Effect of temperature on N_2O emissions from a highly enriched nitrifying granular sludge performing partial nitritation of a low-strength wastewater

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Abstract

In the race to achieve a sustainable urban wastewater treatment plant, not only the energy requirements have to be considered but also the environmental impact of the facility. Thus, nitrous oxide (N_2O) emissions are a key-factor to pay attention to, since they can dominate the total greenhouse gases emissions from biological wastewater treatment. In this study, N_2O production factors were calculated during the operation of a granular sludge airlift reactor performing partial nitritation treating a low-strength synthetic influent, and furthermore, the effect of temperature on N_2O production was assessed. Average gas emission relative to conversion of ammonium was 1.5 ± 0.3% and 3.7 ±

0.5% while the effluent contained 0.5 ± 0.1% and 0.7 ± 0.1% (% N-oxidized) at 10 and 20 ºC, respectively. Hence, temperature increase resulted in higher N_2O production. The reasons why high temperature favoured N_2O production remained unclear, but different theoretical hypotheses were suggested.

Keywords

Nitrous oxide; emission factors; partial nitritation; temperature; Nitrotoga

1. Introduction

The implementation of the autotrophic biological nitrogen removal (BNR) in the mainstream has been proposed as the most promising solution for achieving energy-neutral or even energy-positive urban wastewater treatment plants (WWTPs) (Kartal et al., 2010; Siegrist et al., 2008). Significant efforts have been made to implement such a treatment as a one-stage system, where partial nitritation and anammox process (PN/A) are integrated in one single reactor (De Clippeleir et al., 2013; Gilbert et al., 2014; Lotti et al., 2014a; Wang et al., 2016a; Wett et al., 2013). This is based on the practise of implementing this process for sidestream treatment (Lackner et al., 2014). However the different conditions of low required effluent concentrations, lower temperature and much larger hydraulic loading relative to nitrogen loading might make a different process design more feasible. Two-stage systems have been reported as a successful alternative to face the challenges of efficient autotrophic BNR at mainstream conditions (Isanta et al., 2015b; Ma et al., 2011; Pérez et al., 2015).

In the race to achieve a sustainable urban WWTP not only the energy requirements have to be considered but also the environmental impact of the facility (Morales et al., 2015). Thus, greenhouse gases emissions are a key-factor to pay attention to (Kampschreur et al., 2009). Nitrous oxide (N_2O) is produced in conventional urban WWTPs during the autotrophic nitrification and heterotrophic denitrification and, actually, N_2O emissions can dominate the total greenhouse gases emissions from biological wastewater treatment (Wunderlin et al., 2012). N_2O is an important greenhouse gas with a global warming potential of about 300 times higher than CO_2 on a 100 year time horizon (IPCC, 2013) and a substantial ozone-depleting compound in the stratosphere. Hence, mitigation strategies and control of emissions are essential issues to consider in the implementation of the autotrophic BNR in the mainstream of urban WWTPs.

It is well known that N_2O production in WWTPs is associated with nitrification by ammonia oxidizing bacteria (AOB) and to denitrification by heterotrophic bacteria (Kampschreur et al., 2009; Wunderlin et al., 2012). Furthermore, N_2O emissions can be also produced by abiotic chemical reactions (Harper et al., 2015; Kampschreur et al., 2011; Soler-Jofra et al., 2016). AOB produce N_2O by two different pathways: (i) from intermediates of the biological oxidation of hydroxylamine (NH_2OH), which is an intermediate during the ammonia oxidation to nitrite and (ii) the nitrifier denitrification pathway, which is the reduction of nitrite to N_2O with ammonia, hydrogen or pyruvate as possible electron donors (Wunderlin et al., 2012). Heterotrophic denitrifiers produce N_2O as intermediate in the denitrification so it can be released due to an imbalanced metabolic activity, a nitrite accumulation or a limited availability of biodegradable organic compounds and incomplete denitrification (Kampschreur et al., 2009; Wunderlin et al., 2012).

In the autotrophic BNR process, N_2O emissions will mainly occur in the partial nitritation step since anammox bacteria are not supposed to produce N_2O as it is not involved in their metabolism (Kartal et al., 2011). Actually, very low N_2O emissions were reported in anammox reactors and they were associated with side reactions independent of anammox bacteria (Lotti et al., 2014b), or to abiotic reactions (Kampschreur et al., 2011). In recent years, N_2O gas emissions were widely studied for PN/A systems (either in one-stage systems or in a single partial nitritation reactor) treating high-strength nitrogen wastewaters, mainly reject water (Castro-Barros et al., 2015; Desloover et al., 2011; Kampschreur et al., 2008b; Mampaey et al., 2016; Okabe et al., 2011; Pijuan et al., 2014). There was a huge variability on N_2O emissions values reported in literature, ranging from 1.5% (Rathnayake et al., 2013) to 11% (Desloover et al., 2011) of the ammonium oxidized emitted as N_2O. This variability was due to differences in reactor configurations, type of influent, conditions applied and even the methodology used for quantifying emissions (Bollon et al., 2016). In the case of PN/A systems at mainstream conditions, to the best of the authors' knowledge, only Wang et al. (2016b) and Reino et al. (2016) reported N_2O gas emissions of a nitritation reactor treating a low-strength synthetic influent. Reino et al. (2016) reported very low values (0.36 ± 0.07% of the ammonium oxidized) in a granular sludge reactor performing partial nitritation at 10 °C, compared to N_2O gas emissions reported by Pijuan et al. (2014) (6% of the ammonium oxidized) which used the same control strategy but treating a reject water at 30 °C, and it was suggested that temperature could be an important factor affecting N_2O emissions.

The effect of temperature on N_2O emissions was never deeply studied since most studies were performed for systems treating reject water, which is characterized by high temperatures (30–35 °C). However, wastewater temperature is a key parameter in the nitrification process which affects to mass transfer, chemical equilibrium and growth rate (Van Hulle et al., 2010), so it could be also an important parameter affecting N_2O emissions. Furthermore, N_2O solubility decreases when temperature increases which affects N_2O stripping from wastewater to gas phase resulting in the enhancement of N_2O gas emissions.

Hence, the objective of the present study was to investigate the effect of temperature on the N_2O gas emissions from a granular sludge airlift reactor performing partial nitritation of a low-strength synthetic influent. Hereto, the reactor was operated at three different temperatures: 10, 15 and 20 °C.

2. Materials and Methods

2.1. Configuration and operation phases of the reactor

A lab-scale granular sludge airlift reactor with a working volume of 1.5 L was used. The downcomer-to-separator diameter ratio was 0.57 and the total length-to-downcomer diameter ratio was 8 (Fig. SI–1 in Supporting Information). pH was controlled and maintained at 8.0 ± 0.1 to rule out any potential effects derived from pH changes. Since the effect of pH on nitritation rates is known to be reduced in the range 7.5–8, a pH set point of 8 was selected, as done in a previous study (Reino et al., 2016). Compressed air was supplied through an air diffuser placed at the bottom of the reactor at a flow rate of 2.9 ± 2 L h^{-1}, 2.4 ± 0.3 L h^{-1} and 4.6 ± 0.7 L h^{-1}, during the operation at 10, 15 and 20 °C, respectively. Gas flow was manually manipulated to maintain a dissolved oxygen (DO) concentration in the bulk liquid of 1.6 ± 0.4 mg O_2 L^{-1}. This DO concentration guaranteed an oxygen to ammonium concentrations ratio (DO/NH_4^+) in the reactor bulk liquid of 0.05 ± 0.03 mg O_2 mg^{-1} N. Low DO/NH_4^+ values in the bulk liquid were reported to repress NOB activity in granular sludge (Isanta et al., 2015). DO monitoring and pH control was done by a biocontroller (ADI 1030, Applikon, The Netherlands). The reactor temperature was controlled by means of a cryostat connected to the jacket of the reactor. Continuous operation of the reactor was divided in four different periods. Period I (days 0–7) corresponded to the start-up period. During start-up temperature was maintained at 10 °C and nitrogen loading rate (NLR) was gradually increased until approximately 0.5 g N L^{-1} d^{-1}. Periods II (days 8–40), III (days 41–58) and IV (days 59–65) corresponded to the stable reactor operation at the different temperatures studied: period II at 10 ºC, period III at 20 ºC and period IV at 15 ºC. During the transition between periods temperature was directly changed and the sequence of temperatures tested was not consecutive (from the lowest temperature to the highest temperature) to minimize the effect of biomass acclimation at high temperature on the N_2O emissions. The metabolism of the nitrifying bacteria can trigger an increase in N_2O emissions as a response to dynamic process conditions, compared to emissions during steady-state (Kampschreur et al., 2008). Thus, we used a period of time of at least 3-5 hydraulic retention times for each condition before temperature tests were performed during several days of operation at each temperature (see section 2.3 for N_2O measurement details).

2.2. Inoculum and influent characteristics

The airlift reactor was inoculated with 1L of granular sludge (approximately 2 g VSS) from a granular sludge reactor operated in the long-term performing partial nitritation of a low-strength synthetic wastewater at 10 ºC (Reino et al., 2016). The operational characteristics of the granular reactor at the moment when the inoculum was withdrawn are shown in Table SI–1 in Supporting Information. The inoculum was highly enriched in ammonia oxidizing bacteria (AOB) with more than 90% of abundance of AOB and 1 ± 1% of NOB (specifically *Nitrobacter* spp.) quantified through fluorescence *in situ* hybridization (FISH). *Nitrospira* spp. and genus *Nitrotoga* were not detected in the inoculum.

The granular sludge airlift reactor was fed with a synthetic influent mimicking the pretreated municipal wastewater coming from the mixture of the effluent of a previous high rate reactor for COD removal plus the recirculation of the reject water of the digested sludge, as in an anammox-based WWTP (Isanta et al., 2015b; Kartal et al., 2010). The resulting influent, from now low-strength synthetic influent, contained in average, 70 mg N-NH_4^+ L^{-1}, 45 mg KH_2PO_4 L^{-1}, 784 mg $NaHCO_3$ L^{-1}, 80 mg NaCl L^{-1}, 40 mg $CaCl_2$ L^{-1}, 90 mg $MgCl_2$ L^{-1} and 1 mL of trace elements solution per L of influent (Guerrero et al., 2011).

2.3. Analytical methods and N_2O measurements

Concentrations of ammonium, nitrite and nitrate in influent and effluent were regularly measured off-line with Dr. Lange test kits (Hach Lange, Germany) in previously filtered (0.22 µm pore) samples. Mixed liquor total suspended solids (TSS) and mixed liquor volatile suspended solids (VSS) were analysed according to Standard Methods (APHA, 2005).

Measurements of N_2O concentration in the off-gas were analysed by means of an online analyser (Emerson Rosemount NGA 2000). Off-gas was collected continuously from the reactor headspace and conducted via a gas tube to the online analyser. A moisture filter was installed at the gas inlet of the analyser and a t-shaped tubing joint was fitted on the gas tube connecting the gas outlet of the reactor and the gas analyser, allowing the excess of gas to escape and thus avoiding overpressure in the line. Data were logged every minute for long periods of time (between 4 and 12 hours of N_2O monitoring). In period II (10 °C), two sets of tests were done: (i) during days 17, 18 and 21 of operation, and (ii) during days 30, 31, 32 and 33 of operation. In period III (20 °C) the N_2O measurements were performed during days 44, 56 and 57. And finally, in period IV (15 °C) measurements were performed during days 63 and 64 of operation.

Measurements of N_2O concentration in the bulk liquid of the airlift reactor were not directly measured, but calculated with mass transfer balances as explained in Supporting Information.

2.4. Calculation of the N_2O production factors

Two different N_2O production factors were calculated. One was based on the total amount of N_2O produced in relation to the total ammonium oxidized to nitrite, and the other one was based on the total amount of N_2O produced in relation to the total ammonium of the influent. The way of calculating the N_2O production factors is important to compare different nitrifying systems since, as explained by Pijuan et al. (2014), the production factor relative to the total ammonium oxidized to nitrite is the most adequate factor to compare the N_2O production when the reactor is oxidizing only a certain fraction of the ammonium load (e.g. either full or partial nitritation).

Moreover, in the present study N_2O production factors were also divided in: N_2O gas emission factors and N_2O liquid production factors, depending on the phase where N_2O was present. The N_2O emitted in the gas phase was quantified with N_2O gas emission factors (EF_{gas}), while the dissolved N_2O in the reactor bulk liquid was quantified with N_2O liquid production factors (PF_{liq}). Finally, a total N_2O production factor (PF_{tot}), comprising production in both gas and liquid phases, was calculated by the sum of the EF_{gas} and PF_{liq}. All the calculations used are described below:

$$PF_{tot} = EF_{gas} + PF_{liq} \qquad \text{(Eq. 1)}$$

$$EF_{gas}(per\ NH_4^+\ oxidized) = \frac{[N - N_2O]_{gas} \times Q_{gas}}{[N - NH_4^+]_{oxidized} \times Q_{influent}} \times 100 \qquad \text{(Eq. 2)}$$

$$PF_{liq}(per\ NH_4^+\ oxidized) = \frac{[N - N_2O]_{liq} \times Q_{influent}}{[N - NH_4^+]_{oxidized} \times Q_{influent}} \times 100 \qquad \text{(Eq. 3)}$$

$$EF_{gas}(per\ NH_4^+\ in\ the\ influent) = \frac{[N-N_2O]_{gas}\times Q_{gas}}{[N-NH_4^+]_{influent}\times Q_{influent}}\times 100 \qquad \text{(Eq. 4)}$$

$$PF_{liq}(per\ NH_4^+\ in\ the\ influent) = \frac{[N-N_2O]_{liq}\times Q_{influent}}{[N-NH_4^+]_{influent}\times Q_{influent}}\times 100 \qquad \text{(Eq. 5)}$$

$$[N-N_2O]_{gas}(mg\ N-N_2O\cdot L^{-1}) = \frac{[N-N_2O](ppmv)\times P(atm)\times 28(g\cdot mol^{-1})}{0.082\left(\frac{atm\cdot l}{mol\cdot K}\right)\times T(K)\times 1000} \qquad \text{(Eq. 6)}$$

$$[N-NH_4^+]_{oxidized}(mg\ N-NH_4^+\cdot L^{-1}) = \left([N-NH_4^+]_{influent}-[N-NH_4^+]_{effluent}\right) \qquad \text{(Eq. 7)}$$

where,

$[N\text{-}N_2O]_{gas}$ is the nitrous oxide concentration in the off-gas in mg N L^{-1}; $[N\text{-}N_2O]_{liq}$ is the nitrous oxide concentration in the bulk liquid in mg N L^{-1}; $[N\text{-}NH_4^+]_{influent}$ is the ammonium concentration in the influent in mg N L^{-1}; Q_{gas} and $Q_{influent}$ are the flow rates of aeration and synthetic influent, respectively, in L d^{-1}; P is the atmospheric pressure and T is the temperature of operation.

2.5. Fluorescence *in situ* hybridization (FISH)

Abundances of AOB and NOB were analysed by FISH technique during the entire operation of the reactor. Hybridizations were performed as described in Supporting Information. Sample slides were observed with an epifluorescence microscope (Axioplan 2; Zeiss), and image acquisition was performed with a Leica D350F camera.

3. Results and Discussion

3.1. Operation of the reactor

The airlift reactor was inoculated with granular sludge from another granular sludge airlift reactor which performed stable partial nitritation of a low-strength synthetic influent for 250 days at 10 ºC (Reino et al., 2016). The operation of the granular sludge airlift reactor in the present study was divided in four periods (Fig. 1). Continuous operation took place from the start (inoculation at day 0) with an initial nitrogen loading rate (NLR) of 0.21 ± 0.03 g N L^{-1} d^{-1} and a temperature of 10 ºC. During period I or start-up period (days 0–7), the NLR was gradually increased until achieving an average NLR of 0.60 ± 0.07 g N L^{-1} d^{-1} in period II (days 8–40). From day 15 onwards the nitrate concentration in the effluent started to increase and nitrite concentration decreased. This meant that nitratation activity developed in the granular sludge airlift reactor despite of maintaining a low DO/N-NH_4^+ concentrations ratio (0.06 ± 0.02 mg O_2 mg^{-1} N during periods I and II) which was previously reported to maintain stable partial nitritation with efficient NOB repression in granular systems (Bartrolí et al., 2010; Isanta et al., 2015; Reino et al., 2016). Table 1 shows the average concentrations of ammonium, nitrite and nitrate in the effluent of the airlift reactor during the different periods of operation. Nitrite and nitrate concentrations stabilized at the end of period II (days 30–40) at 10 °C with effluent values of 7 ± 4 mg N-NO_2^- L^{-1} and 11 ± 3 mg N-NO_3^- L^{-1}, between these days. In period III (days 41–58) temperature was increased until 20 ºC and stable operation was achieved with an average NLR of 0.78 ± 0.10 g N L^{-1} d^{-1}. The concentration of the different nitrogen species in the effluent was maintained stable (Fig. 1B and Table 1), even when temperature was decreased again until 15 ºC in period IV (days 59–65) and the NLR decreased until an average value of 0.72 ± 0.08 g N L^{-1} d^{-1}. Specific ammonium oxidation rate (sAOR) increased from 0.31 ± 0.04 g N g^{-1} VSS d^{-1} in period II (10 ºC) until 0.40 ±

0.06 g N g^{-1} VSS d^{-1} in period III (20 ºC), which was expected since biomass activity increases with temperature.

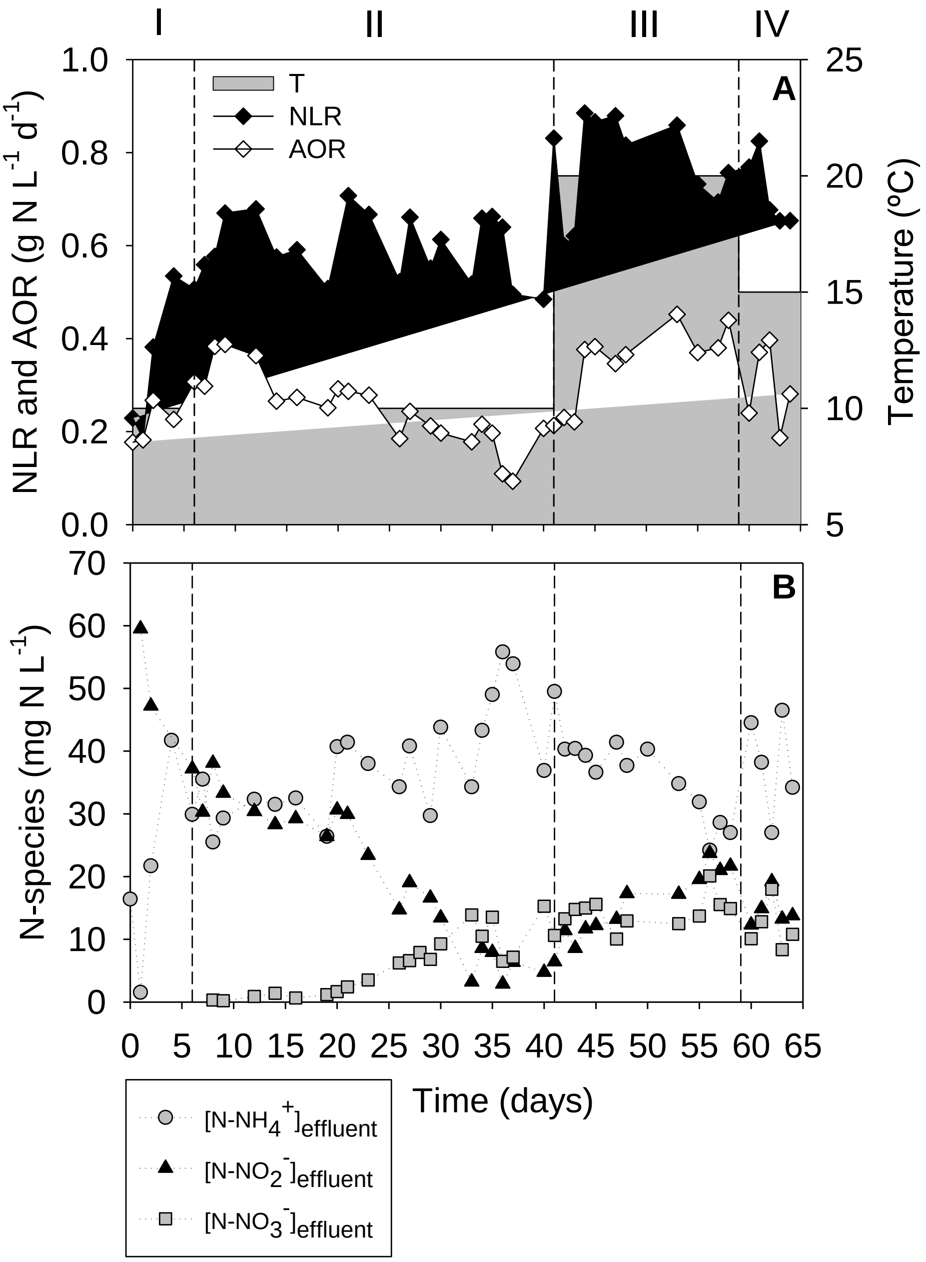

Fig. 1. Continuous operation of the granular sludge airlift reactor treating a low-strength synthetic influent at different temperatures. Operation was divided in four different periods: period I (start-up), period II (operation at 10 °C), period III (operation at 20 °C)

and period IV (operation at 15 °C). (A) Nitrogen Loading Rate (NLR), Ammonium Oxidation Rate (AOR) and temperature (T); (B) Nitrogen compounds concentrations throughout the operation of the granular sludge reactor.

Table 1. Nitrogen loading rate achieved and concentration of the nitrogen species present in the effluent during the different periods of operation of the granular sludge airlift reactor. The low-strength synthetic influent treated contained 66 ± 6 mg $N\text{-}NH_4^+$ L^{-1}. T: temperature; NLR: nitrogen loading rate; n.a.: not analysed.

Period	T (°C)	Gas flow ($L\ h^{-1}$)	NLR ($g\ N\ L^{-1}\ d^{-1}$)	$[N\text{-}NH_4^+]_{eff}$ ($mg\ N\ L^{-1}$)	$[N\text{-}NO_2^-]_{eff}$ ($mg\ N\ L^{-1}$)	$[N\text{-}NO_3^-]_{eff}$ ($mg\ N\ L^{-1}$)
I	10	2.4 ± 0.1	0.4 ± 0.2	25 ± 10	42 ± 10	n.a.
II	10	2.9 ± 0.2	0.60 ± 0.07	38 ± 9	18 ± 10	6 ± 5
III	20	4.6 ± 0.7	0.8 ± 0.1	35 ± 9	15 ± 6	14 ± 3
IV	15	2.4 ± 0.3	0.72 ± 0.08	38 ± 8	15 ± 3	12 ± 4

Biomass concentration in the reactor was maintained stable during the four different periods of operation and resulted in 1.9 ± 0.2 g VSS L^{-1}. Settling properties of the granules were also maintained during the whole operation of the granular sludge airlift reactor, with an average settling velocity of 23 ± 5 m h^{-1} and an average sludge volumetric index (SVI) of 76 ± 4 ml g^{-1} TSS, which are typical values of granular biomass (an image of the nitrifying granules is depicted in Fig. SI–2 in Supporting Information). The hydraulic retention time (HRT) was maintained at 2.4 ± 0.5 h and the solid retention time (SRT) was kept at an average value of 23 ± 10 days.

The measured nitrogen compounds balanced well during the whole operation of the reactor, with an average value of 99 ± 6% detection of the nitrogen load from the influent in the effluent (Fig. SI–3 in Supporting Information). Thus, neither heterotrophic nor autotrophic (anammox process) denitrification was considered to take place in the granular sludge airlift reactor, and consequently the contribution of heterotrophic denitrification to N_2O emissions can be neglected.

3.2. Microbial characterization of the granular sludge

Biomass samples were periodically analysed by FISH technique to identify the nitrifying population present in the granular sludge airlift reactor. Qualitative evaluation of the FISH results (Fig. SI–4 in Supporting Information) indicated that granules were highly enriched in AOB during the entire operation of the reactor, while NOB were barely detected at the beginning but developed during the operation.

NOB were expected to be present in the granules since up to 12 ± 4 mg N-NO_3^- L^{-1} were produced in the reactor at the end of the operation. However, neither genus *Nitrospira* nor *Nitrobacter* spp., which are often the most commonly NOB found in nitrifying sludge (Wagner et al., 2002), were detected in the granules. In contrast, the newly recognized NOB genus *Nitrotoga* (Alawi et al., 2007) was detected in biomass samples from day 15 onwards and its abundance increased during the operation of the reactor (see Fig. SI–4 in Supporting Information). Optimal growth temperature for genus *Nitrotoga* has been reported to be lower than for other NOB species (10ºC, Alawi et al., 2007). Reports also indicated that low temperatures enhance the development of *Nitrotoga* population in engineered ecosystems for water treatment (Alawi et al., 2009; Hüpeden et al., 2016). Recent studies have reported a high abundance of genus *Nitrotoga* in full-scale urban WWTPs in the north of Europe (Lücker et al., 2015; Saunders et al., 2016).The prolonged low temperatures applied in our study could favour the growth of *Nitrotoga* in the granular sludge. Thus, the important role of this novel nitrite oxidizer in nitrifying systems was reaffirmed in the present study.

3.3. Nitrous oxide production

Off-gas was monitored during the periods II, III and IV of operation of the granular sludge airlift reactor in order to calculate the N_2O gas emission factors (EF_{gas}) of the nitrifying sludge treating a low-strength synthetic influent at different temperatures. DO concentration was maintained stable (1.6 ± 0.4 mg O_2 L^{-1}) to avoid a strong influence on the N_2O production as previously reported by Pijuan et al. (2014). Since the granular sludge airlift reactor operated performing partial nitritation, a discussion of the nitrous oxide emission factors based on the N_2O emitted in relation to the total ammonium oxidized by AOB is more appropriate.

Fig. 2 shows the N_2O production factors obtained during the operation of the granular sludge airlift reactor at 10, 15 and 20 ºC. A 2.5-times increase was measured in N_2O gas emissions relative to the oxidized ammonium when temperature increased from 10 °C (EF_{gas} of 1.5 ± 0.3%) to 20 ºC (EF_{gas} of 3.7 ± 0.5%) in period III. It could be argued that after a long period operating at 10 ºC there is an acclimation of the biomass at that temperature, and changing the operation at higher temperature acted as a disturbance triggering higher N_2O emissions. Nevertheless, N_2O gas measurements were performed at the beginning and end of the period III and no diminishment in emissions after operating at 20 °C was observed. In addition, a decrease in N_2O EF_{gas} was observed when temperature decreased again until 15 ºC in period IV (EF_{gas} of 1.5 ± 0.5%), reaching roughly the same value obtained at 10ºC.

As mentioned in section 3.1, the ammonium concentration in the effluent was maintained during N_2O monitoring (37 ± 8 mg N-NH_4^+ L^{-1}), however nitrite and nitrate concentrations changed from day 15 onwards (Fig. 1). It could be thought that the variations of the nitrite and nitrate concentrations in the granular sludge airlift reactor could affect N_2O emissions, however the two sets of measurements on period II were performed at different concentrations of nitrite and nitrate

(29 ± 2 mg N-NO_2^- L^{-1} and 1.5 ± 0.8 mg N-NO_3^- L^{-1} on days 17, 18 and 21 while 11 ± 7 mg N-NO_2^- L^{-1} and 10 ± 4 mg N-NO_3^- L^{-1} on days 30, 31, 32 and 33 of operation) and still they resulted in very similar values (EF_{gas} of 1.5 ± 0.3%, Fig. 2). Furthermore, when the temperature was decreased from 20 to 15 ºC (period IV in Figs. 1 and 2) the emission rates -despite nitrate production- were in agreement with the overall trend, so that the increase in emissions at 20ºC is not linked to the nitrate production.

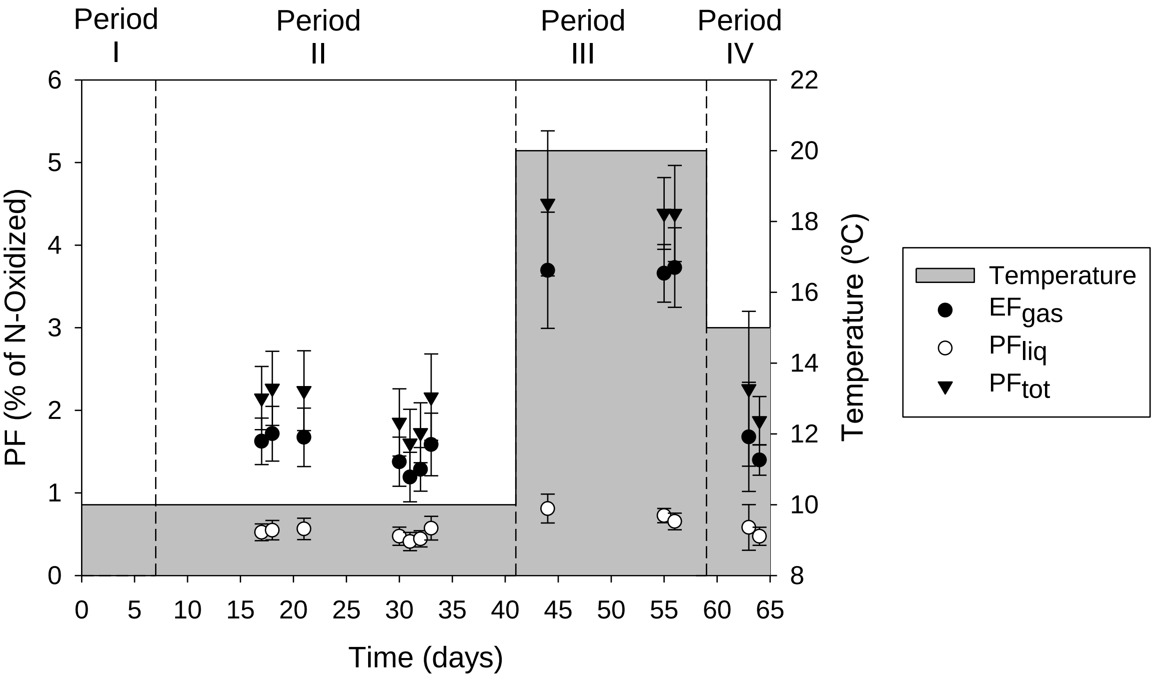

Fig. 2. N_2O production factors relative to the ammonium oxidized to nitrite during the operation of the granular airlift reactor at 10, 15 and 20 °C. EF_{gas}: N_2O gas emission factors; PF_{liq}: N_2O liquid production factors; PF_{tot}: N_2O total production factors.

Hence, a clear increase of N_2O gas emissions was found for temperatures higher than 15 °C. Two reasons could explain this observation: (i) high temperature led to high N_2O production in the granular sludge airlift reactor or (ii) N_2O gas emissions increased because of the increment of stripping of N_2O, since aeration was not constant during the three periods, being the highest aeration at 20 °C.

To assess the overall N_2O production rates at the different temperatures of operation, the N_2O liquid production factors (PF_{liq}) were calculated. Fig. 3 shows the average gas and liquid N_2O production factors at different temperatures, together with the total production factors calculated as the sum of both contributions (liquid and gas). Total N_2O production factors indicated an increase with temperature. PF_{liq} increased when temperature increased despite of N_2O solubility decreases with temperature. N_2O is a very soluble gas (solubility: 1260 mg L^{-1} at 20 °C, (Weiss and Price, 1980)) and, actually, N_2O concentrations in the liquid phase were always higher than in the gas phase, which, in addition, increased when temperature increased (Table 2) despite of N_2O solubility decreases with temperature. Both PF_{liq} and EF_{gas} remained rather constant, with a slight increase from 10 °C to 15 °C although gas flow decreased (gas flow of 2.9 ± 0.2 L h^{-1} at 10°C and 2.4 ± 0.3 L h^{-1} at 15 °C).

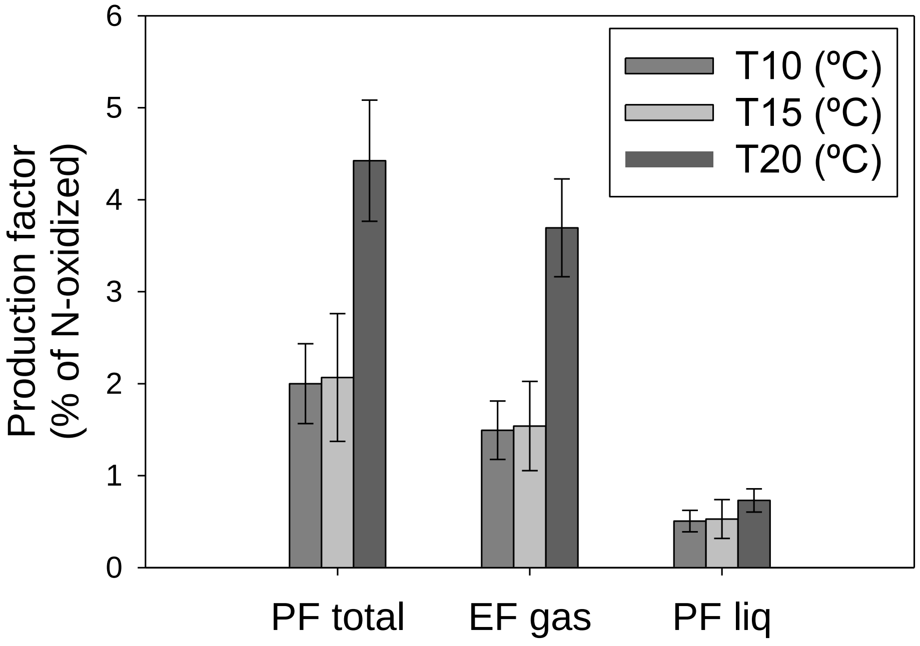

Fig. 3. Average N_2O production factors relative to the total NH_4^+ oxidized to NO_2^- at the different temperatures tested. EF gas: N_2O gas emission factor; PF liq: N_2O liquid production factor; PF total: sum of liquid and gas N_2O production factors; T10, T15 and T20: 10, 15 and 20 °C of temperature, respectively. Error bars represent the standard

deviation of the average values obtained at each temperature.

Table 2. Concentrations of N_2O in the off-gas and the liquid phase during the different periods of operation of the granular sludge airlift reactor. Concentration in the off-gas was directly measured in the reactor headspace, while the concentration in the liquid was calculated as explained in the materials and methods section.

Period	Days	T (°C)	$[N\text{-}N_2O]_{off\text{-}gas}$ (mg L^{-1})	$[N\text{-}N_2O]_{liquid}$ (mg L^{-1})
II	8–40	10	0.08 ± 0.01	0.12 ± 0.02
III	41–58	20	0.18 ± 0.01	0.24 ± 0.03
IV	59–65	15	0.09 ± 0.02	0.11 ± 0.04

Total N_2O production rate at 20 °C was higher than production at lower temperatures and gas phase emissions were highly affected due to the severe stripping occurred. Thus, lower aeration could be proposed to reduce N_2O gas emissions at temperatures higher than 15 °C in the granular sludge reactor. However, nitrifying activity increased with temperature and NLR was increased to maintain the ammonium oxidation throughout the reactor operation. This increase led to high oxygen consumption and, thus, a high aeration was needed to maintain the DO concentration in the granular sludge airlift reactor. If NLR was not increased, more ammonium would be oxidized and effluent characteristics would not be maintained in the reactor. Hence, it was not valid to decrease aeration in this system as a mitigation strategy and the high gas flow needed to maintain the DO concentration, together with the decrease in gas solubility, increased the fraction of N_2O stripped to the gas phase. In any case, in the event that lower aeration flow rates could be used at temperatures higher than 15 °C to avoid the influence of stripping, N_2O gas emissions would be considerably reduced in the gas phase but maintained in the liquid phase, which could transpose the emissions problem to the effluent. Though, the reactor of partial nitritation is not the last reactor before effluent discharge and N_2O could be denitrified in a subsequent reactor resulting in an overall emissions reduction.

The average values of N_2O EF_{gas} obtained for a granular sludge enriched in AOB were in the same order of magnitude of other partial nitritation systems reported before (Table SI–4 in Supporting Information). However, most of the studies available in literature reported EF_{gas} in partial nitritation systems treating high-strength wastewater (e.g. reject water) and little has been published for partial nitritation of low-strength wastewater (e.g. urban wastewater). Overall, bibliographic reports show that nitritation systems treating high-strength wastewater presented higher emission factors than systems treating low-strength influents, although there is a huge variability (Table SI–4 in Supporting Information).

To the best of the authors' knowledge, the effect of temperature on N_2O production in a granular sludge enriched in AOB performing partial nitritation has not been reported before. There are few studies that characterize N_2O emissions from full-scale nitrifying reactors, which present winter and summer campaigns and, thus, high and low temperatures appear as a side parameter. Nevertheless, these studies were not specifically focused on temperature and other side parameters affected the emissions. On the one hand, Bollon et al. (2016) found that PF_{tot} (based on the N-oxidized) from full-scale nitrifying biofilters performing complete nitrification were higher in the winter campaign (PF_{tot} = 4.9 ± 0.5% at 15 °C) than in the summer campaign (PF_{tot} = 2.3 ± 0.5% at 22.5

°C), which contrasts with the results of the present study. However, they suggested that the negative influence of temperature on emissions was not related to the temperature itself, but to the low DO and high nitrite concentrations occurred in winter, which enhance N_2O production. On the other hand, Daelman et al. (2015) reported N_2O emission factors in the long-term operation of a full-scale urban WWTP and did not find any correlation with nitrous oxide emissions and water temperature. In the same way, Ahn et al. (2010) did not directly correlate temperature with N_2O emissions from activated sludge processes of 12 WWTPs across the United States, but expected that emissions were indirectly governed by temperature trough manifestation in ammonium, nitrite or DO concentrations.

3.4. Theoretical implications

The difference in N_2O production rates found at 20, 15 and 10 °C in the reactor suggests that there is a kinetic deactivation of N_2O emissions at low temperatures. In this sense two possible explanations for this observation were hypothesised: (i) the kinetic dependency with temperature of the ammonia monooxygenase (AMO) enzyme catalysing the oxidation of ammonium to hydroxylamine is different than that for the hydroxylamine oxidoreductase (HAO) enzyme catalysing the oxidation of hydroxylamine to nitrite, in such a way that at 20 °C the intermediate hydroxylamine slightly accumulates because the oxidation of hydroxylamine is the limiting step. However, at lower temperature, this situation would be reversed, and the oxidation of hydroxylamine would be faster than the oxidation of ammonia. The decrease in hydroxylamine accumulation could reduce considerably the N_2O emissions because hydroxylamine is the precursor of N_2O in both pathways (hydroxylamine oxidation and nitrifier denitrification) in granular sludge reactors performing partial nitritation (Sabba et al., 2015). Nevertheless, the scarce references found related to the temperature dependence contradict this hypothesis (Tokuyama et al., 2004; Zhang et al., 2015), which would therefore rule out the differential impact of temperature on enzymes activities. (ii) A second possibility to take into account is the temperature dependency of the acid-base equilibrium ammonium-ammonia. The true substrate of AOB is ammonia rather than ammonium (Suzuki et al., 1974). There is an impact of the temperature in the half-saturation coefficient expressed in ammonium concentration units (Suzuki et al., 1974). Since the residual ammonium concentration is kept rather constant among the different temperature tests (37 ± 8 mg $N\text{-}NH_4^+$ L^{-1}), the fraction of ammonia is significantly decreasing with the decrease in temperature at a pH of the bulk liquid of 8 (free ammonia concentration of 0.81, 1.2 and 1.7 mg NH_3 L^{-1} at 10, 15 and 20 °C, respectively). Additionally, a gradient of pH is expected in the biofilm, because ammonia oxidation produces protons (de Beer et al., 1993; Gieseke et al., 2006; Schreiber et al., 2009) which will decrease even more the ammonia concentration in the deeper layers of the granule. With the decrease in temperature if a good fraction of the cells is not saturated in ammonia, the accumulation of hydroxylamine could decrease considerably, and consequently, also the N_2O production, as already discussed.

A third possible explanation which should not be overlooked is the effect of oxygen concentration on the N_2O emissions. It was mentioned before that DO concentration in the bulk liquid was maintained stable during the three periods operating at different temperatures. However, the oxygen penetration into the granules was expected to be different at different temperatures. When the temperature in the reactor decreases, the oxygen penetration depth increases. This could lead to a reduced fraction of AOB in anoxia, which in turn would reduce the N_2O production due to the diminishment of N_2O production from the nitrifier denitrification pathway.

In the present study, the reasons why high temperatures led to high N_2O production in the system remained unclear, although a significant impact of the temperature in the N_2O emissions in a partial nitritation system treating a low-strength synthetic wastewater was demonstrated. Hence, the present study appears as a starting point for further studies investigating the effect of temperature on PN/A systems. The present study is an interesting study to assess the N_2O emissions in a partial nitritation reactor treating a low-strength synthetic influent. Added value comparing to the values obtained in Reino et al. (2016) from grab samples is that online measurements were done at different temperatures for long periods of time (ca. 5–30 HRT) which allowed to avoid both a short-term temperature effect and a substantial change of population).

4. Conclusions

A granular sludge airlift reactor performing partial nitritation at mainstream conditions was operated at high NLR with low N_2O emissions.

The production of N_2O by an enriched nitrifying granular sludge at 10, 15 and 20 ºC was determined and the highest N_2O production rates were observed at the highest temperature.

Temperatures higher than 15 ºC caused an increase of the N_2O gas emissions due to a higher N_2O production rate together with a more severe stripping as a result of the higher aeration requirements.

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