Evaluation of the simultaneous biogas upgrading and treatment of centrates in a high-rate algal pond through C, N and P mass balances

Cynthia Alcántara, Pedro A. García-Encina and Raúl Muñoz

ABSTRACT

The simultaneous capture of CO_2 from biogas and removal of carbon and nutrients from diluted centrates in a 180 L high-rate algal pond (HRAP) interconnected to a 2.5 L absorption column were evaluated using a C, N and P mass balance approach. The experimental set-up was operated indoors at 75 $\mu E/m^2 \cdot s$ for 24 h/d at 20 days of hydraulic retention time for 2 months of steady state, and supported a C-CO_2 removal in the absorption column of 55 ± 6%. Carbon fixation into biomass only accounted for 9 ± 2% of the total C input, which explains the low biomass productivity recorded in the HRAP. In this context, the low impinging light intensity along with the high turbulence in the culture broth entailed a C stripping as CO_2 of 49 ± 5% of the total carbon input. Nitrification was the main NH_4^+ removal mechanism and accounted for 47 ± 2% of the inlet N-NH_4^+, while N removal as biomass represented 14 ± 2% of the total nitrogen input. A luxury P uptake was recorded, which resulted in a P-PO_4^{-3} biomass content over structural requirements (2.5 ± 0.1%). Phosphorus assimilation corresponded to a 77 ± 2% of the inlet dissolved P-PO_4^{-3} removed.

Key words | algal-bacterial consortium, biogas upgrading, bioremediation, luxury P uptake, mass balances, nitrification

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INTRODUCTION

Microalgae are photoautotrophic micro-organisms highly efficient at fixing CO_2 using solar energy, 1.8 kg of CO_2 being required per kg of microalgae produced (Chisti 2007; Lardon *et al.* 2009; Alcántara *et al.* 2013). Despite the inhibitory CO_2 concentration thresholds in microalgae being often low and strain specific, tolerances to CO_2 concentrations of up to 50% have been reported in *Scenedesmus obliquus* strains (Lam *et al.* 2012; Arbib *et al.* 2014). Photoautotrophic microalgae growth can support both the mitigation of greenhouse emissions by capturing CO_2 from industrial gas emission and the removal of nutrients from wastewaters with low C/nutrients ratios, such as anaerobic effluents (Arbib *et al.* 2012). In this context, microalgae-based wastewater treatment in high-rate algal ponds (HRAPs) represents an opportunity to simultaneously remove the CO_2 present in biogas and the residual carbon and nutrients from digestates at low energy costs and environmental impacts (Park & Craggs 2010). Hence, the supply of biogas to HRAPs can provide the additional C source required to boost nutrient removal by assimilation and result in a significant production of biomass that could be further used as a substrate for the subsequent generation of biogas (De Godos *et al.* 2010; Alcántara *et al.* 2013). Likewise, microalgae-based CO_2 removal during biogas upgrading will result in lower transportation costs and a higher biogas energy content, as CO_2 accounts for 25–50% of the biogas on a volume basis (Sialve *et al.* 2009; Hernández *et al.* 2013). However, despite the above-mentioned advantages of photosynthetic biogas upgrading coupled with nutrient removal from diluted centrates, little information is available about the carbon (C), nitrogen (N) and phosphorus (P) removal mechanisms (biotic, abiotic and/or dissimilatory) of this process.

This work was devised to evaluate the mechanisms governing the simultaneous upgrading of biogas and diluted centrate treatment in an HRAP interconnected to an external CO_2 absorption column using a C, N and P mass balance approach. For this purpose, the C, N and P speciation in the influent and effluent streams in this two-stage experimental set-up, along with the removal efficiencies of

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organic and inorganic C, N and P, were assessed in order to elucidate the removal mechanisms in this combined wastewater treatment and CO_2 capture process.

MATERIALS AND METHODS

Experimental set-up

The indoor experimental set-up consisted of a 1.3 m^2 180 L HRAP (202 cm length × 63 cm width × 15 cm depth) interconnected to a 2.5 L external CO_2 bubble column (Ø = 4 cm; height = 195 cm) (Figure 1). The HRAP was continuously agitated using a six-blade paddle wheel, which supported a liquid recirculation velocity of 0.2 m/s at the center of the pond channel. The HRAP was illuminated using a bench of 15 Gro-Lux fluorescent lamps (Sylvania, Germany) providing a cool white fluorescent light of 75 ± 5 μE/m^2·s of photosynthetically active radiation (PAR) at the culture surface for 24 h/d. The initial operational conditions of this experimentation corresponded to the last steady state of the study conducted by Bahr *et al.* (2014), which was maintained for 2 months under steady state to obtain enough experimental data to evaluate the C, N and P mass balances. At the starting point of the experiment, the microalgae/cyanobacteria population (from now on referred to as microalgae) was composed of (percentage of cells) *Phormidium* sp. (71%), *Oocystis* (20%) and *Microspora* sp. (9%). The composition of the bacterial population was not characterized by molecular tools, but microscopic observations confirmed the presence of bacteria in the algal-bacterial consortium initially present in the HRAP. Centrate wastewater was obtained by centrifugation of the anaerobically digested mixed sludge of Valladolid wastewater treatment plant (Spain), which was eight-fold diluted with tap water due to the potential inhibition of microalgae growth by the high NH_4^+ concentrations (González *et al.* 2008). Typically, the vertical light attenuation is associated with different waterborne materials (Xu *et al.* 2005). In this context, total suspended solids (TSS) concentration represents the most important factor controlling the light attenuation coefficient (Kd) (Gallegos 2001; Devlin *et al.* 2008). In our particular case, Kd associated with TSS in diluted centrates (0.17 m^{-1}) was significantly lower than those values associated with light-limiting conditions (above 5 m^{-1}) (Devlin *et al.* 2008). Therefore, a potential light limitation in the culture broth associated with turbidity in the eight-fold diluted centrate cultivation medium was ruled out. The HRAP was continuously fed (Watson Marlow 102 UR pump) with the diluted centrates in order to maintain a hydraulic retention time (HRT) of 20 days. The average total organic carbon (TOC), inorganic carbon (IC), total nitrogen (TN) and $P\text{-}PO_4^{-3}$ concentrations in the diluted centrates during the entire experiment were 11 ± 2 g/m^3, 95 ± 3 g/m^3, 92 ± 3 g/m^3 and 8.9 ± 0.4 g/m^3, respectively. Simulated biogas (Abello Linde, Spain) containing CO_2 (30%) and N_2 (70%) instead of CH_4 due to its potential explosion hazards was supplied to the bubble column at 22 mL/min through a ceramic sparger located at the bottom of the column (HRT_{column} = 1.9 h) co-currently with a recycling microalgal broth stream drawn at 20 mL/min (1 m/h) from the HRAP (Figure 1). The HRAP cultivation broth was daily supplemented with 50 cm^3 of NaOH (20 g/dm^3) to maintain the pH at

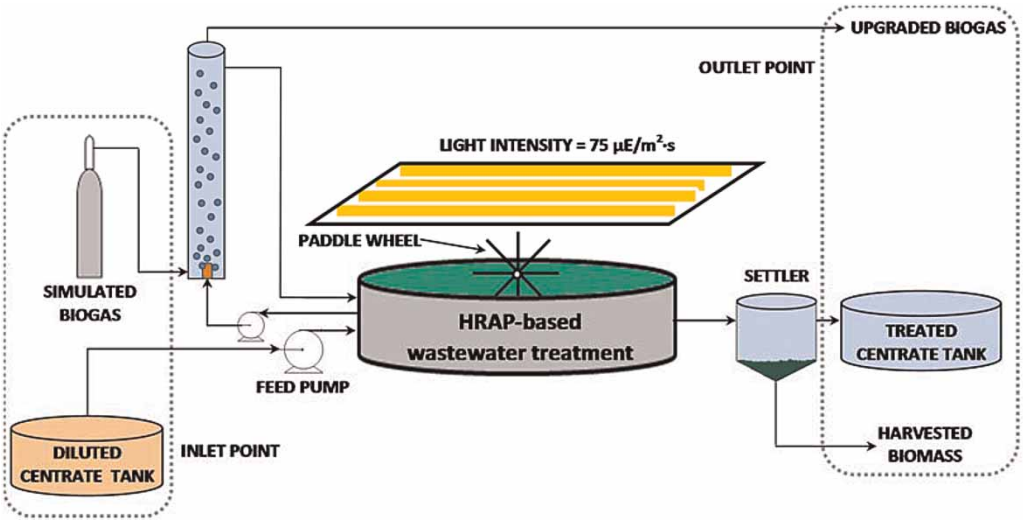

Figure 1 | Schematic of the experimental set-up devoted to the simultaneous biogas upgrading and diluted centrate treatment.

8.1 ± 0.1 in order to promote bacterial nitrification and minimize N losses by NH_3 stripping. The sedimentation of the HRAP cultivation broth was carried out in an 8 L settler located at the outlet of the HRAP (Figure 1) from which biomass harvesting was carried out once a week. The areal biomass productivity was determined according to Posadas *et al.* (2014).

Gas samples of 100 μL were periodically drawn at the inlet and outlet of the absorption bubble column to monitor the CO_2, N_2 and O_2 concentrations using a gas chromatograph coupled with a thermal conductivity detector (GC-TCD). Liquid samples were also drawn twice a week from the diluted centrate (influent) and HRAP effluent to monitor the concentration of dissolved TOC, dissolved IC, dissolved N species (TN, $N\text{-}NH_4^+$, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$ and $N_{organic}$), dissolved P ($P\text{-}PO_4^{-3}$) and biomass concentration as TSS. The temperature, dissolved oxygen (DO) concentration and pH in the HRAP were also recorded. Prior to analysis (except for TSS determination), liquid samples were centrifuged for 10 min at 10,000 rpm and 23 °C (Sorvall, Legend RT Plus centrifuge, Thermo Scientific, USA), and filtered through 0.20 μm nylon filters. The C, N and P content of the algal-bacterial biomass formed was also experimentally determined.

Mass balance calculation

A mass balance calculation was conducted for C, N and P based on the average concentrations of all their chemical species at the inlet ('IN' = diluted centrate feed + inlet biogas) and outlet ('OUT' = treated effluent + settled algal-bacterial biomass waste + upgraded biogas) of the experimental system under steady state (Figure 1). The validity of the experimentation carried out was assessed by means of recovery factors defined as follows:

$$\text{C mass recovery}\,(\%) = \frac{[\text{C-CO}_2 + \text{TOC} + \text{IC} + \text{C}_{biomass}]_{OUT}}{[\text{C-CO}_2 + \text{TOC} + \text{IC} + \text{C}_{biomass}]_{IN}} \times 100 \quad (1)$$

$$\text{N mass recovery}\,(\%) = \frac{[\text{N-NH}_4^+ + \text{N-NO}_2^- + \text{N-NO}_3^- + \text{N}_{biomass} + \text{N}_{organic}]_{OUT}}{[\text{N-NH}_4^+ + \text{N-NO}_2^- + \text{N-NO}_3^- + \text{N}_{biomass} + \text{N}_{organic}]_{IN}} \times 100 \quad (2)$$

$$\text{P mass recovery}\,(\%) = \frac{[\text{P-PO}_4^{-3} + \text{P}_{biomass}]_{OUT}}{[\text{P-PO}_4^{-3} + \text{P}_{biomass}]_{IN}} \times 100 \quad (3)$$

where $C\text{-}CO_2$ is the carbon input in the simulated biogas, TOC is the total dissolved organic carbon in the aqueous phase, IC is the dissolved inorganic carbon in the aqueous phase, $C_{biomass}$ is the particulate carbon in the form of microalgal-bacterial biomass, $N\text{-}NH_4^+$, $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ represent the dissolved ammonium, nitrite and nitrate, respectively, while $N_{biomass}$ and $N_{organic}$ account for the particulate organic nitrogen in the form of biomass and the dissolved organic nitrogen accumulated in the HRAP culture broth, respectively. $P\text{-}PO_4^{-3}$ stands for the phosphorus in the aqueous phase and $P_{biomass}$ for the particulate phosphorus in the form of biomass. All parameters were estimated as the total mass of the target compound over the 2 months of experimentation.

Analytical procedures

The impinging irradiation at the surface of the HRAP was measured as PAR using an LI-250A light meter (LI-COR Biosciences, Germany). The pressure at the bottom and top of the bubble column was measured using a PN 5007 pressure sensor (IFM, Germany). The gas concentrations of CO_2, O_2 and N_2 were determined using a CP-3800 gas chromatograph (Varian, USA) coupled with a thermal conductivity detector and equipped with a CP-Molsieve 5A (15 m × 0.53 mm × 15 μm) and a CP-Pora BOND Q (25 m × 0.53 mm × 15 μm) column. The injector, detector and oven temperatures were maintained at 150 °C, 175 °C and 40 °C, respectively. Helium was used as the carrier gas at 13.7 cm^3/min. TOC, IC and TN concentrations were determined using a TOC-V CSH analyzer equipped with a TNM-1 module (Shimadzu, Japan). $N\text{-}NH_4^+$ concentration was measured using the Nessler analytical method in a U-2000 spectrophotometer (Hitachi, Japan) at 425 nm. $N\text{-}NO_2^-$, $N\text{-}NO_2^-$ and $P\text{-}PO_4^{-3}$ were analyzed by high-performance liquid chromatography–ion chromatography according to Alcántara *et al.* (2013). The soluble P concentration was determined according to Eaton *et al.* (2005) using a U-2000 spectrophotometer (Hitachi, Japan). A Crison micropH 2002 (Crison instruments, Spain) was used for pH determination. DO concentration and temperature were recorded using an OXI 330i oximeter (WTW, Germany). The determination of the TSS concentration of microalgal-bacterial biomass was performed according to Eaton *et al.* (2005). The analysis of $C_{biomass}$ and $N_{biomass}$ was conducted using a LECO CHNS-932, while P biomass was measured using a 725-ICP optical emission spectrophotometer (Agilent, USA) at 213.62 nm. The identification, quantification and biometry measurements of microalgae were carried out by microscopic examination (Olympus

IX70, USA) of microalgal samples (fixed with lugol acid at 5% and stored at 4 °C prior to analysis) according to Sournia (1978). The relative error associated with the counting procedure was ±10% in number of cells (Lund *et al.* 1958). The concentration of the $N_{organic}$ released into the liquid phase was determined as the difference between the TN concentration and the sum of $N\text{-}NH_4^+$, $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$.

RESULTS AND DISCUSSION

Algal-bacterial symbiosis can support a cost-effective wastewater treatment in this combined process as a result of the *in situ* photosynthetic oxygenation of the culture broth (capable of oxidizing both organic matter and $N\text{-}NH_4^+$) and the high nutrient assimilation potential mediated by the high microalgae productivities (Muñoz & Guieysse 2006). In this combined system, the microalgae population uses both centrate IC and the CO_2 contained in the biogas as a C source to assimilate N and P in the form of new biomass (biotic nutrient removal) (Posadas *et al.* 2015). Moreover, $N\text{-}NH_4^+$ can be transformed into $N\text{-}NO_3^-$ by nitrifying bacteria under aerobic conditions, which entails a reduction in the total Kjeldahl nitrogen concentration in the effluent (De Godos *et al.* 2010). NH_4^+ nitrification contributes also to IC biotic removal as nitrifying bacteria are autotrophic micro-organisms. In addition, the high pH induced by microalgal photosynthesis can also enhance both N and P abiotic removal by $N\text{-}NH_4^+$ stripping as NH_3 gas or $P\text{-}PO_4^{-3}$ precipitation in the form of $Ca_5(OH)(PO_4)_3$ (De Godos *et al.* 2009; Posadas *et al.* 2015).

The C, N and P mass balances in the system under steady-state conditions showed recovery factors of 99.8%, 101.7% and 99.0%, respectively, which validated both the experimental protocol followed and the analytical and instrumental methods used in this study. The results obtained were given as the average ± the error at 95% confidence interval ($n = 21$). The specific growth rate of the microalgal-bacterial community was 0.05 d^{-1}, which resulted in an average biomass concentration in the cultivation broth of 1.4 ± 0.0 g TSS/L during the 66 days of steady-state operation. The TSS removal efficiency in the settler of 98 ± 35% entailed effluent suspended solid concentrations of 22 ± 9 mg TSS/L, which were below the maximum permissible TSS discharge limit in EU legislation (35 mg TSS/L) (European Directive 91/271/CEE). The average biomass productivity in the HRAP was 2.2 ± 0.0 g/m^2·d, which agrees with the 2.1 ± 0.6 g TSS/m^2·d obtained by Posadas *et al.* (2014) in a 180 L HRAP treating domestic wastewater under similar indoor cultivation conditions. This biomass productivity was, however, significantly lower than the 10–30 g TSS/m^2·d range typically observed in outdoor full-scale HRAPs treating domestic wastewater and could be attributed to the low impinging irradiation used in this indoor study (Oswald 1988; Tredici 2004; Park & Craggs 2010). The moderate culture broth temperature (25 ± 1°C), together with the high turbulence in the pilot HRAP, mediated evaporation losses of 4.5 ± 0.4 L/m^2·d. This high turbulence associated with pilot-scale HRAPs is often promoted by the use of a high power engine and the absence of guide vanes devoted to softening the change in direction of the cultivation broth (Mendoza *et al.* 2013; Posadas *et al.* 2014).

C speciation

The structural carbon content (on a dry weight basis) of the harvested algal-bacterial biomass remained constant at 41 ± 0%, which was slightly lower than the 43–56% carbon content typically reported for microalgal biomass (Sydney *et al.* 2010; Arbib *et al.* 2014). This carbon was fixed by the algal-bacterial biomass under fully photoautotrophic conditions (as TOC concentration in the influent wastewater only represented 1 ± 0% (11 ± 2 g TOC/m^3) of the total carbon input) and corresponded to 9 ± 2% of the total C input (Figure 2). This assimilation corresponded to a biotic C removal of 0.9 ± 0.4 g $C_{biomass}$/m^2·d, which entailed a CO_2 sequestration of 1.6 g CO_2/g of biomass formed. This poor C fixation was likely due to the low impinging irradiation (75 ± 5 μE/m^2·s) at the cultivation broth surface, which limited microalgae growth throughout the entire process.

Approximately 89 ± 1% of the total IC input (gas $C\text{-}CO_2$ + dissolved IC) was provided by bubbling through

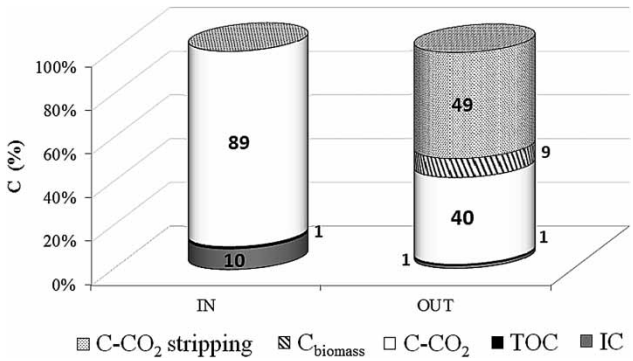

Figure 2 | Carbon distribution in the influent (IN) and effluent (OUT) streams in the experimental set-up.

the absorption column a simulated biogas containing a CO_2 concentration of 171 ± 1 g C-CO_2/m^3. The remaining 10 ± 1% was supplied as dissolved IC in the diluted centrates (95 ± 3 g IC/m^3 of wastewater) (Figure 2). The low liquid recirculation rate from the HRAP through the absorption column resulted in a low C-CO_2 absorption from the biogas into the liquid phase, which supported a C-CO_2 biogas removal of 55 ± 6%. However, additional experiments increasing this recirculation rate from 1 to 10 m/h, which entailed an increase in the liquid/gas recirculation ratio from 0.9 to 9.4, boosted CO_2 removal from 55 ± 6 to 100 ±0%. C-CO_2 stripping was the main mechanism of carbon removal in the system, representing 49 ± 5% of the C output (Figure 2) and corresponding to an abiotic C elimination of 4.7 ± 0.8 g/m^2·d. In this context, the estimated average dissolved C-CO_2 concentration at pH = 8.1 ± 0.1 in the cultivation broth during the 66 operational days was 2.4 ± 0.3 g/m^3, which was significantly higher than the aqueous CO_2 concentration in equilibrium with the atmospheric CO_2 (0.4 g/m^3) and supported the occurrence of an abiotic C removal. In this regard, De Godos *et al.* (2009) reported a 59% loss of IC by stripping in a 464 L HRAP treating 10-fold diluted swine manure. Similarly, up to 50% of the influent carbon was removed by stripping in a 180-L HRAP treating fish farm wastewater (Posadas *et al.* 2014). In our particular study, the high turbulence associated with pilot-scale HRAPs and the low light intensity at the culture broth surface (which hindered CO_2 assimilation into biomass) can explain the high abiotic carbon removal recorded. The C-CO_2 that was not lost by stripping or assimilated into biomass left the system in the outlet biogas stream (40 ± 5%) (Figure 2). In a hypothetical scenario with biogas composed of CH_4 instead of N_2 (initial composition of 30% (v/v) of CO_2 and 70% (v/v) of CH_4) the 55 ± 6% of C-CO_2 removal in the column would result in an enrichment in the biogas CH_4 content up to 87% (v/v). This increase in bio-methane content would correspond with a gain of 19% in the biogas energy content (from 25,067 to 31,040 kJ/Nm^3, assuming a heating value for CH_4 of 50 kJ/g (Alcántara *et al.* 2013)). In this context, regulations for bio-methane injection into natural gas grids of some European countries require a CH_4 content over 95% and O_2 below 0.5% due to its associated explosion hazards (Mandeno *et al.* 2005; Huguen & Le Saux 2010). However, preliminary assays in our laboratory at a liquid to biogas ratio of ≈1 have shown CH_4 removals <1% by absorption but contamination of the upgraded biogas with O_2 and N_2 of up to 3%, which suggests that, despite the fact that photosynthetic CO_2 removal during biogas upgrading can significantly reduce the transportation costs and burning efficiency per cubic metre of biogas, O_2 and N_2 content in the upgraded biogas currently entails a technical limitation to the full-scale implementation of this biotechnology.

N and P speciation

The biomass harvested in the settler presented a structural nitrogen content of 6.6 ± 0.1%, which remained constant during the entire experiment as previously observed with the structural carbon, and entailed a biotic N removal of 0.15 ± 0.1 g N/m^2·d. This N content was in accordance to the typical content reported for microalgae, which ranges from 6.6 to 9.3% (Oswald 1988; Grobelaar 2004). Nitrification was the main NH_4^+ removal mechanism and was supported by the high DO concentration (7.0 ± 0.1 g/m^3) and constant IC supply to the cultivation over the 2 months experimentation. In this context, the inlet N-NH_4^+ (which represented 100 ± 0% of the N input, Figure 3(a)) was totally transformed (99 ± 19%) into N-NO_3^-, $N_{biomass}$ and $N_{organic}$ at average shares of 47 ± 2%, 14 ± 2% and 38 ± 2%, respectively (Figure 3(a)). In our particular study, the limited microalgal photosynthetic nitrogen fixation likely boosted the high nitrifying activity herein observed, which itself prevented ammonia stripping. The 38 ± 2% of

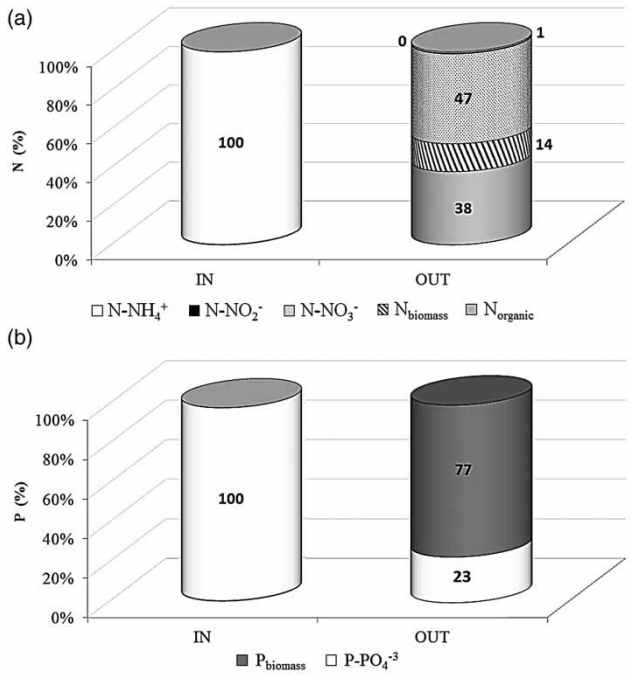

Figure 3 | Nitrogen and phosphorus distribution in the influent (IN) and effluent (OUT) streams in the experimental set-up.

$N_{organic}$ present in the N output (Figure 3(a)) was the result of $N_{organic}$ accumulation in the culture broth during the 66 days of steady-state operation from 13 to 40 g/m^3, likely associated with microalgal-bacterial metabolite excretion during their photoautotrophic growth and cell lysis.

The phosphorus content in the harvested biomass accounted for 2.5 ± 0.1%, which agrees with P range (0.2–3.9) reported by Powell *et al.* (2009). The large variability in P content often reported in microalgae is due to the potential occurrence of a luxury P uptake in some microalgae species, where P is accumulated over structural requirements in order to store energy in the form of polyphosphate (Powell *et al.* 2008, 2009). Thus, the high $P_{biomass}$ herein recorded (2.5 ± 0.1%) was likely the result of the combination of a luxury uptake and a growth-associated P uptake (Powell *et al.* 2008, 2009) since a typical P content of 1% is observed when the uptake of P-PO_4^{-3} occurs preferentially for microalgal biomass formation (Alcántara *et al.* 2013). A possible explanation for this is that the high growth rate induced by the exposure to a high light intensity results in this form of polyphosphate being utilized by the cells for synthesis of cellular constituents at a rate that exceeds replenishment (Powell *et al.* 2008). In this regard, Hessen *et al.* (2002) reported a three-fold increase in the biomass polyphosphate content when decreasing the light intensity from 70 to 10 μE/m^2·s, while an eight-fold increase was observed by Powell *et al.* (2008) when irradiation was decreased from 150 to 60 μE/m^2·s. Therefore, the low light intensity in our particular case likely restricted microalgal P assimilation to form new biomass and therefore promoted P-PO_4^{-3} accumulation over structural requirements. Despite the limited microalgae growth recorded, the removal of dissolved P-PO_4^{-3} by assimilation accounted for 77 ± 2% (0.06 ± 0.0 g P/m^2·d), while the P-PO_4^{-3} that was not incorporated into biomass left the system in the effluent (23 ± 2%) (Figure 3(b)). In addition, algal populations' gross changes seem to suggest that *Microspora* sp. could have replaced *Phormidium* sp. along the process but enough experimental evidence is lacking to fully support this observed change.

Energy and environmental considerations

Despite the low biomass productivity obtained in our particular study as a result of the technical difficulties to supply high irradiances in indoors pilot-scale systems, the upgrading of biogas mediated by CO_2 capture combined with wastewater treatment harbors a valuable potential from an environmental and energy viewpoint. Thus, a conservative biomass productivity of 20 g/m^2·d in full-scale HRAPs would result in a potential bio-methane production of 4.2 g CH_4/m^2·d (assuming 50.6% of structural C and a CH_4 yield of 0.21 g CH_4/g microalgae (Alcántara *et al.* 2013)). The combustion of this bio-methane would entail an energy production of 88 kJ/m^2_{HRAP}·d (≈1 W/m^2) (assuming a CH_4 to electricity conversion efficiency of 41.7% (Lucas 2000)). In this context, Chisti (2013) reported a value of 34 kJ/m^2_{HRAP}·d (0.4 W/m^2) as the minimum power requirement for mixing a 360 m^3 outdoor HRAP (300 m of total loop length × 4 m wide × 0.3 m deep) at a paddle wheel efficiency of 0.17 using a Manning coefficient of 0.012 (polymer-membrane lined smooth raceway channel) with 0.3 m/s of liquid velocity. The energy for mixing the cultivation broth represents the main power consumption during the operation of this combined HRAP–absorption column system, where the energy for external liquid recirculation between both units and for biogas sparging in the absorption column are negligible compared to the energy consumption associated with the mixing the culture broth. This energy represents 40% of the energy obtained from CH_4 combustion, which suggests that the photosynthetic CO_2 removal from biogas (which also reduces the transportation costs of biogas) and further combustion of the bio-methane obtained through anaerobic digestion of the biomass generated in the upgrading process would significantly improve the global energy balance of outdoor HRAPs during this combined process of wastewater treatment and CO_2 capture.

Moreover, based on the nitrogen mass balance, this system released to aquatic ecosystems (purge + clarified) 0.8 g N/g N_{input}, which, compared with the typical gas N-N_2O emission factor in HRAPs of 0.00005 g N-N_2O/g N_{input} (Alcántara *et al.* 2015), clearly represented the main contribution regarding total nitrogen emissions from this system. Considering a N_2O emission factor of 0.00005 g N-N_2O/g N_{input} and a global warming potential of 298 g CO_2/g N_2O, this combined system would produce 7.2·10^{-3} g N_2O/m^3 of wastewater treated (WW treated), equivalent to 2.2 g CO_2/m^3 WW treated. Conversely, the amount of CO_2 fixed by photosynthetic microalgae growth accounts for 521 g CO_2/m^3 WW treated, which confirmed the environmental sustainability of this process in terms of greenhouse gas emission mitigation. Despite a direct comparison with N_2O emissions from activated sludge processes being difficult given the large variation of the rates reported (Ahn *et al.* 2010), wastewater treatment plants with primary and activated sludge treatment present average N_2O emissions of 33·10^{-3} g N_2O/m^3 WW treated (Czepiel *et al.* 1995). In addition, the carbon footprint associated with N_2O emissions in this technology (2.2 g CO_2/m^3)

compared favorably against the indirect carbon footprint from electricity use for aeration and mixing of activated sludge tanks (119–378 g CO_2/m^3) and for mixing HRAPs (3–14 g CO_2/m^3 WW treated). This preliminary analysis suggests that N_2O generation by the microalgal-bacterial biomass present in the HRAP should not challenge the environmental performance of wastewater treatment in HRAPs in terms of global warming mitigation.

CONCLUSIONS

The C, N and P mass balances used to evaluate the removal mechanisms in this combined biogas upgrading and wastewater treatment process showed recovery factors close to 100%, which validated both analytical and instrumental methods used in this study. $C_{biomass}$ only accounted for 9% of the C input to the system, which explains the low biomass productivity recorded in the HRAP. The low light intensity used in this experimentation together with the high turbulence associated with pilot-scale HRAPs supported C-CO_2 stripping as the main mechanism of C removal in the system (≈ 49%). The C-CO_2 removal from biogas in the column (≈ 55%) entailed an increase of 19% in the biogas energy content, which highlighted the potential of this combined wastewater treatment and biogas upgrading process. Nitrification was the main NH_4^+ removal mechanism with $47 \pm 2\%$ of the N-NH_4^+ input transformed into N-NO_3^-, while only $14 \pm 2\%$ of the nitrogen input was converted to $N_{biomass}$. A luxury uptake of P was hypothesized based on the high P biomass content ($2.5 \pm 0.1\%$) and the fact that light limitation often promotes polyphosphate accumulation. P-PO_4^{-3} assimilation into biomass accounted for $77 \pm 2\%$ of the phosphate removed in the process. Finally, a successful suspended solid removal was achieved in the settler (≈ 98%), which entailed effluent suspended solid concentrations below the maximum permissible EU discharge limit.

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