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**Abiotic nitrous oxide (N₂O) production is strongly pH dependent, but
contributes little to overall N₂O emissions in biological nitrogen
removal systems**

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ABSTRACT

Hydroxylamine (NH_2OH) and nitrite (NO_2^-), intermediates during the nitrification process, can engage in chemical (abiotic) reactions that lead to nitrous oxide (N_2O) generation. Here, we quantify the kinetics and stoichiometry of the relevant abiotic reactions in a series of batch tests under different and relevant conditions, including pH, absence/presence of oxygen, and reactant concentrations. The highest N_2O production rates were measured from NH_2OH reaction with HNO_2 , followed by HNO_2 reduction by Fe^{2+} , NH_2OH oxidation by Fe^{3+} , and finally NH_2OH disproportionation plus oxidation by O_2 . Compared to other examined factors, pH had the strongest effect on N_2O formation rates. Acidic pH enhanced N_2O production from the reaction of NH_2OH with HNO_2 indicating that HNO_2 instead of NO_2^- was the reactant. In departure from previous studies, we estimate that abiotic N_2O production contributes little ($< 3\%$ of total N_2O production) to total N_2O emissions in typical nitrification reactor systems between pH 6.5 and 8. Abiotic contributions would only become important at acidic pH (≤ 5). In consideration of pH effects on both abiotic and biotic N_2O production pathways, circumneutral pH set-points are suggested to minimize overall N_2O emissions from nitrification systems.

Keywords: Nitrous oxide; Abiotic reaction; pH; Nitrous acid; Hydroxylamine

INTRODUCTION

Rising atmospheric concentrations of nitrous oxide (N_2O) contribute to global warming and the destruction of stratospheric ozone ¹. Wastewater treatment plants (WWTPs) are point sources for N_2O emissions, which are associated with biological nitrogen removal (BNR) ². In recent years, BNR processes that involve nitrification (aerobic ammonium (NH_4^+) oxidation to nitrite (NO_2^-)), anammox (anaerobic NH_4^+ oxidation with NO_2^- to dinitrogen gas (N_2)), or a combination of partial nitrification plus anammox (PNA) are being implemented as energy and resource-efficient alternatives ³. Compared to traditional nitrification and denitrification, these new BNR processes display lower energy consumption and high resource utilization efficiency ⁴. However, the potential for high N_2O emissions from nitrification reactors may offset the claimed environmental benefits ⁵⁻⁹. N_2O emissions are positively associated with characteristics of operational conditions of nitrification systems, such as low dissolved oxygen (DO) and high NO_2^- concentrations ^{6,10-12}.

Ammonia oxidizing bacteria (AOB) are considered the major contributors to N_2O production, especially in nitrification or PNA systems ^{11,13}. N_2O is produced by AOB as a side product in incomplete oxidation of hydroxylamine (NH_2OH) or via the nitrifier denitrification pathway ¹⁴. In addition, during the nitrification processes, reactive intermediates, like NH_2OH , are enzymatically produced. These intermediates may engage in chemical reactions that yield N_2O , especially in the presence of trace metals (e.g. Fe) ¹⁵. In most previous studies on N_2O production in BNR reactors, abiotic reactions were ignored or deemed unimportant due to the low environmental concentrations, high reactivity or short life-times of reactive nitrogen intermediates ¹⁶. As these intermediates, especially NH_2OH , are only formed in the presence of microbial activity (and ongoing nitrification); biotic and abiotic processes are tightly linked and their individual contributions are difficult to unravel ¹⁶, as recently highlighted by Soler-Jofra et al. and Terada et al. ¹⁷⁻¹⁹.

The five dominant chemical reactions that yield N₂O, relevant under environmental conditions of nitrification reactors, are ²⁰:

(1) The oxidation of NH₂OH by HNO₂ ²¹:

(2) The oxidation of NH₂OH by O₂ ²²:

(3) The disproportionation of NH₂OH ²³:

(4) The reduction of HNO₂ by Fe²⁺ ²⁴:

(5) The oxidation of NH₂OH by Fe³⁺ ²⁵:

Factors known to regulate biological N₂O production, like pH and reactant availability, would also affect abiotic N₂O production rates. pH would influence the speciation of several reactants (HNO₂, NH₂OH, Fe²⁺/Fe³⁺), and acidic pH has been suggested to enhance abiotic N₂O emissions ^{17,18,24,26}. However, the rates of the abiotic N₂O yielding reactions are poorly investigated, and hence their contributions to total (biotic + abiotic) N₂O production are highly uncertain ²⁰. Reports on abiotic N₂O production rates vary widely and the comparison between studies is difficult ^{18,19,24,26,27}. For instance, the N₂O emissions rates through abiotic reaction of NH₂OH with NO₂⁻ were estimated at 0.0057 mM/h (pH = 7) under conditions similar to those observed in a SHARON reactor (i.e., without biomass but at consistent NH₂OH and NO₂⁻ concentrations of 0.02 mM and 46.4 mM, respectively) ¹⁸, while others measured 10- to 100-fold higher abiotic N₂O production rates of 0.05-0.9 mM/h (pH = 7) in the presence and absence of AOB-enriched biomass (at NH₂OH and NO₂⁻ concentrations of 0.07-1.4 mM and 28.6 mM, respectively) ¹⁹. Additionally, kinetic parameters of

abiotic reactions were not estimated in previous abiotic studies on nitrification systems due to incomplete data point under limited range of experimental conditions^{17-19,27}. To better assess the contribution of abiotic reactions to N₂O emissions, nitrogen mass balance and reaction rate constants (*k*) should be developed.

The main objectives of this study were, therefore, to carefully examine N₂O production rates of relevant abiotic reactions (Eq.1-5) and infer reaction kinetics, in the absence of biological reactions and with specific attention to the effect of pH (4-9). Using the estimated reaction rate kinetics, we assessed the contribution of abiotic reactions to overall N₂O emissions from nitrification processes as studied by us and others^{18,19,27}. Overall, we conclude that the quantitative contribution of abiotic reactions to N₂O production under nitrification reactor conditions is smaller than previously estimated.

MATERIALS AND METHODS

Experiments

Conditions similar to those encountered in biological nitrification reactors were applied in batch tests (without biomass) to assess N₂O production rates through a series of chemical reactions (Supporting Information (SI), Table S1). Abiotic batch tests were conducted in a jacketed glass vessel with working volume of 0.4 L at room temperature (24-26°C) under high DO (8-8.4 mg O₂/L) or low DO (< 1 mg O₂/L) conditions. To examine the effect of reactor medium on N₂O production, we performed experiments in deionized water (diH₂O) as well as in a typical synthetic medium in nitrification studies²⁸. The synthetic medium consisted of 169.7 mg/L KH₂PO₄, 751.1 mg/L MgSO₄·7H₂O, 451.6 mg/L CaCl₂·2H₂O, 5 mg/L EDTA, 5 mg/L FeSO₄·7H₂O and a trace element solution including 0.43 mg/L ZnSO₄·7 H₂O, 0.24 mg/L CoCl₂·6H₂O, 0.99 mg/L MnCl₂·4H₂O, 0.25 mg/L CuSO₄·5H₂O, 0.22 mg/L NaMoO₄·2H₂O, 0.19 mg/L NiCl₂·6H₂O and 0.21 mg/L NaSeO₄·10H₂O²⁹. The diH₂O or medium was saturated with nitrogen gas or air, and adjusted to

target pH before each test. The vessel was completely filled with diH₂O or medium and sealed with the insertion of sensors and rubber stoppers. Substrates were then spiked into the vessel to initiate abiotic reactions after sensor signals had stabilized. Samples were collected periodically for chemical analysis (i.e. NO₂⁻, NH₂OH, Fe²⁺ and Fe³⁺). The headspace in the vessel increased (from 0 L) to maximum 0.022 L at the end of the experiment. During experiments, pH was controlled by manually adding 0.5 M HCl and 0.5 M NaHCO₃, and continuous mixing was provided with a magnetic stirrer at 100 rpm.

Two experimental scenarios were used: in Scenario 1, we performed parallel tests at fixed initial pH (pH = 4, 5, 6, 7, 8 and 9) and fixed initial substrate concentrations (17.8 mM NO₂⁻, 0.07 mM NH₂OH, 0.5 mM FeSO₄ and 0.1 mM FeCl₃); in Scenario 2, we performed tests with certain initial concentrations that were subject to stepwise changes (increase in reactants, decrease in pH) by sequential spiking of reactants and acid. Further experimental details are listed in SI Table S1.

Offline chemical analysis and pH, DO and N₂O monitoring

Abiotic tests were conducted without biomass in deionized water or the synthetic medium. NO₂⁻ concentrations were analyzed colorimetrically by Merck kits. NH₂OH was determined spectrophotometrically³⁰ and sulfamic acid was added immediately after sampling to prevent the reaction of NH₂OH with NO₂⁻ (SI Section 1). The modified ferrozine method was applied to sequentially determine concentrations of Fe²⁺ and Fe³⁺, where Fe³⁺ is reduced to Fe²⁺ by NH₂OH under strongly acidic conditions³¹. pH and DO were monitored continuously (WTW GmbH, Weilheim, Germany) with measured limit of detection of DO sensor at 0.02 mg O₂/L. Liquid N₂O was measured online by N₂O-R Clark-type microsensors (UNISENSE A/S, Århus, Denmark) with limit of detection of 0.1 µM and data logged every 30s. The pH sensitivity of the N₂O sensor was measured below 0.2% of the signal, and neither DO levels nor stirring intensity interfered with the

signal (SI Section 2). The response times of N₂O, pH, and DO sensors were < 30s, ≤ 45s, and ≤ 45s, respectively.

Calculations

Free nitrous acid (FNA) concentrations (mM) were calculated as:

$$\text{HNO}_2 = \frac{10^{-\text{pH}} \cdot [\text{NO}_2^- - \text{N}]}{14 \cdot 10^{-\text{pK}_a}} \quad (\text{Eq.6})$$

Where pK_a is the dissociation equilibrium constant of HNO₂ corrected for temperature (pK_a (T) = pK_a (298) + 0.0324 (T-298) (pK_a 3.25 at 298 K)). The NH₂OH depletion rate (r_{NH₂OH}), the Fe²⁺ depletion rate (r_{Fe²⁺}) and the N₂O production rate (r_{N₂O}) (mM/min or mM/h) were estimated from the slope of the measured concentration profiles of Fe²⁺, NH₂OH and N₂O (mM) (n>3), respectively. The total amount of NH₂OH (m_{NH₂OH}, mmol) or Fe²⁺ consumed (m_{Fe²⁺}, mmol) and N₂O produced (m_{N₂O}, mmol) were calculated through integration of the depletion/production profiles multiplied by the working volume of the vessel (0.4 L). The total N₂O production could be calculated based on liquid phase measurements because the liquid-gas transfer of N₂O was minimal due to low stirring intensity and the low head-space volume (0.022 L maximum). Based on Henry's law, the maximum [N₂O_{gaseous}]:[N₂O_{liquid}] molar ratio was 1:419 (H = 0.025 M/atm at 25 °C ³²), resulting in maximum 0.2% of N₂O partitioned in the gaseous phase. The r_{N₂O} and m_{N₂O} of NH₂OH oxidation by HNO₂ and Fe³⁺, respectively, were estimated after correction by subtraction of N₂O production by NH₂OH disproportionation and/or oxidation by O₂, since we assume that the latter reactions co-occur simultaneously with other reactions (Table S2). The N₂O yield relative to the amount of NH₂OH or Fe²⁺ oxidized (X_{N₂O/NH₂OH} or X_{N₂O/Fe²⁺}, %) was calculated through the following equations:

The oxidation of NH₂OH by HNO₂:

$$X_{\text{N}_2\text{O}/\text{NH}_2\text{OH}} = \frac{m_{\text{N}_2\text{O}}}{m_{\text{NH}_2\text{OH}}} \cdot 100 \% \quad (\text{Eq.7})$$

The oxidation of NH₂OH by O₂:

$$X_{\text{N}_2\text{O}/\text{NH}_2\text{OH}} = \frac{m_{\text{N}_2\text{O}} \cdot 2}{m_{\text{NH}_2\text{OH}}} \cdot 100 \% \quad (\text{Eq.8})$$

The disproportionation of NH_2OH :

$$X_{\text{N}_2\text{O}/\text{NH}_2\text{OH}} = \frac{m_{\text{N}_2\text{O}} \cdot 4}{m_{\text{NH}_2\text{OH}}} \cdot 100 \% \quad (\text{Eq.9})$$

The reduction of HNO_2 by Fe^{2+} :

$$X_{\text{N}_2\text{O}/\text{Fe}^{2+}} = \frac{m_{\text{N}_2\text{O}} \cdot 4}{m_{\text{Fe}^{2+}}} \cdot 100 \% \quad (\text{Eq.10})$$

The oxidation of NH_2OH by Fe^{3+} :

$$X_{\text{N}_2\text{O}/\text{NH}_2\text{OH}} = \frac{m_{\text{N}_2\text{O}} \cdot 2}{m_{\text{NH}_2\text{OH}}} \cdot 100 \% \quad (\text{Eq.11})$$

Assuming elementary reaction kinetics and stoichiometry as expressed in Eq.1-5, we estimated N_2O production rate constants using the following rate equations for the different reactions:

The disproportionation of NH_2OH :

$$r_{\text{N}_2\text{O}} = k_1 \cdot [\text{NH}_2\text{OH}]^4 \quad (\text{Eq.12})$$

The oxidation of NH_2OH by O_2 :

$$r_{\text{N}_2\text{O}} = k_2 \cdot [\text{NH}_2\text{OH}]^2 \quad (\text{Eq.13})$$

The oxidation of NH_2OH by HNO_2 :

$$r_{\text{N}_2\text{O}} = k_3 \cdot [\text{HNO}_2] \cdot [\text{NH}_2\text{OH}] \quad (\text{Eq.14})$$

Estimates for reaction rate constants ($\text{L}/\text{mmol}/\text{h}$ or $\text{L}^3/\text{mmol}^3/\text{h}$) were obtained by fitting experimental data to Eq.12-14 by minimizing the normalized root mean square error (NRMSE).

The correlation between reaction rate constants and pH was achieved by nonlinear regression fitting.

The best-fit values were found with the solver function in Excel. Data reported in the literature

^{17,18,24,26,27} were extracted using WebPlotDigitizer (<https://apps.automeris.io/wpd/>), and processed

mathematically as mentioned above.

RESULTS

NH₂OH disproportionation and/or oxidation by O₂

After the addition of NH₂OH solution to diH₂O or medium, both NH₂OH depletion and N₂O production were continuously monitored (SI Figure S1). At initial NH₂OH concentration of 0.07 mM, the maximum $r_{\text{NH}_2\text{OH}}$ (0.0073 mM/h) and $r_{\text{N}_2\text{O}}$ (0.0020 mM/h) occurred at pH = 8 in synthetic medium under high DO conditions (Scenario 1) (Figure 1, SI Figure S1, Table S4). $r_{\text{NH}_2\text{OH}}$ and $r_{\text{N}_2\text{O}}$ in synthetic medium were 2-22 times higher than in diH₂O, indicating that trace concentrations of dissolved metals (e.g. Fe²⁺/Fe³⁺, Cu²⁺, Mn²⁺) accelerated NH₂OH decomposition to N₂O, either by direct participation as reactants or by acting as catalysts^{22,33-35}. While it is not possible to isolate NH₂OH disproportionation from NH₂OH oxidation by O₂ under the examined DO conditions, O₂ had a limited stimulatory effect on N₂O production both in diH₂O or synthetic medium (Figure 1-A). For instance, the $r_{\text{N}_2\text{O}}$ at pH = 8 in diH₂O only increased by 5% under high DO (8-8.4 mg O₂/L) compared to that under low DO (< 1 mg O₂/L) (Figure 1). Furthermore, the N₂O yield (relative to the amount of NH₂OH oxidized) calculated following Eq.8 and 9 was $41 \pm 14\%$ (data not shown) and $82 \pm 28\%$ (Figure 1-C), respectively, indicating that the reactions followed the stoichiometry of NH₂OH disproportionation. Hence, NH₂OH disproportionation was more important than NH₂OH oxidation by O₂. Further measurements of NH₄⁺ production from NH₂OH disproportionation (Eq.3) would help to separate these two reactions. The N₂O yield relative to the amount of NH₂OH did not vary substantially with pH (Figure 1-C).

NH₂OH oxidation by HNO₂

The reaction of NH₂OH with HNO₂ was followed by adding NO₂⁻ to the vessel with an initial NH₂OH concentration of 0.07 mM (Scenario 1). NH₂OH was depleted at a rate of 0.00022-0.39 mM/h after addition of NO₂⁻, with a strong dependency on the HNO₂ concentration (Figure 2). At excess NO₂⁻ concentrations (≥ 13.0 mM), HNO₂ concentrations ranged from 0.0002 to 0.9 mM

191 depending on pH (4.5-8). HNO_2 concentrations remained nearly constant and were unlikely to limit
192 the reaction. N_2O production initiated when both NH_2OH and NO_2^- were spiked, and ceased with
193 depletion of NH_2OH (Figure 2). The $r_{\text{N}_2\text{O}}$ ranged from 0.00014 to 0.78 mM/h at different pH set-
194 points, DO levels and medium types (Figure 3-A). Assuming elementary reaction kinetics (Eq.1),
195 based on the measured NH_2OH and HNO_2 concentrations, k values were estimated in the range of
196 0.92-56 L/mmol/h, with higher rates at lower pH (Figure 3-A).

197 pH significantly affected N_2O formation: the N_2O production rate increased four orders of
198 magnitude, with a consistent (almost four log) decrease in pH (Scenario 1) (Figure 3-A). Also, in
199 the sequential acid addition (Scenario 2), sequential pH drops led to a rapid N_2O production, with
200 $r_{\text{N}_2\text{O}}$ at pH = 6 being more than two orders of magnitude higher than at pH = 8.5 (Figure 2-B). The
201 results indicate that HNO_2 instead of NO_2^- is the actual reactant. Expressing the reaction rate as a
202 function of HNO_2 showed that the reaction rate constant increased slightly with pH decrease ($k =$
203 $8272.5e^{-1.1\text{pH}}$, $R^2 = 0.99$; Figure 3-A). Similar to $r_{\text{N}_2\text{O}}$, $r_{\text{NH}_2\text{OH}}$ significantly increased with decreasing
204 pH, which was ~400 times higher at pH = 4.5 than at pH = 8 (Figure 3-B). Presence/absence of
205 oxygen or medium type had limited effect on either NH_2OH depletion or N_2O formation. The
206 influence of the reactant ($\text{NH}_2\text{OH}/\text{HNO}_2$) concentration on the reaction kinetics was outweighed by
207 the pH effect (Figure 2-B). The N_2O yield (relative to the amount of NH_2OH oxidized) increased
208 from 35% at pH = 8 to nearly 200% at pH = 4.5, suggesting different reaction mechanisms (Figure
209 3-C).

210 **Iron-mediated reduction of HNO_2**

211 After NO_2^- addition, Fe^{2+} was oxidized to Fe^{3+} at a constant rate coupled with N_2O production. Fe^{2+}
212 was depleted at a rate of 0.28 ± 0.04 mM/h, while Fe^{3+} accumulated at a nearly equimolecular rate
213 of 0.29 ± 0.02 mM/h (pH = 4.5) (Eq.4) (Scenario 1) (SI Figure S2-A). The $r_{\text{Fe}^{2+}}$ was two-fold higher
214 than $r_{\text{N}_2\text{O}}$ and N_2O yield relative to the amount of oxidized Fe^{2+} was up to 100%, indicating that

Fe²⁺ reacted with HNO₂ following the stoichiometry of Eq.4 (SI Figure S3). However, at pH = 6 and 8, the ratio between $r_{\text{Fe}^{2+}}$ and $r_{\text{Fe}^{3+}}$ was higher than 1:1 (data not shown), and the N₂O yields from oxidized Fe²⁺ were lower than 50%. This is likely due to Fe²⁺ oxidation by O₂ (0.02-0.5 mg O₂/L) to form Fe³⁺ as precipitate and oxyhydroxide or Fe²⁺ oxidation coupled with HNO₂ reduction to ammonium, which can occur under neutral or alkaline pH ³⁶. Both $r_{\text{Fe}^{2+}}$ and $r_{\text{N}_2\text{O}}$ were strongly dependent on pH but not on Fe²⁺ or NO₂⁻: Fe²⁺ depletion and N₂O production increased steeply when HCl was spiked into the vessel and there were less significant responses to increasing concentrations of NO₂⁻ and Fe²⁺ (SI Figure S2-B).

NH₂OH oxidation by Fe³⁺

The reaction of Fe³⁺ with NH₂OH was only tested under pH = 4.3 due to the formation of precipitates and iron oxyhydroxide species at alkaline pH, which would have resulted in lower rates (Scenario 1). NH₂OH and Fe³⁺ were depleted at rates of 0.003 and 0.005 mM/h, respectively, resulting in production rates of N₂O and Fe²⁺ of 0.001 and 0.005 mM/h, respectively (SI Figure S4). The reacted Fe³⁺ and NH₂OH followed the stoichiometry of Eq.5. The N₂O yield relative to the amount of NH₂OH oxidized was 66% at pH = 4.3.

DISCUSSION

pH as the key factor influencing abiotic N₂O production rates

pH has a significant effect on abiotic N₂O reaction kinetics in the range studied (pH = 4-9) in the presence of HNO₂, NH₂OH and iron (Fe²⁺ and Fe³⁺) (Figure 1-3, SI Figure S1-4). pH is known to change the speciation of NO₂⁻ (by equilibrium with HNO₂), NH₂OH (by equilibrium with NH₃OH⁺) and iron (by formation of different precipitates and iron oxyhydroxide species). Previous abiotic N₂O studies in nitrification systems could not elucidate whether NO₂⁻ or HNO₂ was the actual reactive species: Soler-Jofra et al. (2016) suggested that the N₂O production through NH₂OH

238 oxidation was limited by the concentration of HNO_2 ¹⁸, whereas others regarded NO_2^- as the
239 reactant ^{19,24,27}. In our experiments, stepwise dosing of NO_2^- at constant pH did not stimulate N_2O
240 yield significantly, while sharp N_2O peaks were observed after pH drops that shifted the NO_2^-
241 speciation to HNO_2 (Figure 2-B), indicating HNO_2 instead of NO_2^- as the actual reactant. Combined
242 with the observed dependence of the reaction rate constant on pH ($k = 8272.5e^{-1.1\text{pH}}$, $R^2 = 0.99$),
243 acidic pH enhances N_2O production both by an increase in reaction rate constant and HNO_2
244 speciation. With respect to NH_2OH disproportionation and oxidation by O_2 , higher NH_2OH
245 depletion rates and N_2O production rates were achieved at higher pH.

246 pH also affects the product conversion ratios of chemical reactions (Figure 3-C). We observed a
247 conversion of $35 \pm 9\%$ and $174 \pm 19\%$ of oxidized NH_2OH into N_2O at $\text{pH} \geq 7$ and at $\text{pH} < 7$,
248 respectively, indicating that side reactions may occur at different pH levels. The low recovery of
249 N_2O at $\text{pH} \geq 7$ was in agreement with findings by Soler-Jofra et al. (2018, 2016), in which the
250 conversion ratio of NH_2OH to N_2O ranged from $20 \pm 1\%$ to $40 \pm 2\%$ at $\text{pH} = 7.5 \pm 0.1$ ^{17,18}. The
251 authors attributed this to the presence of a side reaction between NH_2OH and HNO (the monomer
252 of hyponitrous acid, one intermediate of reaction Eq.1) with N_2 as the final product. The higher
253 recovery of N_2O over theoretical value (100%) at acidic pH has not been reported. Since NH_2OH
254 was completely oxidized at the end of experiments, the gap in the nitrogen mass balances cannot be
255 explained by equimolecular use of NH_2OH and HNO_2 . Yet, N_2O could not be detected in the sole
256 presence of HNO_2 (data not shown). The recovered excess N_2O is suspected to be due to a higher
257 stoichiometry in HNO_2 , which has been reported to increase above 1 and can approach 2 under
258 acidic conditions ($\text{pH} = 2$) ³⁷. Transient N_2O peaks were observed immediately after acid additions
259 (Figure 2-B), making it difficult to estimate $r_{\text{N}_2\text{O}}$. Considering low sensitivities of the N_2O sensor
260 towards changes in pH, oxygen and stirring intensity (SI Section 2), the observation of transient
261 N_2O peaks is unlikely caused by uneven mixing, a transient response of the N_2O sensor, or signal

interference by pH changes. The determination of abiotic N₂O production rates during sequential acid additions would require further investigation.

Reaction mechanisms and proposed reaction kinetics

The kinetics and mechanisms of the oxidation of NH₂OH by HNO₂ have been investigated under acidic conditions down to pH = 1³⁷⁻⁴¹. The reaction is believed to occur by an initial O-nitrosation, which leads to the formation of ON·NH₂·OH⁺³⁷. Then ON·NH₂·OH⁺ would readily tautomerise to a mixture of cis- and trans-hyponitrous acids, where cis-hyponitrous acids would decompose rapidly to N₂O and water, leaving a small amount of the stable trans-form³⁷⁻³⁹.

The rate equation has been reported as $r_{N_2O} = k \cdot [NO_2^-] \cdot [NH_2OH]$ or $k \cdot [HNO_2] \cdot [NH_2OH]$ or $k \cdot [H^+] \cdot [HNO_2] \cdot [NH_2OH]$ ^{21,27,37,39,40}, and the rate constant has been shown to depend on pH^{37,40}. For instance, Bennett et al. (1982) observed that *k* values rose with increasing H⁺ but decreased at 2 M (pH = -0.3)⁴⁰. The dependence of *k* value on pH was suggested to be due to a change in the rate-determining step from the nitrosation step that converts the NO⁺ group to ON·NH₂·OH⁺⁴⁰. Alternatively, it could be understood in terms of the effect of pH on the decomposition or rearrangement of ON·NH₂·OH⁺^{37,38}. Our observation of decreasing *k* values at more alkaline pH agrees with Bennett et al. (1982) as the pH range tested (4-9) was far above -0.3 (Figure 3)⁴⁰.

To obtain the best description of the experimental data, different rate equations were compared (SI Table S3). The commonly applied equation - $r_{N_2O} = k \cdot [NO_2^-] \cdot [NH_2OH]$ - presented the largest error with NRMSE 8-34 times higher than the other four rate equations, while expressing *k* as a function of pH and considering HNO₂ as reactant provides the best experimental data fit.

Comparison of reported abiotic and biotic N₂O production

Most studies that have examined abiotic N₂O production did not monitor NH₂OH concentrations and consider the nitrogen mass balance (SI Table S4). The variable N₂O yield relative to the amount

285 of NH_2OH oxidized observed in this study (e.g. 24-192% for NH_2OH oxidation by HNO_2) clearly
286 indicated the presence of side reactions (Figure 3-C).

287 The effect of pH on abiotic N_2O production was examined by Soler-Jofra et al. (2016) and
288 Kampschreur et al. (2011)^{18,24}. Soler-Jofra et al. (2016) observed that NH_2OH depletion rates
289 during reaction with HNO_2 increased at lower pH (4.3-7.6), yet N_2O production rates were not
290 monitored during the tests¹⁸. Here, we comprehensively quantified the effect of pH on N_2O
291 production rates and kinetics of the oxidation of NH_2OH by HNO_2 by continuously following
292 changes of nitrogen species. In contrast to Kampschreur et al. (2011)²⁴ who did not find a clear
293 correlation between pH and N_2O production, we observed that N_2O production from the reduction
294 of HNO_2 by Fe^{2+} was significantly stimulated at acidic pH.

295 In a separate study we quantified N_2O emissions from a nitrification reactor from pH 6.5 to 8.5 and
296 observed that the specific net N_2O production rates and the fractional N_2O yield increased seven-
297 fold from pH = 6.5 to 8, and decreased slightly with further pH increase to 8.5 ($p < 0.05$)⁴². The
298 results were consistent with previous studies: Law et al. (2011) showed that the specific N_2O
299 production rate increased with pH to the maximum at pH = 8 in the investigated pH range of 6.0-8.5
300⁴³, while Rathnayake et al. (2015) reported highest N_2O emission at pH = 7.5 in a PN reactor (pH =
301 6.5-8.5)⁴⁴. Abiotic $r_{\text{N}_2\text{O}}$ in the reactor was estimated from NH_2OH oxidation by HNO_2 because its
302 $r_{\text{N}_2\text{O}}$ was 1-3 orders of magnitude higher compared to other abiotic reactions. Further investigations
303 are required to completely quantify the reaction kinetics of NH_2OH oxidation by Fe^{3+} , and HNO_2^-
304 reduction by Fe^{2+} , and the corresponding contributions to N_2O in nitrification systems. Based on the
305 estimated reaction rate constants (Figure 1, 3, SI Table S4) and the measured NH_2OH and HNO_2
306 concentrations during reactor operation, abiotic N_2O production rates were estimated at different pH
307 considering the oxidation of NH_2OH by HNO_2 and NH_2OH disproportionation plus oxidation by O_2
308 (Table 1). The estimated abiotic $r_{\text{N}_2\text{O}}$ values were 1-5 orders of magnitude lower than the total $r_{\text{N}_2\text{O}}$

value measured in the reactor across the examined pH range. The abiotic contributions accounted for less than 3% of total N₂O production and varied with pH, increasing from 0.025% at pH = 8 to 2.6% at pH = 6.5. Abiotic contributions below 3% of total N₂O production here are consistent with reported proportions of NH₄⁺ converted to N₂O ($\leq 0.12\%$) via extracellular abiotic NH₂OH conversion in pure AOB cultures ²⁶. In contrast, in other studies ^{17–19,27}, both abiotic and biotic routes were suggested to contribute in a comparable degree to N₂O emissions (at pH = 7) (Table 1). For example, Soler-Jofra et al. (2016) concluded that abiotic r_{N₂O} (0.006 mM/h) (measured without biomass) was of the same order of magnitude as total r_{N₂O} (0.017 mM/h) in a nitrification reactor ¹⁸, while Terada et al. (2017) and Harper et al. (2015) indicated abiotic hybrid N₂O production as a dominant pathway in a PN reactor, accounting for approximately 51% of the total N₂O production ^{19,27}. However, Soler-Jofra et al. (2016) performed off-line abiotic tests with initial NH₂OH concentration of 0.02 mM and initial HNO₂ concentration of 0.012 mM to estimate an r_{N₂O} of 0.006 mM/h and did not determine *k* values. This initial r_{N₂O} was used to estimate the abiotic r_{N₂O} in a nitrification reactor despite of a lower NH₂OH in the reactor (0.0043 mM vs 0.02 mM) ¹⁸. The only *k* value (0.049 L/mmol/h) reported in Harper et al. (2015) was expressed with NO₂⁻ as reactant ²⁷, which we suggest as incorrect. Based on the estimated *k* values in this study and the reported experimental conditions, the reported abiotic r_{N₂O} ^{18,19,27} were recalculated and estimated to be 1-2 orders of magnitude lower than those originally reported (Table 1). Hence, we contend that the significance of abiotic N₂O production has been overestimated in previous studies.

Practical implications for nitrogen removal systems

The highest N₂O production rates were measured for NH₂OH oxidation by HNO₂, followed by HNO₂ reduction by Fe²⁺, NH₂OH oxidation by Fe³⁺, and finally NH₂OH disproportionation and/or oxidation by O₂. Typical NH₂OH concentrations measured in nitrification reactors vary from 0.002-0.007 mM ^{18,45}. Compared to low NO₂⁻ concentrations (≤ 0.07 mM) in reactors treating typical

domestic wastewaters ⁴⁶, NO_2^- can reach up to 50 mM in nitrification reactors treating high strength wastewaters ⁴⁷. Hence, abiotic $r_{\text{N}_2\text{O}}$ in nitrification (side-stream) reactors could be 1-3 orders of magnitude higher than in typical (main-stream) treatment reactors (SI Figure S5). Avoiding accumulation of NO_2^- as well as HNO_2 could reduce N_2O production via chemical reactions; such is possible by operating reactors at low NH_4^+ removal rates. In addition, iron mediated reduction of HNO_2 and oxidation of NH_2OH can also contribute quantitatively to abiotic N_2O production in nitrification systems. Hence minimizing iron dosage in earlier stages of WWTPs can prevent N_2O emissions from chemical iron oxidation or reduction.

By applying the estimated abiotic reaction kinetic coefficients, abiotic $r_{\text{N}_2\text{O}}$ in other nitrification systems (pH = 7-8) was estimated to be 1-3 orders of magnitude lower than the reported total $r_{\text{N}_2\text{O}}$ (SI Table S5). The estimated abiotic contribution ranged from 0.07 to 2.31% of total N_2O production, consistent with the observations in our nitrification reactor (SI Table S5). Furthermore, abiotic N_2O production rates would decrease but biotic N_2O production rates would be enhanced at increasing pH (6.5–8.0). In summary, N_2O production in nitrification systems is dominated by biotic pathways but abiotic pathways become important under extremely acidic pH (≤ 5). Therefore, N_2O emissions from nitrification reactors are minimized at circum-neutral pH, considering the effect of pH on both abiotic and biotic N_2O production pathways. In addition, the estimated reaction kinetics for biologically-driven abiotic N_2O production from the reaction of NH_2OH and NO_2^- can easily be incorporated into already established N_2O models to estimate abiotic contributions under other wastewater treatment applications ⁴⁸.

To the best of our knowledge, this is the first study that comprehensively quantifies N_2O production by dominant biotic reactions under environmental conditions relevant to nitrification bioreactors, a representative modern day BNR technology. The contribution of chemical reactions to N_2O emissions appears to have been overestimated in recent studies on nitrification systems. Correct

quantification of abiotic reaction kinetics and careful consideration of pH effects are required to assess the role of abiotic N₂O production in BNR systems.

ASSOCIATED CONTENT

Supporting Information

List of Figure S1-S6, Table S1-S5 and Section 1-2.

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Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) IPCC. *Climate Change 2013: The Physical Science Basis*; Cambridge, United Kingdom and New York, NY, USA, 2013.
- (2) Kampschreur, M. J.; Temmink, H.; Kleerebezem, R.; Jetten, M. S. M.; van Loosdrecht, M. C. M. Nitrous oxide emission during wastewater treatment. *Water Res.* **2009**, *43* (17), 4093–4103.
- (3) Siegrist, H.; Salzgeber, D.; Eugster, J.; Joss, A. Anammox brings WWTP closer to energy autarky due to increased biogas production and reduced aeration energy for N-removal. *Water Sci. Technol.* **2008**, *57* (3), 383.
- (4) Kartal, B.; Kuenen, J. G.; van Loosdrecht, M. C. M. Sewage Treatment with Anammox. *Science*. **2010**, *328* (5979), 702–703.
- (5) Gao, K.; Zhao, J.; Ge, G.; Ding, X.; Wang, S.; Li, X.; Yu, Y. Effect of ammonium concentration on N₂O emission during autotrophic nitrification under oxygen-limited conditions. *Environ. Eng. Sci.* **2017**, *34* (2), 96–102.

- (6) Mampaey, K. E.; De Kreuk, M. K.; van Dongen, U. G. J. M.; van Loosdrecht, M. C. M.; Volcke, E. I. P. Identifying N₂O formation and emissions from a full-scale partial nitrification reactor. *Water Res.* **2016**, *88*, 575–585.
- (7) Desloover, J.; De Clippeleir, H.; Boeckx, P.; Du Laing, G.; Colson, J.; Verstraete, W.; Vlaeminck, S. E. Floc-based sequential partial nitrification and anammox at full scale with contrasting N₂O emissions. *Water Res.* **2011**, *45* (9), 2811–2821.
- (8) Kong, Q.; Liang, S.; Zhang, J.; Xie, H.; Miao, M.; Tian, L. N₂O emission in a partial nitrification system: Dynamic emission characteristics and the ammonium-oxidizing bacteria community. *Bioresour. Technol.* **2013**, *127*, 400–406.
- (9) Lv, Y.; Ju, K.; Wang, L.; Chen, X.; Miao, R.; Zhang, X. Effect of pH on nitrous oxide production and emissions from a partial nitrification reactor under oxygen-limited conditions. *Process Biochem.* **2016**, *51* (6), 765–771.
- (10) Peng, L.; Ni, B.-J.; Ye, L.; Yuan, Z. The combined effect of dissolved oxygen and nitrite on N₂O production by ammonia oxidizing bacteria in an enriched nitrifying sludge. *Water Res.* **2015**, *73* (2), 29–36.
- (11) Kampschreur, M. J.; Tan, N. C. G.; Kleerebezem, R.; Picioreanu, C.; Jetten, M. S. M.; Van Loosdrecht, M. C. M. Effect of dynamic process conditions on nitrogen oxides emission from a nitrifying culture. *Environ. Sci. Technol.* **2008**, *42* (2), 429–435.
- (12) Tallec, G.; Garnier, J.; Billen, G.; Gossailles, M. Nitrous oxide emissions from secondary activated sludge in nitrifying conditions of urban wastewater treatment plants: Effect of oxygenation level. *Water Res.* **2006**, *40* (15), 2972–2980.
- (13) Law, Y.; Ye, L.; Pan, Y.; Yuan, Z. Nitrous oxide emissions from wastewater treatment processes. *Philos. Trans. R. Soc. B Biol. Sci.* **2012**, *367* (1593), 1265–1277.
- (14) Wrage-Mönnig, N.; Horn, M. A.; Well, R.; Müller, C.; Velthof, G.; Oenema, O. The role of nitrifier denitrification in the production of nitrous oxide revisited. *Soil Biol. Biochem.* **2018**, *123*, A3–A16.
- (15) Heil, J.; Vereecken, H.; Brüggemann, N. A review of chemical reactions of nitrification intermediates and their role in nitrogen cycling and nitrogen trace gas formation in soil. *Eur. J. Soil Sci.* **2016**, *67* (1), 23–39.
- (16) Zhu-Barker, X.; Cavazos, A. R.; Ostrom, N. E.; Horwath, W. R.; Glass, J. B. The importance of abiotic reactions for nitrous oxide production. *Biogeochemistry* **2015**, *126* (3), 251–267.
- (17) Soler-Jofra, A.; Picioreanu, C.; Yu, R.; Chandran, K.; van Loosdrecht, M. C. M.; Pérez, J. Importance of hydroxylamine in abiotic N₂O production during transient anoxia in planktonic axenic *Nitrosomonas* cultures. *Chem. Eng. J.* **2018**, *335*, 756–762.
- (18) Soler-Jofra, A.; Stevens, B.; Hoekstra, M.; Picioreanu, C.; Sorokin, D.; van Loosdrecht, M. C. M.; Pérez, J. Importance of abiotic hydroxylamine conversion on nitrous oxide emissions during nitrification of reject water. *Chem. Eng. J.* **2016**, *287*, 720–726.
- (19) Terada, A.; Sugawara, S.; Hojo, K.; Takeuchi, Y.; Riya, S.; Harper, W. F.; Yamamoto, T.; Kuroiwa, M.; Isobe, K.; Katsuyama, C.; Suwa, Y.; Koba, K.; Hosomi, M. Hybrid Nitrous Oxide Production from a Partial Nitrifying Bioreactor: Hydroxylamine Interactions with Nitrite. *Environ. Sci. Technol.* **2017**, *51* (5), 2748–2756.
- (20) Schreiber, F.; Wunderlin, P.; Udert, K. M.; Wells, G. F. Nitric oxide and nitrous oxide turnover in natural and engineered microbial communities: Biological pathways, chemical reactions, and novel technologies. *Front. Microbiol.* **2012**, *3*, 372.

- 422 (21) Döring, C.; Gehlen, H. Über die Kinetik der Reaktion zwischen Hydroxylamin und Salpetriger Säure. *J. Inorg.*
423 *Gen. Chem.* **1961**, 312 (1–2), 32–44.
- 424 (22) Moews, P. C.; Audrieth, L. F. The autoxidation of hydroxylamine. *J. Inorg. Nucl. Chem.* **1959**, 11 (3), 242–246.
- 425 (23) Bonner, F. T.; Hughes, M. N. The Aqueous Solution Chemistry of Nitrogen in Low Positive Oxidation States.
426 *Comments Inorg. Chem.* **1988**, 7 (4), 215–234.
- 427 (24) Kampschreur, M. J.; Kleerebezem, R.; de Vet, W. W. J. M.; van Loosdrecht, M. C. M. Reduced iron induced
428 nitric oxide and nitrous oxide emission. *Water Res.* **2011**, 45 (18), 5945–5952.
- 429 (25) Butler, J. H.; Gordon, L. I. Rates of Nitrous Oxide Production in the Oxidation of Hydroxylamine by Iron(III).
430 *Inorg. Chem* **1986**, 25, 4573–4577.
- 431 (26) Liu, S.; Han, P.; Hink, L.; Prosser, J. I.; Wagner, M.; Brüggemann, N. Abiotic Conversion of Extracellular NH
432 2 OH Contributes to N 2 O Emission during Ammonia Oxidation. *Environ. Sci. Technol.* **2017**, 51 (22), 13122–
433 13132.
- 434 (27) Harper, W. F.; Takeuchi, Y.; Riya, S.; Hosomi, M.; Terada, A. Novel abiotic reactions increase nitrous oxide
435 production during partial nitrification: Modeling and experiments. *Chem. Eng. J.* **2015**, 281, 1017–1023.
- 436 (28) van de Graaf, A. A.; Bruijn, P. de; Robertson, L. A.; Jetten, M. S. M.; Kuenen, J. G. Autotrophic growth of
437 anaerobic ammonium-oxidizing micro-organisms in a fluidized bed reactor. *Microbiology* **1996**, 142 (8), 2187–
438 2196.
- 439 (29) Su, Q.; Ma, C.; Domingo-Félez, C.; Kiil, A. S.; Thamdrup, B.; Jensen, M. M.; Smets, B. F. Low nitrous oxide
440 production through nitrifier-denitrification in intermittent-feed high-rate nitrification reactors. *Water Res.* **2017**,
441 123, 429–438.
- 442 (30) Frear, D. S.; Burrell, R. C. Spectrophotometric Method for Determining Hydroxylamine Reductase Activity in
443 Higher Plants. *Anal. Chem.* **1955**, 27 (10), 1664–1665.
- 444 (31) Viollier, E.; Inglett, P. ; Hunter, K.; Roychoudhury, A. ; Van Cappellen, P. The ferrozine method revisited:
445 Fe(II)/Fe(III) determination in natural waters. *Appl. Geochemistry* **2000**, 15 (6), 785–790.
- 446 (32) Weiss, R. F.; Price, B. A. Nitrous oxide solubility in water and seawater. *Mar. Chem.* **1980**, 8 (4), 347–359.
- 447 (33) Bremner, J. M.; Blackmer, A. M.; Waring, S. A. Formation of nitrous oxide and dinitrogen by chemical
448 decomposition of hydroxylamine in soils. *Soil Biol. Biochem.* **1980**, 12 (3), 263–269.
- 449 (34) Alluisetti, G. E.; Almaraz, A. E.; Amorebieta, V. T.; Doctorovich, F.; Olabe, J. A. Metal-Catalyzed Anaerobic
450 Disproportionation of Hydroxylamine. Role of diazene and nitroxyl intermediates in the formation of N₂, N₂O,
451 NO⁺, and NH₃. *J. Am. Chem. Soc.* **2004**, 126 (41), 13432–13442.
- 452 (35) Anderson, J. H. The copper-catalysed oxidation of hydroxylamine. *Analyst* **1964**, 89 (1058), 357.
- 453 (36) Huang, Y. H.; Zhang, T. C. Nitrite reduction and formation of corrosion coatings in zerovalent iron systems.
454 *Chemosphere* **2006**, 64 (6), 937–943.
- 455 (37) Hughes, M. N.; Stedman, G. Kinetics and mechanism of the reaction between nitrous acid and hydroxylamine.
456 Part I. *J. Chem. Soc.* **1963**, 2824.
- 457 (38) Hussain, M. A.; Stedman, G.; Hughes, M. N. Kinetics and mechanism of the reaction between nitrous acid and
458 hydroxylamine. Part III. The formation of hyponitrous acid. *J. Chem. Soc. B Phys. Org.* **1968**, 597.
- 459 (39) Bothner-By, A.; Friedman, L. The Reaction of Nitrous Acid with Hydroxylamine. *J. Chem. Phys.* **1952**, 20 (3),
460 459–462.

- (40) Bennett, M. R.; Maya, L.; Brown, G. M.; Posey, F. A. Oxidation of hydroxylamine by nitrous and nitric acids. *Inorg. Chem.* **1982**, *21* (6), 2461–2468.
- (41) Morgan, T. D. B.; Stedman, G.; Hughes, M. N. Kinetics and mechanism of the reaction between nitrous acid and hydroxylamine. Part II. The alkyl hydroxylamines. *J. Chem. Soc. B Phys. Org.* **1968**, *0* (0), 344.
- (42) Su, Q.; Domingo-Félez, C.; Zhang, Z.; Blum, J.-M.; Jensen, M. M.; Smets, B. F. The effect of pH on N₂O production through nitrifier-denitrification in intermittently-fed nitrification reactors (under review). **2019**.
- (43) Law, Y.; Lant, P.; Yuan, Z. The effect of pH on N₂O production under aerobic conditions in a partial nitrification system. *Water Res.* **2011**, *45* (18), 5934–5944.
- (44) Rathnayake, R. M. L. D.; Oshiki, M.; Ishii, S.; Segawa, T.; Satoh, H.; Okabe, S. Effects of dissolved oxygen and pH on nitrous oxide production rates in autotrophic partial nitrification granules. *Bioresour. Technol.* **2015**, *197*, 15–22.
- (45) Kinh, C. T.; Ahn, J.; Suenaga, T.; Sittivorakulpong, N.; Noophan, P.; Hori, T.; Riya, S.; Hosomi, M.; Terada, A. Free nitrous acid and pH determine the predominant ammonia-oxidizing bacteria and amount of N₂O in a partial nitrifying reactor. *Appl. Microbiol. Biotechnol.* **2017**, *101* (4), 1673–1683.
- (46) Ni, B.; Ye, L.; Law, Y.; Byers, C.; Yuan, Z. Mathematical Modeling of Nitrous Oxide (N₂O) Emissions from Full-Scale Wastewater Treatment Plants. *Environ. Sci. Technol.* **2013**, *47* (14), 7795–7803.
- (47) van Dongen, U.; Jetten, M. S.; van Loosdrecht, M. C. The SHARON-Anammox process for treatment of ammonium rich wastewater. *Water Sci. Technol.* **2001**, *44* (1), 153–160.
- (48) Domingo-Félez, C.; Calderó-Pascual, M.; Sin, G.; Plósz, B. G.; Smets, B. F. Calibration of the comprehensive NDHA-N₂O dynamics model for nitrifier-enriched biomass using targeted respirometric assays. *Water Res.* **2017**, *126*, 29–39.

FIGURE AND TABLE CAPTION

Figure 1. NH_2OH disproportionation and/or oxidation by O_2 at different pH set-points (Scenario 1).

(A) Averaged N_2O production rate (bar) and rate constant (k) (scatter); (B) Averaged NH_2OH depletion rate; (C) N_2O yield relative to the amount of NH_2OH oxidized (%). k and N_2O yield relative to the amount of NH_2OH oxidized were calculated based on the reaction kinetics and stoichiometry of NH_2OH disproportionation (Eq. 12 and 9, respectively). Gray dot bars represent the tests that were not performed. Error bars indicate standard deviations of measurements.

Figure 2. Chemical dynamics during NH_2OH oxidation by HNO_2 in Scenario 1 (A) and Scenario 2 (B). (A) and (B) were conducted in synthetic medium under low DO condition ($< 1 \text{ mg O}_2/\text{L}$).

Figure 3. NH_2OH oxidation by HNO_2 at different pH set-points (Scenario.1). (A) Averaged N_2O production rate (bar) and rate constant (k) (scatter); (B) Averaged NH_2OH depletion rate; (C) N_2O yield relative to the amount of NH_2OH oxidized (%). Gray dot bars represent the tests that weren't performed. Error bars indicate standard deviations of measurements.

Table 1. The contribution of abiotic reactions to overall N_2O production in nitrification reactors.

Table 1. The contribution of abiotic reactions to overall N₂O production in nitrification reactors.

Reference	Total N ₂ O production in nitrification reactors						Abiotic N ₂ O production					
	Experimental condition					Measured total N ₂ O production rates (mM/h)	Considered pathway	Method ^c	Estimated abiotic N ₂ O production rates (mM/h) ^e		Fraction of abiotic pathway to total N ₂ O production (%)	
	Reactor types	pH	NH ₂ OH (mM)	HNO ₂ (mM)	NO ₂ ⁻ (mM)				Original Estimation	Estimation based on <i>k</i> in this study	Original Estimation	Estimation based on <i>k</i> in this study
This study	Lab-scale, nitrification, SBR	6.5 ^a	4.5±0.58 ×10 ^{-3 a}	4.6±0.87 ×10 ^{-3 a}	11.7±1.8 _a	5.6±2.3 ×10 ^{-3 a}	NH ₂ OH oxidation by HNO ₂ NH ₂ OH disproportionation and/or oxidation by O ₂	Abiotic batch tests without biomass	1.5±0.35 ×10 ⁻⁴		2.6±1.2	
		7.0 ^a	4.1±1.2 ×10 ^{-3 a}	2.1±0.15 ×10 ^{-3 a}	16.6±0.8 _{5^a}	2.0±1.0 ×10 ^{-2 a}	NH ₂ OH oxidation by HNO ₂		2.9±0.91 ×10 ⁻⁵		1.5±0.87 ×10 ⁻¹	
		8.0 ^a	3.7±1.0 ×10 ^{-3a}	2.7±0.080 ×10 ^{-4 a}	20.2±0.1 _{4 ^a}	7.0±1.3 ×10 ^{-2 a}	NH ₂ OH oxidation by HNO ₂ NH ₂ OH disproportionation and/or oxidation by O ₂		1.8×10 ⁻⁵		2.5±0.47 ×10 ⁻²	
Terada et al. (2017)	Bath tests with AOB enriched biomass	7	7.1×10 ⁻² - 1.4 ^b	6.4×10 ^{-3 b}	28.6 ^b	2×10 ⁻¹ - 3.3 _b	NH ₂ OH oxidation by HNO ₂	Abiotic batch tests with/without biomass	5×10 ⁻² - 9×10 ^{-1 b}	1.4×10 ⁻³ - 2.8×10 ^{-2 b}	51	6.9×10 ⁻¹ - 8.3×10 ^{-1 b}
Soler-Jofra et al. (2016)	Full-scale, PN, SHARON, flocs	7	4.3×10 ^{-3 b}	1.2×10 ^{-2 b, d}	46.4 ^b	1.7×10 ^{-2 b}	NH ₂ OH oxidation by HNO ₂	Abiotic batch tests without biomass	1.1×10 ^{-3 b, e}	1.7×10 ^{-4 b}	6.8 ^b	1 ^b
Harper et al. (2015)	Bath tests with AOB enriched biomass	7	7.1 ×10 ⁻³ - 1.4 ^b	6.4×10 ^{-3 b}	28.6 ^b	1.5×10 ⁻² - 8.8×10 ^{-1 b}	NH ₂ OH oxidation by HNO ₂	Abiotic batch tests with/without biomass and combined with model simulations	2×10 ⁻² - 7×10 ^{-1 b}	1.4×10 ⁻⁴ - 2.8×10 ^{-2 b}	/	9.2×10 ⁻¹ - 3.2 _b

^a Numbers were retrieved from Su et al. (2018).^b Numbers were calculated based on original data in literatures.^c The details of experimental methods refer to materials and methods section and Table S1, S3 in Supporting Information.^d HNO₂ was recalculated based on the Eq.6 in this study.^e Estimated abiotic N₂O production rate was calculated based on the equation of $r_{N_2O} = k [HNO_2] [NH_2OH]$.

1 **Supporting Information for:**

2 **Abiotic nitrous oxide (N₂O) production is strongly pH dependent, but**

3 **contributes little to overall N₂O emissions in biological nitrogen**

4 **removal systems**

5

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Figure S1. Chemical dynamics during NH_2OH disproportionation and/or oxidation by O_2 in Scenario 1. The test was conducted in synthetic medium under high DO (8-8.4 mg O_2 /L).

Figure S2. Chemical dynamics during the reduction of HNO_2 by Fe^{2+} in Scenario 1 (A) and Scenario 2 (B). (A) was conducted in diH_2O under low DO ($< 1 \text{ mg O}_2/\text{L}$); (B) was conducted in synthetic medium under low DO ($< 1 \text{ mg O}_2/\text{L}$).

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47 Figure S3. The reduction of HNO₂ by Fe²⁺ at different pH values in Scenario 1. (A) Averaged N₂O

48 production rate (bar); (B) Averaged Fe²⁺ depletion rate; (C) N₂O yield relative to the amount of

49 Fe²⁺ oxidized (%). Error bars indicate standard deviations of measurements.* indicates Fe²⁺

50 oxidation by O₂ or Fe²⁺ oxidation coupling with HNO₂ reduction to ammonium.

Figure S4. Chemical dynamics during the oxidation of NH_2OH by Fe^{3+} in Scenario 1. The test was conducted in diH_2O under low DO (< 1 $\text{mg O}_2/\text{L}$).

		Abiotic $r_{\text{N}_2\text{O}}$ (mM/h)				
NO_2^- (mM)	Reactors treating typical domestic wastewaters	0.04	1.4E-04	3.3E-06	1.5E-07	5.7E-09
		0.07	2.8E-04	6.5E-06	3.0E-07	1.1E-08
	Nitritation reactors	0.4	1.4E-03	3.3E-05	1.5E-06	5.7E-08
		0.7	2.8E-03	6.5E-05	3.0E-06	1.1E-07
		3.6	1.4E-02	3.3E-04	1.5E-05	5.7E-07
		7.1	2.8E-02	6.5E-04	3.0E-05	1.1E-06
		14.3	5.7E-02	1.3E-03	6.1E-05	2.3E-06
		21.4	8.5E-02	2.0E-03	9.1E-05	3.4E-06
		28.6	1.1E-01	2.6E-03	1.2E-04	4.6E-06
		35.7	1.4E-01	3.3E-03	1.5E-04	5.7E-06
		50	2.0E-01	4.6E-03	2.1E-04	8.0E-06
			5	6	7	8
			pH			

54
 55 Figure S5. Predictions of abiotic N_2O production rates ($r_{\text{N}_2\text{O}}$) under operational conditions in
 56 reactors treating typical domestic wastewaters (0.007 mM NH_2OH , 0.04-0.4 mM NO_2^-) and
 57 nitritation reactors (0.007 mM NH_2OH , 0.7-50 mM NO_2^-). $r_{\text{N}_2\text{O}}$ was calculated based on the
 58 equation of $r_{\text{N}_2\text{O}} = k [\text{HNO}_2] [\text{NH}_2\text{OH}]$.

59 Table S1. Abiotic batch experiments conditions. The targeted initial substrate concentrations are
60 17.8 mM NO_2^- , 0.07 mM NH_2OH , 0.5 mM FeSO_4 and 0.1 mM FeCl_3 and targeted pH values were 4,
61 5, 6, 7, 8 and 9. The temperature was 24-26°C during experimental period. Each test was done by
62 duplicate.

Experiment	Reaction types	NH_2OH (mM)	NO_2^- (mM)	FeCl_3 (mM)	FeSO_4 (mM)	pH	DO (mg O_2/L)	Deionized water (diH_2O) / synthetic medium
Scenario 1	NH_2OH oxidation by HNO_2	0.06 ± 0.01	13.0 ± 3.0	/	/	4.4 ± 0.1	< 1	diH_2O
		0.06 ± 0.01	16.3 ± 1.6			5.1 ± 0.3		
		0.07 ± 0.01	16.8 ± 0.7			6.1 ± 0.1		
		0.07	17.1			7.0		
		0.05	16.4			8.2		
		0.05 ± 0.01	13.6 ± 0.7	/	/	4.5 ± 0.03	8-8.4	diH_2O
		0.06	16.4			8.0		
		0.05 ± 0.01	16.3 ± 0.2	/	/	4.7 ± 0.01	< 1	medium
		0.06	19.3			8.0		
		0.05 ± 0.001	15.9 ± 0.5	/	/	4.6 ± 0.1	8-8.4	medium
		0.07	17.1			8.0		
	NH_2OH disproportionation and oxidation by O_2	0.06	/	/	/	4.1	< 1	diH_2O
		0.05				6.2		
		0.05				8.0		
		0.05				8.9		
		0.06	/	/	/	8.0	8-8.4	diH_2O
		0.05	/	/	/	4.0	< 1	medium
		0.06				8.0		
		0.06	/	/	/	4.1	8-8.4	medium
		0.05				8.0		
	NH_2OH oxidation by Fe^{3+}	0.05	/	0.09	/	4.3	< 1	diH_2O
	HNO_2 reduction by Fe^{2+}	/	13.2 ± 0.7	/	0.50 ± 0.21	4.4 ± 0.002	< 1	diH_2O
			19.1		0.26	6.4		
			17.1		0.18	8.2		
Scenario 2	NH_2OH oxidation by HNO_2	$0 \rightarrow 0.01 \rightarrow 0.06$	$0 \rightarrow 3.9 \rightarrow 41.2$	/	/	$9 \rightarrow 6.4 \rightarrow 5.3$	< 1	diH_2O
		$0 \rightarrow 0.05 \rightarrow 0.11$	$0 \rightarrow 21.4 \rightarrow 28.6$	/	/	$7.7 \rightarrow 7.5 \rightarrow 7.1 \rightarrow 7.6$	< 1	medium

		0→0.04→0.07	0→6.8→37.9	/	/	8.6→8.3→7.9 →6.8→6	< 1	medium
	HNO ₂ reduction by Fe ²⁺	/	21.4	0.03	/	8.5→7.6→8.5 →5.8→5.1	< 1	diH ₂ O
		/	20.3	0.03	/	9.1→5.3	< 1	diH ₂ O
		/	17.1	0.12	/	7.6→6.8→5.8	< 1	medium

64 Table S2. Overview of the methodology followed in this study to estimate k values (DO levels < 1; 8-8.4 mg O₂/L).

Timeline	Reaction		Experimental conditions			Ref. in text	Reaction kinetics		Comments / Assumptions	Output
	Number	Stoichiometry	Dataset (measured)	diH ₂ O/synthetic medium	pH		Data and assumed reactions	Data fit and k -value estimated		
Step_1	Reaction (4) HNO ₂ reduction by Fe ²⁺	$2\text{HNO}_2 + 4\text{Fe}^{2+} + 4\text{H}^+ \rightarrow 4\text{Fe}^{3+} + \text{N}_2\text{O} + 3\text{H}_2\text{O}$	HNO ₂ , Fe ²⁺ , Fe ³⁺ , N ₂ O	diH ₂ O	4.4/6/8	Fig S2, S3	Data_Step_1 (rN ₂ O, HNO ₂ , Fe ²⁺ , Fe ³⁺) (pH = 4.4) = [Reaction ₍₄₎]	Data_Step_1 = $k_{(4)} [\text{HNO}_2]^2 [\text{Fe}^{2+}]^4$	Incomplete recovery of N ₂ O from the stoichiometric Fe ²⁺ oxidation at pH = 6.4 (49%) and pH = 8.2 (12%).	$k_{(4)}$ could not be estimated at pH values higher than 4.4 Reaction (4) will not a big impact at neutral pH values.
Step_2	Reaction (3) NH ₂ OH disproportionation Reaction (2) NH ₂ OH oxidation by O ₂	$4\text{NH}_2\text{OH} \rightarrow 2\text{NH}_3 + \text{N}_2\text{O} + 3\text{H}_2\text{O}$ $2\text{NH}_2\text{OH} + \text{O}_2 \rightarrow \text{N}_2\text{O} + 3\text{H}_2\text{O}$	NH ₂ OH, N ₂ O	diH ₂ O; medium	4/6/8/9	Fig 1	Data_Step_2 (rN ₂ O, NH ₂ OH) = [Reaction ₍₂₎ + Reaction ₍₃₎]	Data_Step_2 $\approx$ Reaction ₍₃₎ = $k_l [\text{NH}_2\text{OH}]^4$ (Eq. 12)	The calculated N ₂ O yield (relative to the amount of NH ₂ OH oxidized) for Reaction (3) was much closer to the theoretical than for Reaction (2) r _{NH₂OH} and r _{N₂O} in synthetic medium were 2-22 times higher than in diH ₂ O	NH ₂ OH disproportionation (Reaction 3) shows higher rates than NH ₂ OH oxidation (Reaction 2). The chemical medium enhances N ₂ O production compared to diH ₂ O (at pH = 8).
Step_3	Reaction (5) NH ₂ OH oxidation by Fe ³⁺	$4\text{Fe}^{3+} + 2\text{NH}_2\text{OH} \rightarrow 4\text{Fe}^{2+} + \text{N}_2\text{O} + \text{H}_2\text{O} + 4\text{H}^+$	NH ₂ OH, Fe ²⁺ , Fe ³⁺ , N ₂ O	diH ₂ O	4.3	Fig S4	Data_Step_3 (rN ₂ O, NH ₂ OH, Fe ³⁺) = [Reaction ₍₅₎ + Reaction ₍₃₎]	/	Incomplete recovery of N ₂ O from the stoichiometric NH ₂ OH oxidation at pH = 4.3 (66%)	$k_{(5)}$ could not be estimated even at low pH where Fe is more stable. Reaction (5) will not a big impact at neutral pH values.
Step_4	Reaction (1) NH ₂ OH oxidation	$\text{NH}_2\text{OH} + \text{HNO}_2 \rightarrow \text{N}_2\text{O} + 2\text{H}_2\text{O}$	NH ₂ OH, HNO ₂ , N ₂ O	diH ₂ O	4.5/5/6/7/8	Fig 2, 3	Data_Step_4 (rN ₂ O, NH ₂ OH, HNO ₂) =	Reaction ₍₁₎ = {Data_Step_4 -	Kinetics of Reaction (1) were calculated assuming only Reaction (3)	$k_{(3)}$ was estimated as a function of pH: ($k_{3_pH} = 8272.5e^{-1.1pH}$, R ²

	by HNO_2			$\begin{aligned} & [\text{Reaction}_{(1)} + \text{Reaction}_{(3)}] \\ & = \\ & [\text{Reaction}_{(1)} + k_f [\text{NH}_2\text{OH}]^4] = \end{aligned}$	$\begin{aligned} & k_f [\text{NH}_2\text{OH}]^4 \} \\ & = \\ & k_3 [\text{HNO}_2] [\text{N} \\ & \quad \text{H}_2\text{OH}] \\ & \text{(Eq. 14)} \end{aligned}$	occurred simultaneously	= 0.99),
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65 Table S3. Comparison of different rate equations to describe the oxidation of NH_2OH by HNO_2 in
66 Scenario 1 (pH = 4.5, 5, 6, 7 and 8; diH_2O ; $\text{DO} < 1 \text{ mg O}_2/\text{L}$; $T=25^\circ\text{C}$). The k values were retrieved
67 by fitting experimental data to model predictions by minimizing normalized root mean square error
68 (NRMSE).

Equation	$r_{\text{N}_2\text{O}_2} = k_0 \frac{[\text{NO}_2^-]}{[\text{NH}_2\text{OH}]}$	$r_{\text{N}_2\text{O}_2} = k_0 \frac{[\text{HNO}_2]}{[\text{NH}_2\text{OH}]}$	$r_{\text{N}_2\text{O}_2} = k_0 \frac{[\text{H}^+][\text{HNO}_2]}{[\text{NH}_2\text{OH}]}$	$r_{\text{N}_2\text{O}_2} = k_0 e^{(k_1 \text{pH})} \cdot \frac{[\text{NO}_2^-]}{[\text{NH}_2\text{OH}]}$	$r_{\text{N}_2\text{O}_2} = k_0 e^{(k_1 \text{pH})} \cdot \frac{[\text{HNO}_2]}{[\text{NH}_2\text{OH}]}$
Reference	Harper et al. (2015)	Döring and Gehlen (1961)	Döring and Gehlen (1961)	This study	This study
k_0 (L/mmol/h)	0.20	44.9	$9.4 \times 10^{5*}$	1.6×10^6	867.7
k_1	/	/	/	-3.0	-0.68
NRMSE	0.49	0.058	0.068	0.014	0.015

69 $*L^2/\text{mmol}^2/\text{h}$

70 Table S4. Summary of experimental conditions and calculated kinetic rates of abiotic reactions in Scenario1 and literature.

Reaction		NH ₂ OH (mM)	NO ₂ ⁻ (mM)	HNO ₂ (mM)	Fe ²⁺ (mM)	Fe ³⁺ (mM)	pH	DO (mg O ₂ /L)	Temperature (°C)	Deionized water (diH ₂ O) / synthetic medium	r _{NH₂OH} (mM/h)	r _{N₂O} (mM/h)	N ₂ O/ NH ₂ O H (%)	k (L/mmol /h)
Scenario1	NH ₂ OH oxidation by HNO ₂	0.06±0.01	13.0	0.9±0.01	/	/	4.4±0.1	< 1	24	diH ₂ O	0.4±0.04	0.8±0.1 ^b	167±23	55.8±3.1 _{b, d}
		0.06±0.006	16.3±1.6	0.3±0.1	/	/	5.1±0.3	< 1	24	diH ₂ O	0.1±0.03	0.3±0.1 ^b	185±5	30.9±2.4 _{b, d}
		0.07±0.01	16.8±0.7	0.02±0.003	/	/	6.1±0.1	< 1	25	diH ₂ O	0.004±0.001	0.008±0.001 ^b	134±11	6.9±0.5 _d ^b
		0.07	17.1	0.004	/	/	7.0	< 1	27	diH ₂ O	0.002	0.0008 ^b	34	3.3 ^{b, d}
		0.05	16.4	0.0002	/	/	8.2	< 1	25	diH ₂ O	0.0005	0.0001 ^b	47	0.9 ^{b, d}
		0.05±0.007	13.6±0.7	0.7±0.05	/	/	4.5±0.03	8.6	25	diH ₂ O	0.2±0.007	0.7±0.1 ^b	186±5	47.9±7.5 _{b, d}
		0.06	16.4	0.0003	/	/	8.0	8.4	26	diH ₂ O	0.0002	0.0002 ^b	44	/
		0.05±0.01	16.3±0.2	0.7±0.03	/	/	4.7±0.01	< 1	24	medium	0.3±0.04	0.7±0.3 ^b	178±6	39.6±4.4 _{b, d}
		0.06	19.3	0.0003	/	/	8.0	< 1	25	medium	0.002	0.0007 ^b	26	/
		0.05±0.001	15.9±0.5	0.7±0.09	/	/	4.6±0.1	8.2	25	medium	0.3±0.004	0.7±0.1 ^b	191±12	46.5±3.1 _{b, d}
		0.07	17.1	0.0004	/	/	8.0	7.5	27	medium	0.009	0.002 ^b	24	/
	NH ₂ OH disproportionation and oxidation by O ₂	0.06	/	/	/	/	4.1	< 1	25	diH ₂ O	0	0	/	0.0 ^c
		0.05	/	/	/	/	6.2	< 1	26	diH ₂ O	0.001	0.0002	57 ^c	34.5 ^c
		0.05	/	/	/	/	8.0	< 1	26	diH ₂ O	0.002	0.0002	24 ^c	11.6 ^c
		0.05	/	/	/	/	8.9	< 1	26	diH ₂ O	0.002	0.0002	44 ^c	12.5 ^c
		0.06	/	/	/	/	8.0	8.0	26	diH ₂ O	0.001	0.00009	20 ^c	3.3 ^c
		0.05	/	/	/	/	4.0	< 1	26	medium	0	0	/	0.0 ^c
		0.06	/	/	/	/	8.0	< 1	27	medium	0.003	0.0007	58 ^c	39.8 ^c
		0.06	/	/	/	/	4.1	8.2	25	medium	0.00009	0.00002	47 ^c	1.1 ^c
		0.05	/	/	/	/	8.0	7.8	26	medium	0.007±0.0004	0.002±0.0004	36 ^c	424.4 ^c

	Iron-mediated reduction of HNO ₂	/	13.2±0.7	0.8±0.09	0.5±0.2	/	4.4	< 1	23	diH ₂ O	0.3±0.03	0.2±0.009	98±3	46.4 ± 4.1 ^f
		/	19.1	0.001	0.3	/	6.4	< 1	25	diH ₂ O	0.02	0.002	49	/
		/	17.1	0.0002	0.2	/	8.2	< 1	24	diH ₂ O	0.03	0.002	12	/
	NH ₂ OH oxidation by Fe ³⁺	0.05	/	/	/	0.09	4.3	< 1	24	diH ₂ O	0.003	0.001 ^b	66	/
Harper et al. (2015)	NH ₂ OH oxidation by HNO ₂	0.007-1.4 ^a	28.6 ^a	0.006 ^a	0.01 ^a	/	7	aerobic	/	medium ^e	/	0.02-0.7 ^a	/	0.05 ^d
Terada et al. (2017)		0.07-1.4 ^a	28.6 ^a	0.006 ^a	0.01 ^a	/	7	7.11-8.25	28	medium ^e	/	0.05-0.9 ^a	/	/
Soler-Jofra et al. (2016)		0.02 ^a	9.6/46.4 ^a	0.0075/0.012 ^a	0.15 ^a	/	6.2/7	aerobic	30	medium ^e	0.01/0.02 ^a	0.003/0.006 ^a	22/41	/
Soler-Jofra et al. (2018)		0.004-0.014 ^a	16.4 ^a	0.0004 ^a	/	0.013 % EDTA-Fe ³⁺	7.5 ± 0.1	aerobic	21	medium ^e	/	0.002-0.003 ^a	20-40	/
Terada et al. (2017)	NH ₂ OH oxidation by O ₂	0.07-1.4 ^a	28.6 ^a	0.006 ^a	0.01 ^a	/	7	7.11-8.25	28	medium ^e	/	0.01 ^a	/	/
Kampshreur et al. (2011)	Iron-mediated reduction of HNO ₂	/	0-43	0-0.05 ^a	10	/	6.5	anoxic	35	water	/	0.9 ^a	/	/

^a Numbers were calculated based on original data in literature.

^b r_{N₂O} and k value of NH₂OH oxidation by HNO₂ and Fe³⁺ in this study were estimated by subtracting N₂O produced by NH₂OH disproportionation and/or oxidation by O₂.

^c N₂O/NH₂OH and k value of NH₂OH disproportionation and/or oxidation by O₂ in this study was calculated based on the equation of $X_{N_2O/NH_2OH} = \frac{m_{N_2O} \cdot 4}{m_{NH_2OH}} \cdot 100 \%$

and r_{N₂O} = k [NH₂OH]⁴.

^d k value of NH₂OH oxidation by HNO₂ in this study was calculated based on the equation of r_{N₂O} = k [HNO₂] [NH₂OH]; but k reported in Harper et al. (2015) was based on r_{N₂O} = k [NO₂⁻] [NH₂OH].

^e The compositions of synthetic medium in literatures are similar to the one used in our experiments, which are mainly consisted of KH₂PO₄, MgSO₄·7H₂O, CaCl₂·2H₂O, EDTA, FeSO₄·7H₂O and trace element solution. The exact concentrations of these chemicals can be found in literatures.

^f k value was calculated only for this pH value based on the equation of r_{N₂O} = k [HNO₂]² [Fe²⁺]⁴ (L⁵/mmol⁵/h).

81 Table S5. Predictions of abiotic N₂O production rates and the abiotic contributions to overall N₂O production in nitrification reactors.

Reference	Reactor types	Experimental condition						Total N ₂ O production ^a	Abiotic N ₂ O production		
		T (°C)	DO (mg O ₂ /L)	pH	ALR ^a (g/L/d)	NO ₂ ⁻ ^a (mM)	HNO ₂ ^a (mM)	r _{N2O} (mM/h)	k ^b (L/mmol/h)	r _{N2O} ^c (mM/h)	Fraction of abiotic pathway to total N ₂ O production (%) ^d
Kong et al., 2013 ⁷	Lab-scale, PN, SBR, flocs	32 ± 0.5	1	6-7.5	3	17.7 ± 0.9	8.5 ± 0.41 × 10 ⁻³	0.036	4.67	2.80 × 10 ⁻⁴	0.79
Rathnayake et al., 2013 ⁸	Lab-scale, PN, SBR, granules	/	2.0 ± 0.3	7.4-7.8	1.03 ± 0.06	13.0 ± 2.1	5.9 ± 0.94 × 10 ⁻⁴	0.012	1.94	8.10 × 10 ⁻⁶	0.07
de Graaff et al., 2010 ⁹	Lab-scale, PN, CFR, flocs	25	4.1 ± 0.73	6.8 ± 0.33	0.52	35.7	1.0 × 10 ⁻²	0.015	4.67	3.40 × 10 ⁻⁴	2.31
Okabe et al., 2011 ¹⁰	Lab-scale, PN, CFR, biofilm	35	<2	7.8 ± 0.1	3.5-4.1	5.7 ± 12.9	3.4-7.7 × 10 ⁻⁴	0.006	1.55	6.20 × 10 ⁻⁶	0.10
Kong et al., 2013 ¹¹	Lab-scale, PN, SBBR, biofilm	35 ± 1	<1.5	7.0-7.5	1.25	16.4	3.5 × 10 ⁻³	0.028	2.85	7.10 × 10 ⁻⁵	0.26
Kampschreur et al., 2008 ¹²	Full-scale, PN, SHARON, flocs	32-33	2.5	6.55-7	6.5	36.1	1.8 × 10 ⁻²	0.164	4.67	6.00 × 10 ⁻⁴	0.36
Desloover et al., 2011 ¹³	Full-scale, PN, SBR, flocs	36	0.75 ± 0.01	7.5 ± 0.1	0.18-0.22	8.2 ± 1.8	1.1 ± 0.23 × 10 ⁻³	0.014-0.022	2.16	1.60 × 10 ⁻⁵	0.08

82 Abbreviation: Partial Nitrification (PN); Sequencing Batch Reactor (SBR); Continuous Flow Reactor (CFR); Sequencing Batch Biofilm Reactor (SBBR); Single reactor
 83 system for High activity Ammonium Removal Over Nitrite (SHARON); Ammonium Loading Rate (ALR); N₂O production rate (r_{N2O}); rate constant (k).

84 ^a Numbers were calculated based on original data in literatures.

85 ^b k in literature was calculated based on the function between k and pH (i.e. $k = 8272.5e^{-1.1pH}$) obtained in this study. However, the effect of temperature was not
 86 corrected during k calculation.

87 ^c Estimated abiotic r_{N2O} was calculated based on the equation of $r_{N2O} = k [HNO_2] [NH_2OH]$. As NH₂OH was not measured in these studies, the concentration of
 88 NH₂OH was assumed at 0.007 mM for abiotic r_{N2O} calculation.

89 **Section 1 - NH₂OH analysis**

90 The NH₂OH concentration was measured following the protocol proposed by Frear and Burrell
91 (1955) and Soler-Jofra et al. (2016):

- 92 1. Add 1.6 mL of the filtered sample containing NH₂OH in the range of 0-2.1 mg N/L to 5 mL
93 test tube, follow with 0.2 mL of 0.1g/mL sulfamic acid to and mix.
- 94 2. Add 1 mL of 0.05 M phosphate buffer, 0.2 mL of 12 wt% trichloroacetic acid and 1 mL of 1%
95 8-quinolinol (w/v), then swirl gently.
- 96 3. Add add 1.0 mL of 1.0 M sodium carbonate solution, shake vigorously.
- 97 4. Heat 1 min at 100 °C in a water bath and cool for 15 min, and then read in
98 spectrophotometer at 705 µm.
- 99 5. Carry out simultaneously a blank by replacing the sample volume by the same volume of
100 demineralized.

101 Additionally, 0.1 mL of 3.5 mg N/L NH₂OH stock solution was added in one of triplicate bulk
102 samples as the internal standard to verify the measuring accuracy. Recovery efficiency of 93 ± 6%
103 of the added internal standard (n=12) indicated that the adopted pretreatment and measurement
104 procedures were applicable and precise for NH₂OH quantification of our samples.

105

Section 2 - Measurement of the sensitivity and response time of sensors

The pH sensitivity of N₂O sensor was measured at stepwise increases or decreases of pH values by sequentially spiking alkali or acid (Figure S6). The signal changes in response to pH changes were below 0.2%, indicating a low pH sensitivity of the N₂O sensor. In addition, we performed tests at different DO levels and stir intensities by spiking oxygen saturated liquid or changing stirring rates. No significant changes of N₂O signal were observed.

The response times of N₂O/pH/DO sensors were detected by counting the interval required by the output signal of sensors to display a change in the applied N₂O/pH/DO concentrations. The measured response times of N₂O, pH, and DO sensors were < 30s, ≤ 45s, and ≤ 45s, respectively.

Figure S6. The sensitivity of N₂O sensor towards pH changes.

117 Reference

- 118 (1) Harper, W. F.; Takeuchi, Y.; Riya, S.; Hosomi, M.; Terada, A. Novel abiotic reactions increase nitrous oxide
119 production during partial nitrification: Modeling and experiments. *Chem. Eng. J.* **2015**, *281*, 1017–1023.
- 120 (2) Döring, C.; Gehlen, H. Über die Kinetik der Reaktion zwischen Hydroxylamin und Salpetriger Säure. *J. Inorg.*
121 *Gen. Chem.* **1961**, *312* (1–2), 32–44.
- 122 (3) Terada, A.; Sugawara, S.; Hojo, K.; Takeuchi, Y.; Riya, S.; Harper, W. F.; Yamamoto, T.; Kuroiwa, M.; Isobe,
123 K.; Katsuyama, C.; Suwa, Y.; Koba, K.; Hosomi, M. Hybrid Nitrous Oxide Production from a Partial Nitrifying
124 Bioreactor: Hydroxylamine Interactions with Nitrite. *Environ. Sci. Technol.* **2017**, *51* (5), 2748–2756.
- 125 (4) Soler-Jofra, A.; Stevens, B.; Hoekstra, M.; Picioreanu, C.; Sorokin, D.; van Loosdrecht, M. C. M.; Pérez, J.
126 Importance of abiotic hydroxylamine conversion on nitrous oxide emissions during nitrification of reject water.
127 *Chem. Eng. J.* **2016**, *287*, 720–726.
- 128 (5) Soler-Jofra, A.; Picioreanu, C.; Yu, R.; Chandran, K.; van Loosdrecht, M. C. M.; Pérez, J. Importance of
129 hydroxylamine in abiotic N₂O production during transient anoxia in planktonic axenic *Nitrosomonas* cultures.
130 *Chem. Eng. J.* **2018**, *335*, 756–762.
- 131 (6) Kampschreur, M. J.; Kleerebezem, R.; de Vet, W. W. J. M.; van Loosdrecht, M. C. M. Reduced iron induced
132 nitric oxide and nitrous oxide emission. *Water Res.* **2011**, *45* (18), 5945–5952.
- 133 (7) Kong, Q.; Liang, S.; Zhang, J.; Xie, H.; Miao, M.; Tian, L. N₂O emission in a partial nitrification system:
134 Dynamic emission characteristics and the ammonium-oxidizing bacteria community. *Bioresour. Technol.* **2013**,
135 *127*, 400–406.
- 136 (8) Rathnayake, R. M. L. D.; Song, Y.; Tumendelger, A.; Oshiki, M.; Ishii, S.; Satoh, H.; Toyoda, S.; Yoshida, N.;
137 Okabe, S. Source identification of nitrous oxide on autotrophic partial nitrification in a granular sludge reactor.
138 *Water Res.* **2013**, *47* (19), 7078–7086.
- 139 (9) de Graaff, M. S.; Zeeman, G.; Temmink, H.; van Loosdrecht, M. C. M.; Buisman, C. J. N. Long term partial
140 nitrification of anaerobically treated black water and the emission of nitrous oxide. *Water Res.* **2010**, *44* (7),
141 2171–2178.
- 142 (10) Okabe, S.; Oshiki, M.; Takahashi, Y.; Satoh, H. Development of long-term stable partial nitrification and
143 subsequent anammox process. *Bioresour. Technol.* **2011**, *102* (13), 6801–6807.
- 144 (11) Kong, Q.; Zhang, J.; Miao, M.; Tian, L.; Guo, N.; Liang, S. Partial nitrification and nitrous oxide emission in an
145 intermittently aerated sequencing batch biofilm reactor. *Chem. Eng. J.* **2013**, *217*, 435–441.
- 146 (12) Kampschreur, M. J.; Tan, N. C. G.; Kleerebezem, R.; Picioreanu, C.; Jetten, M. S. M.; Van Loosdrecht, M. C.
147 M. Effect of dynamic process conditions on nitrogen oxides emission from a nitrifying culture. *Environ. Sci.*
148 *Technol.* **2008**, *42* (2), 429–435.
- 149 (13) Desloover, J.; De Clippeleir, H.; Boeckx, P.; Du Laing, G.; Colsen, J.; Verstraete, W.; Vlaeminck, S. E. Floc-
150 based sequential partial nitrification and anammox at full scale with contrasting N₂O emissions. *Water Res.* **2011**,
151 *45* (9), 2811–2821.
- 152 (14) Frear, D. S.; Burrell, R. C. Spectrophotometric Method for Determining Hydroxylamine Reductase Activity in
153 Higher Plants. *Anal. Chem.* **1955**, *27* (10), 1664–1665.