuminated with fluorescent lamps at 420 ± 105 μmol m⁻² s⁻¹ using 16:8 h light:dark cycles. Tap water was supplied to compensate evaporation losses. The composition of the synthetic digestate was (mg L⁻¹): ammonium (NH₄⁺) = 526 ± 132, total nitrogen (TN) = 646 ± 61, total phosphorous (TP) as P-PO₄³⁻ = 53 ± 11, inorganic carbon (IC) = 4458 ± 106 and sulfate (SO₄²⁻) = 317 ± 83. Digestates are characterized by a high alkalinity and nutrient concentrations [16]. The effluent from the HRAP was collected in the settler and the clarified effluent was then pumped to the bottom of the AC at 1.6 m³ m⁻² h⁻¹ (flow rates referred to the AC cross sectional area) co-currently with the biogas sparged (70% CH₄, 29.5% CO₂, 0.5% H₂S, Abello Linde (Barcelona, Spain)) through a metallic diffuser at 1.6 m³ m⁻² h⁻¹. The liquid phase exiting the AC was returned to the HRAP, while the excess of effluent from the system was removed by overflow from the settling tank. This innovative photobioreactor configuration allowed decoupling the hydraulic retention time from the algal bacterial biomass productivity by controlling the rate of settled biomass wasted and returned to the HRAP.
Figure 1. Schematic diagram of the experimental setup used for the continuous upgrading of biogas coupled to digestate treatment.

2.2 Influence of the recycling liquid to biogas ratio on the quality of the upgraded bio-methane.

L/G ratios ranging from 0.5 to 60 were tested in order to maximize CO$_2$ and H$_2$S removal while minimizing O$_2$ and N$_2$ desorption from the recycling liquid to the upgraded biogas. The synthetic biogas was sparged into the AC at 5.3, 16.0, and 31.5 mL min$^{-1}$, while the external liquid recirculation rate was set at 15, 60, 120, 203, and 315 mL min$^{-1}$ for each biogas flow rate tested. The AC was constantly fed with the algal-bacterial broth at a pH of 10 ± 0.3. The absorption system was allowed to stabilize for two times the AC hydraulic retention time (HRT) prior to the monitoring of the upgraded biogas composition by GC-TCD.

2.3 Influence of biomass productivity on biogas upgrading and nutrient recovery.
The HRAP was inoculated with a consortium of cyanobacteria/microalgae composed of *Geitlerinema* sp. (61.5%), *Staurosira* sp. (1.5%) and *Stigeoclonium tenue* (37%) from a previous culture grown in diluted centrate wastewater. The consortium was then acclimated to the digestate for 40 days prior to the experiment start-up. A biomass productivity of 2.2 g m\(^{-2}\) d\(^{-1}\) was set during stage I (days 0-77) by controlling the rate of withdrawal of settled biomass based on the total suspended solids (TSS) concentration in the settler. The biomass productivity was increased to 4.4 g m\(^{-2}\) d\(^{-1}\) during stage II (days 78-159) and to 7.5 g m\(^{-2}\) d\(^{-1}\) during stage III (days 160-202). The latter productivity was selected based on the maximum biomass productivity expected from the TP daily fed into the HRAP (assuming a P biomass content of 1 % according to Alcántara *et al.*, [17]). The experimental system was operated indoors for 202 days.

Liquid samples (100 mL) were collected twice a week from the digestate influent, the treated digestate and the cultivation broth of the HRAP to monitor the pH and concentration of IC, TN, NH\(_4^+\), nitrite (NO\(_2^-\)), nitrate (NO\(_3^-\)), phosphate (PO\(_4^{3-}\)), SO\(_4^{2-}\) and TSS. The TSS concentration of the settled biomass was also determined twice a week to control biomass productivity. The temperature and dissolved O\(_2\) concentration (DO) were monitored in-situ. Gas samples from the inlet and outlet of the biogas absorption column were periodically drawn to monitor the concentrations of CO\(_2\), H\(_2\)S, O\(_2\), N\(_2\), and CH\(_4\). The inlet and outlet gas flow rates in the AC were also measured. An aliquot of 50 ml of algal-bacterial biomass was taken in each steady state to characterize the populations of microalgae and bacteria.

**2.4 Analytical procedures.**

Dissolved IC and TN concentrations were determined using a Shimadzu TOC-VCSH analyzer (Japan) equipped with a TNM-1 chemiluminescence module. NH\(_4^+\) was
measured using an ammonia electrode Orion Dual Star (Thermo Scientific, The Netherlands). NO$_3^-$, NO$_2^-$, PO$_4^{3-}$ and SO$_4^{2-}$ concentrations were analyzed by HPLC-IC according to Serejo et al., [14]. TSS analyses were carried out according to Standard Methods [18]. The pH in the cultivation broth was monitored with a pH meter Eutech Cyberscan pH 510 (Eutech instruments, The Netherlands). The light intensity at the HRAP surface was measured with a LI-250A light meter (LI-COR Biosciences, Germany). The C and N contents of the algal-bacterial biomass were determined using a CHNS analyser (LECO CHNS-932), while P and S contents were determined using an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES, Varian 725-ES) after microwave-acid digestion [19]. The biogas CO$_2$, H$_2$S, O$_2$, N$_2$, and CH$_4$ concentrations were analyzed by GC-TCD according to Posadas et al., [15]. The morphological identification of microalgae was carried out by microscopic observations (OLYMPUS IX70, USA) after sample fixation with 5% of lugol acid. The bacterial community determination was conducted by DGGE-sequencing according to Posadas et al., [15] and the sequences were deposited in GenBank Data Library under accession numbers KU605583-KU605606.

3. Results and discussion.

3.1 Influence of the recycling liquid to biogas ratio on the quality of the upgraded biogas.

The performance of the photosynthetic biogas upgrading process can be optimized by determining the optimum L/G ratio in order to prevent O$_2$ and N$_2$ desorption while boosting the absorption of CO$_2$ and H$_2$S. CO$_2$ mass transfer from the biogas is a function of pH, CO$_2$ concentration, temperature, pressure and ionic strength of the recycling algal-bacterial broth. Since H$_2$S and CO$_2$ are acidic gases, a more efficient
absorption of these biogas pollutants would be expected at a high pH. In our particular study, the pH of the algal-bacterial broth was 10, which supported CO\(_2\) and H\(_2\)S removal efficiencies (REs) of 98.8 ± 0.19\% and 97.1 ± 1.4\%, respectively, regardless of the L/G ratio tested. However, the N\(_2\) and O\(_2\) stripped from the cultivation broth increased linearly at increasing the L/G ratio, to finally stabilize at 25\% and 7\%, respectively (Figures 2a and 2b). In contrast to the results here obtained, Serejo et al., [14] reported a stabilization in the CO\(_2\)-REs at 95 ± 2\% at L/G ratios above 15 (likely due to the relatively low pH of the cultivation broth ≈7.9), with a maximum O\(_2\) concentration in the upgraded biogas of 3 ± 1\%. The higher N\(_2\) and O\(_2\) concentrations here observed were likely due to the increase in the overall mass transfer coefficients in the AC as a result of the higher ionic strength of the cultivation broth (IC =2300 mg L\(^{-1}\)), which prevented the coalescence of the fine bubbles produced by the diffuser. This increased contamination of the upgraded biogas at increasing L/G ratios resulted in a concomitant decrease in CH\(_4\) concentration from 95\% at a L/G of 1 down to 68\% at L/G >15. Similar results were reported by Posadas et al. [15], who despite the high CO\(_2\) and H\(_2\)S REs obtained, observed a decrease in the final CH\(_4\) concentration down to 81 ± 2\% as a result of a high N\(_2\) content in the upgraded biogas. Hence, a L/G ≤1 resulted in CH\(_4\) concentrations over 95\% (Figure 2c), and in H\(_2\)S, CO\(_2\), O\(_2\) and N\(_2\) concentrations lower than 0.007\%, 0.4\%, 0.2\% and 3\%, respectively, which complied with most European bio-methane legislations. Therefore, the recycling liquid to biogas ratio was identified as a key operating factor determining the final quality of the upgraded biogas.
Figure 2. Influence of the recycling liquid to biogas ratio on the concentrations of (a) N$_2$, (b) O$_2$ and (c) CH$_4$ in the upgraded biogas. Vertical bars represent the standard deviation from replicate measurements.

3.2 Influence of biomass productivity on biogas upgrading to bio-methane.
The CO₂ content in the bio-methane gradually decreased during stage I from 3.5% to 1.2% (Figure 3a), concomitantly with the increase in the pH of the cultivation broth in the HRAP up to 9.1 ± 0.1 due to microagal photosynthetic activity (Table 1). These results confirmed that the high CO₂-REs here recorded significantly depended on the pH of the cultivation broth. In spite of the high CO₂ absorption recorded in the AC, only a slight decrease in the pH (0.1-0.3 gradient) from the bottom to the top of the AC was observed. These results were not in agreement with those reported by Meier et al., [20] using a similar two-stage system, who observed a pH gradient of ~1-2 along the column depending on the cultivation broth recycling rate. This difference was attributed to the high buffer capacity of the digestate used in this study for microalgae growth. The average steady CO₂-REs obtained in stages I, II and III were 96.6 ± 1.2%, 98.4 ± 0.8% and 99 ± 0.3%, respectively (Figure 4). The increase in the pH of the cultivation broth from 9.1 to 10.6, likely mediated by the increase in the overall photosynthetic activity (which itself was induced by the increase in biomass productivity), supported the higher CO₂-REs recorded. These values were higher than those recorded by Bahr et al. [8] (RE = 86 ± 5%) using a similar experimental setup operated with a highly carbonated mineral salt medium at a pH of 9.4 and at a L/G ratio of 1. On the other hand, despite similar CO₂-REs (97%) from a synthetic biogas containing 41% of CO₂ were reported by Mann et al. [21], contamination of the upgraded biogas with up to 23.4% of O₂ was also observed in their study.
Figure 3. Time course of the concentration of (a) CO₂, (□) H₂S (▲) and CH₄ (○), and 
b) oxygen (■) and nitrogen (◊) in the upgraded biogas. The horizontal dashed lines 
indicate the maximum CO₂ and O₂ concentrations required for bio-methane injection 
into natural gas grids. Vertical bars represent standard deviation from replicate 
measurements.

An almost complete H₂S removal was recorded regardless of the biomass productivity 
set: 99.0 ± 1.0%, 98.0 ± 1.2% and 98.5 ± 1.0% in stages I, II and III, respectively 
(Figure 4), which was in accordance with those reported by Serejo et al. [14] and 
Posadas et al. [15]. Sulfate formation was observed as a result of the biological H₂S
oxidation. At this point it must be highlighted that no oxygen limitation occurred throughout the entire experimentation. Dissolved oxygen concentration increased from $5.4 \pm 0.8 \text{ mg O}_2 \text{ L}^{-1}$ in stage I to $9.6 \pm 0.4 \text{ mg O}_2 \text{ L}^{-1}$ in stage III as a result of the increase in microalgae productivity. The sulfate concentrations during stages I, II and III were $388 \pm 43 \text{ mg-SO}_4^{2-} \text{ L}^{-1}$, $483 \pm 24 \text{ mg-SO}_4^{2-} \text{ L}^{-1}$ and $386 \pm 52 \text{ mg-SO}_4^{2-} \text{ L}^{-1}$, respectively. The decrease observed during stage III was attributed to the increase in biomass productivity (sulfate assimilation into biomass). The sulfur mass balance revealed that only 40% of the sulfur removed was oxidized to sulfate, the remaining 60% being likely present (dissolved or in suspension) as S-intermediates such as $S^0$, thiosulfate or sulfite. Partial oxidation of the $H_2S$ transferred from biogas has been previously reported [22], however a further analysis of the sulfur compounds present in the cultivation broth is necessary in order to elucidate the fate of the $H_2S$ removed.

**Figure 4.** Time course of the removal efficiencies of $CO_2$ (▲) and $H_2S$ (□). Vertical bars represent standard deviation from replicate measurements.
The biological oxidation of CH\textsubscript{4} resulted in average CH\textsubscript{4} losses of 4.9 ± 2.4% (on a mass basis) during stage I, no methane losses being recorded afterwards. The CH\textsubscript{4} content in the upgraded biogas was 95.8 ± 0.8%, 96.9 ± 0.7% and 97.2 ±0.2% in stages I, II and III, respectively (Figure 3a). These values are comparable to those achieved by water scrubbing technologies, where CH\textsubscript{4} losses by dissolution in the pressurized water of 3-5% result in CH\textsubscript{4} purities of 80-99%, depending on the N\textsubscript{2} and O\textsubscript{2} content of the upgraded biogas [23].

The O\textsubscript{2} demand in the absorption column resulting from the biological oxidation of H\textsubscript{2}S caused an oxygen content in the upgraded biogas of 0.1 ± 0.2% in stages I and II and 0.03 ± 0.04% in stage III (Figure 3b). These O\textsubscript{2} concentrations recorded in the bio-methane were lower than the values obtained by Meier et al. [20] (1.2%) and Posadas et al., [15] (0.7-1.2%), and remained significantly below those reported in literature during biogas upgrading in algal photobioreactors (10-24%) [21, 24]. Finally, the N\textsubscript{2} stripped out from the recycling cultivation broth resulted in average concentrations of 2.6 ± 0.9%, 2.4 ± 0.5% and 2.4 ± 0.2% during stages I, II and III, respectively (Figure 3b), due to the low L/G ratio applied in this study (which limited the amount of N\textsubscript{2} potentially desorbed). Higher N\textsubscript{2} concentrations in the upgraded biogas of 6-8% were recorded by Posadas et al., [15] and Serejo et al., [14] during photosynthetic biogas upgrading at a L/G of 10. In this context, the optimum bio-methane composition was obtained at the highest microalgae productivity evaluated (0.4 ± 0.1% CO\textsubscript{2}, 0.03 ± 0.04% O\textsubscript{2}, 2.4 ± 0.2% N\textsubscript{2} and 97.2 ± 0.2% CH\textsubscript{4}), which complied with the regulatory limits of most European legislations for bio-methane injection in natural gas grids (Figures 3a and 3b). For instance, the injection of bio-methane into the Spanish network...
allows up to 0.3% of O₂ provided that CO₂ concentration does not exceed 2% and CH₄ concentration remains over 95% [25].

3.3 Influence of biomass productivity on nutrient removal and nutrient recovery.

Most recent life cycle analyses have shown that the use of wastewater as a low-cost nutrients and water source can reduce the overall energy requirements and improve the environmental sustainability of microalgae mass production [26, 27]. In our particular case, microalgae production using the N and P present in anaerobically digested wastewaters can significantly decrease the operating costs of the biogas upgrading process, while preserving fresh water resources and recovering these nutrients in the form of a microalgae biomass that can be further valorized as a bio-fertilizer. Despite the potential of microalgal biotechnology to fix nutrients from digestates, abiotic removal still represents an important mechanism for nutrient removal from wastewater in algal-bacterial processes. Thus, N removal by stripping can account for up to 82% [28] and P removal by precipitation for up to 63% [29] of the total nutrients supplied. Nonetheless, the monitoring of this abiotic nutrient removal in HRAPs is often disregarded [12].

| **Table 1.** Average dissolved oxygen concentration, pH, temperature, total suspended solid concentration and biomass productivity recorded during the three operational stages. |
|-----------------|-------|-------|----------------|-----------------|-----------------|
| **Stage**      | **T<sub>HRAP</sub> (°C)** | **pH<sub>HRAP</sub>** | **DO (mg O₂ L⁻¹)** | **TSS HRAP (g L⁻¹)** | **Productivity (g m⁻² d⁻¹)** |
| I              | 22 ± 3 | 9.1 ± 0.1 | 5.4 ± 0.8 | 1.6 ± 0.1 | 2.2 ± 1.4 |
| II             | 25 ± 2 | 9.6 ± 0.3 | 7.5 ± 1.4 | 1.2 ± 0.4 | 4.4 ± 1.5 |
| III            | 28 ± 1 | 10.6 ± 0.1 | 9.6 ± 0.4 | 0.9 ± 0.1 | 7.5 ± 0.1 |

17
The high buffer capacity of the cultivation broth as a result of the high IC concentrations present in the digestate and the high water evaporation losses, together with the high photosynthetic activity in the system, maintained high pH values during the three operational stages without an automatic pH control (Table 1). The temperature of the algal–bacterial broth slightly increased concomitantly with the seasonal variation of the ambient temperature, but remained close to optimum values for microalgae and bacteria cultivation. Apart from the impinging radiation, other variables such as the nutrients load (determined by the flow rate and nutrients concentration of the target wastewater) and biomass concentration in the cultivation broth (determining light penetration) influence microalgae productivity in HRAPs devoted to wastewater treatment. For instance, low biomass concentrations (~0.5 g L\(^{-1}\)) are typically encountered in open ponds treating domestic wastewaters at HRTs of 5-10 days. Biomass productivity can be thus boosted by increasing the nutrients load into the HRAPs, provided that light supply does not limit the process. However, while an increase in wastewater flow rate might induce microalgae washout, the use of wastewaters with high nutrient concentrations (such as digestates) would entail very dense microalgae cultures, which would ultimately limit microalgae productivity as a result of an excessive mutual shading. In this context, the decoupling between the hydraulic retention and biomass retention time (inversely related to microalgae productivity) represents an innovative strategy for maximizing biomass productivity during microalgae cultivation in high-strength wastewaters. The control of biomass productivity via regulation of the settled biomass wastage rate would allow maximizing nutrient recovery from wastewaters. A TSS concentration of 1.6 ± 0.1 g L\(^{-1}\) was recorded in the HRAP when operating at an average productivity of 2.2 g m\(^{-2}\) d\(^{-1}\) in stage I. This TSS concentration decreased to 1.2 ± 0.4 g L\(^{-1}\) and 0.9 ± 0.1 g L\(^{-1}\) under
operation at 4.4 g m\(^{-2}\) d\(^{-1}\) and 7.5 g m\(^{-2}\) d\(^{-1}\), respectively. The results clearly showed that an increase in the rate of biomass wastage from the settler resulted in lower TSS concentrations, which likely improved the overall photosynthetic efficiency as a result of an enhanced light penetration. In addition, the control of biomass productivity was supported by the good settling properties of the algal-bacterial biomass present in the HRAP. However, a decrease in the TSS removal efficiency of the settler from 95 ± 3% in stage I to 84 ± 4% in stage III was recorded, which was attributed to the shift in microalgae population observed in stage II (see section 3.4). Unfortunately, the effluent TSS concentrations (70 ± 50 mg L\(^{-1}\)) remained always over the maximum discharge limit in European Union legislation (35 mg L\(^{-1}\)) [30].

### Table 2. Average removal efficiencies of total nitrogen, ammonium, phosphorus, inorganic carbon and total suspended solids recorded during the three operational stages.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Removal efficiencies (%)</th>
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<tbody>
<tr>
<td></td>
<td>TN</td>
</tr>
<tr>
<td>I</td>
<td>91 ± 4</td>
</tr>
<tr>
<td>II</td>
<td>92 ± 4</td>
</tr>
<tr>
<td>III</td>
<td>98 ± 2</td>
</tr>
</tbody>
</table>

A complete removal of ammonium was observed during all stages, while TN-REs increased from 91 ± 4% up to 98 ± 2% when biomass productivity increased from 2.2 to 7.5 g m\(^{-2}\) d\(^{-1}\) (Table 2). Despite the slight influence of biomass productivity on TN-REs, the share of the inlet TN assimilated into biomass varied from 19 ± 13 % at the lowest microalgae productivity to 83 ± 9% at the highest productivity (Table 3). In this context, the low nitrification activity recorded along with the high pH value supported a significant N-NH\(_4^+\) removal by stripping, which decreased from 75 ± 12% in stage I to 13 ± 9% in stage III. On the other hand, phosphorus removal remained stable regardless of the biomass productivity set, with REs of 77 ± 16%, 63 ± 18% and 73 ± 19% in
stages I, II and III, respectively. Serejo et al. [14] recorded similar phosphorous REs (71 ± 3%) at a comparable biomass productivity (7.1 ± 0.8 g m⁻² d⁻¹) during the treatment of anaerobically digested vinasse coupled to biogas upgrading. Similar to the share of TN assimilated, the increase in biomass productivity resulted in an increase in the contribution of P assimilation to the TP removal from 22 ± 12% to 100%. The absence of PO₄³⁻ volatilization, together with the high pH prevailing in the cultivation broth throughout the entire experimental period, suggested that precipitation was the main phosphorous removal mechanism under low biomass productivities. Therefore, the control of biomass productivity via regulation of the biomass wastage rates allowed maximizing nutrient recovery in the form of algal biomass in detriment of the abiotic nutrients removal mechanisms.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Nutrient recovery as biomass (%)</th>
<th>Biomass elemental composition (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>C</td>
<td>P</td>
</tr>
<tr>
<td>I</td>
<td>6 ± 3</td>
<td>22 ± 12</td>
</tr>
<tr>
<td>II</td>
<td>16 ± 5</td>
<td>50 ± 19</td>
</tr>
<tr>
<td>III</td>
<td>30 ± 1</td>
<td>100</td>
</tr>
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3.4 Consortia of cyanobacteria/microalgae and bacteria.

The microalgae and cyanobacteria species initially present in the inoculum were gradually replaced along the three operational stages. The cyanobacterium prevailing in the inoculum (Geitlerinema sp.) was not observed under steady state conditions in stages I, II and III. Thus, the cyanobacteria/microalgae consortium was mainly composed of Limnothrix planktonica (32.9%), Acutodesmus obliquus (2.6%), Chlorella vulgaris (2.6%), Mychonastes homosphaera (5.9%), Navicula sp. (0.7%), Phormidium sp. (19.7%) and Stigeoclonium tenue (35.5%) during stage I. This high diversity was
similar to that reported in indoor HRAP treating digestates [14, 15]. Surprisingly, this high microalgae diversity disappeared in stage II with the establishment of an unialgal culture of the Chlorophyta Mychonastes homosphaera. This unialgal culture remained dominant throughout stage III likely due to the extreme environmental conditions prevailing in this study (high pH and salinity as a result of the high water evaporation losses). In addition, the control of biomass productivity via regulation of the settled biomass wastage rate applied might have also influenced the dominant species and algal/bacterial ratio since the increase in biomass productivity likely induced the development of fast growing microorganisms. Mychonastes homosphaera (Skuja) Kalina & Puncochárová is currently regarded as a taxonomic synonym of Chlorella minutissima Fott & Novákova. The potential of this microalga for wastewater treatment [31], heavy metal removal [32] and biodiesel production has been consistently demonstrated, Mychonastes homosphaera being capable of storing a desirable fatty acid profile under nitrogen starvation [33]. The valorization of this microalga into high-added value chemicals or biofuels such as syngas, bioethanol or bio-oil using a biorefinery approach will certainly enhance the sustainability and economic viability of microalgae-based biogas upgrading [34].

The high diversity revealed by microscopic observation was confirmed by the Shannon-Wiener diversity indexes obtained, which ranged from 1.5 to 3.5 (Figure 1, supplementary material). The slight decrease of this index from 3.2 in stage I to 2.9 in stage II also confirmed the shift in algae diversity microscopically observed. Likewise, the analysis of the Pearson similarity coefficients showed a high similarity between the microbial communities present in stages II and III (99%), which was in agreement with the above mentioned establishment of a dominant microalga specie. The DGGE analysis (Figure 1, supplementary material) showed 24 bands, which were sequenced. Six
different phyla were retrieved from the RDP database: Cyanobacteria/Chloroplast (10 bands), Acidobacteria (4 bands), Proteobacteria (4 bands), Deinococcus-thermus (1 band), Chloroflexi (1 band), Actinobacteria (1 band) (Table 1, supplementary material). The morphological identification of Mychonastes homosphaera was confirmed by bands 8, 9 and 10, which belonged to the genus Chlorophyta and were related to Chlorella species. The phyla Acidobacteria (bands 12 and 13), Proteobacteria (bands 15 and 16) and Actinobacteria (band 21) were found in the three operational stages, while the phylum Chloroflexi was detected in the inoculum and stages II and III. Bacteria from the genus Blastocatella (band 11) and the Gammaproteobacteria class (band 15), which have been identified in activated sludge [35] and HRAPs treating piggery wastewater [36], respectively, likely supported the aerobic biodegradation of the organic matter and ammonia contained in the digestate. Finally, the identification of the genus Thioalbus (band 16) confirmed the biological nature of H₂S oxidation [37]. To the best of our knowledge, this is the first time that sulfur-oxidizing bacteria (facultative microorganisms that can use O₂ or NO₃⁻ as electron acceptors) have been found in these photosynthetic biogas upgrading processes.

3.5 Conclusions.

This study confirmed the potential of photosynthetic biogas upgrading to support a cost-efficient bio-methane production coupled to nutrient recovery from digestate. To the best of our knowledge, this is the first experimental study reporting biological biogas upgrading to a bio-methane complying with most European legislations for biogas injection into natural gas grids. An almost complete removal of H₂S and CO₂, and concentrations of O₂ and CH₄ in the upgraded biogas <0.1% and >95%, respectively, were achieved regardless of the biomass productivity set. The innovative HRAP
Operational strategy here developed allowed enhancing nutrient recovery by shifting from an abiotic-based nutrients removal to an assimilatory-based removal. Furthermore, the extreme cultivation conditions established in the HRAP expedited the dominance of the microalga *Mychonastes homosphaera*, and supported the growth of sulfur-oxidizing bacteria. The presence of sulfur-oxidizing bacteria from the genus *Thioalbus* confirmed, for the first time, the biological nature of H2S oxidation during biogas upgrading in algal-bacterial photobioreactors.

**References.**


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