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Assessing nitrous oxide emissions from algal-bacterial photobioreactors devoted to biogas upgrading and digestate treatment

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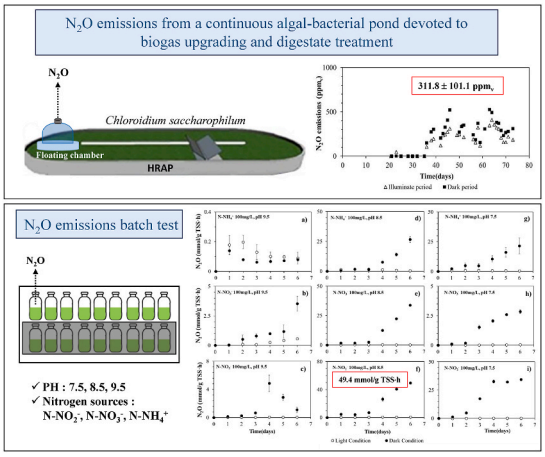
HIGHLIGHTS

- N_2O emission was assessed in an algal pond dominated by *Chloroidium saccharophilum*.
- 312 ± 101 ppm_v of N_2O gas were recorded in the dark period in a HRAP.
- The HRAP achieved complete removal of H_2S and CO_2 removals of 30–68%.
- N_2O emission peaked at 49.4 mmol g^{-1} TSS·h under pH 8.5 and 100 mg $N–NO_2^-$/L in dark.

GRAPHICAL ABSTRACT



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ABSTRACT

Nitrous oxide (N_2O) emissions in High Rate Algal Ponds (HRAP) can negatively affect the sustainability of algal-bacterial processes. N_2O emissions from a pilot HRAP devoted to biogas upgrading and digestate treatment were herein monitored for 73 days. The influence of the pH (7.5, 8.5, and 9.5), nitrogen sources (100 mg L^{-1} of $N–NO_2^-$, $N–NO_3^-$, and $N–NH_4^+$) and illumination on N_2O emissions from the algal-bacterial biomass of the HRAP was also assessed in batch tests. Significantly higher N_2O gas concentrations of 311.8 ± 101.1 ppm_v were recorded in the dark compared to the illuminated period (236.9 ± 82.6 ppm_v) in the HRAP. The batch tests revealed that the highest N_2O emission rates (49.4 mmol g^{-1} TSS·h^{-1}) occurred at pH 8.5 in the presence of 100 mg $N–NO_2^-$/L under dark conditions. This study revealed significant N_2O emissions in HRAPs during darkness.

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List of abbreviations

AOB	Ammonia-oxidizing bacteria
CAPEX	Capital expenditure
DO	Dissolved oxygen
HRAPs	High-rate algal ponds
IC	Inorganic carbon
LED	Light-emitting diodes
L/G ratio	Liquid/gas ratio
NOB	Nitrite-oxidizing bacteria
OPEX	Operational expenditure
PAR	Photosynthetic active radiation
PVC	Polyvinyl Chloride
PCBs	Printed circuit boards
TN	Total nitrogen
TOC	Total organic carbon
TSS	Total suspended solids
VSS	Volatile suspended solids

1. Introduction

Today, there is a worldwide concern about the emissions of N_2O from agriculture, industry, wastewater treatment and transportation. The Intergovernmental Panel on Climate Change claims that N_2O is responsible for approximately 6% of the total global warming caused by greenhouse gas emissions, and its atmospheric concentration has increased by 20% since pre-industrial times (IPCC, 2019). The European Union (EU) has committed to gradually reduce N_2O emissions through its policy and legislation, including the National Emission Ceilings Directive and the Effort Sharing Regulation. These policies aim at setting limits on emissions from individual EU countries and encourage the use of cleaner technologies and practices to reduce N_2O emissions (Brémond et al., 2021; UNEP, 2021).

Wastewater treatment represents a major contributor to N_2O emissions due to the inherent biotransformation of nitrogen species during wastewater purification. In this context, the most typical photobioreactor design used for wastewater treatment is the HRAP based on their low capital expenditure (CAPEX) and operational expenditure (OPEX) as well as simple operation, but they can be a source of nitrous oxide (N_2O) emissions due to their open nature and the potential of algal metabolism to release N_2O (Gao et al., 2021). HRAPs are a type of algal pond typically used for domestic wastewater treatment and designed to maximize algal productivity and growth rates, thus reducing the hydraulic retention time and supporting higher nutrient loading rates (Park et al., 2011). HRAPs were originally proposed in the 1960s to treat domestic wastewater at low operating cost based on the symbiosis between algae and bacteria powered by solar energy. Algae utilize wastewater nutrients and carbon dioxide to grow and produce oxygen and organic metabolites through photosynthesis. Thus, algal metabolism supports the aerobic bacteria and protozoa growth, which results in the ultimate removal of organic pollutants and nutrients from the water (Chan et al., 2022; Jiang et al., 2021).

In the past ten years, HRAPs have become a common and successful technology platform for the treatment of wastewater and the generation of algal biomass (Craggs et al., 2015; de Godos et al., 2010; Sutherland et al., 2021). HRAPs of 1–2 ha have been constructed with the framework of European projects SABANA and ALLGAS for the treatment of wastewaters. N_2O emissions from HRAPs are often lower than those from activated sludge treatment in wastewater treatment plants (Aboobakar et al., 2013; Maktabifard et al., 2022). Indeed, N_2O emissions from activated sludge tanks are typically higher due to the presence of N_2O-producing bacteria in the sludge, higher organic loading, continuous aeration, and the occurrence of incomplete denitrification (Ali et al., 2014; Maktabifard et al., 2022; H. Wang et al., 2016). Conversely, the ability of algae to assimilate nitrogen lowers the extent of N_2O production during denitrification, which represents an advantage of HRAPs. However, nitrogen assimilation by microalgae does not necessarily prevent the potential total emissions of N_2O due to the inherent ability of microalgae to synthesize N_2O (Bellido-Pedraza et al., 2022; Burlacot et al., 2019; Plouviez and Guieysse, 2020). The mass of N_2O emitted from an algal pond can vary depending on factors such as temperature, pH, nutrient concentrations and algal biomass concentration (Alcántara et al., 2015a).

The use of HRAPs has now been expanded to biogas upgrading in combination with digestate treatment, which represents a cutting-edge method of wastewater treatment and resource recovery (Montemezzani et al., 2015; Posadas et al., 2017; Vargas-Estrada et al., 2023a). The CO_2 and H_2S present in the biogas are transferred to the algal-bacterial broth in an external absorption column interconnected to the HRAP(Rodero et al., 2019). While CO_2 is photosynthetically fixed by microalgae in the pond, H_2S is oxidized by bacteria in the absorption column, and both metabolic processes can be supported by the nitrogen and phosphorous present in the digestate (Sepúlveda-Muñoz et al., 2023).

The number of studies devoted to evaluating N_2O emissions from HRAPs simultaneously treating biogas and digestate is low, despite the relevance of N_2O emissions during microalgal-based wastewater treatment. This study assessed for the first time N_2O emissions during a continuous algal-bacterial process devoted to biogas upgrading to biomethane and digestate treatment at pilot level under indoor conditions. In addition, the influence of the nitrogen source, pH and illumination on N_2O emissions from the algal-bacterial biomass of the HRAP was evaluated in separate batch assays.

2. Materials and methods

2.1. Photosynthetic biogas upgrading set-up

At the University of Valladolid (Spain), the indoor prototype photobioreactor was operated at the Institute of Sustainable Processes. The system's main component was a 180-L HRAP with a 1.2 m^2 illuminated area that was coupled to an 8-L conical settler and sequentially to a 2.5-L biogas bubble absorption column (Fig. 1). Using a six-blade paddle wheel, the open algal pond experienced agitation at 20 cm s^{-1}. It was exposed to light for 16 h a day at a photosynthetic active radiation (PAR) of 1368.8 ± 88.6 μmol m^{-2} s^{-1}, provided by six LED PCBs from Phillips SA, Spain. The absorption column was interconnected to the HRAP via recirculation of supernatant of the settler at a liquid to biogas flowrate ratio of 1. The absorption column was operated with a 2 μm stainless steel biogas diffuser located at the bottom of the column, which was operated co-currently.

A floating chamber was designed and installed by the research group to assess the emissions of N_2O from the HRAP. The floating structure was made of expanded polystyrene foam (22 cm width × 16.2 cm long) and equipped with a central cylindrical PVC chamber (6.1 cm internal diameter × 7 cm tall) with a gas sampling septum on the top (Fig. 1). A gas tight syringe of 100 μL was used to inject the gas sample in a GC without sample loop. The corresponding calibrations were conducted using the gas tight syringe. Considering the open nature of the HRAP and the absence of an active gas flow from the culture broth surface, the N_2O emissions from HRAP were reported in concentration units of parts per million by volume (ppm_v) in the headspace in equilibrium with the liquid phase.

2.2. Operation of the photosynthetic biogas upgrading system

The dominant microalgal species in the HRAP at the beginning of the experiment was *Chloroidium saccharophilum*. It was the most dominant photosynthetic microorganism in the HRAP, with an average level of

3.25×10^9 ind/L during the experiment. 5 L d^{-1} of digestate/centrate fed into the system daily, kindly provided by the Valladolid wastewater treatment plant in Spain. The average composition of the centrate was: 579 ± 34 mg L^{-1} of inorganic carbon (IC), 686 ± 24 mg L^{-1} of total nitrogen (TN) and 141 ± 31 mg L^{-1} of total organic carbon (TOC). $S\text{–}SO_4^{2-}$ concentration in centrate was negligible. Each day settled biomass was eliminated from the bottom of the settler to ensure consistent biomass productivity of 22.5 g m^{-2} d^{-1}, which resulted in consistent microalgae growth as Méndez et al. (2022) described. Following a zero-effluent technique, the removed settled biomass was centrifuged (5800 rpm for 10 min) and the supernatant was then added back to the algal open pond. Tap water was consistently fed to the algal open pond to balance out water losses caused by evaporation from the HRAP surface.

Daily monitoring was conducted for dissolved oxygen (DO) in HRAP, pH in HRAP, centrate and the absorption column, and temperature in ambient and HRAP. Twice a week, from the HRAP culture and centrate, 150 mL was drawn to determine the concentration of TN, TOC, IC, $N\text{–}NH_4^+$, $N\text{–}NO_2^-$, $N\text{–}NO_3^-$, $S\text{–}SO_4^{2-}$, $P\text{-}PO_4^{3-}$ and total suspended solids (TSS) and volatile suspended solids (VSS) from the HRAP and settler. Microalgae population structure was determined once a month by collecting samples from the HRAP and preserving them with formaldehyde (10%) and Lugol iodine solution (5%). In addition, gas was taken twice a week from the inlet and outlet of the absorption column to measure the CO_2, CH_4, N_2, O_2, and H_2S composition of the raw synthetic biogas and biomethane. Synthetic biogas was composed of 0.5% H_2S, 29.5% CO_2 and 70% CH_4 (Abello, Linde; Spain)

2.3. Influence of pH, nitrogen source and illumination on N_2O emissions

Batch tests were carried out to evaluate the influence of the cultivation broth pH (7.5, 8.5, and 9.5) and N-source ($N\text{–}NO_2^-$, $N\text{–}NO_3^-$, and $N\text{–}NH_4^+$) on N_2O emissions under light and dark conditions in duplicate. The selected nitrogen concentrations were based on typical concentrations found in domestic wastewaters, which guarantee optimal growth conditions for microalgae. Domestic wastewater typically contains total nitrogen concentrations ranging from 20 to 100 mg L^{-1} (Robertson et al., 2012). 50 mL of algal bacterial broth in HRAP was centrifuged at 5800 rpm for 4 min and resuspended with a mineral salt medium containing 0.94 g K_2HPO_4, 0.02 g $CaCl_2{\cdot}2H_2O$, 0.005 g $FeSO_2{\cdot}7H_2O$, 0.10 g $MgSO_4{\cdot}7H_2O$ and 5 mL of a micronutrient solution (composed of 0.10 g $ZnSO_4{\cdot}7H_2O$, 0.10 g $MnCl_2{\cdot}4H_2O$, 0.20 g H_3BO_3, 0.02 g $Co(NO_3)_2{\cdot}6H_2O$, 0.02 g $Na_2MoO_4{\cdot}2H_2O$, 0.0005 g $CuSO_4{\cdot}5H_2O$, 0.70 g $FeSO_4{\cdot}7H_2O$ and 1.02 g EDTA·2Na·$2H_2O$ per liter of distilled water) (Marín et al., 2020). Then the resuspension was poured into the 120-mL serum bottles. The pH was adjusted with $NaHCO_3$ and Na_2CO_3 salts. The total concentration of the inorganic carbon contained in the mineral salt medium (from $NaHCO_3$ and Na_2CO_3) was fixed at 500 mg L^{-1}, mimicking total carbonate concentration in centrate. Three nitrogen sources were tested at 100 mg L^{-1} of N concentration, which corresponded to 0.493g $NaNO_2$ L^{-1} ($N\text{–}NO_2^-$), 0.607g $NaNO_3$ L^{-1} ($N\text{–}NO_3^-$) and 0.382g NH_4Cl L^{-1} ($N\text{–}NH_4^+$). The initial biomass concentration was 1.2 ± 0.1 g VSS L^{-1} of all conditions, in order to guarantee limited variations during the assay. Aluminium caps and butyl septa were used to instantly seal the serum bottles, then incubated at 25 ± 0.8 °C under constant agitation at 200 rpm in the dark or under continuous illumination (900 μmol $m^{-2} \bullet s^{-1}$ of PAR) for 6 days.

Gas Chromatography with Electron Capture Detection (GC-ECD) was used to monitor N_2O headspace concentrations by taking 100 μL of gas samples every day. A gas tight syringe of 100 μL was used to inject the gas sample in a GC without sample loop. The corresponding calibrations were conducted using the gas tight syringe. At the start and end of the tests, the pH of the culturing broth was assessed. Equations (1) and (2) were used to determine the concentration of dissolved N_2O (C_L) and its total mass in the serum bottle, respectively:

$$C_L = H \bullet C_G \quad \text{(Eq. 1)}$$

$$M_a = C_G \bullet V_G + C_L \bullet V_L \quad \text{(Eq. 2)}$$

where, C_G is the N_2O headspace concentration (mg L^{-1}), H represents the dimensionless Henry's Law constant for N_2O under conditions of 25 °C and 1 atm, with a value of 2. Equation (1) is based on the Henry's Law, which states that the concentration of a gas in a liquid is proportional to its partial pressure in the gas phase at a constant temperature. M_a represents the total N_2O mass in the liquid phase and headspace. V_G is the volume of the headspace (70 mL) and V_L is the liquid phase volume (50 mL). N_2O emissions in batch tests were calculated as the mass of N_2O produced per unit of time divided by the mass of biomass in the bottles.

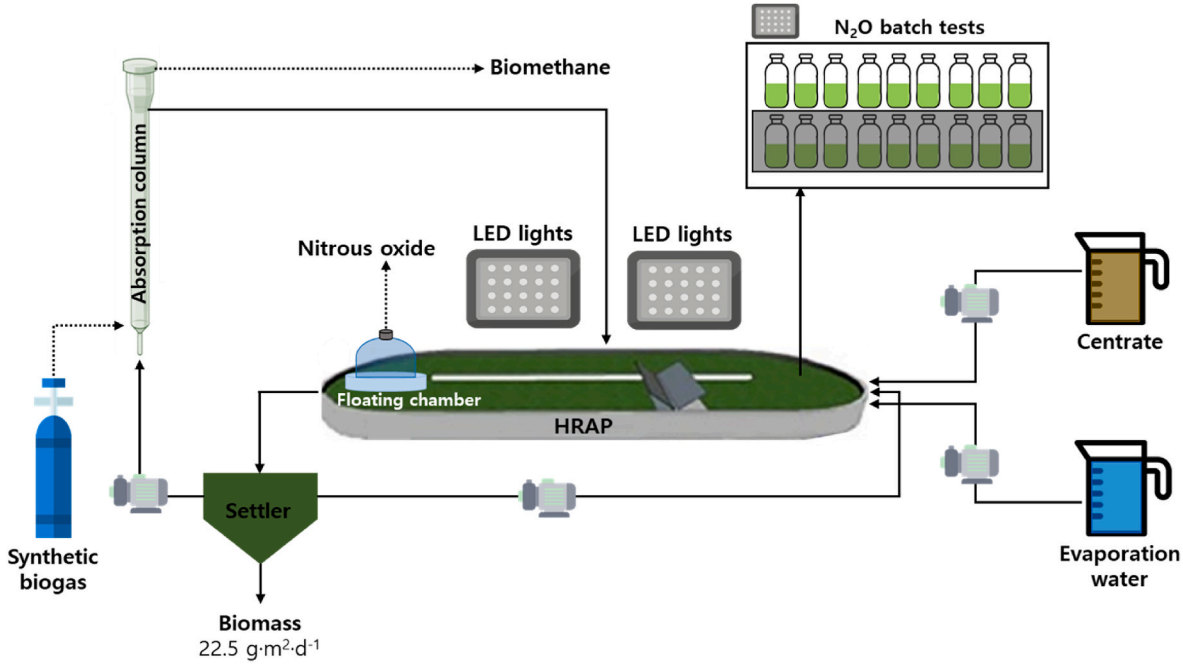

Fig. 1. Schematic of the experimental set-up.

Abiotic N_2O generation was not taken into consideration in the experimental design of the influence of pH, nitrogen source and illumination on N_2O emissions due to their low kinetics compared to biological mechanisms (Kampschreur et al., 2009).

2.4. Analytical procedures

An OXI 3310 oximeter (WTW, Germany) was used to measure the temperature and DO of the culture broth. The pH was assessed with a SensION™ + PH3 pH meter (HACH, Spain). In a Shimadzu TOC-VCSH

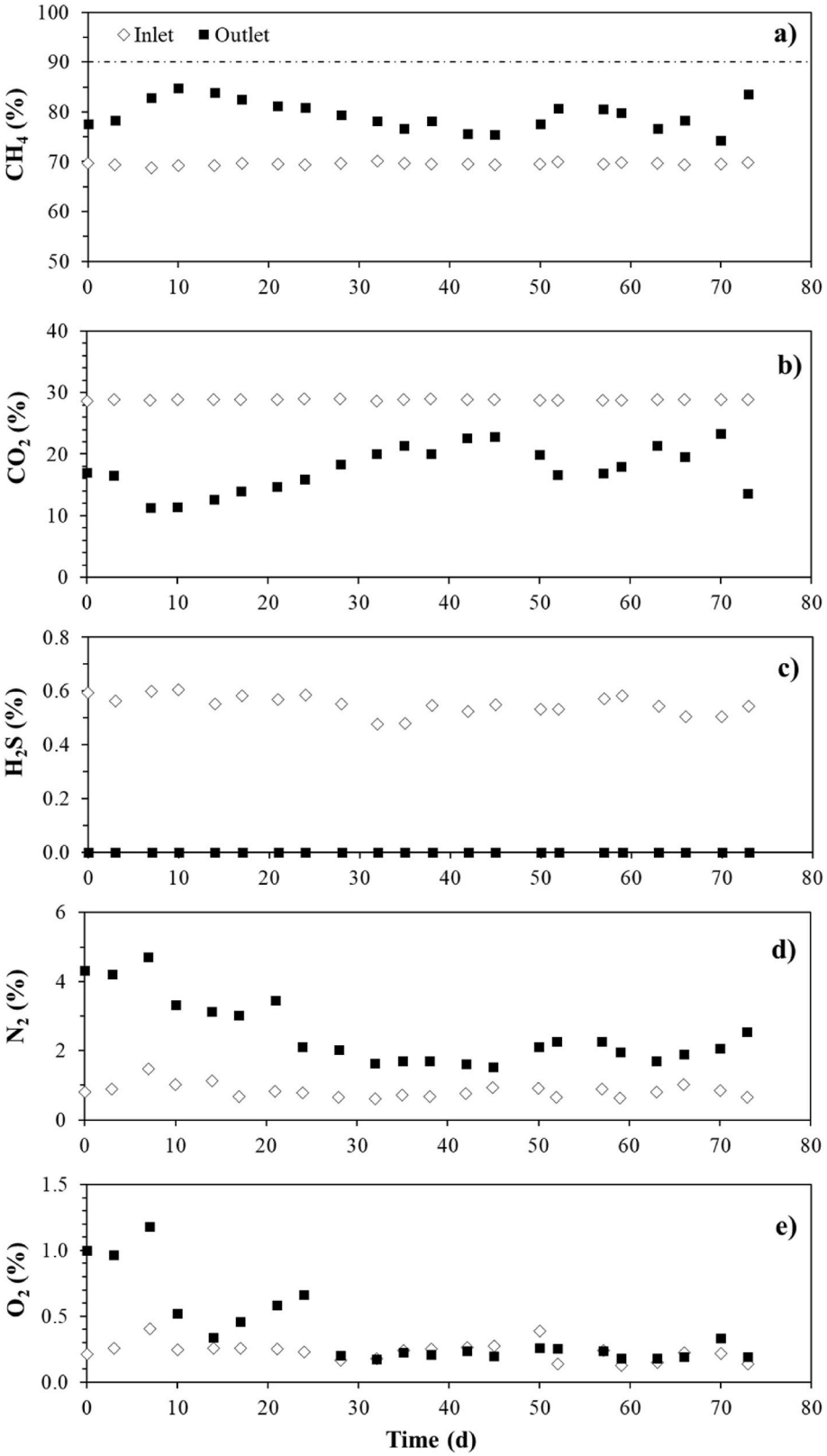

Fig. 2. Time course of the concentration of (a) CH_4, (b) CO_2, (c) O_2, (d) H_2S and (e) N_2 in the raw synthetic biogas (diamonds) and biomethane (squares).

analyzer (Japan) fitted with a Total Nitrogen chemiluminescence module TNM-1, the IC, TN, and TOC concentrations were measured.

Using the standard methods (APHA-AWWA-WPCF, 1999; Vargas-Estrada et al., 2023b), the concentrations of TSS and VSS were determined. A Li-250 A light meter (Li-COR Biosciences, Germany) was used to measure PAR. Using a UV-2550 spectrophotometer (Shimadzu, Japan), the Nessler method was used to measure the N–NH_4^+ concentration at 425 nm. By using HPLC-IC (Waters 432, conductivity detector, USA), the concentrations of S–SO_4^{2-}, N–NO_2^-, N–NO_3^-, and P-PO_4^{3-} in the centrate and HRAP cultivation broth were assessed. According to (Posadas et al., 2015), using a gas chromatograph with thermal conductivity detection (Bruker, USA), the levels of CO_2, CH_4, N_2, O_2 and H_2S in the biogas and biomethane were measured. A Bruker Scion 436 gas chromatograph (Palo Alto, USA) was used to determine the concentration of N_2O gas. This chromatograph was equipped with an electron capture detector and a HS-Q packed column (1 m × 2 mm ID × 3.18 mm OD). Temperatures were set at 100, 300, and 40 °C for the injector, detector, and oven. Nitrogen was flowed as a carrier gas at 20 mL min^{-1}(Frutos et al., 2015). Microalgae population structure was identified and quantified as described elsewhere (Méndez et al., 2022).

3. Results and discussion

3.1. HRAP performance during biogas upgrading and centrate treatment

The ambient and HRAP temperature averaged 24.6 ± 1.0 and 24.4 ± 1.4 °C, respectively. The temporal evolution of the different parameters during HRAP operation is shown in Figs. 2 and 3. Microalgae such as *Chloroidium saccharophilum* (dominant species, throughout the experiment) is known to play a key role in algal ponds devoted to biogas upgrading, since they can assimilate CO_2 in high strength media and maintain the high pH required to facilitate an effective CO_2 gas-liquid mass transfer during biogas scrubbing in the absorption column (Posadas et al., 2015). The pH in the HRAP initially was 8.0 and increased to 9.4 during the first week (Fig. 3a). After that, it gradually decreased until stabilizing at 8.0 ± 0.1 during the last ten days of the experiment. The pH in the centrate (supplemented with 3.6 g d^{-1} of sodium carbonate) remained constant at 8.3 ± 0.1 throughout the experiment. In Fig. 3a, the transfer of CO_2 and H_2S to the recirculating culture broth resulted in a more acidic medium, resulting in a lower pH at the outlet of the absorption column than in the algal pond as reported by Franco-Morgado et al. (2018); Méndez et al. (2022). The CH_4 and CO_2 composition in the biomethane during the experiment ranged between 74.2-84.8% and 11.3–23.4% (CO_2 removal efficiency of 30–68%) (Fig. 2a–b). The poor upgrading performance was likely due to the low liquid/gas (L/G) ratio in the absorption column of 1.0 and the low buffer capacity of the culture broth (IC = 349.1 ± 85.9 mg L^{-1}) (Rodero et al., 2018). If the L/G ratio in the absorption column increases, the biogas upgrading performance would be improved (Marín et al., 2019). A higher L/G ratio would provide a greater volume of liquid for CO_2 absorption, increasing the contact between the gas phase and the liquid phase, and consequently increasing the efficiency of CO_2 removal. However, a higher L/G ratio would entail to a greater O_2 and N_2 stripping, which would increase the proportion of these gases in the biomethane, thus decreasing its quality.

Complete removal of H_2S was achieved throughout the entire study due to the high solubility of H_2S in water (Fig. 2c). The DO in the HRAP cultivation broth averaged 9.3 ± 3.5 mg O_2 L^{-1} during the entire experiment, even in the dark period without photosynthesis. The high DO concentration present in the HRAP cultivation broth supported the complete biological oxidation of H_2S to sulfate by sulfur-oxidizing bacteria (González-Sánchez and Revah, 2007).S–SO_4^{2-} concentration in HRAP increased from 166 to 279 mg L^{-1} in the HRAP, though S–SO_4^{2-} concentration in centrate was negligible, as a consequence of the continuous oxidation of H_2S to SO_4^{2-} and the accumulation of the latter due to the zero effluent strategy herein implemented (Meier et al., 2018).

The N_2 concentration in the biomethane averaged 2.1 ± 0.5 % (Fig. 2d). The nitrogen content in the biomethane was mediated by the desorption of the dissolved nitrogen present in the recirculating liquid, which itself was continuously absorbed in the HRAP from the atmosphere. Therefore, this N_2 stripping was linearly dependent on the operational L/G ratio in the biogas absorption column. On the other hand, the O_2 content in the upgraded biogas averaged 0.3 ± 0.1% (Fig. 2e), which was the result of O_2 stripping from the recirculating broth and mitigated by the continuous consumption by sulfur-oxidizing bacteria to oxidize the dissolved H_2S to sulfate in the absorption column (Hou et al., 2018).

The TOC concentration in the HRAP increased from 142 to 314 mg L^{-1} during the 73 days. The TOC over the concentration in centrate would be attributed to CO_2 sequestration. One significant contributor to this increase is likely the process of CO_2 sequestration by microalgae during photosynthesis. Microalgae absorb CO_2 from the surrounding environment as a carbon source for growth and biomass production. As the microalgae grow, they incorporate carbon into their cellular structures, contributing to the overall increase in TOC concentration in the HRAP (Eloka-Eboka and Inambao, 2017; H. Li et al., 2023a). Additionally, organic carbon compounds present in the influent centrate may also contribute to the TOC concentration in the HRAP. The centrate, which serves as a nutrient-rich medium for microalgae cultivation, contains organic compounds that can be utilized by microorganisms within the HRAP. These organic compounds may undergo biological degradation and transformation processes, leading to the release of carbonaceous substances into the HRAP, further contributing to the observed increase in TOC concentration. Overall, the rise in TOC concentration reflects the dynamic interplay between carbon assimilation by microalgae, organic carbon inputs from the centrate, and microbial processes within the HRAP.

The TN concentration in the open algal pond gradually increased from 757 to 1161 mg L^{-1} throughout the experiment (Fig. 3b). *Chloroidium saccharophilum* grew and photosynthesized, thus assimilating the nitrogen present in the centrate into their biomass (Piasecka and Baier, 2022). This increase in TN concentration was due to an excess of ammonium (NH_4^+) in the centrate (compared to the nitrogen demand for microalgae growth)(Salbitani and Carfagna, 2021), which was nitrified as observed by the gradual increase in the nitrate (NO_3^-) concentration in the HRAP from 512 to 1130 mg L^{-1} (Fig. 3d).

P-PO_4^{3-} concentration in the centrate averaged 117 ± 17 mg L^{-1}, and increased from 32 to 114 mg L^{-1} in the cultivation broth of the HRAP. In this regard, microalgae growth entails lower needs of P-PO_4^{3-} than of nitrogen based on the lower P content in the biomass (0.5–2 %). In addition, Praveen et al. (2018) found that high concentrations of N–NO_3^- reduce the phosphorus requirements of algae. Under abundant nitrate conditions, algae prioritize nitrate uptake. This could occur because nitrate is typically less energetically favourable nitrogen source for algae compared to ammonium or other nitrogen compounds (Carvalho et al., 2007; Erratt et al., 2018; Zhou et al., 2010). Thus, the higher energy requirement of nitrate assimilation does not cause a direct decrease in phosphorus assimilation, and when nitrate is abundant, algae direct their energy resources toward assimilating the more energetically demanding form of nitrate, which in turn influences the demand for phosphorus assimilation indirectly.

Ultimately, the algal open pond had a consistent biomass concentration of 1.4 ± 0.1 g VSS L^{-1} from day 15 until the end of the experiment. This biomass was mainly composed by microalgae and in a lesser extent by nitrifying and heterotrophic bacteria. The results of the HRAP operation were in agreement with other experiences reported in literature (Plouviez et al., 2019; Rodero et al., 2018) and showed the typical behavior and capabilities of HRAP systems during photosynthetic biogas upgrading.

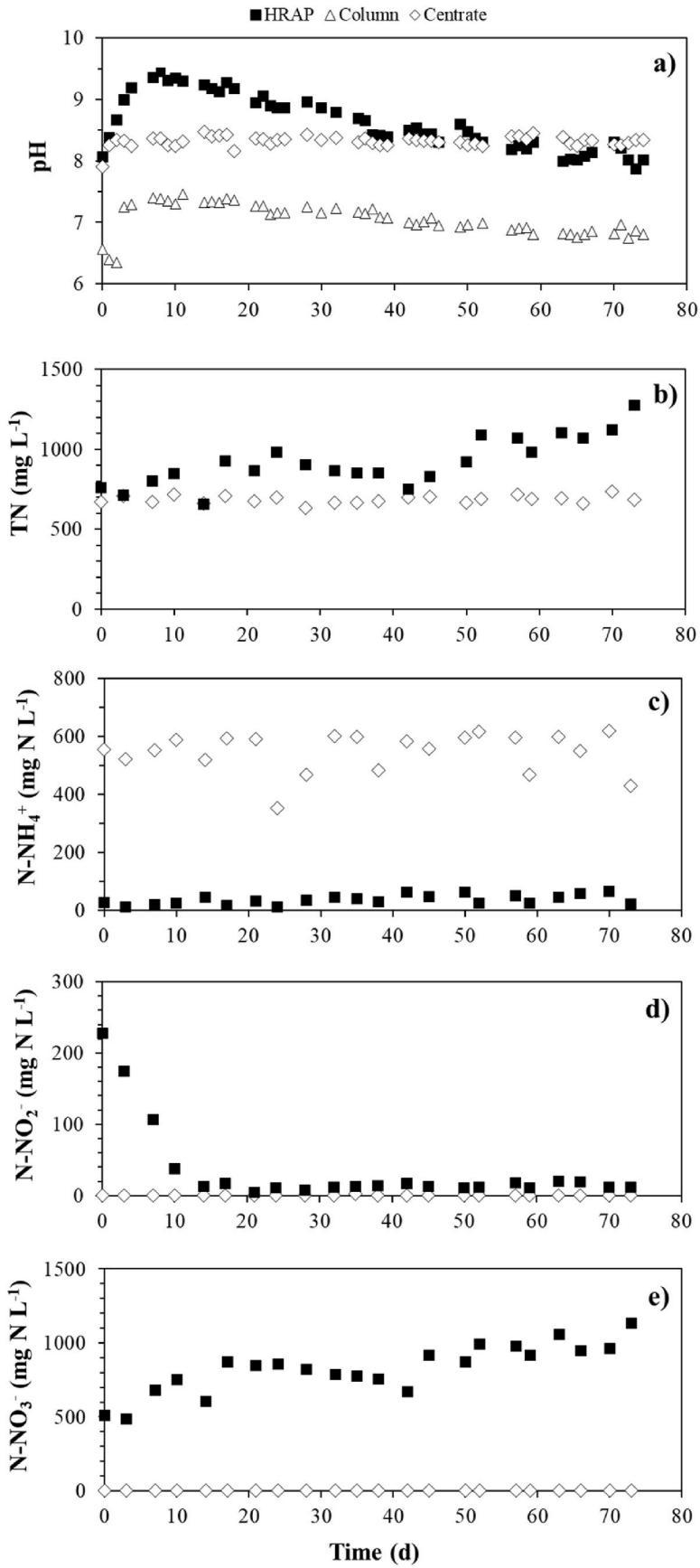

Fig. 3. Time course of (a) pH in the HRAP, centrate and in the recirculation both at the outlet of the biogas absorption column, and concentrations of (b) TN, (c) $N–NH_4^+$, (d) $N–NO_2^-$ and (e) $N–NO_3^-$ in the HRAP cultivation broth (squares) and centrate (diamonds).

3.2. N_2O emissions during biogas upgrading and digestate treatment in a HRAP

N_2O emissions were monitored during the illuminated and dark periods of the HRAP from day 21. N_2O emissions reached a steady state by day 36 (Fig. 4). N_2O emissions were significantly higher in the dark period than in the illuminated period (Fig. 4). The average steady state N_2O emissions during the dark and illuminated period averaged 311.8 ± 101.1 ppm_v and 236.9 ± 82.6 ppm_v, respectively. N_2O emissions from HRAP were reported in earlier studies. Plouviez et al. (2019) showed 0.36–1029 nmol $N_2O{\cdot}g^{-1}$ TSS using real domestic wastewater with TN (9–74 mg L^{-1}), NO_3^- (0.02–2.26 mg L^{-1}), NO_2^- (0–17 mg L^{-1}), and NH_4^+ (1.5–30 mg L^{-1}). Similarly, Alcántara et al. (2015a) reported emissions rates of 34–5685 nmol $N_2O{\cdot}g^{-1}$ TSS·h^{-1} in the presence of NO_3^- (8.24 mmol L^{-1}) and NO_2^- (12 mmol L^{-1}). Compared to these literature results, the emissions here recorded (2469–6606 nmol $N_2O{\cdot}g^{-1}$ TSS) were higher likely due to the use of real centrate.

Previous studies have shown that nitrification or denitrification is associated with N_2O emissions from HRAP (Fagerstone et al., 2011; Florez-Leiva et al., 2010). Algae also contribute to N_2O emissions through their interactions with nitrogen compounds and microbial communities (Zhang et al., 2023). By releasing organic matter and oxygen via photosynthesis, algae influence microbial activity and nutrient cycling in HRAPs (Liu et al., 2020), potentially affecting N_2O production pathways indirectly. However, in this study, the contribution of algae to N_2O emissions was found to be insignificant compared to bacterial processes. In this study, N_2O emissions were significantly higher during the dark period, correlating with reduced photosynthesis and microbial activity. Despite observing nitrification in the HRAP, $N–NH_4^+$ concentrations were negligible in the photobioreactor where algae were present, suggesting algae play a minor role in N_2O emissions. Previous literature studies evaluated the involvement of specific groups of autotrophic bacteria, such as ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), in nitrification processes and concluded that those bacterial processes, rather than algae, are primarily responsible for nitrification and potentially N_2O emissions in HRAPs (despite the potential of microalgae to synthesize N_2O) (Yao and Peng, 2017). In this context, the oxygen released via photosynthesis maintains aerobic conditions in the cultivation broth, thus inhibiting denitrification and minimizing N_2O emissions (H. Wang et al., 2016). In this experiment, DO levels in the HRAP culture broth, averaging 9.3 ± 3.5 mg O_2 L^{-1}, remain relatively high, even during dark periods, due to oxygen saturation achieved during the light period and the natural oxygenation. This ensures that denitrification and its associated N_2O emissions are prevented, even during periods when photosynthesis is inactive.

Recent research (Burlacot et al., 2019; Fabisik et al., 2023) provided new insights into the mechanisms of N_2O synthesis in microalgae, where several enzymatic activities and biochemical pathways contribute to the synthesis of N_2O. In this context, NO serves as a crucial intermediate in this process. NO is produced within microalgal cells through the reduction of NO_3^- to NO_2^- by the enzyme nitrate reductase (narB). Subsequently, NO_2^- can undergo further reduction to NO via enzymatic reactions or chemical processes within the cell. The flavodiiron proteins play a pivotal role in this conversion process, exhibiting a dual functionality to reduce both NO and O_2 (Deng et al., 2023). During photosynthesis, oxygen production may influence N_2O synthesis by competing with NO in the reduction mediated by flavodiiron enzymes. Additionally, other enzymes with nitric oxide reductase activity may contribute to the conversion of NO to N_2O.

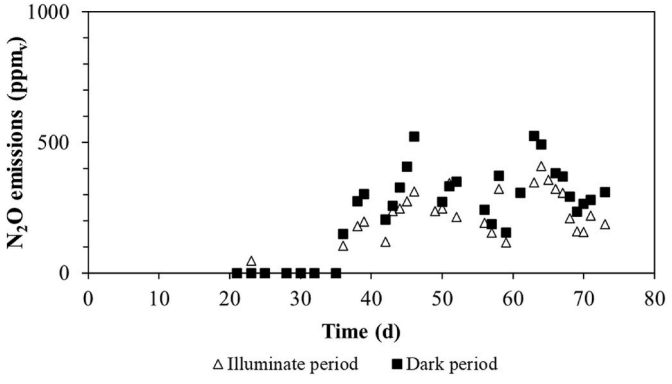

Fig. 4. Time course of N_2O gas concentrations in equilibrium with the N_2O dissolved in the HRAP during the illuminated (triangles) and dark period (squares).

During nitrification, the conversion of NH_4^+ to nitrite (NO_2^-) and NO_3^- is mediated by specific groups of autotrophic bacteria, such as ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB)(Yao and Peng, 2017). Based on the redox state of nitrogen, $N–NH_4^+$ is the preferred form of nitrogen for green microalgae growth (X. Li et al., 2019). Microalgal uptake of NH_4^+ along with nitrification of $N–NH_4^+$ to $N–NO_2^-$ and $N–NO_3^-$ were likely responsible for the intense consumption of $N–NH_4^+$ (Fig. 3c). The occurrence of nitrification in the HRAP was confirmed by the levels of $N–NO_2^-$ (Fig. 3d) and $N–NO_3^-$ (Fig. 3e) in the culture medium of the algal open pond.

$N–NH_4^+$ in the centrate averaged 546.7 ± 77.8 mg L^{-1}, while in the photobioreactor the concentrations of NH_4^+ were negligible (Fig. 3c). $N–NO_2^-$ and $N–NO_3^-$ concentrations were not detected in the centrate (Fig. 3d–e). Similarly, nitrite concentrations in the algal open pond remained negligible from day 14 to the end of the experiment because the temperature in the cultivation broth remained <28 °C and DO > 2 mg L^{-1} (Fig. 3d). These conditions are known to promote the growth of nitrite-oxidizing bacteria versus ammonia oxidizing-bacteria, thus preventing the accumulation of NO_2^- (Francis et al., 2005).

3.3. Influence of pH, nitrogen source and illumination on N_2O emissions

Several studies have investigated the impact of environmental parameters on N_2O emissions in algal-bacterial processes (Aboobakar et al., 2013; Y. Wang et al., 2022; Zhang et al., 2023). Batch tests were carried out in this study to evaluate the influence of pH and different nitrogen sources under both dark and light conditions on the N_2O emissions in gas-tight serum bottles of 120 mL. The initial biomass concentration was 1.2 ± 0.1 g VSS L^{-1} of all conditions. It is well known that these variables influence N_2O emissions in biological treatment systems, especially when it comes to wastewater treatment and HRAPs. The availability and utilization of nitrogen compounds by microorganisms is strongly influenced by the nitrogen source, which in turn affects N_2O production. pH has a significant impact on the balance between nitrification and denitrification processes, which are essential to produce N_2O emissions, and is crucial for the activities of nitrogen-transforming microorganisms. Furthermore, light conditions can affect the availability of organic matter and oxygen, which in turn affects the microbial processes responsible for N_2O emissions. Light conditions are also essential for algal photosynthesis in HRAPs. N_2O emissions were higher under dark conditions, which was consistent with the results obtained in the continuous algal-bacterial process (Fig. 5). At this point it should be also highlighted that the 2–3 days lag phase in N_2O production could be due to the acclimation period of the algal-bacterial community to the mineral salt medium, which exhibited significant differences with the broth of the HRAP used as inoculum in terms of pH, salinity, illumination, nutrient concentration, alkalinities, etc. DO levels remained low under dark conditions in batch tests and a more anaerobic environment favourable to denitrification was created (Fagerstone et al., 2011). As a result, the biological conversion of NO_3^- back to nitrogen gas (N_2), with nitrous oxide (N_2O) as a potential intermediate or end product, was fostered in the absence of photosynthesis (Q. Li et al., 2023b). Denitrification can directly produce N_2O as an

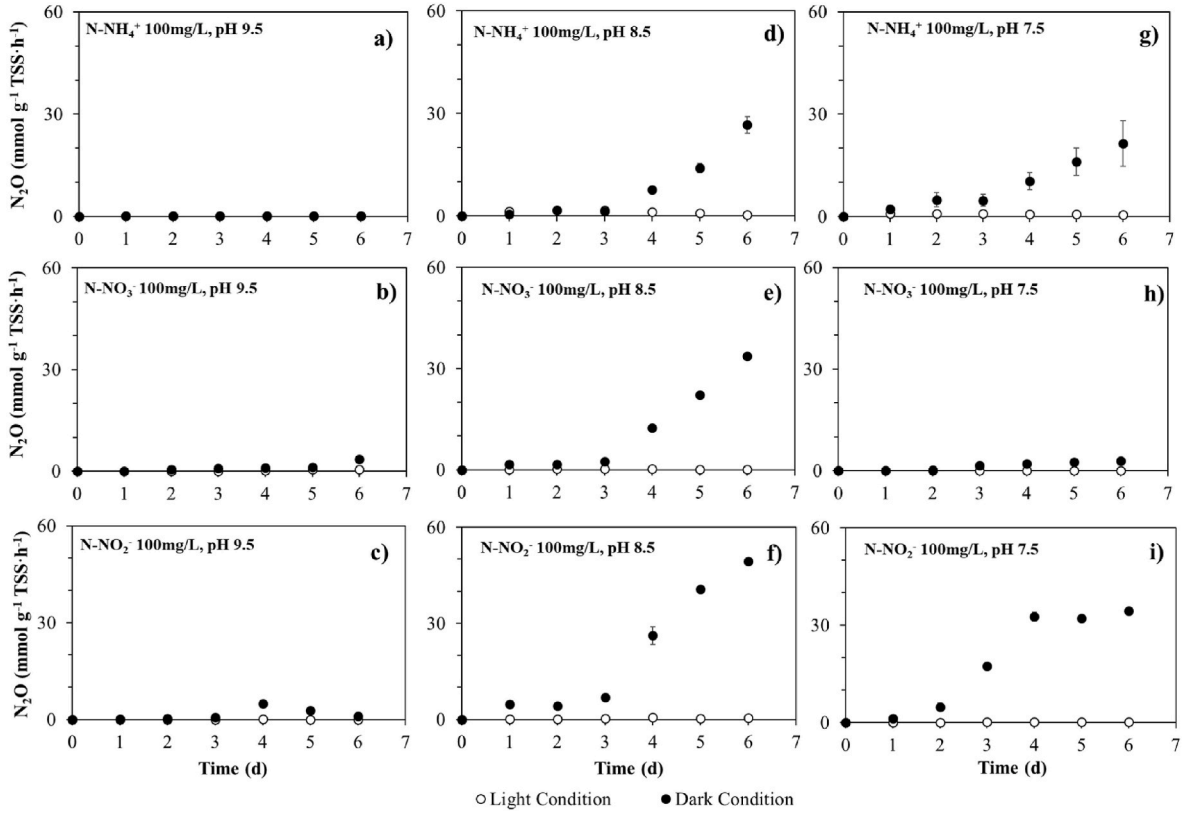

Fig. 5. Influence of the nitrogen source and pH on the specific N_2O emission rates from the algal-bacterial biomass present in the cultivation broth of the HRAP under light (open circles) and dark conditions (solid circles).

intermediate product, whereas nitrification produces N_2O as a byproduct (Florez-Leiva et al., 2010).

The source of nitrogen in algal ponds has been found to have a significant impact on N_2O emissions (Alcántara et al., 2015b). Overall, higher emissions of N_2O have been reported in algal ponds fed with nitrogen in the form of ammonium ($N–NH_4^+$) compared to those fed with nitrate ($N–NO_3^-$) or nitrite ($N–NO_2^-$). This finding can be explained by the fact that nitrification converts $N–NH_4^+$ into $N–NO_2^-$ and $N–NO_3^-$, and typically produces N_2O as a byproduct, leading to higher N_2O emissions in ponds treating $N–NH_4^+$ laden effluents (Alcántara et al., 2015b; Plouviez et al., 2019). However, in this batch study, $N–NO_2^-$ has been shown to have a significant impact on N_2O emissions (Fig. 5c, f and 5i). Maximum N_2O emission rates accounted for 4.9 ± 1.2 mmol g^{-1} TSS·h^{-1}, 49.4 ± 0.08 mmol g^{-1} TSS·h^{-1} and 34.5 ± 0.05 mmol g^{-1} TSS·h^{-1} in the assays conducted with nitrite at pH of 9.5, 8.5, and 7.5, respectively, under dark conditions. These outcomes aligned with Alcántara et al. (2015a). Nitrite (NO_2-) can potentially result in higher N_2O emissions because this oxidized nitrogen form acts as a key intermediate or byproduct during denitrification and nitrification (Kong et al., 2013). In denitrification, nitrite can be reduced to N_2O directly and under a broader range of conditions compared to NO_3^- (Alcántara et al., 2015b). In nitrification, *Nitrosomonas* can produce N_2O from nitrite under certain environmental conditions, such as low oxygen levels or high nitrite concentration (Law et al., 2013; Ni et al., 2014; Zhu et al., 2013). Therefore, when nitrite is the main nitrogen source, it may lead to higher N_2O emissions because it bypasses the initial steps of nitrification and denitrification, fostering the direct pathways for N_2O production (Zhao et al., 2022). In contrast, when ammonia (NH_3) or NO_3^- are the primary nitrogen sources, they undergo more steps to be transformed into N_2O.

The highest N_2O emissions were recorded at pH 8.5 regardless of the nitrogen source. The highest N_2O emission rates were 26.7 ± 2.4 mmol g^{-1} TSS·h^{-1}, 33.8 ± 0.86 mmol g^{-1} TSS·h^{-1} and 49.4 ± 0.08 mmol g^{-1} TSS·h^{-1} when using $N–NH_4^+$, $N–NO_3^-$ and $N–NO_2^-$, respectively, under dark conditions (Fig. 5d, e and 5f). In contrast, N_2O emissions were consistently lower at pH 9.5, where the highest N_2O emission rates recorded accounted for 0.2 ± mmol g^{-1} TSS·h^{-1} (light condition), 3.5 ± 0.63 mmol g^{-1} TSS·h^{-1} and 4.9 ± 1.2 mmol g^{-1} TSS·h^{-1} for nitrogen source of $N–NH_4^+$, $N–NO_3^-$ and $N–NO_2^-$, respectively (Fig. 5a, b and 5c).

Table 1 shows the initial and final pH values in the cultivation broths used in the batch N_2O emission experiments. pH increases under dark conditions at initial pH 7.5 and 8.5 would result from CO_2 stripping and denitrification. The pH of the cultivation broth can influence N_2O emissions in HRAPs by affecting the nitrification and denitrification processes, and by altering the chemical form and availability of the nitrogen compounds (Shaaban et al., 2020). The optimal pH for nitrification ranges from 7.5 to 8.5 (Campos et al., 2007). If pH is too low (acidic conditions), the nitrification process can be inhibited, leading to the accumulation of nitrite, which itself fosters N_2O emissions. The optimal pH for denitrification typically ranges from 7.0 to 7.5 (Saleh-Lakha et al., 2009). If the pH is too high (alkaline conditions), the denitrification process can be potentially limited, thus leading to an increased N_2O production. In addition, pH influences the solubility and share of nitrogen compounds such as NH_4^+ and NH_3. For example, NH_3 remains in equilibrium with NH_4^+ in water, and the ratio between them is determined by the pH. As pH increases, more NH_3 (which can be directly converted to N_2O via nitrification) is present compared to NH_4^+ (Blum et al., 2018). However above pH 9.5, the efficiency of both nitrification and denitrification can be severely inhibited (He et al., 2018; Yue et al., 2023). Overall, dark conditions and pH 8.5 resulted in the highest N_2O emission rates, creating detrimental synergism for N_2O synthesis. Of then, the presence of nitrite specifically resulted in the highest N_2O emissions as previously reported in literature (Alcántara et al., 2015a). Conversely, lower N_2O emissions were consistently recorded under light conditions and pH 9.5, indicating a potential inhibition of both nitrification and denitrification processes.

Table 1
Initial and final pH in the cultivation broths of the batch tests conducted to evaluate N_2O emission under different nitrogen sources, initial pH values and dark and light conditions.

N-source	Initial pH	Illumination Conditions	End pH
$N–NH_4^+$	9.48	Dark	9.13 ± 0.00
		Light	9.05 ± 0.01
	8.56	Dark	12.01 ± 0.06
		Light	7.56 ± 0.14
	7.55	Dark	12.04 ± 0.01
		Light	7.41 ± 0.06
$N–NO_3^-$	9.42	Dark	9.18 ± 0.23
		Light	9.00 ± 0.06
	8.56	Dark	12.10 ± 0.05
		Light	7.69 ± 0.04
	7.58	Dark	12.12 ± 0.01
		Light	7.47 ± 5.28
$N–NO_2^-$	9.51	Dark	11.98 ± 0.04
		Light	8.70 ± 0.06
	8.48	Dark	11.73 ± 0.06
		Light	7.63 ± 0.00
	7.52	Dark	11.81 ± 0.01
		Light	7.58 ± 0.01

On the other hand, it must be highlighted that batch assays often experience rapid anoxic or anaerobic conditions in the dark, which may not accurately reflect the oxygen dynamics prevailing in the cultivation broth of the HRAPs. This difference is significant because oxygen availability strongly influences microbial processes, including nitrification and denitrification, which are key drivers of N_2O emissions. Future work should consider integrating molecular techniques, such as metagenomic or metatranscriptomic analysis, to provide new insights into microbial community dynamics and metabolic pathways driving N_2O emissions in HRAPs.

4. Conclusions

This study represents the first assessment of N_2O emissions from a continuous algal-bacterial pond devoted to photosynthetic biogas upgrading and digestate treatment at pilot scale indoors. The dominant microalga was *Chloroidium saccharophilum*, crucial for CO_2 assimilation, keeping pH stable at 8.0. N_2O emissions were greater in the dark than in light. Batch tests showed maximum N_2O emissions at pH 8.5, in darkness with nitrite as the nitrogen source. To minimize N_2O emissions and promote sustainable algal cultivation, it is important to regulate nutrient loading rates or implement a basal aeration to prevent nitrite build-up. Additionally, buffering the pH is recommended, especially during the dark hours when algae are not actively photosynthesizing.

CRediT authorship contribution statement

Yura Jo: Writing – original draft, Visualization, Methodology, Conceptualization. **Edwin G. Hoyos:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Saúl Blanco:** Validation, Investigation, Data curation. **Sang-Hyoun Kim:** Project administration, Investigation. **Raúl Muñoz:** Writing – review & editing, Validation, Supervision, Data curation.

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.

Data availability

Data will be made available on request.

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References

Aboobakar, A., Cartmell, E., Stephenson, T., Jones, M., Vale, P., Dotro, G., 2013. Nitrous oxide emissions and dissolved oxygen profiling in a full-scale nitrifying activated sludge treatment plant. Water Res. 47 (2), 524–534. https://doi.org/10.1016/j.watres.2012.10.004.

Alcántara, C., Muñoz, R., Norvill, Z., Plouviez, M., Guieysse, B., 2015a. Nitrous oxide emissions from high rate algal ponds treating domestic wastewater. Bioresour. Technol. 177, 110–117. https://doi.org/10.1016/j.biortech.2014.10.134.

Alcántara, C., Domínguez, J.M., García, D., Blanco, S., Pérez, R., García-Encina, P.A., Muñoz, R., 2015b. Evaluation of wastewater treatment in a novel anoxic-aerobic algal-bacterial photobioreactor with biomass recycling through carbon and nitrogen mass balances. Bioresour. Technol. 191, 173–186. https://doi.org/10.1016/j.biortech.2015.04.125.

Ali, T.U., Ahmed, Z., Kim, D.J., 2014. Estimation of N2O emission during wastewater nitrification with activated sludge: effect of ammonium and nitrite concentration by regression analysis. J. Ind. Eng. Chem. 20 (4), 2574–2579. https://doi.org/10.1016/j.jiec.2013.10.042.

APHA-AWWA-WPCF, 1999. Standard methods for the examination of water and wastewater. In: American Journal of Public Health and the Nations Health. https://doi.org/10.2105/ajph.56.4.684-a, 20th ed.

Bellido-Pedraza, C.M., Calatrava, V., Llamas, A., Fernandez, E., Sanz-Luque, E., Galvan, A., 2022. Nitrous oxide emissions from nitrite are highly dependent on nitrate reductase in the microalga chlamydomonas reinhardtii. Int. J. Mol. Sci. 23 (16) https://doi.org/10.3390/ijms23169412.

Blum, J.M., Jensen, M.M., Smets, B.F., 2018. Nitrous oxide production in intermittently aerated Partial Nitritation-Anammox reactor: oxic N2O production dominates and relates with ammonia removal rate. Chem. Eng. J. 335, 458–466. https://doi.org/10.1016/j.cej.2017.10.146.

Brémond, U., Bertrandias, A., Steyer, J.P., Bernet, N., Carrere, H., 2021. A vision of European biogas sector development towards 2030: trends and challenges. J. Clean. Prod. 287 https://doi.org/10.1016/j.jclepro.2020.125065.

Burlacot, A., Richaud, P., Gosset, A., Li-Beisson, Y., Peltier, G., 2019. Algal photosynthesis converts nitric oxide into nitrous oxide. Proc. Natl. Acad. Sci. USA. https://doi.org/10.1073/pnas.1915276117/-/DCSupplemental.

Campos, J.L., Garrido, J.M., Mosquera-Corral, A., Méndez, R., 2007. Stability of a nitrifying activated sludge reactor. Biochem. Eng. J. 35 (1), 87–92. https://doi.org/10.1016/j.bej.2007.01.002.

Carvalho, G., Lemos, P.C., Oehmen, A., Reis, M.A.M., 2007. Denitrifying phosphorus removal: linking the process performance with the microbial community structure. Water Res. 41 (19), 4383–4396. https://doi.org/10.1016/j.watres.2007.06.065.

Chan, S.S., Khoo, K.S., Chew, K.W., Ling, T.C., 2022. Recent advances biodegradation and biosorption of organic compounds from wastewater: microalgae-bacteria consortium - a review. Bioresour. Technol. 344 https://doi.org/10.1016/j.biortech.2021.126159. Show, P. L.

Craggs, R., Park, J., Sutherland, D., Heubeck, S., 2015. Economic construction and operation of hectare-scale wastewater treatment enhanced pond systems. J. Appl. Phycol. 27 (5), 1913–1922. https://doi.org/10.1007/s10811-015-0658-6.

de Godos, I., Blanco, S., García-Encina, P.A., Becares, E., Muñoz, R., 2010. Influence of flue gas sparging on the performance of high rate algae ponds treating agro-industrial wastewaters. J. Hazard Mater. 179 (1–3), 1049–1054. https://doi.org/10.1016/j.jhazmat.2010.03.112.

Deng, W.H., Zhou, T.P., Liao, R.Z., 2023. Computational exploration of enzyme promiscuity: mechanisms of O2 and NO reduction activities of the desulfovibrio gigas flavodiiron protein. ACS Catal. 13 (24), 16318–16336. https://doi.org/10.1021/acscatal.3c03451.

Eloka-Eboka, A.C., Inambao, F.L., 2017. Effects of CO2 sequestration on lipid and biomass productivity in microalgal biomass production. Appl. Energy 195, 1100–1111. https://doi.org/10.1016/j.apenergy.2017.03.071.

Erratt, K.J., Creed, I.F., Trick, C.G., 2018. Comparative effects of ammonium, nitrate and urea on growth and photosynthetic efficiency of three bloom-forming cyanobacteria. Freshw. Biol. 63 (7), 626–638. https://doi.org/10.1111/fwb.13099.

Fabisik, F., Guieysse, B., Procter, J., Plouviez, M., 2023. Nitrous oxide (N2O) synthesis by the freshwater cyanobacterium Microcystis aeruginosa. Biogeosciences 20 (3), 687–693. https://doi.org/10.5194/bg-20-687-2023.

Fagerstone, K.D., Quinn, J.C., Bradley, T.H., De Long, S.K., Marchese, A.J., 2011. Quantitative measurement of direct nitrous oxide emissions from microalgae cultivation. Environ. Sci. Technol. 45 (21), 9449–9456. https://doi.org/10.1021/es202573f.

Florez-Leiva, L., Tarifeño, E., Tarifeño, T., Cornejo, M., Kiene, R., Farías, L., 2010. High production of N_2O, CH_4 and DMSP in phytoplankton culture High production of nitrous oxide (N_2O), methane (CH_4) and dimethylsulphoniopropionate (DMSP) in a massive marine phytoplankton culture High production of N2O, CH4 and DMSP in phytoplankton culture. Biogeosci. Discuss. 7, 6705–6723. https://doi.org/10.5194/bgd-7-6705-2010.

Francis, C.A., Roberts, K.J., Beman, J.M., Santoro, A.E., Oakley, B.B., 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proc. Natl. Acad. Sci. USA. 102 (41), 14683–14688. https://doi.org/10.1073/pnas.0506625102.

Franco-Morgado, M., Toledo-Cervantes, A., González-Sánchez, A., Lebrero, R., Muñoz, R., 2018. Integral (VOCs, CO_2, mercaptans and H_2S) photosynthetic biogas upgrading using innovative biogas and digestate supply strategies. Chem. Eng. J. 354, 363–369. https://doi.org/10.1016/j.cej.2018.08.026.

Frutos, O.D., Arvelo, I.A., Pérez, R., Quijano, G., Muñoz, R., 2015. Continuous nitrous oxide abatement in a novel denitrifying off-gas bioscrubber. Appl. Microbiol. Biotechnol. 99 (8), 3695–3706. https://doi.org/10.1007/s00253-014-6329-816.

Gao, L., Liu, G., Zamyadi, A., Wang, Q., Li, M., 2021. Life-cycle cost analysis of a hybrid algae-based biological desalination – low pressure reverse osmosis system. Water Res. 195 https://doi.org/10.1016/j.watres.2021.116957.

González-Sánchez, A., Revah, S., 2007. The effect of chemical oxidation on the biological sulfide oxidation by an alkaliphilic sulfoxidizing bacterial consortium. Enzym. Microb. Technol. 40 (2), 292–298. https://doi.org/10.1016/j.enzmictec.2006.04.017.

He, T., Ye, Q., Sun, Q., Cai, X., Ni, J., Li, Z., Xie, D., 2018. Removal of nitrate in simulated water at low temperature by a novel psychrotrophic and aerobic bacterium. Pseudomonas taiwanensis Strain J. BioMed Res. Int. https://doi.org/10.1155/2018/4984087.

Hou, N., Xia, Y., Wang, X., Liu, H., Liu, H., Xun, L., 2018. H2S biotreatment with sulfide-oxidizing heterotrophic bacteria. Biodegradation 29 (6), 511–524. https://doi.org/10.1007/s10532-018-9849-6.

Jiang, L., Li, Y., Pei, H., 2021. Algal–bacterial consortia for bioproduct generation and wastewater treatment. Renew. Sustain. Energy Rev. 149 https://doi.org/10.1016/j.rser.2021.111395.

Kampschreur, M.J., Temmink, H., Kleerebezem, R., Jetten, M.S.M., van Loosdrecht, M.C. M., 2009. Nitrous oxide emission during wastewater treatment. Water Res. 43 (17), 4093–4103. https://doi.org/10.1016/j.watres.2009.03.001.

Kong, Q., Zhang, J., Miao, M., Tian, L., Guo, N., Liang, S., 2013. Partial nitrification and nitrous oxide emission in an intermittently aerated sequencing batch biofilm reactor. Chem. Eng. J. 217, 435–441. https://doi.org/10.1016/j.cej.2012.10.093.

Law, Y., Lant, P., Yuan, Z., 2013. The confounding effect of nitrite on N2O production by an enriched ammonia-oxidizing culture. Environ. Sci. Technol. 47 (13), 7186–7194. https://doi.org/10.1021/es4009689.

Li, H., Feng, X., Xiong, T., Shao, W., Wu, W., Zhang, Y., 2023a. Particulate organic carbon released during macroalgal growth has significant carbon sequestration potential in the ocean. Environ. Sci. Technol. 57 (48), 19723–19731. https://doi.org/10.1021/acs.est.3c04959.

Li, Q., Xu, Y., Liang, C., Peng, L., Zhou, Y., 2023b. Nitrogen removal by algal-bacterial consortium during mainstream wastewater treatment: transformation mechanisms and potential N_2O mitigation. Water Res. 235 https://doi.org/10.1016/j.watres.2023.119890.

Li, X., Li, W., Zhai, J., Wei, H., Wang, Q., 2019. Effect of ammonium nitrogen on microalgal growth, biochemical composition and photosynthetic performance in mixotrophic cultivation. Bioresour. Technol. 273, 368–376. https://doi.org/10.1016/j.biortech.2018.11.042.

Liu, J., Pemberton, B., Lewis, J., Scales, P.J., Martin, G.J.O., 2020. Wastewater treatment using filamentous algae – a review. Bioresour. Technol. 298 https://doi.org/10.1016/j.biortech.2019.122556.

Maktabifard, M., Blomberg, K., Zaborowska, E., Mikola, A., Mąkinia, J., 2022. Model-based identification of the dominant N_2O emission pathway in a full-scale activated sludge system. J. Clean. Prod. 336 https://doi.org/10.1016/j.jclepro.2021.130347.

Marín, D., Carmona-Martínez, A.A., Lebrero, R., Muñoz, R., 2020. Influence of the diffuser type and liquid-to-biogas ratio on biogas upgrading performance in an outdoor pilot scale high rate algal pond. Fuel 275. https://doi.org/10.1016/j.fuel.2020.117999.

Marín, D., Ortíz, A., Díez-Montero, R., Uggetti, E., García, J., Lebrero, R., Muñoz, R., 2019. Influence of liquid-to-biogas ratio and alkalinity on the biogas upgrading performance in a demo scale algal-bacterial photobioreactor. Bioresour. Technol. 280, 112–117. https://doi.org/10.1016/j.biortech.2019.02.029.

Meier, L., Stará, D., Bartacek, J., Jeison, D., 2018. Removal of H2S by a continuous microalgae-based photosynthetic biogas upgrading process. Process Saf. Environ. Protect. 119, 65–68. https://doi.org/10.1016/j.psep.2018.07.014.

Méndez, L., García, D., Perez, E., Blanco, S., Munoz, R., 2022. Photosynthetic upgrading of biogas from anaerobic digestion of mixed sludge in an outdoors algal-bacterial photobioreactor at pilot scale. J. Water Process Eng. 48 https://doi.org/10.1016/j.jwpe.2022.102891.

Montemezzani, V., Duggan, I.C., Hogg, I.D., Craggs, R.J., 2015. A review of potential methods for zooplankton control in wastewater treatment High Rate Algal Ponds and algal production raceways. Algal Res. 11, 211–226. https://doi.org/10.1016/j.algal.2015.06.024.

Ni, B.J., Peng, L., Law, Y., Guo, J., 2014. Modeling of nitrous oxide production by autotrophic ammonia-oxidizing bacteria with multiple production pathways. Environ. Sci. Technol. 48 (7), 3916–3924. https://doi.org/10.1021/es405592h. Yuan, Z.

Park, J.B.K., Craggs, R.J., Shilton, A.N., 2011. Wastewater treatment high rate algal ponds for biofuel production. Bioresour. Technol. 102 (1), 35–42. https://doi.org/10.1016/j.biortech.2010.06.158.

Piasecka, A., Baier, A., 2022. Metabolic and proteomic analysis of *Chlorella sorokiniana, Chloroidium saccharofilum*, and *Chlorella vulgaris* cells cultured in autotrophic, photoheterotrophic, and mixotrophic cultivation modes. Molecules 27 (15). https://doi.org/10.3390/molecules27154817.

Plouviez, M., Chambonnière, P., Shilton, A., Packer, M.A., Guieysse, B., 2019. Nitrous oxide (N_2O) emissions during real domestic wastewater treatment in an outdoor pilot-scale high rate algae pond. Algal Res. 44 https://doi.org/10.1016/j.algal.2019.101670.

Plouviez, M., Guieysse, B., 2020. Nitrous oxide emissions during microalgae-based wastewater treatment: current state of the art and implication for greenhouse gases budgeting. Water Sci. Technol. 82 (6) https://doi.org/10.2166/wst.2020.304.

Posadas, E., Marín, D., Blanco, S., Lebrero, R., Muñoz, R., 2017. Simultaneous biogas upgrading and centrate treatment in an outdoors pilot scale high rate algal pond. Bioresour. Technol. 232, 133–141. https://doi.org/10.1016/j.biortech.2017.01.071.

Posadas, E., Serejo, M.L., Blanco, S., Pérez, R., García-Encina, P.A., Muñoz, R., 2015. Minimization of biomethane oxygen concentration during biogas upgrading in algal-bacterial photobioreactors. Algal Res. 12, 221–229. https://doi.org/10.1016/j.algal.2015.09.002.

Praveen, P., Guo, Y., Kang, H., Lefebvre, C., Loh, K.C., 2018. Enhancing microalgae cultivation in anaerobic digestate through nitrification. Chem. Eng. J. 354, 905–912. https://doi.org/10.1016/j.cej.2018.08.099.

Robertson, W.D., Moore, T.A., Spoelstra, J., Li, L., Elgood, R.J., Clark, I.D., Schiff, S.L., Aravena, R., Neufeld, J.D., 2012. Natural attenuation of septic system nitrogen by anammox. Ground Water 50 (4), 541–553. https://doi.org/10.1111/j.1745-6584.2011.00857.x.

Rodero, M. del R., Lebrero, R., Serrano, E., Lara, E., Arbib, Z., García-Encina, P.A., Muñoz, R., 2019. Technology validation of photosynthetic biogas upgrading in a semi-industrial scale algal-bacterial photobioreactor. Bioresour. Technol. 279, 43–49. https://doi.org/10.1016/j.biortech.2019.01.110.

Rodero, M. del R., Posadas, E., Toledo-Cervantes, A., Lebrero, R., Muñoz, R., 2018. Influence of alkalinity and temperature on photosynthetic biogas upgrading efficiency in high rate algal ponds. Algal Res. 33, 284–290. https://doi.org/10.1016/j.algal.2018.06.001.

Salbitani, G., Carfagna, S., 2021. Ammonium utilization in microalgae: a sustainable method for wastewater treatment. Sustainability 13 (2), 1–17. https://doi.org/10.3390/su13020956.

Saleh-Lakha, S., Shannon, K.E., Henderson, S.L., Goyer, C., Trevors, J.T., Zebarth, B.J., Burton, D.L., 2009. Effect of pH and temperature on denitrification gene expression and activity in Pseudomonas mandelii. Appl. Environ. Microbiol. 75 (12), 3903–3911. https://doi.org/10.1128/AEM.00080-09.

Sepúlveda-Muñoz, C.A., de Godos, I., Muñoz, R., 2023. Wastewater treatment using photosynthetic microorganisms. Symmetry 15, 2. https://doi.org/10.3390/sym15020525.

Shaaban, M., Wu, Y., Wu, L., Hu, R., Younas, A., Nunez-Delgado, A., Xu, P., Sun, Z., Lin, S., Xu, X., Jiang, Y., 2020. The effects of pH change through liming on soil N_2O emissions. Processes 8 (6), 1–13. https://doi.org/10.3390/pr8060702.

Sutherland, D.L., Park, J., Ralph, P.J., Craggs, R., 2021. Ammonia, pH and dissolved inorganic carbon supply drive whole pond metabolism in full-scale wastewater high rate algal ponds. Algal Res. 58 https://doi.org/10.1016/j.algal.2021.102405.

United Nations Environment Programme, 2021. Making Peace with Nature: A Scientific Blueprint to Tackle the Climate, Biodiversity and Pollution Emergencies. https://www.unep.org/resources/making-peace-nature.

Vargas-Estrada, L., Hoyos, E.G., Sebastian, P.J., Muñoz, R., 2023a. Influence of mesoporous iron based nanoparticles on Chlorella sorokiniana metabolism during photosynthetic biogas upgrading. Fuel 333. https://doi.org/10.1016/j.fuel.2022.126362.

Vargas-Estrada, L., Hoyos, E.G., Méndez, L., Sebastian, P.J., Muñoz, R., 2023b. Boosting photosynthetic biogas upgrading via carbon-coated zero-valent iron nanoparticle addition: a pilot proof of concept study. Sustain Chem Pharm 31. https://doi.org/10.1016/j.scp.2022.100952.

Wang, H., Guan, Y., Pan, M., Wu, G., 2016. Aerobic N_2O emission for activated sludge acclimated under different aeration rates in the multiple anoxic and aerobic process. J. Environ. Sci. 43, 70–79. https://doi.org/10.1016/j.jes.2015.08.010.

Wang, Y., Cao, W., Guo, J., Zhang, M., 2022. Effects of increasing pH on nitrous oxide and dinitrogen emissions from denitrification in sterilized and unsterilized forest soils. Forests 13 (10). https://doi.org/10.3390/f13101589.

Yao, Q., Peng, D.C., 2017. Nitrite oxidizing bacteria (NOB) dominating in nitrifying community in full-scale biological nutrient removal wastewater treatment plants. Amb. Express 7 (1). https://doi.org/10.1186/s13568-017-0328-y.

Yue, X., Liu, H., Wei, H., Chang, L., Gong, Z., Zheng, L., Yin, F., 2023. Reactive and microbial inhibitory mechanisms depicting the panoramic view of pH stress effect on common biological nitrification. Water Res. 231 https://doi.org/10.1016/j.watres.2023.119660.

Zhang, Y., Wang, J.H., Zhang, J.T., Chi, Z.Y., Kong, F.T., 2023. The long overlooked microalgal nitrous oxide emission: characteristics, mechanisms, and influencing factors in microalgae-based wastewater treatment scenarios. Sci. Total Environ. 856 https://doi.org/10.1016/j.scitotenv.2022.159153. Zhang, Q.

Zhao, J., Zhao, J., Yang, W., Hu, B., Huang, T., Xie, S., Lei, S., Hou, W., 2022. Mechanisms of NO and N_2O production by enriched nitrifying sludge in a sequencing batch reactor: effects of hydroxylamine. J. For. Environ. 316 https://doi.org/10.1016/j.jenvman.2022.115237.

Zhou, S., Zhang, X., Feng, L., 2010. Effect of different types of electron acceptors on the anoxic phosphorus uptake activity of denitrifying phosphorus removing bacteria. Bioresour. Technol. 101 (6), 1603–1610. https://doi.org/10.1016/j.biortech.2009.09.032.

Zhu, X., Burger, M., Doane, T.A., Horwath, W.R., 2013. Ammonia oxidation pathways and nitrifier denitrification are significant sources of N_2O and NO under low oxygen availability. Proc. Natl. Acad. Sci. U.S.A. 110 (16), 6328–6333. https://doi.org/10.1073/pnas.1219993110, 2013.