Nitrous oxide emissions during microalgae-based wastewater treatment: current state of the art and implication for greenhouse gases budgeting

Maxence Plouviez and Benoit Guieysse

ABSTRACT

Microalgae can synthesise the ozone depleting pollutant and greenhouse gas nitrous oxide (N_2O). Consequently, significant N_2O emissions have been recorded during real wastewater treatment in high rate algal ponds (HRAPs). While data scarcity and variability prevent meaningful assessment, the magnitude reported (0.13–0.57% of the influent nitrogen load) is within the range reported by the Intergovernmental Panel on Climate Change (IPCC) for direct N_2O emissions during centralised aerobic wastewater treatment (0.016–4.5% of the influent nitrogen load). Critically, the ability of microalgae to synthesise N_2O challenges the IPCC's broad view that bacterial denitrification and nitrification are the only major cause of N_2O emissions from wastewater plants and aquatic environments receiving nitrogen from wastewater effluents. Significant N_2O emissions have indeed been repeatedly detected from eutrophic water bodies and wastewater discharge contributes to eutrophication via the release of nitrogen and phosphorus. Considering the complex interplays between nitrogen and phosphorus supply, microalgal growth, and microalgal N_2O synthesis, further research must urgently seek to better quantify N_2O emissions from microalgae-based wastewater systems and eutrophic ecosystems receiving wastewater. This future research will ultimately improve the prediction of N_2O emissions from wastewater treatment in national inventories and may therefore affect the prioritisation of mitigation strategies.

Key words | climate change, eutrophic ecosystems, greenhouse gas, IPCC, microalgae biotechnology, nitrous oxide

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HIGHLIGHTS

- Direct N_2O emissions occur during microalgae-based wastewater treatment.
- N_2O emissions during microalgae-based wastewater treatment have been estimated as 0.13–0.57% of the influent N load but more data are needed.
- The significance of indirect microalgal N_2O emissions following discharge is potentially high, but unknown.
- Specific methodologies must be used to capture microalgal N_2O synthesis.

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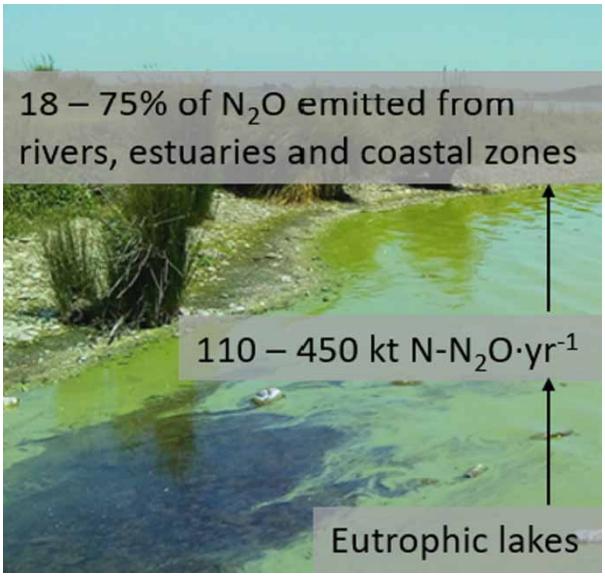
GRAPHICAL ABSTRACT



INTRODUCTION

Nitrous oxide (N_2O) is a greenhouse gas (GHG) and a potent ozone depleting atmospheric pollutant (Ravishankara *et al.* 2009) that is biologically produced via numerous pathways (Ciais *et al.* 2013; Plouviez *et al.* 2019b). The Intergovernmental Panel on Climate Change (IPCC) considers that wastewater treatment generates significant N_2O emissions via two routes: (1) directly via nitrification or nitrification–denitrification during centralised aerobic wastewater treatment; and (2) indirectly via the discharge of nitrogen (N) triggering bacterial nitrification and denitrification in aquatic environments (Box 1, based on Bartram *et al.* 2019). The ability of microalgae to synthesise N_2O however challenges this 'bacterial centric' approach to computing N_2O emissions. Hahn & Junge (1977) early hypothesised that N_2O was the by-product generated during the reductive assimilation of nitrate (NO_3^-) by phytoplankton, and Weathers and co-authors (Weathers 1984; Weathers & Niedzielski 1986) later demonstrated the ability of axenic eukaryotic microalgae and cyanobacteria to synthesise N_2O in the presence of nitrite. More recently, Guieysse *et al.* (2013) showed axenic *Chlorella vulgaris* could synthesise N_2O via nitrate reduction under oxia and several routes for N_2O synthesis in *Chlamydomonas reinhardtii* were established by Plouviez *et al.* (2017b) and Burlacot *et al.* (2020).

The occurrence of studies reporting N_2O emissions from microalgae-based systems has also intensified and N_2O emissions have now been reported from a range of cultivation systems representative of the microalgal industry (see Plouviez *et al.* 2019b for examples). In light of this knowledge and the current popularity of microalgae-based wastewater treatment as a potential sustainable biorefinery platform (Javed *et al.* 2019; Pancha *et al.* 2019), this review summarises recent advances on N_2O emissions during microalgae-based wastewater treatment and discusses the implications of these findings. This work seeks to inform researchers and policymakers of a potential new source of N_2O emissions that must be better understood and, if deemed to be significant, must be addressed in GHG budgeting, climate change policy, and the assessment of the environmental credentials of microalgae-based wastewater treatment technologies.

DIRECT N_2O EMISSION DURING MICROALGAE-BASED WASTEWATER TREATMENT

High rate algal ponds (HRAPs) are broadly heralded as sustainable processes that enable combining wastewater treatment with economic biomass cultivation for resource

recovery (Craggs *et al.* 2012; Ángeles *et al.* 2019). Challenging this view, two studies have investigated N_2O emissions during microalgae-based wastewater treatment: Alcántara *et al.* (2015) first recorded average N_2O emission of 23 μg $N\text{-}N_2O{\cdot}m^{-2}{\cdot}d^{-1}$ during synthetic wastewater treatment in bench scale HRAPs. Based on their average value, these authors concluded that at large scale the N_2O emissions potentially generated from HRAPs treating domestic wastewater would have a low environmental impact. However, the authors also acknowledged that monitoring under full-scale conditions would still be needed to confirm their findings. Plouviez *et al.* (2019a) more recently quantified N_2O emissions from an outdoor pilot HRAP fed primary domestic wastewater. Over a year of monitoring, these authors recorded highly variable N_2O emissions of 70–18,300 μg $N\text{-}N_2O{\cdot}m^{-2}{\cdot}d^{-1}$. Based on the 25–75% data range emission recorded (2,250–9,700 μg $N\text{-}N_2O{\cdot}m^{-2}{\cdot}d^{-1}$), these emissions represented 0.13–0.57% of the influent N load when the pond was operated at a hydraulic retention time of 7.5 days (see Plouviez *et al.* (2019a) for further details). In comparison, the IPCC documents N_2O emissions representing 0.016–4.5% of the influent N load and the IPCC recommends a default value of 1.6% for 'centralised aerobic wastewater treatment' (Bartram *et al.* 2019). While a meaningful assessment is currently not possible given the scarcity and variability of the data available, the current data show HRAPs have the potential to generate significant amounts of N_2O despite harbouring a very different ecology than advanced centralised systems. This challenges the IPCC assumption that direct N_2O emissions only take place during centralised aerobic treatment systems (aerobic shallow ponds are considered as 'unlikely source of CH_4 and N_2O') and raises the question if other microalgae-based treatment system also release N_2O because microalgae can abound in the waste stabilisation ponds used by countless farms all over the world (Shilton & Walmsley 2005). Glaz *et al.* (2016) indeed reported median emissions of 0.04 and 0.53 μg $N\text{-}N_2O{\cdot}m^{-2}{\cdot}d^{-1}$ from ponds located in Australia and Quebec, while Hernandez-Paniagua *et al.* (2014) reported emissions of 8–605 μg $N\text{-}N_2O{\cdot}m^{-2}{\cdot}d^{-1}$ representing 0.0001–0.01% of the influent N input load (1,715 g $TN{\cdot}d^{-1}$) from ponds located in Mexico. Although these emissions appears to be lower than the emissions reported during advanced wastewater treatment, caution is needed given the very limited number of studies available and the inherent challenges associated with detecting microalgae N_2O synthesis (see further discussion below).

INDIRECT N_2O EMISSIONS FROM EUTROPHIC AQUATIC ECOSYSTEMS

As explained in Box 1, the IPCC estimates indirect N_2O emissions from wastewater discharge based on the assumptions that a fraction of the N discharged is emitted as N_2O via bacterial nitrification and/or denitrification. This assumption must be challenged in view of the ability of microalgae to synthesise N_2O in ecosystems receiving wastewater effluents. Interestingly, as part of its Tier 3 methodology, the IPCC proposes to use an EF of 0.019 kg $N\text{-}N_2O{\cdot}kg\ N^{-1}$ for 'nutrient-impacted' aquatic environments (against 0.005 kg $N\text{-}N_2O{\cdot}kg\ N^{-1}$ in the Tier 1 methodology). This is critical because N_2O emissions from eutrophic ecosystems have been documented for years (see the review of Plouviez *et al.* 2019b) and Delsontro *et al.* (2018) found that N_2O emissions from lakes and impoundments are expected to increase as a function of lake size and chlorophyll a (a proxy for the presence of microalgae). These authors therefore predicted global N_2O estimates of 190–450 kt $N\text{-}N_2O{\cdot}yr^{-1}$ for lakes, which is equivalent to 32–75% of the current IPCC estimate of 600 kt $N\text{-}N_2O{\cdot}yr^{-1}$ for N_2O emitted from all rivers, estuaries and coastal zones (Ciais *et al.* 2013). Focusing on eutrophic lakes, Plouviez *et al.* (2019b) conservatively estimated that eutrophic lakes and reservoirs could generate 110 kt $N\text{-}N_2O{\cdot}yr^{-1}$ (or 18% of the current IPCC estimate for all rivers, estuaries and coastal zones). In contrast, Webb *et al.* (2019) showed that 67% of 101 constructed agricultural reservoirs monitored ($<0.01\ km^2$) acted as N_2O sinks despite of their highly eutrophic status ($99 \pm 289\ \mu g{\cdot}L^{-1}$ chlorophyll a). While we cannot explain these contradictory findings based on the limited amount of relevant data in the field, the magnitude of the potential emissions from eutrophic ecosystems is critical to properly assess indirect N_2O emissions from wastewater discharges because nitrate pollution triggers microalgae growth and microalgae can synthesise N_2O when fed nitrate (Plouviez *et al.* 2017a). It is therefore possible that N discharge from wastewater effluents is fuelling both algae growth and N_2O synthesis. There is also increasing evidence that the combination of N and phosphorus (P) is critical to trigger microalgae growth in eutrophic ecosystems (Conley *et al.* 2009; Xu *et al.* 2010; Paerl *et al.* 2016; Smith *et al.* 2016). This means that, in comparison with the current methodology used by the IPCC, a new methodology based on both N and P loads on receiving waters may be needed to accurately compute indirect N_2O emissions from wastewater effluent in GHG inventories.

Box 1 | N_2O emissions from wastewater treatment and discharge

The Intergovernmental Panel on Climate Change (IPCC) currently considers that only bacterial nitrification and denitrification causes N_2O emission during wastewater treatment (Bartram *et al.* 2019). These emissions can occur directly during centralised aerobic wastewater systems, and indirectly in aquatic environments receiving nitrogen (N)-laden effluents (Bartram *et al.* 2019).

The IPCC proposes a tiered methodology approach to estimate N_2O emissions during centralised wastewater treatment and discharge. Countries with limited data should follow the Tier 1 method and therefore use default values for the emission factor and activity parameters. The Tier 2 method is similar to the Tier 1 method but allows for country-specific emission factors and activity data. Finally, countries with good data and advanced methodologies (e.g. plant specific emissions factors), can follow a country-specific Tier 3 method.

Direct N_2O emissions are computed based on the amount of nitrogen found in wastewater and the degree of utilisation of treatment/discharge pathways or systems:

$$N_2O\ Plants_{DOM} = \left[\sum_{i,j}(U_i \cdot T_{ij} \cdot EF_j)\right] \cdot TN_{DOM} \cdot \frac{44}{28}$$

where $N_2O\ Plants_{DOM}$ is the amount of N_2O emitted from wastewater treatment plants (kg $N_2O{\cdot}yr^{-1}$); TN_{DOM} is the amount of total nitrogen in wastewater (kg $N{\cdot}yr^{-1}$); U_i is the fraction of population in income group i; T_{ij} is the degree of utilisation of treatment/discharge pathway or system j, for each income group fraction i; i is the income group (rural, urban high income and urban low income); j is each treatment/discharge pathway or system; EF_j is the emission factor for treatment/discharge pathway or system j (default = 0.016 kg N_2O-$N{\cdot}kg\ N^{-1}$); and $\frac{44}{28}$ is the conversion factor of kg N-N_2O into kg N_2O. The value of TN_{DOM} can be computed as the sum of the total annual amount of nitrogen in wastewater for each treatment pathway:

$$TN_{DOM\_j} = (P_{treatment\_j} \cdot Protein \cdot F_{NPR} \cdot N_{HH} \cdot F_{NON-CON} \cdot F_{IND-COM})$$

where TN_{DOM_j} is the total annual amount of nitrogen in wastewater for treatment pathway j (kg $N{\cdot}yr^{-1}$); $P_{treatment\_j}$ is the human population who are served by the treatment pathway j (person$\cdot yr^{-1}$); *Protein* is the annual per capita protein consumption (kg protein$\cdot$person$^{-1}\cdot yr^{-1}$, estimated as a fraction of protein consumed and the annual per capita protein supply (kg protein$\cdot$person$^{-1}yr^{-1}$)); F_{NPR} is the fraction of nitrogen in protein (default = 0.16 kg $N{\cdot}kg\ protein^{-1}$); N_{HH} is the additional nitrogen from household products added to the wastewater (default value of 1.1); $F_{NON-CON}$ is the factor for nitrogen in non-consumed protein disposed in sewer system (kg $\cdot kg\ N^{-1}$, value of 1 if food waste is disposed with solid waste) and $F_{IND-COM}$ is the factor for industrial and commercial co-discharged protein into the sewer system (default value of 1.25 kg $N{\cdot}kg\ N^{-1}$ for centralised treatment and 0 for decentralised treatment or untreated wastewater discharge). Indirect emissions from effluent discharges are computed based on the fraction of the N emitted as N_2O as:

$$N_2O_{EFFLUENT,\,DOM} = N_{EFFLUENT\ DOM} \cdot EF_{EFFLUENT} \cdot \frac{44}{28}$$

where $N_2O_{EFFLUENT,\,DOM}$ is the total indirect N_2O emissions (kg N-$N_2O{\cdot}yr^{-1}$); $N_{EFFLUENT,\,DOM}$ is the nitrogen in the effluent discharged to aquatic environments (kg $N{\cdot}yr^{-1}$); and $EF_{EFFLUENT}$ is the emission factor for N_2O emissions from discharged wastewater (kg N-$N_2O{\cdot}kg\ N^{-1}$). While the default value for $EF_{EFFLUENT}$ is set at 0.005 kg N-$N_2O{\cdot}kg\ N^{-1}$ for a Tier 1 method, it is set at 0.019 kg N-$N_2O{\cdot}kg\ N^{-1}$ for a Tier 3 method (i.e. for wastewater discharged in nutrient-impacted (eutrophic) or hypoxic aquatic environments). The value of $N_{EFFLUENT,\,DOM}$ is computed as:

$$N_{EFFLUENT,\,DOM} = \sum_j [(TN_{DOM} \cdot T_j) \cdot (1 - N_{REM,\,j})]$$

where T_j is the degree of utilisation of treatment system j (from all income groups); j is each wastewater treatment type used; and $N_{REM,j}$. is the fraction of total wastewater N removed during wastewater treatment per treatment type j (including transfer to sludge and nitrification–denitrification with concomitant N loss to the atmosphere).

IMPLICATIONS FOR GHG BUDGETING

The large variability in the emissions factors reported from centralised aerobic wastewater treatment and microalgae-based systems (Table 1) generates a large uncertainty in the estimation of direct emissions from wastewater treatment. This means that both centralised and microalgae-based systems may generate amounts of N_2O that are significantly different than what is currently estimated, and that further research must seek to improve the accuracy of predictions (Vasilaki *et al.* 2019). Wastewater discharge also contributes to fuel both microalgae growth and N_2O synthesis by microalgae so further research must therefore seek to reduce the considerable uncertainty associated with indirect emissions factors (Table 1) and to define specific EFs for ecosystems affected by different degree of eutrophication. To our opinion, further research must finally also establish how P discharge contribute to indirect emissions as this has the potential to dramatically change the way indirect N_2O emission are quantified and addressed.

Microalgal N_2O synthesis is influenced by factors such as the cell biology and the type and concentration of the nitrogen source microalgae receive (Plouviez *et al.* 2019b), meaning extensive monitoring (i.e. long-term with wide spatial coverage and high sampling frequency) of several microalgae-rich environments may be required to improve the accuracy of N_2O emissions from these systems. In addition, microalgal N_2O synthesis is influenced by light supply (Plouviez *et al.* 2019b), meaning that sampling methodologies may need to be adapted to capture this 'microalgal activity'. For example, the rate of microalgal N_2O synthesis can vary greatly over short time scales (minute) due to, in particular, changes in solar radiation (Plouviez *et al.* 2017a). Frequent grab samples should therefore be preferred to, for example, the use of static floating chambers in order to capture temporal variability and prevent issues such as shading of the microalgae or N_2O re-dissolution when N_2O production is intermittent. Based on the recent identification of key genes involved during microalgal N_2O synthesis (Plouviez *et al.* 2017b; Burlacot *et al.* 2020), the use of genomics could also help to generate new and comprehensive insights into the occurrence and ecological implications of microalgal N_2O synthesis.

Table 1 | Variabilities in N_2O emissions factors from wastewater treatments based on current literature

% of N influent load	Centralised domestic wastewater treatment	Waste stabilisation pond	HRAP
Direct	0.016–4.5[a]	0.0001–0.01[b]	0.13–0.57[c]
Indirect – Tier 1[d]	0.05–7.5	0.05–7.5	0.05–7.5
Indirect – Tier 3[e]	0.41–9.1	0.41–9.1	0.41–9.1

[a]Bartram *et al.* (2019).
[b]Calculated based on data reported by Hernandez-Paniagua *et al.* (2014).
[c]Plouviez *et al.* (2019a).
[d]Freshwater, estuarine, and marine environments (Bartram *et al.* 2019).
[e]Nutrient-impacted and/or hypoxic freshwater, estuarine, and marine environments (Bartram *et al.* 2019).

CONCLUSIONS

While aerobic shallow ponds are currently considered as 'unlikely source of N_2O' (Bartram *et al.* 2019), the magnitude of N_2O emissions during real wastewater treatment in HRAPs (0.13–0.57% of the influent N load) is within the range reported for direct emissions during centralised aerobic wastewater treatment (0.016–4.5%; Bartram *et al.* 2019) and indirect emissions from 'nutrient-impacted' aquatic environments (0.41–9.1%; Bartram *et al.* 2019). Monitoring using methodologies specifically designed to capture microalgal N_2O synthesis must now be conducted. This work will be critical to better understand the mechanism of microalgal N_2O synthesis and assess its significance. If significant, this mechanism should then be addressed in GHG budgeting, climate change policy, and the assessment of the environmental credentials of microalgae-based wastewater treatment technologies.

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