Kinetics of nitrous oxide production from hydroxylamine oxidation by birnessite in seawater

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ABSTRACT

This study characterized the kinetics of abiotic production of the greenhouse gas nitrous oxide (N2O) by chemical oxidation of the nitrification intermediate hydroxylamine (NH2OH) in seawater at circumneutral pH (6.2–8.3). The oxidant was birnessite, a ubiquitous manganese oxide mineral in a variety of marine environments. Experiments using microsensor electrodes for high-resolution measurements of N2O production combined with the simultaneous measurements of the removal of NH2OH using spectrophotometric techniques revealed that the reaction was overall first order with the rate law d[N2O]/dt = k[NH2OH]¹⁰[O2]¹²[H⁺]¹⁰ where k is 0.01 s⁻¹. Birnessite consistently oxidized 80–100% of NH2OH to N2O within 3 min. Mass balance on nitrogen indicated rapid formation and disappearance of an intermediate species that was evidently involved in the formation of N2O. In the presence of a nitroxyl (HNO) scavenger, N2O production rates and yield were suppressed by 17–59% and ~50%, respectively, suggesting that HNO is an intermediate in NH2OH oxidation to N2O. These results support a mechanism whereby Mn(IV) is reduced to Mn(III) with the formation of an amine (NH2OH) radical as the first product of NH2OH oxidation, which donates a second electron to another Mn(IV) center, or reduces the same Mn(III), to release Mn²⁺ and HNO in solution. The final step is predicted to be HNO dimerization to N2O given the complete oxidation of NH2OH to N2O at steady-state. The experimentally-derived second-order rate constant for the dimerization step suggests that adsorption of HNO onto the excess solid surface controls the rate of N2O formation. Our findings suggest that abiotic NH2OH oxidation could be an important source of N2O in coastal ecosystems such as open oceans and oxygen minimum zones as well as sediment ecosystems wherever nitrification occurs in the presence of particulate metal oxides.

1. Introduction

Nitrous oxide (N2O) is a potent greenhouse gas with over 250 times the warming potential of carbon dioxide per 100 year timescale (IPCC 2014). Bacterial nitrification (NH4⁺ → NH3·H2O → NO → N2O), denitrification (NO3⁻ → NO2⁻ → NO → N2O → N2), and nitrifier denitrification (NH4⁺ → NH3·H2O → NO → NO2⁻ → NO → N2O) all contribute to global N2O emissions (Caranto and Lancaster 2017; Stein 2011), while archael nitrification may be an important source of N2O from the oceans (Santoro et al. 2011).

Recent studies have challenged the assumption that N2O is produced purely by enzymatic pathways, and highlighted the importance of understanding the mechanisms of coupled biotic-abiotic interactions between redox-active nitrogen and metal species (Kozlowski et al. 2016; Liu et al. 2017; Luther et al. 1997; Zhu-Barker et al. 2015). One such reaction is hydroxylamine (NH2OH) oxidation coupled to the reduction of manganese (Mn) oxides such as Mn(IV)O2:

\[2\text{NH}_2\text{OH} + 2\text{Mn(IV)}\text{O}_2 + 4\text{H}^+ \rightarrow \text{N}_2\text{O} + 2\text{Mn}^{2+} + 3\text{H}_2\text{O}\]  

(1)

The reaction is overall first order with the rate law

\[d[N_2O]/dt = k[NH_2OH]^{1.0}[O_2]^{1.2}[H^+]^{1.0}\]

where \(k\) is 0.01 s⁻¹. Birnessite consistently oxidized 80–100% of NH2OH to N2O within 3 min. Mass balance on nitrogen indicated rapid formation and disappearance of an intermediate species that was evidently involved in the formation of N2O. In the presence of a nitroxyl (HNO) scavenger, N2O production rates and yield were suppressed by 17–59% and ~50%, respectively, suggesting that HNO is an intermediate in NH2OH oxidation to N2O. These results support a mechanism whereby Mn(IV) is reduced to Mn(III) with the formation of an amine (NH2OH) radical as the first product of NH2OH oxidation, which donates a second electron to another Mn(IV) center, or reduces the same Mn(III), to release Mn²⁺ and HNO in solution. The final step is predicted to be HNO dimerization to N2O given the complete oxidation of NH2OH to N2O at steady-state. The experimentally-derived second-order rate constant for the dimerization step suggests that adsorption of HNO onto the excess solid surface controls the rate of N2O formation. Our findings suggest that abiotic NH2OH oxidation could be an important source of N2O in coastal ecosystems such as open oceans and oxygen minimum zones as well as sediment ecosystems wherever nitrification occurs in the presence of particulate metal oxides.

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flocs suspended above oxyclines (Nameroff et al. 2002), at seawater-sediment interfaces (Lin and Taillefert 2014; Luther et al. 1997), and in ferromanganese nodules in deep sea sediments (Mallik 1980; Shiraishi et al. 2016). In this study, we characterized the kinetics of N2O oxidation by birnessite in synthetic seawater at circumneutral pH as a first step towards understanding the importance of abiotic N2O production in marine ecosystems.

2. Methods

2.1. Synthetic ocean water preparation

All experiments were performed in 2.4 mL Eppendorf tubes to quantify N2O consumption and potential production of other dissolved N species. Hydroxylamine (100 μM) was added to SOW containing 0, 300, or 1000 μM MnO2. Samples were collected every 5–30 s for 3 min and filtered through 0.45 μm cellulose acetate syringe filters (VWR International). Nitrate (NO3−) and nitrite (NO2−) concentrations were analyzed using the modified Griess method (Garca-Rodrigo et al. 2014). Measurement of NH4+ by the phenol hypochlorite method (Solórzano 1969) was infeasible due the NH4OH interference (Riley 1953).

A variety of NH4OH spectrophotometric analyses were tested to determine the optimal procedure for our experimental conditions. When used with SOW, the reagents in the quinolinol method developed by Frear and Burrell (1955) formed a thick, cloudy precipitate, preventing further use. Instead, we optimized the iodine method, originally described by Fiadeiro et al. (1967) and improved in Strickland and Parsons (1972), for use in small volumes of SOW. Specifically, 1 mL sample was placed in a 2.0 mL Eppendorf tube, and 40 μL of sulfanilic acid and 20 μL of iodine solution were added and allowed to react for 3 min. Then, 20 μL of a sodium arsenite solution was added and allowed to react for 2 min. Finally, 20 μL of N-(1-Napthyl)-ethylenediamine was added, color was allowed to develop for 15 min, and the absorbance at 543 nm was read by an UV–Vis spectrophotometer. Calibration curves were made using filtered MnO2 solutions in SOW with a 0.45 μm cellulose acetate syringe filter to account for possible interferences from Mn.

3. Results

3.1. Mass balance

To determine the percent of NH4OH oxidized to N2O as a function of MnO2 concentration, we quantified the consumption of NH4OH (initial concentration 100 μM) and production of N2O at pH 7.8. No detectable NH4OH consumption, and no more than 6 μM N2O (10% yield) production was observed in the absence of MnO2 (Fig. 1a). In the presence of MnO2, ≥50% NH4OH was consumed within 5 s of NH4OH addition, and 97–100% NH4OH was consumed within 210 s, exhibiting a pseudo-first order decay with a half-life of 20–25 s (Fig. 1b–f; Table 1). NO3− production was minimal in all experiments (< 5 μM; data not shown). Mass balance indicates that an unknown intermediate species is produced initially, but consumed over time (Fig. 1b–f). After accounting for the 2:1 substrate-to-product ratio, we found that 89–100% of NH4OH was converted to N2O at steady-state (Table 1), and that N2O yield from NH4OH was inversely proportional to the initial NH4OH concentration (Tables 1, 2). When HNO scavenger N-acetyl-L-cysteine was added, N2O production rates decreased by 17–59% (Fig. 2) while N2O production yields decreased by 41–52% (not shown). Minimal N2O was produced when NH4OH was replaced with NH4+ (Fig. 2).

3.2. Kinetic experiments to determine the overall order of the reaction and the rate constant

To determine the overall order and rate constant of the reaction, a general rate law for NH4OH oxidation by MnO2 was developed:

\[ \frac{d[NH_4OH]}{dt} = -k[NH_4OH][MnO_2][F^+] \]

(2)

where a, b, and c represent the order of the reaction with respect to each reactant, and k is the rate constant.

A parallel set of experiments were performed in 2.0 mL Eppendorf tubes to quantify N2O consumption and potential production of other dissolved N species. Hydroxylamine (100 μM) was added to SOW containing 0, 300, or 1000 μM MnO2. Samples were collected every 5–30 s for 3 min and filtered through 0.45 μm cellulose acetate syringe filters (VWR International). Nitrate (NO3−) and nitrite (NO2−) concentrations were analyzed using the modified Griess method (Garca-Rodrigo et al. 2014). Measurement of NH4+ by the phenol hypochlorite method (Solórzano 1969) was infeasible due the NH4OH interference (Riley 1953).

A variety of NH4OH spectrophotometric analyses were tested to determine the optimal procedure for our experimental conditions. When used with SOW, the reagents in the quinolinol method developed by Frear and Burrell (1955) formed a thick, cloudy precipitate, preventing further use. Instead, we optimized the iodine method, originally described by Fiadeiro et al. (1967) and improved in Strickland and Parsons (1972), for use in small volumes of SOW. Specifically, 1 mL sample was placed in a 2.0 mL Eppendorf tube, and 40 μL of sulfanilic acid and 20 μL of iodine solution were added and allowed to react for 3 min. Then, 20 μL of a sodium arsenite solution was added and allowed to react for 2 min. Finally, 20 μL of N-(1-Napthyl)-ethylenediamine was added, color was allowed to develop for 15 min, and the absorbance at 543 nm was read by an UV–Vis spectrophotometer. Calibration curves were made using filtered MnO2 solutions in SOW with a 0.45 μm cellulose acetate syringe filter to account for possible interferences from Mn.
3.2.1. Order with respect to NH₂OH

To determine the order with respect to NH₂OH, the isolation method was used, in which initial NH₂OH concentrations were varied while MnO₂ and pH were kept constant, such that Eq. (2) can be rearranged to give:

\[
\frac{d[NH₂OH]}{dt} = -k_{obs}[NH₂OH]^a
\]

(3)

where

\[k_{obs} = k \left[MnO₂\right]^b[H⁺]^c\]

(4)

If the unknown intermediate species is rapidly produced and consumed, the steady-state hypothesis can be used to relate the rate of NH₂OH consumption to the rate of N₂O production measured by the microsensor such that Eq. (3) becomes:

\[-\frac{d[NH₂OH]}{dt} = \frac{2d[N₂O]}{dt} = R = k_{obs}[NH₂OH]^a\]

(5)

where \(R\) is the rate of N₂O production. As the rate of N₂O production was linear over most of the reaction (Fig. 3a), it was possible to use the initial rate method to obtain \(a\) after rearranging Eq. (5) to:

\[\log(R) = \log(k_{obs}) + a \times \log([NH₂OH])\]

(6)

where \([NH₂OH]\) is the initial concentration of NH₂OH.

We found that initial N₂O production rates increased linearly with increasing NH₂OH concentrations (Fig. 3a). The slope of Eq. (6) gives \(a\), the order of reaction with respect to NH₂OH, which was found to be first order \((a = 0.9 ± 0.07; \text{Fig. 4a})\).

3.2.2. Order with respect to MnO₂

To determine the order of the reaction with respect to MnO₂, experiments were performed by injecting 100 μM NH₂OH into 361, 414, 426, 677, and 915 μM MnO₂ at pH 7.8 and monitoring NH₂OH concentrations as a function of time (Fig. 1b–f). Because the order of the reaction with respect to NH₂OH was ~1 (Fig. 4a), Eq. (2) can be rearranged to:

\[-\frac{d[NH₂OH]}{dt} = k_{obs}[NH₂OH]\]

(7)

where

\[k_{obs} = k \left[MnO₂\right]^b[H⁺]^c\]

(8)

In this case, the initial rate method was used to calculate the \(k_{obs}\) from Eq. (7) for each experiment. The order of the reaction, \(b\), was then determined from the linearized form of Eq. (8) by representing log \(k_{obs}\) at each concentration of MnO₂ as a function of the initial concentration of MnO₂ at fixed pH:

\[\log(k_{obs}) = \log(k[H⁺]^c) + b \times \log([MnO₂])\]

(9)

Using this approach, a slope \((b)\) equal to the order of reaction with respect to MnO₂, of 0.3 ± 0.08, was found (Fig. 4b).

3.2.3. Order with respect to proton concentration

To determine the order of reaction with respect to the proton concentration, experiments were carried out by injecting 100 μM NH₂OH into 300 μM MnO₂ at pH 6.2, 6.7, 7.0, 7.8, and 8.3, and monitoring N₂O concentrations as a function of time (Fig. 3c). The same method used for determining the order of reaction with respect to MnO₂ was used for determining the order of reaction with respect to the proton.

### Table 1

<table>
<thead>
<tr>
<th>[MnO₂] (μM)</th>
<th>[NH₂OH]° (μM)</th>
<th>[NH₂OH] (μM)</th>
<th>[N₂O] (μM)</th>
<th>% NH₂OH consumption</th>
<th>% NH₂OH conversion to N₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>364.5 ± 4.9</td>
<td>103.0 ± 16.7</td>
<td>2.6 ± 1.0</td>
<td>48.8 ± 5.4</td>
<td>97.5</td>
<td>97.2</td>
</tr>
<tr>
<td>468.5 ± 60.1</td>
<td>107.3 ± 1.7</td>
<td>0.8 ± 0.2</td>
<td>56.2 ± 0.2</td>
<td>99.3</td>
<td>94.3</td>
</tr>
<tr>
<td>661.0 ± 22.6</td>
<td>95.5</td>
<td>0.4 ± 0.2</td>
<td>46.9 ± 9.1</td>
<td>99.6</td>
<td>98.6</td>
</tr>
<tr>
<td>717.5 ± 102.5</td>
<td>95.5</td>
<td>0.4 ± 0.2</td>
<td>42.5 ± 6.5</td>
<td>99.6</td>
<td>89.4</td>
</tr>
<tr>
<td>949.5 ± 48.8</td>
<td>88.6 ± 1.7</td>
<td>0.3 ± 0.3</td>
<td>44.0 ± 13.1</td>
<td>99.7</td>
<td>99.7</td>
</tr>
</tbody>
</table>

Fig. 1. Complete conversion of NH₂OH to N₂O in various experiments. Mass balance conserved between NH₂OH and N₂O from experiments conducted with 100 μM NH₂OH with varied concentrations of MnO₂ at pH 7.8. Conversion to N₂O ranged from 89 to 100%.
The slope of the initial rate of N\textsubscript{2}O production presented as a function of the initial concentration of NH\textsubscript{2}OH (Eq. 7) was used to calculate the pseudo-first order rate constant (k\textsubscript{obs}) at each pH. The order of the reaction with respect to proton concentration, c, was then determined by linearizing Eq.(8) to:

$$\log k\textsubscript{obs} = \log(k\textsubscript{MnO}) + c \times \log[H^+]$$

The slope of the log of the k\textsubscript{obs} as a function of the pH (Eq.(10)) indicates a zero order reaction with respect to pH ($c = 0.007 \pm 0.011$) at circumneutral pH (Fig. 4c).

**3.2.4. Rate law**

The orders of each reactant were substituted into Eq. (2) to give Eq. (11):

$$\frac{dN\textsubscript{2}O}{dt} = k [NH\textsubscript{2}OH]^{0.9}[MnO\textsubscript{2}]^{0.3}$$

When the production rates of N\textsubscript{2}O and initial concentrations of NH\textsubscript{2}OH and MnO\textsubscript{2} from each experiment (Table 2) are substituted into Eq. (11), the overall first order rate constant, k, is found to be $0.009 \pm 0.002$ s\textsuperscript{-1}. When the overall first order rate constant is calculated using NH\textsubscript{2}OH oxidation rates, k is found to be $7.45 \pm 0.59$ for pH 7.33 ± 0.42.

**Table 2**

| [MnO\textsubscript{2}] (×10\textsuperscript{-6} M) | [NH\textsubscript{2}OH] (×10\textsuperscript{-6} M) | pH | Rate\textsubscript{NH2O} (D1) (×10\textsuperscript{-7} M s\textsuperscript{-1}) | Rate\textsubscript{NH2O} (D2) (×10\textsuperscript{-7} M s\textsuperscript{-1}) | k\textsubscript{obs} (D1) (×10\textsuperscript{-3} s\textsuperscript{-1}) | k\textsubscript{obs} (D2) (×10\textsuperscript{-3} s\textsuperscript{-1}) | Rate\textsubscript{NH2OH} (D1) (×10\textsuperscript{-3} s\textsuperscript{-1}) | Rate\textsubscript{NH2OH} (D2) (×10\textsuperscript{-3} s\textsuperscript{-1}) | k\textsubscript{NH2OH} (D1) (×10\textsuperscript{-3} s\textsuperscript{-1}) | k\textsubscript{NH2OH} (D2) (×10\textsuperscript{-3} s\textsuperscript{-1}) | Mean yield (%) |
|-----------------|-----------------|-----|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|----------------|
| 790 | 100 | 7.8 | 2.40 | 2.02 | 8.14 | 6.85 | 7.45 ± 0.59 | 7.33 ± 0.42 | 91.3 |
| 150 | 7.21 | 3.08 | 7.57 | 3.34 | 4.65 | 13.9 | 19.3 | 100 |
| 200 | 7.38 | 5.26 | 7.38 | 7.82 | 80.1 |
| 250 | 6.05 | 6.08 | 7.64 | 7.68 | 80.4 |
| 300 | 6.2 | 2.14 | 2.21 | 10.1 | 10.5 | 100 |
| 6.7 | 1.99 | 2.33 | 9.13 | 10.7 | 100 |
| 7.0 | 2.36 | 2.26 | 10.9 | 10.5 | 96.9 |
| 7.8 | 2.18 | 2.45 | 9.73 | 10.9 | 100 |
| 8.3 | 2.59 | 2.28 | 11.3 | 9.94 | 100 |
| 300 | 100 | 6.2 | 2.14 | 2.21 | 10.1 | 10.5 | 100 |
| 6.7 | 1.99 | 2.33 | 9.13 | 10.7 | 100 |
| 7.0 | 2.36 | 2.26 | 10.9 | 10.5 | 96.9 |
| 7.8 | 2.18 | 2.45 | 9.73 | 10.9 | 100 |
| 8.3 | 2.59 | 2.28 | 11.3 | 9.94 | 100 |
| Mean | 7.69 ± 1.45 | 8.89 ± 1.52 | 10.2 ± 0.87 | 10.5 ± 0.37 | 12.9 ± 3.5 |

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**Fig. 2.** Addition of N-acetyl-L-cysteine decreases N\textsubscript{2}O production rates, and there is no noticeable N\textsubscript{2}O production from NH\textsubscript{4}+\textsuperscript{+}. Nitrous oxide production rates decrease with 300–900 μM N-acetyl-L-cysteine addition (3× NH\textsubscript{2}OH concentration) at pH 7.8. Error bars, when present, show range of data (n = 2, except for 100 μM NH\textsubscript{2}OH into 1103 μM MnO\textsubscript{2} where n = 3). No error bars represent single experiments (n = 1).

**Fig. 3.** Nitrous oxide production rates increase with increasing NH\textsubscript{2}OH. Averaged N\textsubscript{2}O production rate from (a) varied initial NH\textsubscript{2}OH concentrations into 790 μM MnO\textsubscript{2} at pH 7.8 ± 0.1, (b) 100 μM NH\textsubscript{2}OH into varied initial MnO\textsubscript{2} concentrations at pH 7.8 ± 0.1, and (c) 100 μM NH\textsubscript{2}OH into 300 μM MnO\textsubscript{2} at varied but constant pH. The control (open triangles) represents 100 μM NH\textsubscript{2}OH into SOW with no MnO\textsubscript{2}. Slope and standard error of the averaged best fit line (n = 2) are shown below varied parameters.
0.013 ± 0.004 s\(^{-1}\). The good agreement between these two calculations indicates that the steady-state hypothesis used to calculate the order of the reaction with respect to NH\(_2\)OH and proton concentrations was appropriate.

4. Discussion

4.1. Mineral effects on NH\(_2\)OH reactivity

To our knowledge, this is the first study that measures the kinetics of NH\(_2\)OH oxidation by an environmentally-relevant mineral substrate (MnO\(_2\)). In previous studies with enzyme-bound Mn(IV) or ligand-bound Mn(III) in pure water or acidic perchlorate solution (Table \(3\)), NH\(_2\)OH was suggested to interact with the Mn(III, IV) center by outer-sphere complexation (Banerjee et al. 2002; Salem 1995). In this study, NH\(_2\)OH oxidation takes place at the MnO\(_2\) mineral surface, where high surface area, small particle size, and highly reactive surface sites mediate rapid reaction. In seawater, abundant cations may enter the interlayer region of birnessite and replace water molecules, leading to strong aggregation (Holland and Walker 1996; Villalobos et al. 2003). Thus, the small decrease in N\(_2\)O production rates observed at increasing MnO\(_2\) concentration (Table \(2\)) may be due to aggregation.

4.2. Known competing reactions

We considered the following abiotic processes to explain the findings reported above.

4.2.1. Nitrosation

Nitrosation involves the transfer of a NO group from nitrous acid compound to a nitrogenous nucleophilic center. For example, NH\(_2\)OH and HNO can react to form N\(_2\):

\[ \text{HNO} + \text{NH}_2\text{OH} \rightarrow \text{N}_2 + 2\text{H}_2\text{O} \]

\( \Delta G^\circ = -328.78 \text{ kJ mol}^{-1} \) (Latimer 1952; Shaﬁrovich and Lymar 2002).

At low pH, NH\(_2\)OH may be oxidized by HNO\(_2\) to form N\(_2\)O (Hussain et al. 1968; Soler-Jofra et al. 2016):

\[ \text{NH}_2\text{OH} + \text{HNO}_2 \rightarrow \text{N}_2\text{O} + 2\text{H}_2\text{O} \] (13)

\[ \text{NH}_2\text{O}^+ + \text{HNO}_2 \rightarrow \text{N}_2\text{O} + \text{H}_2\text{O} + \text{H}_3\text{O}^+ \] (14)

Nitroxyl (HNO) may also dimerize to produce N\(_2\)O:

\[ \text{HNO} + \text{HNO} \rightarrow \text{N}_2\text{O} + \text{H}_2\text{O} \] (15)

The importance of Eq. (12) has been demonstrated at high NH\(_2\)OH:Na\(_2\)N\(_2\)O\(_3\) (HNO donor) ratios and may explain the lower N\(_2\)O yields with high NH\(_2\)OH (Table \(2\)), but the reaction is slower than HNO dimerization (Eq. (15); Fehling and Friedrichs 2011; Shaﬁrovich and Lymar 2002), and is unlikely to be important in natural waters with low NH\(_2\)OH (see below). Eqs. (13) and (14) are unlikely to have occurred in this study because all experiments were performed above the pKa of NH\(_2\)OH (5.9) and HNO\(_2\) (2.8), and NO\(_2^-\) concentrations did not significantly change during the reaction (data not shown). Together with the fact that the addition of a HNO scavenger decreased rates (Fig. \(2\)) and yields (not shown) of N\(_2\)O production, these considerations suggest that HNO dimerization is the most likely nitrosation reaction to occur in these experiments.

4.2.2. Hydroxylamine disproportionation

Hydroxylamine disproportionation (or autoxidation) to NH\(_4^+\), N\(_2\)O or N\(_2\) (Bari et al. 2010; Bonner et al. 1978) is described by the following reactions:

\[ 2\text{NH}_2\text{OH} + \text{H}^+ \rightarrow \text{NH}_4^+ + \text{HNO} + 3\text{H}_2\text{O} \] (16)

\[ 4\text{NH}_2\text{OH} + 2\text{H}^+ \rightarrow \text{N}_2\text{O} + 2\text{NH}_4^+ + 3\text{H}_2\text{O} \] (17)

\[ 3\text{NH}_2\text{OH} + \text{H}^+ \rightarrow \text{N}_2 + \text{NH}_4^+ + 3\text{H}_2\text{O} \] (18)

These reactions are catalyzed by copper and other reduced metals (Anderson 1964; Butler and Gordon 1986; Moews Jr and Audrieth, 1959). Hydroxylamine disproportionation was ruled out as a significant contributor to N\(_2\)O production in previous studies with enzyme-bound Mn(IV) or ligand-bound Mn(III, IV) centers. In this study, however, NO\(_2^-\) was present in the reaction mixture, and yields (not shown) of N\(_2\)O production, these considerations suggest that HNO dimerization is the most likely nitrosation reaction to occur in these experiments.

4.2.3. Nitrous oxide reduction by Mn\(^{2+}\)

The reduction of N\(_2\)O by Mn\(^{2+}\) was considered a possible mechanism for a general decrease in N\(_2\)O production rates with increasing MnO\(_2\) concentrations (Figs. 3b; Table \(2\)). In the investigated reaction (Eq. \(1\)), Mn\(^{2+}\) would be produced simultaneously with N\(_2\)O, and this Mn\(^{2+}\) could, in theory, reduce N\(_2\)O to N\(_2\):

\[ \text{N}_2\text{O} + \text{Mn}^{2+} + \text{H}_2\text{O} \rightarrow \text{N}_2 + \text{MnO}_2 + 2\text{H}^+ \]

\( \Delta G^\circ = -52.06 \text{ kJ mol}^{-1} \)

Eq. (19) could account for the small fraction of missing N in our mass balance. If N\(_2\) produced by this reaction was the missing nitrogen fraction, however, it should have increased as a function of time, as Mn\(^{2+}\) was progressively produced during the reduction of MnO\(_2\) by NH\(_2\)OH. As the missing nitrogen fraction was immediately produced and progressively removed from solution (Fig. \(1\)), this reaction was likely not significant in these experiments.

4.2.4. Nitroxyl binding by Mn\(^{3+}\)

A more likely explanation for the increased rates of NH\(_2\)OH consumption with increasing MnO\(_2\), without significant change in rates of
Table 3

<table>
<thead>
<tr>
<th>Source</th>
<th>NH₂OH oxidation and other reactions</th>
<th>Measurement method</th>
<th>pH</th>
<th>Overall order</th>
<th>Rate constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banerjee et al. (2002)</td>
<td>Tri-bridged Mn(IV,IV) dimer</td>
<td>Double-distilled water</td>
<td>4.0</td>
<td>−</td>
<td>15.0 × 10³ s⁻¹</td>
</tr>
<tr>
<td>Davies and Kustin (1969)</td>
<td>Mn(III)</td>
<td>Acidic perchlorate media</td>
<td>0.5-0.2</td>
<td>2</td>
<td>1.5 × 10⁻¹ M⁻¹ s⁻¹</td>
</tr>
<tr>
<td>Hynes et al. (1993)</td>
<td>Bis(pentane-2,4-dionato)diaquo manganese(III)</td>
<td>Double-distilled water</td>
<td>5.02</td>
<td>2</td>
<td>5.70 × 10⁻² M⁻¹ s⁻¹</td>
</tr>
<tr>
<td>Shafovich and Lymar (2002)</td>
<td>Mn(III)-bis(salicylaldimine) complexes</td>
<td>Double-distilled water</td>
<td>5.2</td>
<td>2</td>
<td>0.16 M⁻¹ s⁻¹</td>
</tr>
<tr>
<td>Jackson et al. (2009)</td>
<td>HNO reduction by NH₂OH</td>
<td>Deionized water with chelator</td>
<td>7.45</td>
<td>2</td>
<td>4.0 ± 0.3 × 10⁻⁹ M⁻¹ s⁻¹</td>
</tr>
<tr>
<td>Shafovich and Lymar (2002)</td>
<td>HNO trapping by ligand-bound Mn³⁺ center</td>
<td>0.1 M phosphate buffer with EDTA</td>
<td>7 or 10</td>
<td>2</td>
<td>4 × 10⁻⁹ M⁻¹ s⁻¹</td>
</tr>
</tbody>
</table>

4.3. Proposed reaction sequence and mechanisms of NH₂OH oxidation to N₂O by MnO₂

Here we propose a reaction sequence for the oxidation of NH₂OH to N₂O by MnO₂ consistent with our experimental findings and mass balance. Birnessite is well known to have low zero point charge (e.g. ~2.25; Balistrieri and Murray 1982; Murray 1974), making the surface charge negative across a wide range of pH, including the circumneutral pH values used in this study. This surface property, combined with its high surface area and presence of structural vacancy sites, makes birnessite highly reactive for the sorption of various species, especially positively charged species such as metal cations. The lone electron pair on N in NH₂OH is likely to introduce a polar effect; thus, the molecule can be attracted to charged surfaces such as the birnessite surface. Although the exact location (e.g. vacancy site vs. edge site) and mechanism (e.g. inner-sphere vs. outer-sphere) of the interaction is unclear, NH₂OH as a polar molecule can possibly form an adsorbed complex with the birnessite surface, transferring an electron to a Mn(IV) atom (Fig. 5a), reducing Mn(IV) to Mn(III), and also resulting in aminoxyl radical (Banerjee et al. 2002; Davies and Kustin 1969; Salem 1995) that may further reduce a Mn(IV) metal center to Mn(III), and either scavenge HNO, or reduce the same Mn(III) intermediate to release both Mn²⁺ and HNO into solution. Release of HNO to solution is likely followed by dimerization to N₂O. Although these mechanistic details will be difficult to distinguish experimentally, the high N₂O yield at steady-state (Tables 1 and 2) indicates the sequestration of HNO onto the solid phase was likely not significant.
4.4. Environmental implications

Based on our findings, rapid N$_2$O production from the coupled biotic-abiotic reaction of microbial NH$_4^+$ oxidation to NH$_2$OH, followed by abiotic NH$_2$OH oxidation by Mn(III, IV) oxides, could occur wherever active nitrifying and Mn$^{2+}$-oxidizing bacteria coexist (Clement et al. 2009; Tebo 1991). Relevant environments include oxyclines above oxygen minimum zones (Beman et al. 2012; Bouskill et al. 2012; Bruland et al. 1994; Löscher et al. 2012; Neretin et al. 2003; Newell et al. 2011; Shiller and Gieskes 1985; Trefry et al. 1984), sediment-water interfaces (Anschutz et al. 2005), and deep sea sediments that contain ferromanganese nodules (Blöthe et al. 2015; Shiraishi et al. 2016). In oceanic environments, particulate Mn tends to be low in concentration, which would decrease the likelihood of aggregation, and potentially lead to faster rates of N$_2$O production than observed in our experiments.

Hydroxylamine is likely the limiting substrate for Eq. (1) in nature because NH$_2$OH occurs at low concentrations and is an essential intermediate in nitrification. During periods of intense nitrification in coastal waters, NH$_2$OH can accumulate up to ~200 nM (Butler et al. 1987, 1988; Gebhardt et al. 2004; Schweiger et al. 2007). The presence of NH$_2$OH in natural waters indicates that it leaks out of cells, and suggests that the fast N$_2$O production from abiotic NH$_2$OH oxidation with Mn(III/IV) oxides could be easily confused with N$_2$O from biological nitrification (Kozlowski et al. 2016). Using representative conservative concentrations from the oxycline Baltic Sea, 31 nM MnO$_2$ (Neretin et al. 2003) and 18 nM NH$_2$OH (Schweiger et al. 2007) and Eq. (11), we calculated that rates of abiotic N$_2$O production from MnO$_2$ oxidation of NH$_2$OH would be ~500 nM d$^{-1}$, whereas measured rates were 135 nM d$^{-1}$ from denitrifying bacterial cultures obtained from the Baltic Sea (Rönner and Sörensson 1985). We acknowledge that fluctuating substrate concentrations could produce a wider range of rates, but this simple calculation suggests that abiotic N$_2$O production could contribute significantly to N$_2$O production in marine oxyclines.

The finding that nitroxyl (HNO), and possibly the aminoxyl radical (H$_2$NO$^+$), are intermediates in the abiotic NH$_2$OH oxidation by Mn(III/IV) oxides suggests that alternative reactive N species may be involved in coupled biotic/abiotic interactions in the nitrogen cycle. Although

Fig. 5. Proposed mechanism for NH$_2$OH oxidation by birnessite. (a) Electron transfer from NH$_2$OH to the Mn(IV) and deprotonation of the hydroxyl group allow formation the aminoxyl radical (H$_2$NO$^+$) and reduction of the Mn(IV) to Mn(III). The electron from the H$_2$NO$^+$ is transferred to either (b) the Mn(III) to produce nitroxyl (HNO) and Mn$^{2+}$ or (c) to another Mn(IV) to produce another Mn(III) and HNO which then could adsorb onto the Mn(III) product. (d) Nitroxyl dimerizes to form cis-ON(H)N(H)O, which deprotonates to cis-ON(H)NO$^−$. (e) O-protonation occurs and forms cis-HONN(O)H, which deprotonates to the unstable cis-hyponitrite anion (HONNO$^−$). (f) The cis-hyponitrite anion loses its hydroxyl group to form N$_2$O.

Fig. 6. Nitroxyl dimerization exhibits pseudo-second order characteristics. Concentrations of HNO calculated from mass balance of varied MnO$_2$ experiments in Fig. 1b-f. The second-order rate constant of HNO dimerization, $k_2$, is found to be $239 \pm 93$ M$^{-1}$ s$^{-1}$ in the experiments. The line represents the best linear regression fit of all data sets.
HNO may also be produced as an enzymatic intermediate in the nitrogen cycle (Bykov et al. 2014; Hooper and Terry 1979; Komarov et al. 2000; Xia et al. 2000), it is commonly assumed that it remains bound to the enzyme and is rapidly converted to NO$_2^-$, N$_2$O, or NH$_4^+$ during nitrification, denitrification, and dissimilatory nitrate reduction to ammonium, respectively. Free HNO, however, is essential in nitric oxide reduction by nitric oxide synthase (Turk and Hollocher 1992), and Mn$^{2+}$ inhibits the dehydrogenation of HNO by hydroxylamine dehydrogenase (formerly hydroxylamine oxidoreductase) to produce N$_2$O instead of NO$_2^-$ (Hooper and Terry 1979). Thus, biologically produced HNO could dimerize with HNO from abiotic NH$_2$OH oxidation to enhance N$_2$O production.

Although the detection limit of the N$_2$O microsensor (1 μM) used in this study required use of higher concentrations of NH$_2$OH than typically found in nature, the rate constants measured here should apply to lower substrate concentrations as well. The proposed rate law and the overall first order rate constant could be used in biogeochemical models to account for the proportion of N$_2$O that is abiotically produced in a variety of environments.

5. Conclusions

To our knowledge, this is the first study that characterized the kinetics of NH$_2$OH oxidation by Mn(IV) oxides in seawater at circumneutral pH. We demonstrated that the reaction is overall first order with respect to NH$_2$OH concentrations. The reaction is rapid at circumneutral pH ($k = 0.01 \text{ s}^{-1}$), with complete oxidation occurring within minutes (half-life of ~23 s). Nitroxyl (HNO) was found to be an important intermediate in abiotic NH$_2$OH oxidation, as mass balance considerations indicate rapid formation and disappearance of an intermediate manganese species, and N$_2$O production was reduced in the presence of a HNO scavenger. Our findings suggest the potential for a novel biotic-abiogenic pathway by which any NH$_2$OH that leaks from the enzyme and is rapidly converted to NO$_2^-$ can account for the proportion of N$_2$O that is abiotically produced in a variety of environments.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marchem.2018.03.002.

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