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Photosynthetic upgrading of biogas from anaerobic digestion of mixed sludge in an outdoors algal-bacterial photobioreactor at pilot scale

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ABSTRACT

Anaerobic digestion can biotransform the biodegradable fraction of sewage sludge into biogas, while the symbiotic action of algal-bacterial consortia can remove both the CO_2 and H_2S from biogas and nutrients from digestate. A 100 L anaerobic digester operated at 20 days of retention time coupled with a 180 L high-rate algal pond (HRAP) engineered to upgrade the biogas and treat the liquid fraction of the pilot digester was optimized along four operational stages: (I) operation with a greenhouse during winter; (II) operation without greenhouse; (III) process supplementation with $NaHCO_3$; (IV) process supplementation with Na_2CO_3. The biogas produced was composed of 63.7 ± 2.9% CH_4, 33.7 ± 1.9% CO_2, 0.5 ± 0.3% O_2 and 1.6 ± 1.1% N_2. An average methane productivity of 324.7 ± 75.8 mL CH_4 g $VSin^{-1}$ and total COD removals of 48 ± 20% were recorded in the digester. The CH_4 concentration in the biomethane gradually decreased to 87.6 ± 2.0% and 85.1 ± 1.3% at the end of stage I and II, respectively, attributed to the loss of inorganic carbon in the HRAP. The supplementation of $NaHCO_3$ and Na_2CO_3 mediated an increase in the CH_4 content to 90.4 ± 1.5 and 91.2 ± 0.7% in stages III and IV, respectively. Steady state CO_2 removals of 90% and 88% in stages I and II, and 95.7 and 97.6% in stages III and IV, respectively, were recorded. A constant biomass productivity of 22 g m^{-2} d^{-1}, set by daily harvesting 26.5 g dry algal-bacterial biomass from the bottom of the settler, was maintained concomitantly with a complete removal of the N and P supplied via centrate.

1. Introduction

Municipal wastewater treatment plants (WWTPs) generate primary and secondary sludge as a result of wastewater management. Sludge disposal is a major concern and operational cost in most WWTPs as a result of the large volumes of sludge annually generated [1,2]. In this context, anaerobic digestion (AD) is a well stablished biotechnology where specialized microorganisms bioconvert the biodegradable fraction of WWTP sludge into a biogas mainly composed of CH_4 and CO_2, and a nutrient rich effluent is also generated. This process is carried out in four consecutive steps namely hydrolysis, acidogenesis, acetogenesis and methanogenesis in the absence of oxygen [3,4]. The AD of mixed sludge minimizes WWTP costs as a result of the significant sludge volume reduction and electricity/heat generation from biogas [1].

The biogas generated from sewage sludge anaerobic digestion has been typically used for heat and/or power (CHP) generation since it contains high concentrations of CH_4 (55–65%), but also significant concentrations of contaminants such as CO_2 (30–40%), N_2 (0–3%), H_2O (5–10%), O_2 (0–1%) and H_2S (0–10.000 ppm_v) [1,5]. Raw biogas requires a partial or active purification prior use in internal combustion engines or turbines, and a stricter purification when used as a transportation fuel or injected into natural gas grids. The composition of the upgraded biogas, commonly named biomethane, depends on national or regional standards that typically require $CH_4 \geq$ 90–95%, $CO_2 \leq$ 2–4%,

Abbreviations: AC, absorption column; AD, anaerobic digestion; COD, chemical oxygen demand; DO, dissolved oxygen; HRAP, high rate algal pond; HRT, hydraulic retention time; IC, inorganic carbon; L/G, liquid to biogas ratio; OLR, organic loading rate; PAR, photosynthetic active radiation; sCOD, soluble chemical oxygen demand; tCOD, total chemical oxygen demand; TN, total nitrogen; TOC, total organic carbon; TS, total solid; TSS, total suspended solids; RE, removal efficiency; VFA, volatile fatty acids; VS, volatile solid; VSS, volatile suspended solids; WWTP, wastewater treatment plant.

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$O_2 \leq 1\%$ and negligible amounts of H_2S [6]. A number of physical-chemical technologies such as pressurized water scrubbing, organic scrubbing, chemical scrubbing, membrane separation or adsorption, have been developed for biogas upgrading and currently dominate the market. However, these technologies are highly energy and chemicals intensive, which can jeopardize the economic and environmental sustainability of biomethane [2,5]. For instance, the electricity demand and cost of biogas upgrading range from 0.2–0.3 kWh Nm^{-3} and 0.15–0.2 € Nm^{-3}, while the CO_2 separated from biogas is typically discarded to the atmosphere.

In recent years, biotechnologies based on the symbiotic action of algal-bacterial consortia have attracted an increasing attention as a result of their potential for CO_2 fixation in the form of biomass and their low operating costs and environmental impacts. Indeed, the CO_2 present in biogas is bioassimilated by photosynthetic microorganisms (microalgae and cyanobacteria) by using solar radiation, while sulfur oxidizing bacteria rapidly oxidize the H_2S contained in biogas using the oxygen generated by photosynthetic activity. Algal-bacterial photobioreactors have been effectively used for bioremediation purposes such as organic matter stabilization or nutrient removal from wastewaters. In fact, the N, P and other trace elements present in the liquid fraction of digestates can be used as nutrient source to support the growth of microalgae and bacteria [7–10]. Thus, centrates, the liquid fraction of digestates obtained from the AD of mixed WWTP sludge, represent an inexpensive cultivation broth for microalgae and sulfur oxidizing bacteria. In this context, photosynthetic biogas upgrading coupled with nutrient recovery from centrate has been validated under indoor and outdoor conditions in multiple photobioreactor configurations interconnected to external biogas scrubbing units, where biogas was sparged into a recirculating algal cultivation broth to promote gas-liquid mass transfer [11–14]. Nevertheless, most of these works were conducted using synthetic biogas or mineral salt media mimicking digestates. A recent techno-economic analysis of this technology compared to an activated carbon filter coupled with a pressurized water scrubber showed a reduction in the electricity demand from 0.3 to 0.08 kWh Nm^{-3} and a decrease in operating cost from 0.2 to 0.03 € Nm^{-3} [15].

This work aimed at validating, for the first time, the integration of WWTP sludge digestion with photosynthetic biogas upgrading at pilot scale under outdoor conditions in order to obtain biomethane and algal biomass as the main by-products from mixed sludge stabilization. The influence of photobioreactor operation inside a greenhouse, and the pH and alkalinity of the centrate, on the quality of biomethane was investigated under long term operation.

2. Materials and methods

2.1. Inocula and feedstock

The algal-bacterial photobioreactor was inoculated with a microalgae cake containing 127.6 g Total Solids (TS)·kg^{-1} and 64.6 g Volatile Solids (VS)·kg^{-1}. This inoculum was initially grown in mineral salt medium with a high carbonate content under outdoors conditions in a 270 m^2 high rate algal pond (HRAP) located at URBASER Technological Innovation Center of Zaragoza (Spain). The microalgae population in the HRAP was composed of *Chlorella* sp. (15%), *Tetradesmus obliquus* (33%), *Lindavia* sp. (24%) and *Synechococcus* sp. (28%) (percentages expressed in number of cells). The mineral media used as a nutrient and water source for the cultivation of microalgae and bacteria in the photobioreactor was centrate daily obtained from the centrifugation (10,000 rpm for 5 min) of the effluent from the pilot anaerobic digester treating mixed WWTP sludge. Centrate from the WWTP of Valladolid was used to compensate the differences observed between centrifugation processes among laboratory scale and industrial scale. The average composition of the centrate was: total organic carbon (TOC) concentration of 323.0 ± 84.3 mg·L^{-1}, inorganic carbon (IC) concentration of 736.9 ± 130.1 mg·L^{-1} and total nitrogen (TN) concentration of 791.0 ± 137.9 mg·L^{-1}.

Fresh anaerobic broth from the full-scale digester of the WWTP located in the city of Valladolid (Spain) was herein used as inoculum of the pilot anaerobic digester. The anaerobic inoculum exhibited a VS/TS ratio of 66%. Mixed (primary + secondary) sludge from Valladolid WWTP was used as feedstock in the pilot anaerobic digester. Fresh mixed sludge was obtained every two weeks and stored at 4 °C prior to use. The mixed sludge presented an average total solid (TS) and volatile solid (VS) concentration of 34.5 ± 10.0 and 22.3 ± 6.1 g·L^{-1}, respectively, and a chemical oxygen demand (COD) of 49.0 ± 14.7 g O_2·L^{-1}. The variations observed in the sludge composition during the 8-month experimental period were ascribed to the typical fluctuations in real WWTP sludge.

2.2. Experimental set-up

The experimental pilot plant was located at the Institute of Sustainable Processes of Valladolid University (Spain) under outdoors conditions. The experimental set-up was composed of an anaerobic digester with a total volume of 130 L and a working volume of 100 L of anaerobic broth. The digester was operated under mesophilic conditions (35 ± 2 °C) with a constant mixing of the anaerobic broth using a recirculation pump (621 series, Watson Marlow) working at 1.4 L min^{-1}. The anaerobic digester was coupled to the algal-bacterial photobioreactor, which was engineered as an outdoors HRAP with a working volume of 180 L and an illuminated surface area of 1.2 m^2 (length = 170 cm; depth = 15 cm; width = 82 cm). The HRAP was interconnected to a 1.8 m biogas absorption column (AC) of 2.5 L through a conical settler of 8 L, providing a total volume of ≈190 L for the entire algal system. A metallic diffuser of 2 μm pore size was installed at the bottom of AC to maximize the mass transfer of CO_2 and H_2S to the recirculating algal broth [6]. The biomass accumulated at the bottom of the settler was continuously recirculated to the HRAP using a Watson Marlow recirculation pump (100 series, Watson Marlow, USA) at a flow rate of 7.2 L·d^{-1}. The cultivation broth inside the HRAP was recirculated by a 6-blade paddlewheel at a velocity of 20 cm·s^{-1} (Fig. 1).

2.3. Operational conditions and sampling procedures

The anaerobic digester was initially inoculated with 100 L of fresh anaerobic sludge with an initial VS concentration of 18.2 g·kg^{-1}. Mixed sludge was daily fed to the anaerobic digester at a flow rate of 5 L·d^{-1} to maintain a hydraulic retention time (HRT) of 20 days and an organic loading rate (OLR) of 1.1 ± 0.3 g VS·L^{-1} d^{-1}. The flow rates of biogas and digestate produced in the anaerobic digester were daily measured. 100 mL of mixed sludge and digestate were drawn twice a week in order to determine the concentrations of TS and VS, chemical oxygen demand (COD), as well as the concentrations of IC, TOC, TN, N-NO_3^-, N-NO_2^-, P-PO_4^{3-}, S-SO_4^{2-} and volatile fatty acids (VFA) in the digestate.

Four operational stages were defined as a function of the operational conditions implemented (namely I, II, III and IV) (Table 1). The HRAP was initially inoculated with 2 L of microalgal inoculum in order to reach an initial biomass concentration of 760 mg VSS ·L^{-1}. Biomass productivity was set to 22.5 g m^{-2} d^{-1} by daily harvesting 26.5 g dry algal-bacterial biomass from the bottom of the settler in order to provide a constant growth of microalgae, with the exception of the first week, where no biomass removal from the bottom of the settler was performed to allow the adaptation of the algal-bacterial biomass. The settled algal-bacterial biomass daily removed was centrifuged and the supernatant was returned into the HRAP, in order to maintain a zero-effluent operation. Centrate from the pilot anaerobic digester was used as a nutrient and water source in the HRAP at a daily flowrate of 4 L d^{-1}. Additionally, 0.5 L·d^{-1} of centrate from Valladolid WWTP was used to compensate the differences between centrifugation processes, where 0.9 L of centrate per L of sludge are produced in full scale WWTPs and only 0.8 L of centrate per L of sludge are produced in the pilot plant

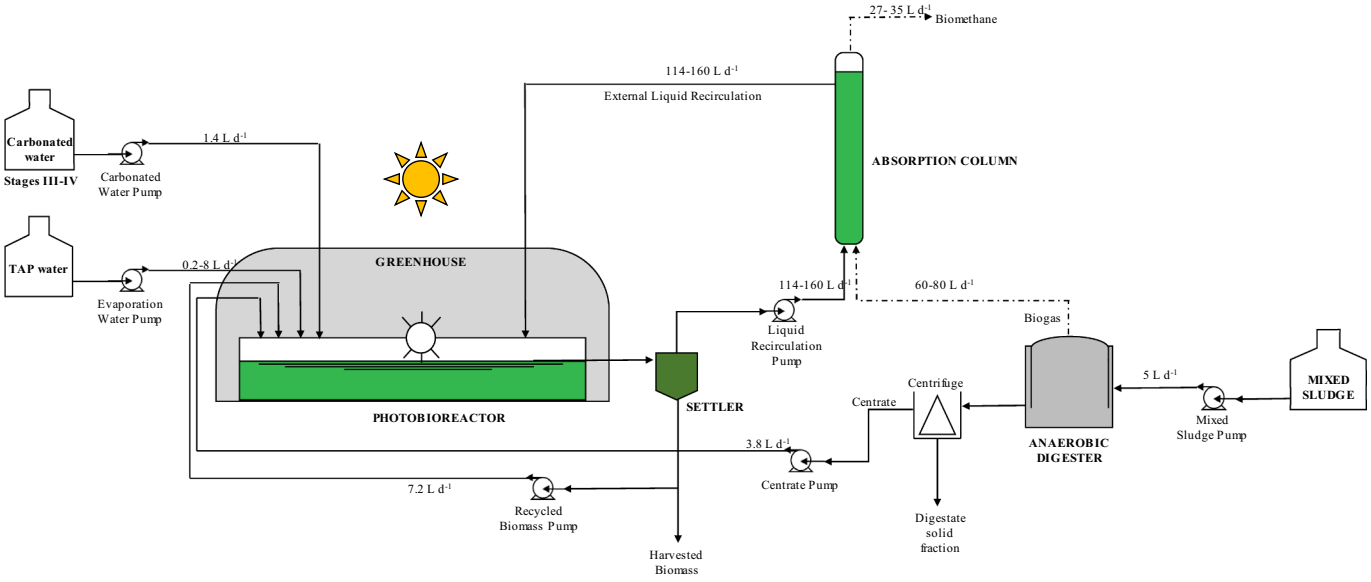

Fig. 1. Schematic diagram of the outdoors pilot experimental set-up.

Table 1
Environmental and operational parameters of the algal-bacterial photobioreactor during the experimental period.

Parameters	Stage I	Stage II	Stage III	Stage IV
Period	19-Feb–17-May	18-May–15-July	16-July–02-Sept	03-Sept–11-Oct
Use of greenhouse	Yes	No	No	No
Morning dissolved oxygen ($mg{\cdot}L^{-1}$)*	2.6 ± 2.9	1.6 ± 1.3	1.3 ± 1.0	5.1 ± 1.9
Afternoon dissolved oxygen ($mg{\cdot}L^{-1}$)*	6.7 ± 4.7	6.0 ± 3.9	8.7 ± 3.3	14.6 ± 3.9
Morning environmental temperature (°C)*	7.6 ± 2.6	15.2 ± 2.9	18.6 ± 2.8	11.9 ± 2.1
Afternoon environmental temperature (°C)*	16.7 ± 3.8	25.1 ± 4.6	30.1 ± 4.1	22.1 ± 4.6
Morning HRAP temperature (°C)*	10.9 ± 2.4	13.9 ± 2.4	16.3 ± 1.7	12.0 ± 2.4
Afternoon HRAP temperature (°C)*	23.2 ± 4.1	26.6 ± 3.8	29.0 ± 3.1	19.5 ± 3.6
Evaporation rate ($L{\cdot}m^{-2}{\cdot}d^{-1}$)*	2.5 ± 1.4	6.1 ± 2.6	7.7 ± 3.5	2.6 ± 3.2
HRAP initial IC ($mg{\cdot}L^{-1}$)	1600	600	1000	1000
Carbonate/bicarbonate addition (pH)	No	No	Yes (9.5)	Yes (11.5)
Harvested biomass productivity ($g{\cdot}m^{-2}{\cdot}d^{-1}$)	22.5	22.5	22.5	22.5

* Values are given as the average ± standard deviation.

herein used.

The raw biogas obtained from the pilot anaerobic digester was injected into the AC at flow rates of 30–86 L d^{-1} under co-current liquid flow operation at a L/G ratio of 2.0 during the entire experimental period. The HRAP was installed inside a greenhouse during Stage I (Feb–May) in order to mitigate the low temperatures in the algal cultivation broth and therefore enhance algal activity during winter conditions. Tap water was supplied to the photobioreactor at a flow rate between 0 and 12 $L{\cdot}d^{-1}$ in order to compensate water evaporation losses during stages II to IV. In Stage II (May–July) the greenhouse was dismantled as a result of the more favorable environmental conditions. In stage III (July–Sept), the cultivation broth of the HRAP was supplemented with 5.2 $g{\cdot}L^{-1}$ of $NaHCO_3$ and 2.2 $g{\cdot}L^{-1}$ of Na_2CO_3 in order to achieve an initial concentration of 1000 mg $IC{\cdot}L^{-1}$. In addition, the HRAP was daily supplemented with 1.4 $L{\cdot}d^{-1}$ of a solution containing 5.3 $g{\cdot}L^{-1}$ Na_2CO_3 and 7.9 $g{\cdot}L^{-1}$ $NaHCO_3$ in order to maintain a stable IC concentration in the HRAP. In stage IV (Sept-Oct), centrate was supplemented with an IC solution containing 15 $g{\cdot}L^{-1}$ Na_2CO_3 in order to increase its pH to 11.5.

The pH of the cultivation broth in the HRAP and digestate were daily measured at 9:00 am. Daily monitoring at 9:00 a.m. and 4:00 p.m. was carried out for photosynthetic active radiation (PAR) and temperature, both measured outdoors and inside the greenhouse, as well as temperature and dissolved oxygen (DO) concentration in the HRAP culture broth. Gas samples of 100 μL were taken in duplicate at 10:00 a.m. twice a week in order to determine CH_4, CO_2, H_2S, N_2 and O_2 concentrations in the raw biogas and biomethane. 100 mL of liquid samples of the HRAP cultivation broth were drawn twice a week in order to determine the concentrations of Total Suspended Solids (TSS), Volatile Suspended Solids (VSS), IC, TOC, TN, N-NO_3^-, N-NO_2^-, P-PO_4^{3-} and S-SO_4^{2-}. A sample of the HRAP cultivation broth was monthly taken and preserved in Lugol iodine solution (5%) and formaldehyde (10%) in order to determine the structure of microalgae population. All results are expressed as the average ± standard deviation.

2.4. Analytical procedures

PAR was measured with a Li-250A light meter (Li-COR Biosciences, Germany). DO and temperature in the algal cultivation broth were recorded using an OXI 3310 oximeter (WTW, Germany). An Eutech Cyberscan pH 510 was used for pH determination. Biogas and biomethane flow rates were determined using the Mariotte method. Biogas and biomethane composition were recorded using a Varian CP-3800 GC-TCD (Palo Alto, USA) according to Marín et al. [16]. The removal efficiencies (REs) of CO_2 and H_2S were calculated according to Eq. (1):

$$RE = \frac{(Q_{in} \times C_{in}) - (Q_{out} \times C_{out})}{Q_{in} \times C_{in}} \times 100 \quad (1)$$

where Q_{in} represents the inlet biogas flowrate ($L{\cdot}d^{-1}$), Q_{out} the outlet biomethane flow rate (L d^{-1}), and C_{in} and C_{out} are the inlet and outlet concentrations (%) of the target biogas pollutants, respectively. VFA concentrations were analyzed in an Agilent 7820A GC-FID (Agilent Technologies, Santa Clara, USA) according to López et al. [17]. N-NO_3^-, N-NO_2^-, P-PO_4^{3-} and S-SO_4^{2-} concentrations were quantified by HPLC-IC (Waters 432, conductivity detector, USA). TOC, IC and TN

concentrations were analyzed using a Shimadzu TOC-L analyzer (Japan). N-NH_4^+ concentration was quantified using the Nessler analytical method in a spectrophotometer SpectroStar Nano (BGM Labtech) at 425 nm. COD, TSS, VSS, TS and VS concentrations were determined according to APHA [18]. The harvested algal-bacterial biomass productivity (W) ($g{\cdot}m^{-2}{\cdot}day^{-1}$) was quantified according to Eq. (2):

$$W = \frac{TSS_{settler} \times Q_{wout}}{S} \quad (2)$$

where $TSS_{settler}$ is the biomass concentration at the bottom of the settler; Q_{Wout} is the flowrate of the biomass harvested and S is the HRAP surface. Algal-bacterial biomass was centrifuged 10,000 rpm for 5 min at 4 °C (Sorvall Legend RT +, Thermo Scientific). Finally, the structure of microalgae population was identified and quantified by microscopic examination (OLYMPUS IX70, USA) of the HRAP cultivation broth according to Sournia [19].

3. Results and discussion

3.1. Anaerobic digestion of mixed sludge

The biogas produced in the anaerobic digester, which was continuously pumped into the biogas absorption column, presented a constant composition of 63.7 ± 2.9% CH_4, 33.7 ± 1.9% CO_2, 0.45 ± 0.28% O_2 and 1.59 ± 1.05% N_2. Interestingly, H_2S was not detected in the raw biogas. The biogas yield and production rate during the treatment of mixed sludge accounted for 969–239 L·kg VS_{in}^{-1} and 92–22 L d^{-1}, respectively, with an average methane production yields of 324.7 ± 75.8 mL CH_4 g VS_{in}^{-1} (Fig. 2a). Biogas production rate averaged 70.3 ± 12.1 L·d^{-1} throughout most of the operational period and decreased to 22 L·d^{-1} at the end of the experiment concomitantly with a sudden reduction in feedstock organic matter content. Interestingly, biogas composition was not affected by the organic matter fluctuations inherent to mixed sludge variability in real WWTP [20,21]. These results were in accordance with literature [22]. For instance, Cho et al. [23] reported yields of 120–150 L biogas·kg VS_{in}^{-1} when using raw waste activated sludge, which increased by a factor of 8.3 under alkaline pretreatment. The anaerobic biodegradability of waste activated sludge is often restricted by the recalcitrance of the bacterial cell walls, the low C/N ratio of this organic feedstock and the presence of inorganic material [24]. Hence, co-digestion with other organic substrates is typically recommended [25]. Mahdy et al., [26] obtained 3.3-fold higher methane yields when digesting primary sludge compared with secondary sludge. This synergy might explain the higher biogas yields obtained in this work using mixed sludge as a substrate of anaerobic digestion.

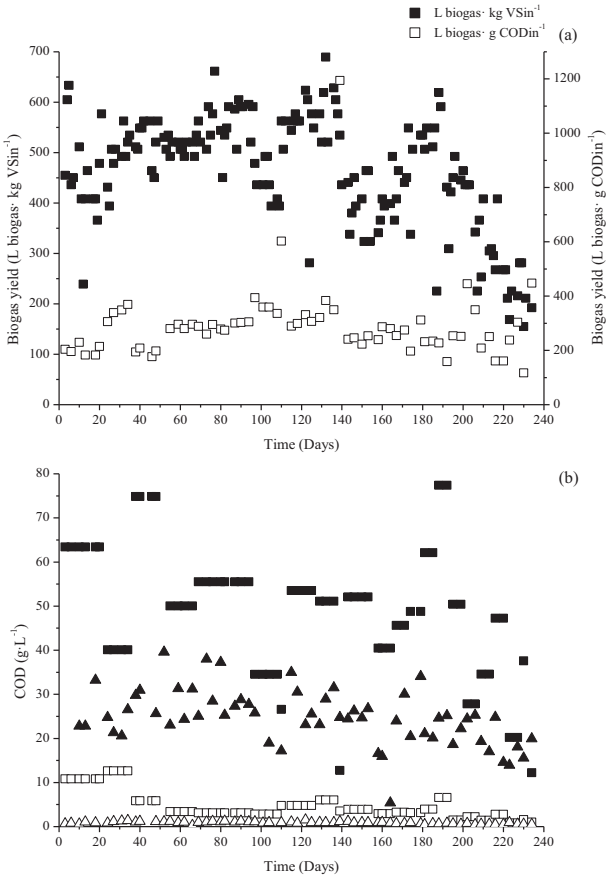

Fig. 2. Time course of (a) biogas production yields and (b) concentration of total chemical oxygen demand (solid symbols) and soluble chemical oxygen demand (empty symbols) in the mixed sludge (squares) and digestate (triangles).

The mixed sludge fed into the anaerobic digester exhibited a total COD (tCOD) ranging from 12 to 77 g O_2 L^{-1} and soluble COD (sCOD) from 1 to 13 g O_2 L^{-1} (Fig. 2b). These fluctuations observed are typically encountered in primary and secondary sludge in real WWTPs, which are subjected to large variations in the composition and flow of the wastewater to be treated and in the seasonal climatic conditions [27]. In this context, tCOD in the mixed sludge remained approximately constant at 52.3 ± 12.8 g O_2 L^{-1} during the first 200 days of experiment and gradually decreased to 12.2 g O_2 L^{-1}. Interestingly, sCOD in the mixed sludge remained stable throughout the entire operational period at 4.7 ± 3.2 g O_2 L^{-1}. tCOD and sCOD in the digestate remained relatively constant at 26.0 ± 5.3 and 0.9 ± 0.2 g O_2 L^{-1}, respectively, while a slight decrease to 17.9 ± 3.5 g O_2 L^{-1} was recorded at the end of the operational period concomitantly with the lower OLR. In this context, tCOD-REs and sCOD-REs of 48 ± 20 and 76 ± 12%, were obtained respectively. Similar values were reported by Houtmeyers et al., [24] during the anaerobic digestion of waste activated sludge (tCOD in the digestate of 27.3 g O_2 L^{-1} and tCOD-REs of 33%).

The mixed sludge exhibited TS and VS content of 34.5 ± 10 and 22.3 ± 3.1 g·kg^{-1} respectively, while the anaerobic digestate was characterized by 23.6 ± 5.0 g TS·kg^{-1} and a 57.5% of VS content. The VS concentrations of the effluent remained stable throughout the experimental period, where slight fluctuations were observed mainly ascribed to feedstock variations that did not compromise the stability of the anaerobic digester. The anaerobic digester exhibited average VS-REs of 41.1 ± 14.1%. Higher VS-REs were obtained by Carballa et al. [28], who reported removals of 64 ± 0.2% in a CSTR anaerobic digester operated under mesophilic conditions with mixed sludge at a HRT of 20d.

NH_4^+ and free ammonia (NH_3) are the most abundant forms of inorganic nitrogen present in anaerobic digesters, free NH_3 being the most toxic since it can permeate through the cell membrane. In this context, high pH values in the digester broth would entail the shift in the NH_4^+/NH_3 equilibrium towards NH_3, which can ultimately result in process instability leading to an increase in the VFA concentrations in the anaerobic broth [1]. In present work, TN concentration in the centrate gradually decreased from 1053 mg N·L^{-1} at day 23 to 500 mg N·L^{-1} by the end of the experiment. Additionally, N-NH_4^+ concentration ranged from 411 to 1055 mg·L^{-1}, with a gradual decreasing trend throughout the operational period. Low VFA concentrations, ranging from 0 to 2200 mg·L^{-1} (Med = 16.8 mg·L^{-1}, concentration calculated on an equivalent acetic acid basis) were recorded throughout the main operational period, which agreed with the stability observed in the pH of the digestate. A gradual acetic acid increase was observed from day 40 to 100, reaching a maximal concentration of 2200 mg·L^{-1}, which did not affect the anaerobic digestion performance. Likewise, a slight increase in butyric, isobutyric, valeric and isovaleric acids concentrations, which ranged 5.2–641 mg·L^{-1} (Med = 103.3 mg·L^{-1}), was observed from day 195 onwards.

3.2. Environmental parameters in the HRAP

Environmental parameters experienced significant variations according to the seasonal climatic conditions (Table 1). The average environmental temperature increased from stage I (February) to stage III (July), with a significant decrease in stage IV (September). The ambient temperatures averaged 7.6, 15.2, 18.6 and 11.9 °C in the morning and 16.7, 25.1, 30.1 and 22.1 °C in the afternoon, in stages I, II, III and IV, respectively (Fig. A.1). The low temperatures prevailing during winter season required the use of a greenhouse in order to avoid freezing of the culture broth during the night [7,29]. In this context, the temperatures recorded inside the greenhouse during stage I ranged from 4 to 48 °C. The latter values were in accordance with those recorded by Marín et al. [13] during a similar seasonal period (−2.0 to 43.0 °C). On the other hand, the temperature in the HRAP culture broth during stages I to IV ranged from 10.9 to 23.2, 13.9 to 26.6, 16.3 to 29.0 and 12.0 to 19.5 °C. The similar range of temperatures recorded in the cultivation broth of the HRAP in Stage I and II confirmed the effectiveness of the greenhouse in maintaining the temperature of the culture broth.

The ambient PAR recorded in stages I, II, III and IV ranged from 42 to 1774, 48 to 1804, 31 to 1708 and 38 to 1580 μmol·m^{-2} s^{-1}, respectively (Fig. A.2). The values herein observed were in accordance to those previously reported by Marín et al. [13] under similar climatic conditions in the same location. The PAR recorded inside the greenhouse during Stage I ranged from 18 to 1388 μmol·m^{-2} s^{-1}, thus providing a reduction of 40–50% of ambient PAR.

Significant changes in the dissolved oxygen concentration and evaporation rate were recorded within and between stages since these parameters are highly influenced by variations in PAR and temperatures. In this context, the water losses by evaporation in the HRAP averaged 2.5 ± 1.4, 6.1 ± 2.6, 7.7 ± 3.5 and 2.6 ± 3.2 L·m^{-2} d^{-1} in stages I, II, III and IV, respectively (Table 1). The evaporation rates (Fig. A.3) herein recorded were similar to those values previously reported by Marín et al. [15] and Posadas et al. [29] in a 180 L outdoors HRAP located at Valladolid during comparable climatic conditions. These rates were attributed to the temperatures of the cultivation broth and turbulence at the HRAP surface caused by the paddlewheel. The maximum DO values (Fig.A.4) in the culture broth were observed in the afternoon: 6.7 ± 4.7, 6.0 ± 3.9, 8.7 ± 3.3 and 14.6 ± 3.9 mg O_2·L^{-1} in stages I, II, III and IV, respectively. DO values remained below inhibitory levels for microalgae activity (>20–25 mg O_2·L^{-1}) [31,32]. The minimum DO concentrations (2.6 ± 2.9, 1.6 ± 1.3, 1.3 ± 1.0 and 5.1 ± 1.9 mg O_2·L^{-1}) were measured during the morning as a result of the absence of photosynthetic activity during nighttime and the concomitant oxygenic respiration of the algal-bacterial biomass [33]. The slight increase observed in Stage IV was attributed to the decrease of temperatures during autumn, which likely resulted in a reduction in microbial aerobic respiration [34] combined with an increase in the O_2 solubility in the cultivation broth [14,35,36].

3.3. Centrate treatment in the HRAP

The pH of the centrate remained constant throughout the entire experimental period with an average value of 7.6 ± 1.3. The pH of the HRAP culture broth, pH which was initially set at 9.0, slowly decreased during stage I till 8.3 and continued decreasing during stage II to 7.1 (Fig. 3a). The drop observed in pH was correlated with the gradual decrease in IC concentration (Fig. 3b) observed during stages I and II, with an average loss of 13 mg IC·L^{-1}·d^{-1}. This gradual IC depletion under continuous biogas supply was attributed to an active photosynthetic fixation since microalgae were able to use different carbon species present in the HRAP cultivation broth and convert them to organic forms through the Calvin cycle [37]. Likewise, a gradual reduction of pH was also observed in the biogas absorption column concomitantly to that observed in the HRAP, which was always lower than the pH of the algal-bacterial broth due to the acidification of the CO_2 directly transferred from the biogas to the culture broth [38].

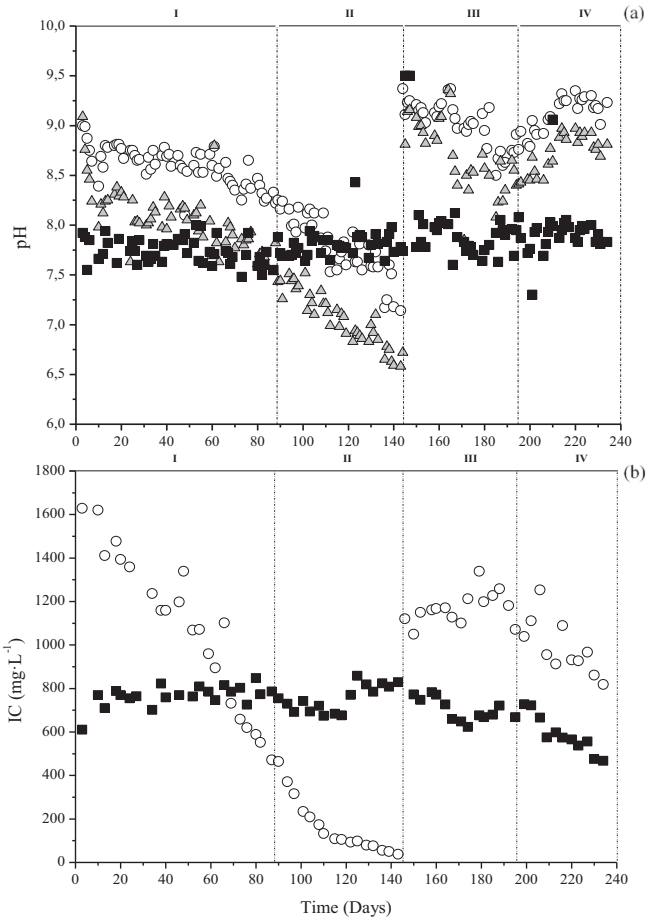

Fig. 3. Time course of the (a) pH of the centrate (solid squares), HRAP culture broth (empty circles) and absorption column (solid triangles), and (b) inorganic carbon concentration of the centrate (solid squares) and HRAP culture broth (empty circles).

Stage III was characterized by an external IC supplementation to the HRAP, in order to increase the buffer capacity of the HRAP culture broth and additionally to compensate the daily loss of IC. During this stage, the pH in the HRAP and AC increased up to 9.3 and 9.2, both experiencing a slight decrease throughout Stage III down to 8.7 and 8.4, respectively. In contrast, the pH recorded in the HRAP and AC during Stage IV increased up to a final value of 9.2 and 8.8, respectively, due to the higher pH provided by the carbonate solution. The IC concentration during Stage III remained constant at an average concentration of 1169.1 ± 73.8 mg IC·L^{-1} and slightly decreased in Stage IV to reach a final value of 818 mg IC·L^{-1}. In this context, this unexpected reduction observed in IC concentration despite the external Na_2CO_3 supplementation was likely due to the decrease in IC concentration of the centrate.

The dissolved TOC concentration in the centrate remained stable in stages I and II at average values of 367.3 ± 50.8 mgC·L^{-1} and decreased during stages III and IV down to 140 mgC·L^{-1} at the end of the experimental period (Fig. A.5). In contrast, the dissolved TOC concentrations in the algal-bacterial broth remained stable during Stage I and gradually increased from stage II until the beginning of stage IV, where a slight decrease was observed concomitantly to the rapid decrease in TOC concentration in the centrate. The accumulation of dissolved TOC in the HRAP confirmed the recalcitrant nature of the organic compounds present in the centrate [30].

On the other hand, centrate TN concentration steadily decreased from 1053 mg N·L^{-1} at day 23 to 500 mg N·L^{-1} by the end of stage IV.

However, the TN concentration recorded in the HRAP steadily increased from 700 mg N·L^{-1} at the beginning of Stage I up to 1100 mg·L^{-1} at the end of stage I (Fig. 4a), reaching the steady state by day 40 with TN-REs of 100% as a result of the zero effluent operation of the HRAP, since neither TN accumulation nor depletion occurred in the HRAP culture broth. A rapid increase was then observed during Stage II, reaching a value of 1600 mg N·L^{-1}, and during Stage III, where a maximum TN concentration of 1800 mg N·L^{-1} was achieved. This accumulation of TN in the HRAP indicated a nitrogen input into the system greater than the rate of nitrogen consumption by microorganisms and stripping. The increase in TN concentration recorded in stage II and III was likely due to the higher biomass concentrations, which entailed higher cell lysis and organic nitrogen accumulation, and the higher nitrification activity, which prevented NH_3 stripping and fixed nitrogen in the form of nitrate. However, the decrease in TN concentration of the centrate mediated a gradual decrease in the TN concentration of the cultivation broth to 1240 mg N·L^{-1} by the end of Stage IV. The percentage of ammonia stripping was calculated based on a maximum ammonia conversion of 57% observed in the afternoon (when temperatures higher). At this point, it should be noted that despite the higher pH in Stage IV, ammonia stripping was not enough to be considered as a nitrogen limiting mechanism or a cause of TN depletion since similar values were observed in Stage III with no TN reduction, as well as the increase in nitrification observed throughout the total experimental period. Thus, NH_4^+ supply was sufficient to support NO_2^-/NO_3^- formation. The N-NH_4^+ concentration of the centrate averaged 768 ± 167 mg·L^{-1} and slightly decreased throughout the experimental period (Fig. 4b). On the other hand, the N-NH_4^+ concentration in the HRAP culture broth remained almost negligible in the four operational stages, with an average value of 20 ± 18 mg N·L^{-1}. This low ammonium concentration was mainly supported by microbial nitrogen fixation, since NH_4^+ is one of the preferred sources of nitrogen for microalgae [37], and by nitrification of NH_4^+ to NO_2^- and NO_3^- [32]. The key role of nitrification was confirmed by the high N-NO_3^- concentrations in the HRAP culture broth (Fig. 4d). A preliminary nitrogen mass balance estimated nitrogen recoveries in the form of biomass of 56.9 ± 7.7%, 46.9 ± 12.8%, 52.5 ± 4.8% and 62.9 ± 6.2% in stages I, II III and IV respectively (based on an experimental nitrogen algal-bacterial biomass content of 6.8 ± 1.1%). In this context, N-NO_2^- concentration in the HRAP culture broth accounted for 582.0 ± 18.7 mg ·L^{-1} during the first 62 days of experiments and was completely depleted at the beginning of Stage II. A concomitant increase in N-NO_3^- concentration was observed as result of NO_2^- oxidation up to 940, 1540 and 1690 mg N-NO_3^-·L^{-1} at the end of stages I, II and III, respectively. N-NO_3^- concentration gradually decreased to 1160 mg·L^{-1} during Stage IV mediated by the lower TN concentrations in the centrate. In this context, Marín et al. [13] also reported a similar partial nitrification of NH_4^+ under oxygen sufficient conditions followed by a rapid conversion of NO_2^- into NO_3^-. However, the mechanisms underlying this shift in nitrification patterns still remain unclear [39].

Finally, P-PO_4^{3-} concentration remained stable in the culture broth throughout Stage I (87 ± 6 mg·L) with P-REs of 100% mediated by the zero-effluent operational strategy, slightly increased during stage II up to steady concentrations of 109 ± 8 mg·L^{-1}, and gradually decreased along stages III and IV to 40 mg·L^{-1}. This reduction in P-PO_4^{3-} concentration was likely mediated by the decrease in the P-PO_4^{3-} concentration of the centrate. Similarly, S-SO_4^{2-} concentration remained constant along the entire operational time with an average concentration of 41.2 ± 18.4 and 9.2 ± 5.4 mg·L^{-1} in the HRAP culture broth and centrate, respectively.

3.4. Algal-bacterial biomass in the HRAP

The biomass concentration in the HRAP averaged 1.39 ± 0.04, 1.58 ± 0.18, 1.80 ± 0.18 and 1.13 ± 0.18 g VSS L^{-1} in stages I, II, III and IV, respectively (Fig. 5a). The HRAP was initially inoculated at 0.76 g

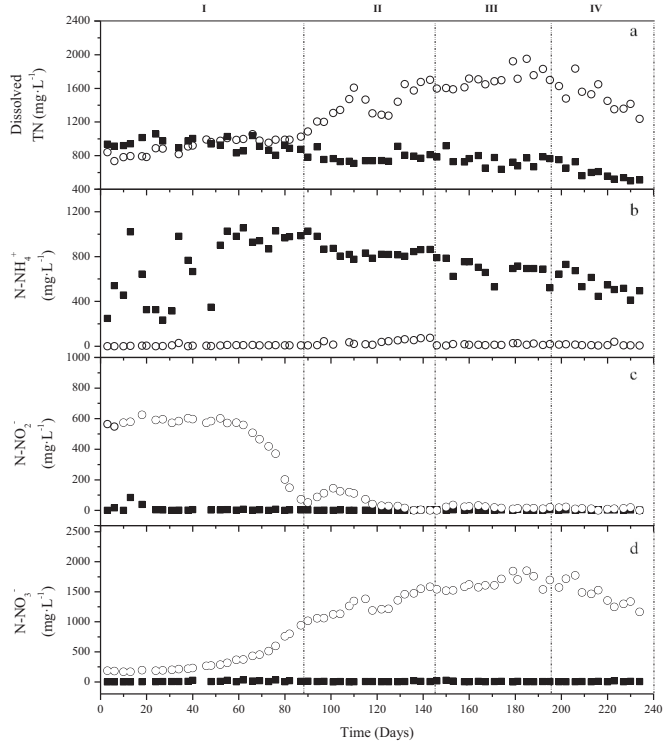

Fig. 4. Time course of the (a) TN, (b) N-NH_4^+, (c) N-NO_2^- and (d) N-NO_3^- concentrations in the centrate (solid square) and HRAP culture broth (empty circles).

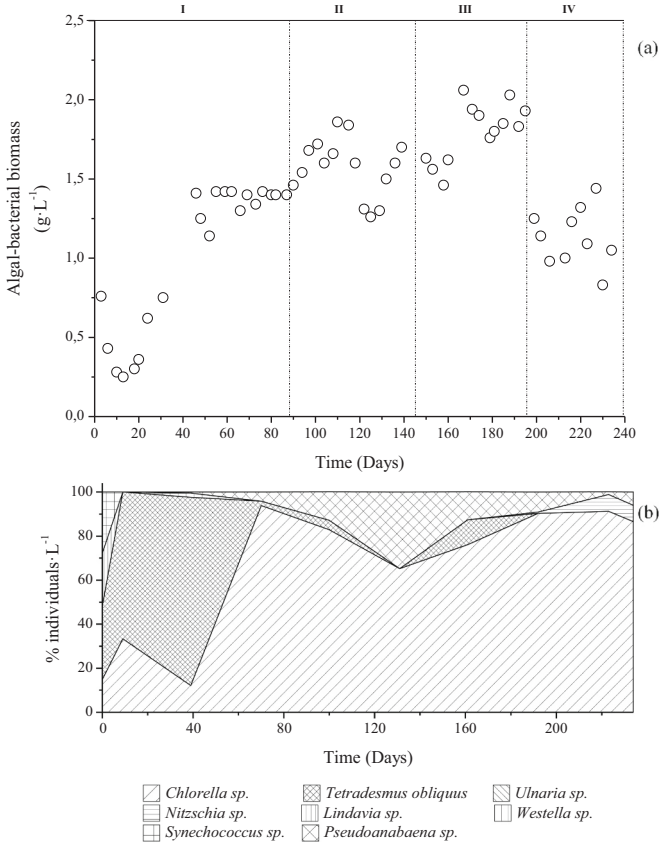

Fig. 5. (a) Time course of the volatile suspended solids concentration and (b) structure of microalgae population in the culture broth of the HRAP.

VSS·L^{-1} with an algal-bacterial consortium, whose concentration drastically decreased to 0.25 g VSS·L^{-1} in the following 13 days. This sharp reduction was attributed to the initial adaptation of the biomass to the cultivation broth since an increase was subsequently observed from day 13 to day 60 of operation. The removal of the greenhouse during stage II entailed an increase in biomass from 1.4 to 1.84 g VSS L^{-1} caused by the greater PAR impinging in the culture broth of the HRAP. A sharp decrease in biomass concentration was observed between days 110 and 120 due to a decrease in ambient temperature. However, the algal-bacterial biomass rapidly grew to finally stabilize at 1.7 g VSS L^{-1} by the end of Stage III. The reduction in the number of hours of solar irradiations, temperature and PAR induced a gradual decrease in VSS concentration in the HRAP in stage IV.

Interestingly, the inoculated mixture of microalgae was mainly replaced by *Tetradesmus obliquus* during stage I (Fig. 5b), which gradually increased its share from 33% to 85% during the first 40 days of operation. The second and third most abundant genera in the initial microalgae consortium, *Lindavia* sp. and *Synechococcus* sp., completely disappeared in less than 10 days. Interestingly, *Chlorella* sp. became the major species (93%) after 60 days of operation. The dominance of *Chlorella* sp. on *T. obliquus* could be explained by the gradual increase in the temperature of the culture broth and therefore a better adaptation of this microalga. In Stage II, a rapid reduction in the number of individuals of *Chlorella* sp., which continued during Stage III, was observed. This transient decrease in *Chlorella* sp. population, concomitant with an increase of the cyanobacteria *Pseudoanabaena* sp. (35%), was probably mediated by the higher irradiations during this period. It has been previously reported that cyanobacteria exhibit a competitive advantage over chlorophyte due to their ability to prevent sedimentation in warmer waters and resistance to grazing [40,41]. During stage III, *Chlorella* sp. accounted for 91% of microalgae population while *Pseudoanabaena* sp. represented only 9% at the end of this stage. The abundance of both species may have been caused by the increased temperatures combined with the increased alkalinities, since both photosynthetic species are able to grow at high pH and carbonate concentrations [42,43]. Finally, at the end of stage IV, *Chlorella* sp., *Pseudoanabaena* sp., and *Nitzschia* sp. represented 87%, 6% and 7% of the total population.

3.5. Biogas upgrading

CH_4 concentration averaged 89.9 ± 1.3% during the first 21 days of operation and gradually decreased to 87.6 ± 2.0 and 85.1 ± 1.3% at the end of stage I and II, respectively (Fig. 6a). The reduction in methane content was attributed to the gradual loss of IC in the HRAP culture broth and the gradual reduction of pH, which hindered CO_2 absorption in AC during the first stages. Thus, the decrease in the pH of the cultivation broth shifted the equilibrium of carbonate species from HCO_3^- towards CO_2 in the aqueous medium [29], which combined with the reduction of gas solubility derived from the gradual increase in temperatures (according to the Henry's Law constant) [14,30,35], reduced the gas-liquid CO_2 concentration gradient. Indeed, CO_2-REs decreased from an initial 95.5 ± 1.1% at the beginning of the experiment to 91.3 ± 1.2% and 89.6 ± 1.2% at the end of stages I and II, respectively, concomitantly to the reduction in CH_4 content in the upgraded biogas. The biomethane CO_2 concentrations in stages I and II accounted for 4.8 ± 1.5 and 6.7 ± 1.7%, respectively. During stage III, an increase in the CH_4 content to 90.4 ± 1.5% concomitant with a decrease in CO_2 content to 2.6 ± 0.9% (corresponding to a CO_2-RE of 95.7%) was recorded as a result of the IC addition, which mediated an increase in the pH and buffer capacity of the HRAP culture broth. These phenomena enhanced CO_2 mass transfer from the biogas into the liquid broth. During stage IV, where the pH of the HRAP culture broth increased, the CH_4 content increased and remained at 91.2 ± 0.7% along with an enhancement in CO_2-REs up to 97.6% and a biomethane CO_2 concentration of 1.6 ± 0.7%. Taking into account the extra cost derived from the use of carbonate solution in order to compensate IC losses, an economic estimate of the additional expenses was carried out. In this context, considering a standard industrial cost of Na_2CO_3 of 200 € per ton, the cost was increased to 0.062 € · Nm^{-3} treated biogas.

The O_2 content in the raw biogas ranged from 0.15 to 1.75% during all experimental period, which slightly increased to 0.20–2.77% in the

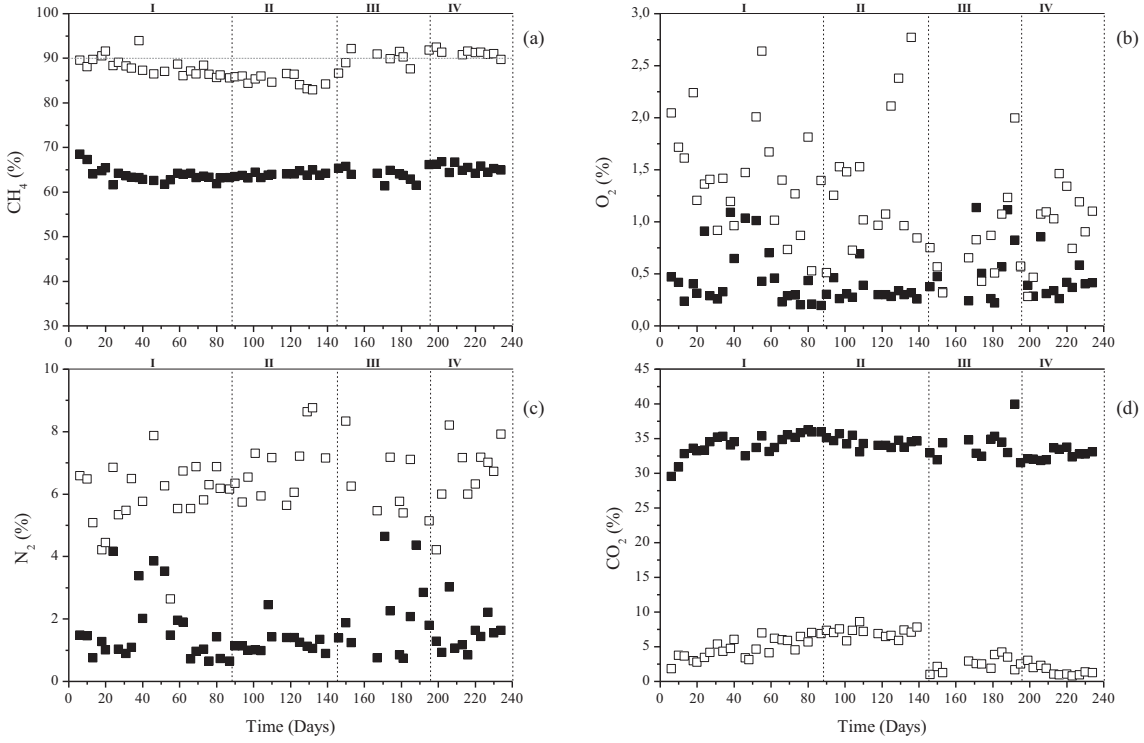

Fig. 6. Time course of the concentration of (a) CH_4, (b) O_2, (c) N_2 and (d) CO_2 in the raw biogas (solid symbols) and biomethane (empty symbols).

biomethane as a result of O_2 stripping from the algal cultivation broth in the biogas scrubbing column (Fig. 6b). The O_2 stripping in AC was more severe during Stage I as a result of the higher DO observed in the HRAP culture broth. This value slightly decreased during stage II onwards. The O_2 concentration in the upgraded biogas did not comply with international regulations ($\leq$1%) during most of the experiment, which requires further optimization of the technology. In this context, an increased pH in the microalgal liquor along with a reduction in the liquid to biogas ratio would support a reduction in the O_2 content in the treated biogas. Additionally, it has been reported that the presence of H_2S in the biogas would entail sulfur oxidation in presence of O_2, even in the absence of sulfur oxidizing bacteria, which would also entail a reduction in the O_2 content in the upgraded biogas [44]. H_2S is a typical contaminant of biogas when digesting protein or sulphate rich feedstocks.

N_2 concentration in the raw biogas remained constant throughout the entire experimental period at average values of 1.5 $\pm$ 0.8% with minor fluctuations. However, the biomethane N_2 content did not present a clear tendency despite the different operational conditions tested, averaging 6.7 $\pm$ 2.3% (Fig. 6c). Similar values of N_2 content in the biomethane were reported by Posadas et al. [30] at a L/G ratio 2 in a similar experimental set-up, while increased N_2 desorption was obtained at higher ratios. It is worth mentioning that N_2 levels higher than 4% were recorded in the raw biogas likely due to the pilot nature of the anaerobic digester.

4. Conclusions

The photosynthetic upgrading of the biogas produced from the anaerobic digestion of WWTP mixed sludge coupled with nutrient recovery from centrates was confirmed for the first time at pilot scale under outdoors conditions. The pilot mesophilic anaerobic digester supported methane yields of 324.7 $\pm$ 75.8 mL CH_4 g VS_{in}^{-1}, and tCOD-REs and sCOD-REs of 48 $\pm$ 20 and 76 $\pm$ 12%, respectively. The high photosynthetic activity of microalgae during stage I and II mediated an active consumption of alkalinity and a concomitant decrease in pH, which decreased CH_4 concentration in the upgraded biogas to 85%. The external supplementation of inorganic carbon supported stable CH_4 contents of 91%. Process operation under a zero effluent strategy, in addition to increased IC and pH, entailed a complete removal of the nitrogen and phosphorous present in the centrate. The IC content and pH in the absorption column were key parameters governing biomethane quality and suggests that this technology can be fully optimized via pH control, adjustment of the liquid to biogas ratio, cultivation broth degassing and enhanced microalgae activity via nanoparticle supplementation. Despite *Tetradesmus obliquus* dominated the culture broth during stage I, *Chlorella* sp. represented the most abundant photosynthetic species during the rest of the experiment.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jwpe.2022.102891.

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