NITROUS OXIDE (N_2O) EMISSIONS DURING WASTEWATER TREATMENT IN HIGH-RATES ALGAE PONDS.

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ABSTRACT

Algae-based wastewater treatment processes are often heralded for their high sustainability. However, this view is challenged by recent demonstrations of the ability of axenic microalgae to synthesize nitrous oxide (N_2O), a powerful greenhouse gas and ozone-depleting atmospheric pollutant. To determine if N_2O can also be released during algae-based wastewater treatment, N_2O emissions from high rate algae ponds (HRAPs) treating synthetic wastewater were quantified under various operating conditions. Low emissions rates (0-7 nmole/g TSS-h) with minor impact of the process carbon footprint were recorded under 'normal' operation. In contrast, considerable emissions (up to 500 nmole/g TSS-h) occurred in batch assays supplied with exogenous nitrite (12 mM). Despite evidence of denitrification and nitrification occurring when the HRAPs were operated under high loadings of nitrate and ammonium, respectively, the accumulation of nitrite in the reactors was not followed by an increase in N_2O emissions. Instead, the changes in operational regimes were followed by significant changes in the rates of N_2O emissions in the presence of a high concentration of exogenous nitrite. Given the high sensitivity of N_2O emissions to process operation, further research is needed to improve the understanding of the mechanisms involved and monitor HRAP treating real wastewater outdoor. Refinement of impact assessment is also needed, especially to quantify the impact of N_2O release of ozone depletion potential.

KEYWORDS

Microalgae, climate change, HRAP, N_2O, wastewater treatment.

1 INTRODUCTION

The use of wastewater as nutrient source is regarded as one of the best alternatives to minimize microalgae cultivation costs in high rate algal ponds (HRAPs). However, the environmental sustainability of this practice might be compromised by the ability of microalgae to produce significant amounts of N_2O (Weathers, 1984; Guieysse et al., 2013), a powerful greenhouse gas and ozone-depleting pollutant. To address this critical knowledge gap, this study quantified N_2O emissions from two HRAPs treating synthetic sewage wastewater (SSW) using *Chlorella vulgaris* and a bacterial consortium.

2 METHODS

Two identical lab-scale HRAPs (7 L working volume) were operated indoors (22±2ºC) and artificially illuminated 12 hours/day using cool white tubes (photosynthetically active radiation of 280 μE/m^2·s at the culture surface). During the first 60 days of operation (Period I), the HRAPs were semi-continuously fed with synthetic sewage (SSW, OECD 303A, 1996) containing 48±1 mg N-NH_4^+ /L. The reactors were then inoculated with a nitrifying culture and fed with the same SSW for an additional 45 days (Period II). During the last 45 days of operation (Period III), HRAP A was supplied with 100 mg N-NO_3^-/L and HRAP B was supplied with 100 mg N-NH_4^+/L. Algal-bacterial concentration (TSS) was measured according to Standard Methods (Eaton et al., 2005). N_2O generation by HRAP microcosms was quantified using *in vivo* batch assays according to Guieysse et al. (2013): In brief, aliquots of 50 mL of HRAP culture were transferred into 120 mL sealed serum flasks and incubated under various conditions and N_2O atmospheric concentration was quantified between 4-24 hr of incubation using a GC-ECD (Shimadzu GC-2010 plus, Japan).

3 CONCLUSIONS

The HRAPs performance was consistent with expectations based on the literature, with a soluble COD removal of 89±1.2 %, total biomass productivities of 4.6 ± 0.1 and 5.1 ± 0.2 g TSS/m^2·d during Periods I and II, respectively, and estimated photosynthetic efficiencies of 3.2 - 3.6 %. As can be seen in Figure 1, N_2O was not significantly generated by HRAP microcosms when NO_2^- was not externally supplied (< 2 nmol N_2O/g TSS·h, 0.0046% of N input). The significant N_2O production reported following addition of 12 mM in darkness (up to 294 ± 84 nmol N_2O/g TSS·h, Figure 1) and the inhibition of these emissions by light (data not shown) corroborates past studies linking microalgal N_2O synthesis to NO_2^- reduction by nitrate oxidase (Guieysse et al., 2013). These observations, together with PCR analysis of reactor biomass DNA (data not shown) and results from antibiotic-laden *in vivo* assays, provided strong evidence N_2O was released by microalgae rather than nitrifying or denitrifying prokaryotes.

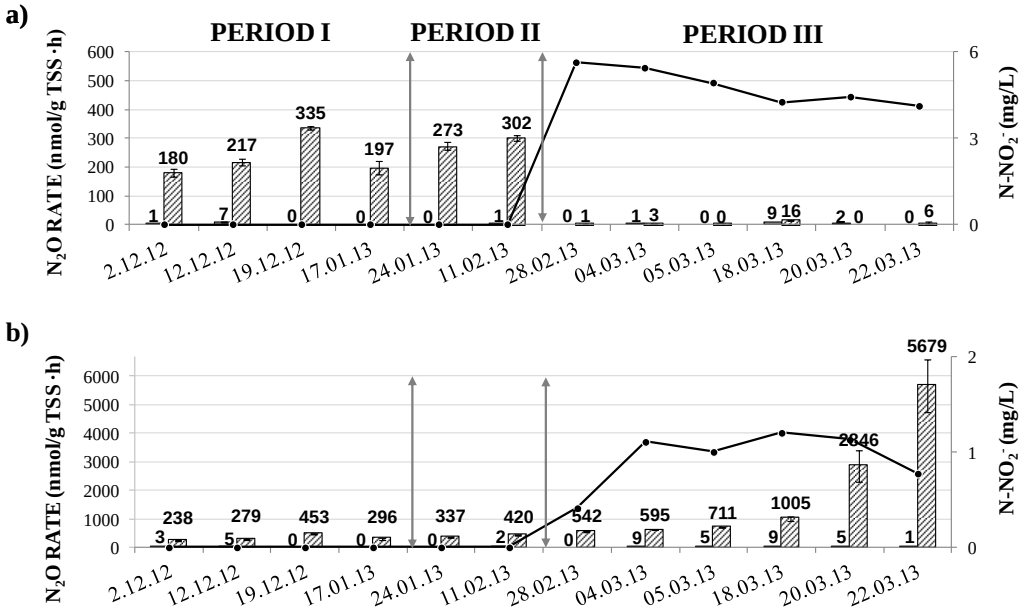

Figure 1: N_2O generation by microcosms withdrawn from HRAP A (a) and HRAP B (b), with (white columns) and without (grey columns) the addition of 12 mM of N-NO_2^- under dark conditions. N-NO_2^- concentration in the effluent (–●–) of HRAP A (a) and HRAP B (b)

As a result of a partial NH_4^+ nitrification, N-NO_2^- concentrations up to 35±8 mg/L have been recorded in HRAPs treating diluted swine manure (de Godos et al. 2010). Given the ability of the HRAP microcosms to generate

N_2O following the addition of 12 mM NO_2^- in darkness, the accumulation of NO_2^- in HRAPs operated at high loadings might trigger the release of N_2O at nighttime. In the present study however, N_2O emissions from HRAP microcosms remained insignificant even under conditions favoring direct bacterial N_2O emissions via denitrification (NO_3^- supply in HRAP A during period III) or nitrification (high NH_4^+ loading during Period III in HRAP B) or 'indirect' algal emissions following nitrite generation during denitrification or nitrification (Figure 1). If these results must be verified under relevant conditions, they provide the first confirmation of the low environmental impacts of algae cultivation during wastewater treatment in HRAP with regards to N_2O atmospheric emissions.

Surprisingly, the ability of HRAP A microcosms to generate N_2O in the presence of NO_2^- was lost during Period III (Figure 1a), while N_2O generation by HRAP B microcosm increased up to 5679 ± 929 nmol N_2O/g TSS·h (Figure 1b). The pathway of N_2O synthesis in microalgae is complex, potentially involving reactive precursors and highly-regulated enzymes. More research is needed to understand the results herein presented.

ACKNOWLEDGEMENTS

This research was supported by the regional government of Castilla y Leon and the European Social Fund (Contract No. E-47- 2011-0053564 and project Ref. GR76), the Spanish Ministry of Economy and Competitiveness (CONSOLIDER-CSD 2007-00055 and RYC-2007-01667), Massey University, and the Marsden Fund Council from New Zealand Government administrated by the Royal Society of New Zealand.

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