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Nitrous oxide emissions from high rate algal ponds treating domestic wastewater

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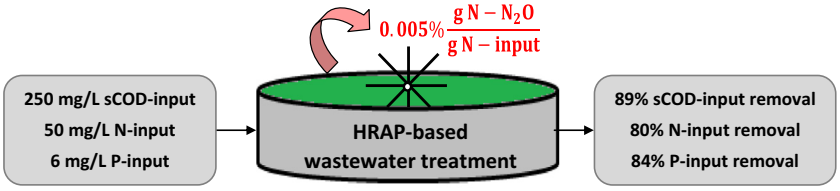
HIGHLIGHTS

- Under normal operation, HRAP treating wastewater released <2 nmol N_2O/g TSS h.
- These N_2O emissions (⩽0.0047% N input) generated a low carbon footprint.
- External supply of nitrite significantly boosted N_2O production in darkness.
- Ammonium overloading was associated with N_2O emissions up to 11 nmol/g TSS h.
- *C. vulgaris* was the most likely significant N_2O producer in the HRAP microcosms.

GRAPHICAL ABSTRACT



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ABSTRACT

This study investigated the generation of N_2O by microcosms withdrawn from 7-L high rate algal ponds (HRAPs) inoculated with *Chlorella vulgaris* and treating synthetic wastewater. Although HRAPs microcosms demonstrated the ability to generate algal-mediated N_2O when nitrite was externally supplied under darkness in batch assays, negligible N_2O emissions rates were consistently recorded in the absence of nitrite during 3.5-month monitoring under 'normal' operation. Thereafter, HRAP A and HRAP B were overloaded with nitrate and ammonium, respectively, in an attempt to stimulate N_2O emissions via nitrite *in situ* accumulation. Significant N_2O production (up to 5685 ± 363 nmol N_2O/g TSS h) was only recorded from HRAP B microcosms externally supplied with nitrite in darkness. Although confirmation under full-scale outdoors conditions is needed, this study provides the first evidence that the ability of microalgae to synthesize N_2O does not affect the environmental performance of wastewater treatment in HRAPs.

1. Introduction

The treatment of wastewater in high rate algal ponds (HRAPs) arguably provides one of the most cost and resource efficient means to mass produce microalgae biomass for biofuel generation. In addition, these photobioreactors allow for a simultaneous nitrogen (N) and phosphorus (P) elimination through biomass assimilation at relatively short hydraulic retention time (HRT), which represents an important advantage in comparison with conventional wastewater treatment systems (De Godos et al., 2010). However, the benefits brought about by the use of wastewater as a source of nutrients and water might be compromised by the ability of microalgae and associated bacteria to synthesize N_2O (Weathers, 1984; Fagerstone et al., 2011; Albert et al., 2013; Guieysse et al.,

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2013), a critical greenhouse gas and ozone-depleting atmospheric pollutant (Ravishankara et al., 2009). For example, Florez-Leiva et al. (2010) detected N_2O emissions of 1–500 μmol of N_2O/m^2 d during *Nannochloris* cultivation in a 48 m^3 full-scale HRAP and Fagerstone et al. (2011) reported a N_2O emission factor of 0.0024% N-N_2O/N-input during *Nannochoropsis salina* cultivation in a bench-scale HRAP. While associated bacteria were suspected to cause N_2O emission in these past studies, recent findings have confirmed direct N_2O synthesis by axenic *Chlorella vulgaris* (Guieysse et al., 2013).

Despite the potential significance of N_2O emissions during algae cultivation, little is known about the mechanisms of N_2O synthesis by algae and/or associated microorganisms. Identifying key 'factors' and putative N_2O production routes is especially difficult during algae-based wastewater treatment because the bacterial, archaeal and algal pathways potentially involve similar precursors and enzymes (Hatzenpichler, 2012; Guieysse et al., 2013; Fig. 1). Hence, in view of the current lack of knowledge on the populations, mechanisms, and culture conditions involved in N_2O production from HRAPs, a better understanding of the role of microalgae in N_2O emissions during algae-based wastewater treatment in HRAPs is required before this cultivation platform can be scaled-up for biofuel production.

This work constitutes a study to assess the potential significance of N_2O emissions from two identical HRAPs inoculated with *C. vulgaris* and semi-continuously supplied with synthetic sewage. For this purpose, HRAP microcosms were periodically withdrawn and tested for N_2O production under various conditions using batch assays. Emphasis was given to the determination of the role of microalgae in N_2O emissions. The impact of the N-source and its loading on N_2O production was also assessed.

2. Methods

2.1. Microorganisms

The microalgae *C. vulgaris* was obtained as described by Novis et al. (2009) and cultivated in 250 mL E-flasks containing 125 mL of buffered BG-11 medium (pH ≈ 7.2; Guieysse et al., 2013) under a CO_2-enriched air atmosphere at 2% CO_2. E-flasks were incubated for 5 days at 25 ± 1.0 °C under continuous illumination (PAR = 92 μE/m^2 s) and shaking (160 rpm) using an orbital shaker (INFORS-HT Minitron incubation shaker, Switzerland).

A heterotrophic microbial inoculum was obtained by aerobically incubating a sample of soil (Palmerston North, New Zealand) in synthetic sewage water (SSW) composed of (per liter of deionized water): 160 mg peptone, 110 mg meat extract, 30 mg urea, 28 mg K_2HPO_4, 7 mg NaCl, 4 mg $CaCl_2{\cdot}2H_2O$ and 2 mg $MgSO_4{\cdot}7H_2O$ (OECD 303A, 1996). This SSW contained 250 ± 2 mg/L of chemical oxygen demand (COD), 50 ± 1 mg/L of total nitrogen (TN) and 5.7 ± 0.1 mg P-PO_4^{3-}/L. This culture was incubated for 7 days at 25 ± 1 °C under continuous darkness and magnetic agitation at 200 rpm.

A nitrifying culture was obtained by aerobically incubating a soil sample (Palmerston North, New Zealand) in Winogradsky Medium composed of (per liter of deionized water): 2 g $(NH_4)_2SO_4$, 1 g K_2HPO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 2 g NaCl, 0.4 $FeSO_4{\cdot}7H_2O$, 0.01 g $CaCO_3$. This culture was incubated for 7 days at 25 ± 1 °C under continuous darkness and magnetic agitation at 200 rpm. The presence of nitrite and nitrate in the medium after 7 days was confirmed by Ionic Chromatography analysis, which verified the nitrifying activity of the inoculum. The culture was centrifuged and the pellet was resuspended in SSW prior to inoculation in HRAPs.

2.2. High rate algal ponds: set-up and operating conditions

The experimental set-up consisted of two identical stainless steel HRAPs (A and B) with an individual working culture volume of 7 L (0.5 m long × 0.3 m wide × 0.06 m deep) and an illuminated area of 0.1 m^2. The reactors were illuminated using nine 36 W cool daylight fluorescent tubes (TLD 36 W/865, Philips) providing a PAR of 280 μE/m^2 s at the culture surface (measured with a 383274 data logger multimeter from EXTECH instruments, USA, equipped with a 401020 light adapter from the same manufacturer) applied using a 12:12 h light–dark cycle. In each reactor, the culture was mixed with a five-bladed paddle wheel operated at 28 rpm, which supported a liquid recirculation velocity of 0.1 m/s at the center of the pond channel. The HRAPs were initially filled with 7 L of BG-11 medium and inoculated with fresh *C. vulgaris* culture at an initial concentration of 81 ± 1 mg TSS/L. The heterotrophic microbial inoculum was resuspended in SSW and added to the HRAPs when the microalgae concentrations reached 414 ± 13 mg TSS/L. Following this, semi-continuous operation was started by daily replacing 1 L of HRAP culture with freshly prepared SSW in order to maintain a HRT of 7 days, which represents a conservative HRT for domestic wastewater treatment in HRAPs (García et al., 2006). Water evaporation losses were daily recorded and compensated with distilled water to avoid salt accumulation. The HRAPs were thus fed with SSW containing 50 ± 1 mg TN/L during the first 60 days of operation (Period I) before being inoculated with the nitrifying culture and further fed with the same SSW for an additional 45 days period (Period II). Hence, HRAP A and B were identically operated during Periods I and II. Thereafter, the influents semi-continuously fed to HRAP A and HRAP B were further supplemented with 100 mg N-NO_3^-/L_{SSW} and 100 mg N-NH_4^+/L_{SSW}, respectively, over the last 45 days of operation (Period III). Nitrate and ammonium addition at high concentration during Period III was performed to stimulate

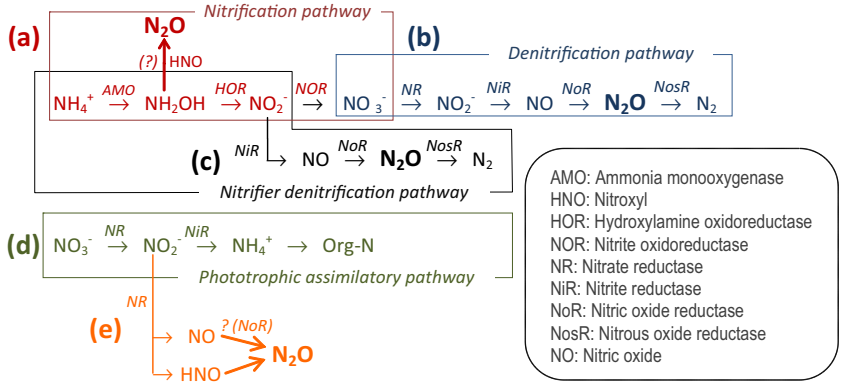

Fig. 1. Potential N_2O production metabolic pathways occurring in HRAPs ((?) = unclear pathway and/or putative enzyme type).