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

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## HIGHLIGHTS

- Abiotic reaction of $NH_2OH$ with $HNO_2$ can contribute to $N_2O$ emissions in nitritation.
- The abiotic $N_2O$ emission was measured at the conditions of sidestream nitritation.
- Residual $NH_2OH$ concentration was measured in a full scale nitritation reactor.

## ARTICLE INFO

## ABSTRACT

The spontaneous abiotic (or chemical) reaction of hydroxylamine ($NH_2OH$) at low concentrations (<0.4 mg N/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 mg N/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 mg N/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.

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## 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 μg N/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 mg N/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 (WWTPs). 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

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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 [13,15]. 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 [13–19]. 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 [20]). 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) [21]:

$$NH_2OH + HNO_2 \rightarrow N_2O + 2H_2O \qquad (1)$$

The precursor of $N_2O$ in reaction 1 is known to be the hyponitrous acid ($H_2N_2O_2$) – the dimer of HNO [21]. More recently, the chemical production of $N_2O$ from $NH_2OH$ and nitrite has also been investigated through site-specific $^{15}N$ isotopic signatures [19]. However, Heil et al. [19] 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. [15]. 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 [23]: 1 mL of the sample containing hydroxylamine (range 0.00–0.25 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 min at 100 °C 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 mg N/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 $H_2O$ [24]. The $NH_2OH$ concentration was then immediately measured in samples with or without sulfamic acid. After keeping different aliquots overnight at room temperature, 4 °C and −20 °C, 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 $(NH_4)_2SO_4$, 5 mL/L of an iron solution containing 9.14 g $FeSO_4$ and 5 g EDTA in 1 L of demineralized water, 1.25 mL/L of a magnesium solution containing 160 g $MgSO_4 \cdot 7H_2O$ in 1 L of demineralized water, 0.625 mL/L of a calcium solution containing 240 g $CaCl_2 \cdot 2H_2O$ in 1 L of demineralized water, and 2.5 mL/L of a trace element solution containing 15 g 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 liter 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 21 °C). 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 0.2 mL 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 1.5 L 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 tests 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 mg N/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