Isotopic evidence for nitrous oxide production pathways in a partial nitritation-anammox reactor

Eliza Harris [a, *], Adriano Joss [b], Lukas Emmenegger [a], Marco Kipf [b], Benjamin Wolf [a, c], Joachim Mohn [a], Pascal Wunderlin [b]

[a] Laboratory for Air Pollution and Environmental Technology, Empa, Überlandstrasse 129, 8600 Dübendorf, Switzerland
[b] Eawag, Swiss Federal Institute of Aquatic Science and Technology, Überlandstrasse 133, 8600 Dübendorf, Switzerland
[c] Institute of Meteorology and Climate Research (IMK-IFU), Karlsruhe Institute of Technology, Kreuzeckbahnstrasse 19, 82467 Garmisch-Partenkirchen, Germany

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ABSTRACT

Nitrous oxide (N_2O) production pathways in a single stage, continuously fed partial nitritation-anammox reactor were investigated using online isotopic analysis of offgas N_2O with quantum cascade laser absorption spectroscopy (QCLAS). N_2O emissions increased when reactor operating conditions were not optimal, for example, high dissolved oxygen concentration. SP measurements indicated that the increase in N_2O was due to enhanced nitrifier denitrification, generally related to nitrite build-up in the reactor. The results of this study confirm that process control via online N_2O monitoring is an ideal method to detect imbalances in reactor operation and regulate aeration, to ensure optimal reactor conditions and minimise N_2O emissions.

Under normal operating conditions, the N_2O isotopic site preference (SP) was much higher than expected — up to 40‰ — which could not be explained within the current understanding of N_2O production pathways. Various targeted experiments were conducted to investigate the characteristics of N_2O formation in the reactor. The high SP measurements during both normal operating and experimental conditions could potentially be explained by a number of hypotheses: i) unexpectedly strong heterotrophic N_2O reduction, ii) unknown inorganic or anammox-associated N_2O production pathway, iii) previous underestimation of SP fractionation during N_2O production from NH_2OH, or strong variations in SP from this pathway depending on reactor conditions. The second hypothesis — an unknown or incompletely characterised production pathway — was most consistent with results, however the other possibilities cannot be discounted. Further experiments are needed to distinguish between these hypotheses and fully resolve N_2O production pathways in PN-anammox systems.

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1. Introduction

In recent years, interest in wastewater treatment with anaerobic ammonium oxidizers (anammox) has increased, resulting in significant technological developments (van der Star et al., 2007; Joss et al., 2009, 2011; Imajo et al., 2004; Fernández et al., 2008; Gilbert et al., 2013), as the process offers the potential for reductions of >50% in energy consumption compared to traditional nitrification–denitrification, without decreasing nitrogen removal efficiency (Siegrist et al., 2008). The anammox reaction, which involves conversion of nitrite (NO_2^-) and ammonium (NH_4^+) to form N_2, plays an important role in a diverse range of artificial, marine and terrestrial environments (Mulder et al., 1995; Thamdrup and Dalsgaard, 2002; Kuypers et al., 2003, 2005; Vymazal, 2007). Wastewater treatment with anammox involves partial nitritation (PN), where ~50% of NH_4^+ is first oxidised to NO_2^- by ammonia oxidizing bacteria (AOBs) under oxic conditions, coupled to the anoxic anammox reaction to produce N_2. As an organic carbon source is not required, PN-anammox is particularly suited for the treatment of high NH_4^+, low carbon wastes, such as digester liquor from conventional wastewater treatment.

For process stability in single-reactor PN-anammox systems, it is critical to balance AOB and anammox activity while minimising growth of nitrite-oxidising bacteria (NOBs). This is achieved by

Table 1
Known N_2O production and consumption pathways that may be occurring in a partial nitritation-anammox reactor, and the site preference and ^{15}N fractionation factors associated with the pathways. AOB = NH_4^+ oxidizing bacteria, HET = heterotrophic denitrifiers.

Pathway	Population	Reaction	Site preference (‰)	$\Delta\delta^{15}N$ (‰)
NH_2OH oxidation	AOB	$NH_2OH \rightarrow NO \rightarrow N_2O$[a]	30 to 36[b]	−37 to −17[c,d]
Nitrifier denitrification	AOB	$NO_2^- \rightarrow NO \rightarrow N_2O$[a]	−10 to 0[b,e]	−68 to −30[b,c,d]
NO_3^-/NO_2^- reduction	HET	$NO_3^- \rightarrow NO_2^- \rightarrow N_2O$	−5 to 0[b,f,g]	−39 to −12[f]
N_2O reduction	HET	$N_2O \rightarrow N_2$	−16.4 to −2.9[i]	−39 to −12[f]
Abiotic production	−	Various[f,h]	29.5 to 35[f,h]	−27 to 1.4[h]

References:
[a] Chandran et al. (2011).
[b] Wunderlin et al. (2013a).
[c] Sutka et al. (2003).
[d] Sutka et al. (2004).
[e] Sutka et al. (2006).
[f] Toyoda et al. (2005).
[g] Toyoda et al. (2011b).
[h] Heil et al. (2014).
[i] Reduction favours N_2O with low site preference as the $^{14}N–O$ bond is more easily broken than the $^{15}N–O$ bond, thus the site preference of the remaining N_2O pool is increased. The values −2.9 to −16.4‰ refer to $\varepsilon(SP)$, the site preference fractionation factor for reduction (see Table S2 for references).

controlling the air supply rate to be the rate-limiting factor for AOB activity and therefore for the entire process, thus avoiding nitrite accumulation (Joss et al., 2011). Anammox bacteria are inhibited by dissolved oxygen (*DO*) concentrations higher than 0.2 mg L^{-1} (6.25 μM) (Joss et al., 2011) and potentially also by high NO_2^- concentrations (eg. > 600 mg L^{-1} or 10 mM) (Dapena-Mora et al., 2007). If AOB activity is reduced, *DO* can increase as less oxygen is consumed in the reactor, inhibiting anammox and interrupting operation. Thus the maximal sustainable treatment rate depends on the sludge activity, which − according to experience from long-term full-scale operations - may not be assumed constant. Online monitoring of NO_2^- has the potential to serve as control parameter to monitor activity. However, as no robust, suitable electrode is available for the continuous, online measurement of NO_2^- in pilot- or full-scale applications, online measurements of N_2O mixing ratio[1] have been suggested to act as a proxy for NO_2^-, thus presenting an ideal method to monitor and control reactor dynamics (Wunderlin et al., 2013b).

Wastewater treatment is an important, growing source of N_2O, through a number of pathways summarised in Table 1. The wastewater sector currently contributes ~6% of N_2O emissions globally, and best estimates predict that emissions from the sector will increase by >25% between 1990 and 2020 (Bogner et al., 2007). N_2O is a potent greenhouse gas with a global warming potential 298 times higher than CO_2 (100-yr) (Forster et al., 2007) and the most important ozone depleting substance currently released (Crutzen, 1970; Ravishankara et al., 2009). Online N_2O monitoring for process stability results in control and reduction of N_2O emissions, thus providing an additional benefit in terms of greenhouse gas (GHG) release.

N_2O production by AOBs can be envisaged as a 'leaky pipeline' (Firestone and Davidson, 1989) because N_2O is an unwanted by-product during the goal reaction, oxidation of NH_4^+ to NO_2^-. Two major pathways are implicated in the emission of N_2O by AOBs: Nitrifier denitrification, and NH_2OH oxidation (see Table 1). When the overall turnover of NH_4^+ by AOBs increases, the 'leaking' of N_2O also increases − thus N_2O emissions can roughly indicate AOB activity. Biotic and abiotic N_2O production by hydroxylamine (NH_2OH) oxidation can be minimised by keeping the concentration of

NH_4^+ relatively low (e.g. by intermittent dosing of wastewater); previous studies suggest that even at high NH_4^+ concentrations this pathway contributes less than 20–30% of total N_2O emissions in PN-anammox reactors (Ni et al., 2014; Ishii et al., 2014). In contrast to AOBs, it was generally agreed until recently that anammox bacteria do not produce significant amounts of N_2O (Kampschreur et al., 2009; Okabe et al., 2011); results from Lotti et al. (2014) suggest however that up to 0.2% of nitrogen removed by anammox may in fact be released as N_2O. This is much lower than the percent of ammonium released as N_2O by AOBs during imbalances in the reactor, thus in a PN-anammox reactor, nitrifier denitrification is expected to dominate N_2O production. Online N_2O measurements can therefore act as a proxy for NO_2^- concentration, which is an indicator for the relative activity of AOBs compared to anammox.

A major challenge for process regulation via N_2O measurements is the activity of NOBs, which reduce N_2O emissions from nitrifier denitrification by consuming NO_2^-. Monitoring the net NO_3^- production to NH_4^+ consumption ratio (hereafter $r(NO_3^-)/r(NH_4^+)$) − which should be approximately 10–11% due to NO_3^- production by anammox when NOBs are not active (Strous et al., 1997; Bürgmann et al., 2011) − to detect NOB activity is therefore important for process control. In addition, NOB activity should be minimised, eg. with regular washout (Joss et al., 2011). Heterotrophic denitrifiers (HET) also release N_2O during the stepwise reduction of NO_3^- to N_2, however HET activity is minimal in PN-anammox systems due to low organic carbon concentrations (Joss et al., 2009; Rathnayake et al., 2013).

N_2O can be the most important greenhouse gas released from wastewater treatment and in addition, shows great potential as an online parameter to monitor process conditions in PN-anammox systems. However, a thorough understanding of N_2O production pathways is lacking, which is necessary both to mitigate emissions and to effectively use online N_2O measurements for process control. N_2O isotopic composition is particularly useful to quantify N_2O production via different pathways (Table 1). The most abundant four isotopocules of N_2O are $^{14}N\,^{14}N\,^{16}O$, $^{14}N\,^{14}N\,^{18}O$, $^{14}N\,^{15}N\,^{16}O$ (α) and $^{15}N\,^{14}N\,^{16}O$ (β). The bulk ^{15}N isotopic composition refers to the average $\delta^{15}N$ at both positions:

$$\delta^{15}N^{bulk} = \frac{\delta^{15}N^\alpha + \delta^{15}N^\beta}{2} \tag{1}$$

The N_2O 'site preference' (SP) refers to the difference in ^{15}N isotopic composition of the central (α) position N compared to the terminal (β) position N:

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[1] Mixing ratio is the ratio of the component of interest to the total of all other constituents in a mixture. In this paper, mixing ratio always refers to the *molar* mixing ratio, ie. ppm = parts per million = moles of X per million moles of the total mixture.