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Importance of abiotic hydroxylamine conversion on nitrous oxide emissions during nitritation of reject water

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Abstract

The spontaneous abiotic (or chemical) reaction of hydroxylamine (NH_2OH) at low concentrations (<0.4 mgN/L) with free nitrous acid (HNO_2) was investigated at the conditions of partial nitritation of reject water. An abiotic batch reactor test was used to quantitatively assess the kinetics of the nitrous oxide (N_2O) emission. The estimated chemical N_2O emission rate was 0.16 mgN/L/h. In addition, the concentration of NH_2OH in a full scale nitritation reactor, Single reactor High Activity ammonium Removal over Nitrite (SHARON) was measured in the range ca. 0.03-0.11 mgN/L. The presence of NH_2OH in the SHARON reactor together with the abiotic N_2O emissions rate (assessed in the abiotic batch reactor test) points towards a significant contribution of the abiotic N_2O emission in the full scale reactor.

An equivalent emission factor (N emitted as N_2O / N oxidized in nitritation) of 1.1% was estimated to be linked to the abiotic pathway, which is around one third of the total measured N_2O emission rate in the SHARON reactor.

Keywords: abiotic N_2O emissions; ammonium-oxidizing bacteria; partial nitrification; sidestream; NH_2OH; chemical reaction.

1. Introduction

Hydroxylamine (NH_2OH) is an intermediate in the biological oxidation of ammonia to nitrite by ammonia-oxidizing bacteria (AOB) and in the dissimilatory nitrate reduction (DNRA) pathway. One of the first experimental indications that NH_2OH is an intermediate in nitrification was reported by Lees [1]. By adding NH_2OH at a very low concentration (1.5 µgN/L) to an aerated *Nitrosomonas* sp. culture, the consumption of NH_2OH in time was measured. At pH of 8.4 and 30°C, the measured NH_2OH consumption rate was found to be even higher than that of ammonium consumption at the same conditions.

In the past, the extracellular release of NH_2OH during the oxidation of ammonia into nitrite by AOB was considered doubtful [2]. However, several publications also pointed out that NH_2OH is a measurable intermediate in the bulk liquid of reactors performing either nitritation (oxidation of ammonium into nitrite) or nitrification (oxidation of ammonium into nitrate)[3-7]. The values reported for the NH_2OH concentration are in the range of 0.01-1 mgN/L. Hydroxylamine is not one of the compounds regularly followed in N_2O emission or nitrification studies neither in laboratory scale reactors nor in full scale wastewater treatment plants (WWTP). Some of these investigations were carried out with the specific goal of clarifying the N_2O emissions pathways, quantification of rates or even setting-up mathematical models for the description of the process (among many others [8-12]). In addition, several mathematical models calibrated with experimental data included NH_2OH as intermediate (for instance Ni et al. [12]), but the measurements were limited to ammonium, nitrite and N_2O, whereas actual NH_2OH concentrations were never measured. Overall, there is a lack of knowledge regarding the formation of NH_2OH in WWTP or laboratory reactors performing nitritation or nitrification.

Hydroxylamine and nitrite are known to be precursors for abiotic (or spontaneous) production of nitric oxide (NO) and nitrous oxide (N_2O) (see a review by Schreiber et al.[13]). Therefore, the presence of significant concentrations of NH_2OH and/or nitrite was postulated as potential environment for the chemical production of NO and N_2O in wastewater or natural aquatic ecosystems [13, 14]. The possible chemical reactions of NH_2OH and nitrite to produce nitrogen oxides are numerous and with a rather complex chemistry. Moreover, the rates can be accelerated by the presence of trace metals (Harper et al., 2015) [13]. The still reduced knowledge on the potential contribution of abiotic pathways on the overall N_2O emissions from nitrite, ammonia and NH_2OH during wastewater treatment has been previously emphasized [13-18]. Schreiber et al. [13] highlighted a few relevant environments in which the chemical production of N_2O could manifest in WWTP, and cited as main example the nitritation of reject water in two-stage nitrogen removal systems (e.g. the Single reactor High Activity ammonium Removal over Nitrite, SHARON rector [19]). In particular, the chemical reaction between the NH_2OH and nitrite (nitrosation of NH_2OH) has been reported to produce N_2O, with overall reaction (1) (Döring and Gehlen, 1961) [20]:

$$NH_2OH+HNO_2 \rightarrow N_2O+2H_2O \tag{1}$$

The precursor of N_2O in reaction 1 is known to be the hyponitrous acid ($H_2N_2O_2$) - the dimer of HNO [20]. More recently, the chemical production of N_2O from NH_2OH and nitrite has also been investigated through site-specific ^{15}N isotopic signatures [18]. However, Heil et al. [18] did not assess the kinetics of N_2O emissions, but rather focused on the isotopic signature of this reaction. They found that microbial and abiotic processes share the same intermediate steps, and therefore it was not possible to use the isotopic signature to assess the contribution of either biological or abiotic N_2O emissions.

The occurrence of reaction 1 during biological nitritation has been already highlighted by Harper et al. (2015). These researchers firstly analyzed the abiotic formation of N_2O and secondly used model fitting in a biological reactor to assess the contribution of the abiotic pathway to the overall N_2O production.

In this study, we investigated the chemical reaction of NH_2OH (at low concentrations) with nitrite and its potential contribution to N_2O emissions in wastewater treatment plants. To this end, some of the experiments targeted specific conditions found in nitritation reactors in two-stage nitrogen removal process applied to reject water (SHARON). This process was selected because: (i) there, the microbial growth rate of AOB is close to the maximum specific growth rate triggering NH_2OH release into the bulk liquid [4], and (ii) the high nitrite concentrations. We complemented this study with NH_2OH measurements in the full scale SHARON reactor in Rotterdam (The Netherlands), to assess the abiotic N_2O emission pathway in that type of nitrogen-converting bioreactors.

2. Materials and Methods

2.1 Analysis of NH_2OH concentration

The NH_2OH concentration was measured following spectrophotometric procedure [21]: 1 mL of the sample containing hydroxylamine (range 0.00 to 0.25 μM of hydroxylamine) was added to 1 mL of 0.05 M phosphate buffer, 0.80 mL of demineralized water, 0.2 mL of 12 wt% trichloroacetic acid, 1 mL of 1% 8-quinolinol (w/v) and 1 mL of 1 M Na_2CO_3. After shaking vigorously the mixture was heated 1 minute at 100ºC in a water bath and cooled for 15 min before measuring absorbance at 705 nm (Novaspec III Amersham Biosciences). A blank was prepared by replacing the sample volume by the same volume of demineralized water.

For the samples withdrawn from the SHARON reactor, a variation of the protocol described was used, since no demineralized water was added, and 1.8 mL of sample were used instead. This was done to increase the absolute value of absorbance of samples. For more details regarding the sampling procedure in the SHARON reactor, see below the specific section 2.4.

2.2 Small-scale reaction tests

2.2.1 Sampling and storage

The NH_2OH reactivity was tested under different conditions in 2 mL Eppendorf tubes. For each test 1.6 mL of medium was mixed with 0.2 mL of a 3.5 mgN/L hydroxylamine solution. Afterwards, 0.2 mL of 10% sulfamic acid was added in some of the tests, as far as it is reacting 1:1 stoichiometrically with nitrite to form $N_{2,}$ H_2SO_4 and H_2O [22]. The NH_2OH concentration was then immediately measured in samples with or without sulfamic acid. After keeping different aliquots overnight at room temperature, 4ºC and -20ºC, with and without sulfamic acid, NH_2OH concentration was also measured.

The mineral medium used contained: 0.330 g/L $NaNO_2$, 0.344 g/L $(NH_4)_2SO_4$, 5mL/L of an iron solution containing 9.14g $FeSO_4$ and 5g EDTA in 1L of demineralized water, 1.25 mL/L of a magnesium solution containing 160 g $MgSO_4 \cdot 7H_2O$ in one litre of demineralized water, 0.625mL/L of a calcium solution containing 240g $CaCl_2 \cdot 2H_2O$ in one litre of demineralized water, and 2.5 mL/L of a trace element solution containing 15g EDTA, 0.43 g $ZnSO_4 \cdot 7H_2O$, 0.24 g $CoCl_2 \cdot 6H_2O$,1.0 g $MnCl_2 \cdot 4H_2O$, 0.25 g $CuSO_4 \cdot 5H_2O$, 0.22 g $(NH_4)Mo_7O_{24} \cdot 4H_2O$, 0.20 g $NiCl_2 \cdot 6H_2O$, 0.10 g $NaSeO_4$, 0.014 g H_3BO_3, 0.050 g $NaWO_4 \cdot 2H_2O$ per litre of Mili-Q water and pH adjusted to 6 with NaOH.

2.2.2 Preliminary characterization of the reaction kinetics

The NH_2OH depletion rates were measured in a set of 2 mL Eppendorf tubes at room temperature (approximately 21ºC). The above mentioned medium was added to a set of 6-7 Eppendorf tubes, then NH_2OH was added to all of them to reach a final volume of 1.8 mL. Finally 0.2mL of 10% sulfamic acid was added to each Eppendorf tube at a different time, in order to stop the reaction (by removing nitrite). Finally, the NH_2OH concentration remaining after different reaction times was measured. For the sample at time zero, sulfamic acid was added first followed by the medium, in order to remove nitrite before NH_2OH addition. The pH was measured before adding HA using a pH meter (827 pH lab Methrom Swiss made).

2.3 Reactor set up for the chemical reaction and gas analysis

Experiments were conducted in a 1.5L lab-scale glass fermenter, equipped with a Teflon-coated magnetic stirrer. Air was added using a gas-diffuser with the flow rates 0.316 and 0.214 L min^{-1} for batch reactor test 1 and 2, respectively. Off-gas was measured on-line (Servomex 4900 infrared gas analyzer), allowing to follow continuously oxygen, carbon dioxide, N_2O and NO. Temperature was controlled with an external jacket and pH was followed, using a pH meter in samples without sulfamic acid, but not controlled, because it was stable.

Reaction mixture contained 1.9 mL and 3.8 mL of trace element and iron solutions, respectively, both described previously. 150 mL of a 3.5 mgN/L NH_2OH solution was added to demineralized water containing the desired amount of metals, reaching a total liquid volume of 1.5 L, finally solid $NaNO_2$ was added to reach desired concentration in each test (Table 1). Samples were withdrawn from the reactor at different times using a syringe and immediately mixed with 0.2 mL of 0.1g/mL sulfamic acid. Hydroxylamine concentration was followed in time in the samples using the procedure mentioned in section 2.1.

2.4 Measurements of NH_2OH concentration in a SHARON reactor

Samples from the SHARON reactor in Sluisjesdijk-Dokhaven (Rotterdam, the Netherlands) were withdrawn directly from the bulk liquid at the WWTP. Following the protocol described in sections 2.1 and 2.2, 1mL of 0.1 g/mL of sulfamic acid was added immediately after filtration of the collected sample through cotton and 0.45mm filter and analyzed in less than 2 hours. A fraction of the sample was not mixed with the sulfamic acid solution to let the NH_2OH react completely with nitrite and therefore quantify the background absorbance of the supernatant.

The SHARON reactor contains flocculent sludge and the process conditions are summarized in Table 2.

2.5 Calculations

From acid-base equilibria the HNO_2 (free nitrous acid, FNA, in mgN/L) concentration can be estimated for a given nitrite concentration (TNN, in mgN/L), pH and temperature (T) (Anthonisen et al., 1976)

$$FNA = \frac{TNN}{10^{pH} \cdot \exp\left(\frac{-2300}{273+T}\right)+1} \cdot \frac{14}{47} \tag{2}$$

The total amount of N_2O produced (m_{N2O}, g N) was calculated through integration in time of the curve of N_2O emission. The conversion of NH_2OH into N_2O ($\chi_{NH2OH/N2O}$, %) can be calculated by using the initial NH_2OH concentration ($C_{NH2OH,ini}$, gN/L), the stoichiometry in reaction 1 and the total volume of reaction (V, L):

$$\chi_{NH_2OH/N_2O} = m_{N_2O} \frac{1g\ NH_2OH - N\ reacted}{2g\ N_2O - N\ produced} \cdot \frac{1}{C_{NH2OH,ini} \cdot V} \tag{3}$$

3. Results and Discussion

3.1 Fast screening of NH_2OH reactivity in Eppendorf tubes

Since NH_2OH is known to be a very reactive short-lived compound, some preliminary tests were carried out to determine the best strategy for sample storage. To simulate a likely medium composition, the tests were carried out by using a synthetic wastewater containing ammonium, nitrite, metals and trace elements. To prevent the oxidation of NH_2OH, the addition of sulfamic acid was tested in order to remove nitrite as nitrogen gas. Nitrite and NH_2OH can react [20] , and the acid reduces the pH increasing the stability of NH_2OH [23, 24]. In fact, removal of nitrite is known to improve the hydroxylamine analysis [24]. When NH_2OH was determined through the colorimetric method immediately after the preparation of the solution (i.e. mixing the NH_2OH standard solution with the synthetic wastewater) the concentration matched the theoretical expected value (0.14±0.01 mgN/L, Fig.1). When repeating the same procedure but including the addition of sulfamic acid, the NH_2OH concentration was not significantly different (0.14±0.02 mgN/L, Fig. 1). Therefore, the sulfamic acid addition did not alter significantly the results of the analysis.

When sulfamic acid was not added, the NH_2OH was found to disappear completely when the sample was kept overnight either at room temperature, at 4ºC or frozen at -20ºC. When sulfamic acid was added, the NH_2OH concentration was rather stable overnight, showing less than 10% decrease (Fig. 1). Storage at 4ºC after addition of the sulfamic acid produced only a slight improvement (Fig. 1). Storage at -20ºC was found to be not applicable because of a 50% decrease in the concentration (Fig. 1).

It was clear that after addition of sulfamic acid, the NH_2OH was stable for several hours, therefore the addition of sulfamic acid was adopted as a preliminary step for sample preservation. Nevertheless, the NH_2OH stability could be linked to either (i) the pH of the solution (i.e., sulfamic acid solution has a very low pH, so that the sample pH dropped from

6.76 to 1.35 after the addition of sulfamic acid), (ii) the absence of nitrite, or (iii) a combination of both.

3.2 Characterizing the extent of N_2O emissions through chemical reaction

A reactor vessel was used to study the coupling of the conversion of NH_2OH and nitrite with the formation of NO and N_2O. Two different experiments were carried out.

In the first experiment, the reaction vessel contained initially NH_2OH at a concentration of ca. 0.35 mgN/L, pH 6.2 and at 20 ºC with the synthetic wastewater containing chelated iron and trace elements, but without nitrite. The reactor was aerated at 0.316 L min^{-1}. Hydroxylamine remained rather constant for more than 200 min and no significant NO or N_2O emissions were recorded (Fig. 2A). Therefore this indicates how NH_2OH cannot disappear at a significant rate if nitrite is not present.

After 220 min, $NaNO_2$ was added to reach a nitrite concentration of 135 mgN/L in the reactor vessel. A decrease in the NH_2OH concentration was immediately recorded and N_2O was simultaneously emitted, as detected through the gas analyzer (Fig. 2A). No nitric oxide (NO) emission was noticeable. Hydroxylamine decreased after nitrite addition at a rate of 0.20±0.02 mgN/L/h (Fig. 2A, Table 1). The maximum N_2O emission rate was ca. 0.2 mgN/h (Fig. 2A). Therefore this indicates that NH_2OH and nitrite react together following reaction 1, as expected.

In a second experiment, the reaction was carried out in the conditions commonly applied in SHARON nitritation bioreactors treating reject water (T=30ºC, pH=7, concentration of nitrite 650 mgN/L, [25]) (see Fig. 2B). The reactor was aerated at 0.214 L min^{-1}. In this case, the initial abiotic reaction mixture contained synthetic wastewater, including nitrite and chelated iron, but without hydroxylamine. Neither N_2O (Fig. 2B) nor NO emissions were detected. This showed how no significant reduction of nitrite into either NO or N_2O was happening.

After 68 minutes a pulse of NH_2OH was added to reach a final concentration of 0.3 mgN/L, which resulted in immediate N_2O emission (but not NO) and decrease in NH_2OH concentration with an average conversion rate of ca. 0.27 ± 0.07 mgN/L/h and a maximum N_2O emission rate at ca. 0.25 mgN/h was maintained for at least 50 minutes. The initial NH_2OH concentration (ca. 0.3 mgN/L) was selected because a similar concentration was measured in a chemostat culture of *Nitrosomonas* sp. (Jiang et al., 2015).

These experiments showed that only when both NH_2OH and nitrite were present, in the conditions tested, one of the reaction products was N_2O (as expected from reaction 1).

The measured N_2O emission rate was integrated in time to determine the reaction yield of N_2O from NH_2OH. Theoretically, the overall reaction would require equimolecular amounts of NH_2OH and HNO_2 (see reaction 1). The experimental results indicated a conversion of 22% and 41% of removed NH_2OH into N_2O in the first and second experiment, respectively. However, for the first experiment, the % of NH_2OH converted is underestimated, since we did not record the complete curve of N_2O emission. The low recovery of N_2O indicates that a fraction of the reacted NH_2OH resulted in a different reaction product. Some of the NH_2OH might have reacted with one of the intermediates of reaction 1 (HNO, the monomer of hyponitrous acid) to produce N_2 [13, 26]:

$$NH_2OH+HNO \rightarrow N_2+2H_2O \qquad (4)$$

Although the presence of HNO was not verified in the course of this research, reaction 4 could explain the gap in the nitrogen mass balances. The influence of alternative reactions of NH_2OH (e.g., disproportionation, autoxidation, oxidation by Fe(III),[13]) is likely very limited at the conditions tested, because before the addition of nitrite in the first experiment, the NH_2OH concentration was stable for more than two hours (Fig. 2A). The same reasoning would apply for the potential nitrite reactivity to produce N_2O (e.g., nitrite reduction by Fe (II),[17]) since in the second experiment no N_2O emissions were noticeable until NH_2OH was added (Fig. 2B).

Given the measured N_2O emission rate from reaction 1, in case NH_2OH is leaking to the bulk liquid in the SHARON reactor, the contribution of the abiotic pathway to the total N_2O emission rate would be relevant. Therefore, the presence of NH_2OH in the SHARON reactor was also investigated (see section 3.3).

A complete characterization of the effects of pH and temperature on the abiotic N_2O emission rate (i.e. the reaction kinetics) would be desirable to clarify the contribution of the abiotic pathway in other nitritation systems used in wastewater treatment.

3.3 Free nitrous acid: the limiting compound

Due to the variability found for the rate of conversion of NH_2OH, the effects of pH and temperature on the conversions were measured. Table 1 and Fig. 3 give the conversion rates determined as a function of the initial free nitrous acid (HNO_2) concentration. The results indicate that the rate of reaction of NH_2OH with nitrite to produce N_2O is in fact limited by the HNO_2 concentration. Temperature and pH were not followed in the Eppendorf tube tests, making it difficult to directly link the rate to an initial HNO_2 concentration. However for the wide range of pH (4.3-7.6) tested the positive influence of lower pH on the reaction was clear.

Within the tested range of conditions, more acidic pH values would enhance the N_2O emission when NH_2OH and nitrite are present in WWTP reactors. Higher nitrite concentrations in the wastewater would mean higher HNO_2 concentrations (at a given pH and temperature), which in turn would result in higher N_2O emissions due to the chemical reaction between NH_2OH and HNO_2. Although a pH of 7 was used here to mimic the SHARON reactor conditions, often the reactor operates at lower pH values. For example, in the pH range 6.6-6.7, as reported by Mampaey et al.[27],higher reaction rates would be expected.

It is important to stress that in those tests the temperature was the same (21ºC), the concentration of nitrite was the same (55±2 mgN/L) as well as initial NH_2OH concentration (0.31±0.05 mgN/L). Therefore, the change in pH was the single parameter affecting reaction rate. This was measured as NH_2OH depletion rate, which ranged from 0.02 mgN/L/h (at pH 7.6) to 0.6 mgN/L/h (at pH=4.3) (Table 1).

In fact these results (Table 1) are in full agreement with findings by Döring and Gehlen [20], in which the reaction rate depended more strongly on the HNO_2 concentration than on the NH_2OH concentration. The doubling of reaction rate when increasing temperature from 20 to 30°C found by Döring and Gehlen could explain the increase in NH_2OH depletion rate between the second and the first batch reactor tests (Table 1).

Table 1. Influence of conditions (pH and temperature) and free nitrous acid (HNO_2) concentration in the NH_2OH reaction rate.* A phosphate buffer solution was added.

Eppendorf tube test No.	pH	T (°C)	NH_2OH (mgN/L)	Nitrite (mg N/L)	HNO_2 (mgN/L)	NH_2OH depletion rate (mgN/L/h)
1	4.3	21	0.27	54	1.8	0.56±0.09
2	5.1	21	0.35	56	0.3	0.29±0.03
3	7.6*	21	0.30	54	0.001	0.02±0.02
Batch reactor						

test No.						
1	6.2	20	0.35	135	0.065	0.20±0.02
2	7.0*	30	0.30	650	0.038	0.27±0.07

3.4 Hydroxylamine presence in a full scale SHARON reactor

The presence of NH_2OH in large scale biological nitritation reactors would allow the abiotic N_2O formation. The sampling campaign at the SHARON reactor in Rotterdam (five measurements during January and June 2015) indicated the presence of NH_2OH in the range of 0.03-0.11 mgN/L (average 0.06 mgN/L) during the aerated phase of operation. The standard deviation of the determinations based on triplicates was for all samples below 15 %, and for most of them lower than 5%. Notably, in the range of NH_2OH concentrations measured in the SHARON reactor, the abiotic tests still indicated a high N_2O emission rate (see Fig. 2B). However, the measured absorbance of the sample background was in average 76 % of that of the sample containing NH_2OH. Given the high absorbance of the sample background, a more precise analytical method would be desirable to confirm the absolute values of NH_2OH concentration here reported.

In addition to our measurements in the SHARON reactor, residual NH_2OH concentration in the bulk liquid has been reported by several researchers in similar conditions to those in the SHARON reactor [3, 4, 6, 7]. Low NH_2OH concentrations are still compatible with significant N_2O production (and emission) rates through the abiotic reaction between NH_2OH and nitrite (i.e., reaction 1). In many occasions, reaction intermediates can be at low concentrations but the reaction still proceeds. For instance, nitrite is usually not measured at high concentrations in WWTP, however nitrification of ammonium into nitrate is known to be happening.

Hydroxylamine released to the bulk liquid was detected when the growth rate of batch nitrifying cultures was close to the maximum [3, 4]. For example, hydroxylamine concentration was measured in the range of 0.03-0.06 mgN/L, during the exponential growth in batch culture of *Nitrosomonas* sp [4]. Therefore, in the SHARON reactor performing partial nitritation at high specific growth rate, NH_2OH release into the bulk liquid would be expected. In that sense, the NH_2OH concentration in the flocculent sludge could locally be even higher than that measured in the bulk liquid, depending on the biomass density and the diameter of the flocs (Sabba et al., 2015). Additionally, Jiang et al. [6] measured sustained levels of NH_2OH in the bulk liquid of a *Nitrosomonas* sp. chemostat culture, in the range 0.2-0.3 mgN/L. The decrease in availability of inorganic carbon (e.g. from 40 times to 4 times excess) was reported to trigger increasing NH_2OH levels [6]. The alkalinity levels of reject water (typically correlated to inorganic carbon) are not enough as to allow oxidation of all ammonium to nitrite [25]. Therefore, in SHARON reactors the availability of inorganic carbon is reduced (although no to the level to limit the partial nitritation), which might also be a reason why sustained NH_2OH levels were detected in the bulk liquid. Also Ma et al. [7] measured concentrations of NH_2OH in the range 0.09 and 0.15 mgN/L for a pilot scale partial nitritation / anammox reactor, with a bulk ammonium concentration of ca. 10-15 mgN/L. In summary, there are several reports of NH_2OH detected at relevant concentrations in nitritation reactors, all in agreement with the results found in the present study.

The residual NH_2OH concentration in the bulk liquid will be stablished as the balance between the NH_2OH leakage rate by AOB, and the amount of NH_2OH reacting to produce N_2O (either by abiotic or biotic routes). This is why we stress that the measured NH_2OH is just the *residual* concentration stablished in the reactor, as a result of the balances between these rates: NH_2OH leakage and (abiotic and biotic) N_2O production.

3.5 Comparing chemical with biological N_2O emission rates

To put the observed abiotic conversion into the context of overall N_2O emissions from nitritation, a direct comparison to reported cases has been carried out (Table 2). Since the SHARON reactor was monitored in the past [28] this was the first reference case. Nevertheless, given the abundance of data reported in the literature, other key cases have been used to compare and discuss the significance of chemical N_2O emissions.

The N_2O emissions rates found through the chemical reaction of NH_2OH and HNO_2 are in the same order of magnitude with those reported in the SHARON reactor and in similar type of biological lab-scale reactors (Table 2). This strengthened the idea that the contribution of the chemical pathway in the SHARON reactor is of major importance (as hypothesized by Schreiber et al. [13]). The rate estimated in the second batch reactor test (Fig. 2b) was 0.16 mgN/L/h, i.e. an N_2O emission factor (N emitted as N_2O / N oxidized in nitritation) of 1.1% of the converted ammonium under the conditions in the SHARON reactor.

Following to our assessment of the rate of N_2O emission by the abiotic pathway and given the N_2O emissions reported for the SHARON reactor (Table 2), we conclude that, in those conditions, both routes, the abiotic and biotic pathways, contribute significantly. Which is in agreement with the conclusions in Harper et al. (2015), who also assessed N_2O emissions in partial nitrification reactors as of hybrid origin, with contribution of both the abiotic and the biotic routes.

The direct comparison of our estimated rate for the chemical reaction with those reported for the biological conversion in an AOB batch reactor in Law et al.[8] at high nitrite (400-1000 mgN/L) and high ammonium (500 mgN/L) concentrations showed very similar values (Table 2). Although that study initially considered the possible abiotic production of N_2O, this was in the end ruled out based on an abiotic test with 1 $mgNH_2OH$-N/L and nitrite concentrations in the range (0-1000 mgN/L). However, the conditions of that test (pH and temperature) were not detailed and results were not shown. Therefore their chemical test cannot be directly compared with our results.

Wunderlin et al. [9] checked the chemical production of N_2O with NH_2OH and nitrite in a solution prepared with tap water. They assessed the abiotic contribution as very reduced, which agrees with our results since the concentration of HNO_2 was much lower in those conditions (Table 2). However, the use of tap water could make a difference from our experiments, in which Fe(II) together with other metals were added at a known concentrations.

In addition, the chemical nitrite reduction by ferrous iron Fe(II) was reported to be a relevant N_2O emission pathway in a full scale Anammox reactor [17]. Iron is present in the reactor influent as Fe(III)-precipitate due to dosage of $FeCl_3$ in earlier stages of the wastewater treatment plant [17]. The production of Fe(II) necessary for chemical N_2O formation through the nitrite reduction is thought to be mediated by biological activity. In a laboratory reactor mimicking the conditions of an anammox reactor, with Fe(III) and formate added, the Fe(III) reduction rate was 19 µmol/g DW/h [17]. Despite Fe(II) presence in the medium (Table 1, Figs. 2&3), this pathway could be neglected in our abiotic tests because no significant NO or N_2O emissions were measured before the addition of NH_2OH in the batch test No. 2 (Fig. 2B). Moreover, the Fe(II) is expected to be quickly oxidized in the aerobic conditions used.

Our results agree with those reported by Harper et al. (2015): the abiotic reaction might be overlooked when calibrating N_2O emission models (e.g. Ni et al., 2014; among others) and therefore the potential contribution of this pathway remains unclear for many biological systems in which partial nitritation is taking place. In addition, we would like to point out that the effects of pH and temperature regulating the concentration of HNO_2 (free nitrous acid) and the residual NH_2OH concentration in the bulk liquid are discussed and measured in this contribution. The concentrations of NH_2OH (up to 20 mgN/L) used in Harper et al (2015) are doubtful to be present in biological systems, and their model fitting used to describe the biological tests has as a main weak point that NH_2OH was added to those high concentrations but its concentration was never measured.

Table 2. Comparing conditions and emission rates of several biological reactors with abiotic tests. *measured in this study. AOB = ammonia-oxidizing bacteria.

Reactor (reference)	pH	T (°C)	NO_2^- (mgN/L)	HNO_2 (mgN/L)	Ammonium (mgN/L)	NH_2OH (mgN/L)	N_2O emission rate (mgN/L/h)
SHARON [28]	7	30	650	0.04	650	0.06*	0.47
AOB, batch [8]	7	33	400-1000	0.02-0.05	500	?	0.13
Batch, abiotic test [8]	Not described	Not described	0-1000	?	0	1	0? / not changing
Batch, abiotic test [9]	7	20	10	0.0008	0	10	0.03
Batch, abiotic test (this study)	7	30	650	0.04	0	0.3	0.16

3.6 Practical implications for the SHARON reactor

Given the dependence of the reaction rate on pH, N_2O emissions from the chemical pathway here described could be mitigated by increasing the pH. However, increasing the pH may have some consequences to the operation of the reactor. In the SHARON reactor nitrate production is voided by keeping a dilution rate higher than the maximum specific growth rate of nitrite-oxidizing bacteria [19]. The reactor is operated with alternating aerobic-anoxic periods in such a way that a mean aerobic retention time of 1.35 days is established [27], regardless the influent flow rate. Since no pH control is applied and the alkalinity is limited, the ammonium oxidation rate is reduced by the rather low pH. Addition of base to increase the pH will therefore produce an effluent with a lower ratio NH_4^+/NO_2^-, which would not be convenient for the subsequent Anammox reactor. One possibility would be to reduce the length of the aerated cycles, to decrease the ammonium oxidation rate achieved at a higher pH. In turn, the overall anoxic period will be longer and this may induce higher N_2O emissions coming from the nitrite reduction induced by the iron present in the reactor [17]. Although the chemical nitrite reduction to N_2O was indicated as significant in the Anammox reactor and mediated by the Anammox capacity to reduce iron, when anoxic conditions are imposed in the SHARON reactor, it is unknown if this pathway would also be of relevance.

For new installations, designing the SHARON reactor at a slightly higher (controlled) pH of operation (i.e. assuming a higher ammonium oxidation rate) should result in a lower N_2O emission rate from the chemical reaction between NH_2OH and nitrite. Further research would be required to find out whether an optimal pH set point could be found in which N_2O emissions would be minimized.

4. Conclusions

- A significant production of N_2O from the abiotic reaction between NH_2OH and HNO_2 was measured at the process conditions of partial nitritation of reject water in two-stage N-removal treatments.
- Therefore the N_2O emissions in those treatments are of hybrid origin, coming from biotic and abiotic routes.

- The abiotic N_2O emission rate measured was 0.16 mgN/L/h at T=30°C, pH=7 and nitrite in excess (650 mgN/L), which are typical operating conditions reported for the SHARON reactor.
- An average residual NH_2OH concentration of 0.06 mgN/L was measured in the SHARON reactor, which supports that the N_2O emissions in that installation are produced from biotic and abiotic routes.

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FIGURE CAPTIONS

Figure 1. Preliminary tests establishing the sampling procedure at 0.14 mgN/L NH_2OH in the samples: RT=room temperature; I=instantaneous measurement; O=overnight measurement; Fr=fridge storage; Fe=freezer storage; SA=sulfamic acid addition.

Figure 2. Chemical reaction of NH_2OH and nitrite in a 1.5 L batch reactor. Results of the linearization are shown in Table 1, as batch reactor tests 1&2. **A.** Conditions used were T=20°C, pH=6.2, nitrite was added at time 220 min at a concentration of 135 mgN/L. Air flow-rate 0.316 L min^{-1}. **B.** Conditions used were T=30°C, pH=7, nitrite concentration 650 mgN/L (already in the reaction vessel at time zero) and NH_2OH added at time 68 min. Air flow-rate 0.214 L min^{-1}.

Figure 3. Eppendorf tube tests for determination of the NH_2OH depletion rate at different pH conditions.