Importance of the operating pH in maintaining the stability of anoxic ammonium oxidation (anammox) activity in moving bed biofilm reactors

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A B S T R A C T

Two bench-scale parallel moving bed biofilm reactors (MBBR) were operated to assess pH-associated anammox activity changes during long term treatment of anaerobically digested sludge centrate pretreated in a suspended growth partial nitrification reactor. The pH was maintained at 6.5 in reactor R1, while it was allowed to vary naturally between 7.5 and 8.1 in reactor R2. At high nitrogen loads reactor R2 had a 61% lower volumetric specific nitrogen removal rate than reactor R1. The low pH and the associated low free ammonia (FA) concentrations were found to be critical to stable anammox activity in the MBBR. Nitrite enhanced the nitrogen removal rate in the conditions of low pH, all the way up to the investigated level of 50 mg NO 2-N/L. At low FA levels nitrite concentrations up to 250 mg NO 2-N/L did not cause inactivation of anammox consortia over a 2-days exposure time.

1. Introduction

The discovery of anoxic ammonium oxidation (anammox) provided a cost effective nitrogen removal alternative for the treatment of high ammonium and low organic carbon wastewater (Fux and Siegrist, 2004). Anammox bacteria (related to Planctomycetes, Schmid et al., 2003) form a group of several genera of autotrophic organisms that convert ammonium and nitrite to dinitrogen gas and a small amount of nitrate. The anoxic nature of this reaction permits significant savings in aeration and organic carbon addition, yielding low excess biomass production (Eq. (1), Strous et al., 1998; van der Star et al., 2007). Overall, 60% of oxygen can be saved when about half of the ammonium is oxidized to nitrite and the nitrite route. Based on the Eq. (1), it should be noted that an increase of the pH in the anammox reactor may occur as hydrogen ions are consumed:

\[ \begin{align*}
&1 \text{NH}_4^+ + 1.32 \text{NO}_2^- + 0.066 \text{HCO}_3^- + 0.13 \text{H}^+ \\
&\quad \rightarrow 1.02\text{N}_2 + 0.26\text{NO}_3^- + 0.066\text{CH}_2\text{O} + 0.15\text{H}_2\text{O} + 2.03\text{H}_2\text{O}.
\end{align*} \]

(1)

The anammox process has been widely studied over the past two decades, however many inconsistencies appear among the obtained results. It was observed that the volumetric specific nitrogen removal rate (NRR) was highly variable for different and similar configurations (see Table 1). The nitrogen removal rate calculated per amount of volatile suspended solids (VSS) was also highly variable (Table 1). These inconsistencies in the specific anammox activity (SAA) were most likely related to such parameters as pH, substrate concentration, degree of enrichment and species of anammox organism enriched for, and inhibitory components, however these have not been investigated in significant detail.

The most widely accepted critical factor in the anammox system stability has been nitrite concentration, important to stability and responsible for severe inhibition under provided experimental conditions (Wett et al., 2007; Bettazzi et al., 2010). However, the effect of nitrite has not been clearly defined, with reported threshold concentrations varying between 5 and 274 mg N/L, under different experimental conditions and operating modes (Wett et al., 2007; Kimura et al., 2010).

Some researchers have suggested that NH 3 (or free ammonia – FA), may be one of the factors associated with deterioration of anammox activity deterioration, even at levels as low as 1.7 mg NH 3-N/L (Jung et al., 2007), however more recent literature does not consider FA to be an important parameter at FA concentrations below 13–15 mg NH 3-N/L (Fernández et al., 2008; Tang et al., 2010; Plaza et al., 2011). According to Martínez et al. (1996), FA diffuses through the cell membrane into the cell and changes the inner pH, neutralizing the membrane potential, thereby causing, in the worst case scenario, cell death. In
anammox cells, the riboplasm is alkaline and negatively charged relative to anammoxosome, thus giving a proton motive force (Fuerst et al., 2006). On the other hand, passive diffusion of protons across the biological membrane leads to high energy losses (van Niftrik et al., 2004). With a pKa of 9.24, the proportion of FA relative to ammonium \( \left( \text{NH}_4^+ \right) \) is pH-dependent, and increases greatly (about 24 times) within the pH range (pH between 7 and 8.5) reported to be optimal for the anammox process (Strous et al., 1997b).

Biofilm processes have proved to be reliable for nitrogen removal having some advantages over suspended growth activated sludge processes (Yang et al., 2009). The moving bed biofilm reactor (MBBR) originated from Europe and was preliminary designed for cold climate operation, where slow growing organism were protected from wash-out (Ødegaard, 2006). The fundamental principle of the MBBR is to immobilize biomass on carrier media, eliminating the need for sludge settling and return in a continuous operation system.

MBBRs have been used in past research to investigate a variety of operational strategies for nutrient removal systems. Studies included the evaluation of energy recovery options through mechanical mixing (Phattaranawik and Leiknes, 2011) and assessing the effect of aeration on the concentration of extracellular polymeric substances (EPS) (Rahimia et al., 2011). Reactor stability under changing hydraulic residence time (HRT) (Li et al., 2011) or the presence of concentrated organic substrates (Wanga et al., 2009) was also investigated in literature.

There has been limited research utilizing MBBRs for anammox processes (Thole et al., 2005; Szatkowska et al., 2007). These studies focused on the overall feasibility of nitrogen removal in MBBR reactor systems using anammox organisms, without emphasis on process optimization. Important operating parameters which affect system performance and stability, such as pH, free ammonia concentration, and the nitrite concentration have not been studied.

Research targeting nitrite and pH associated FA threshold concentration separately, considering the possibility that their inhibitory effects might be overlapping. This current work aimed at elucidating the role of nitrite and pH in long term anammox reactor operation, under high pH (naturally occurring) and low (controlled) pH conditions.

2. Methods

2.1. Reactors set-up

A two stage configuration was used as shown in Fig. 1. The first part of the system consisted of one continuously-fed sequencing batch reactor (SBR) for the partial nitritation process, with a working volume of 20 L. A Masterflex peristaltic pump was used to feed anaerobic digester centrate from a local wastewater treatment plant (North End Water Pollution Centre NEWPCC, Winnipeg, MB, Canada) to the reactor during the reaction phase. Centrate had an average total ammonia concentration of 743 mg N/L (std. deviation 58). Centrate was delivered twice a week from the plant, settled to remove solids, and stored at a constant temperature of 5°C.

The second part of the system (where all tests were conducted) consisted of two moving bed biofilm reactors (MBBR), R1 and R2, with 3 L working volume, each. The fill ratio with media was

![Figure 1](https://example.com/figure1.png)
50%. Anammox bacteria were cultivated on plastic carrier media (Kaldnes rings – K1). The pH was controlled in reactor R1 at 6.5 with 1 N sulphuric acid. In R2 the pH oscillated naturally in a range between 7.5 and 8, for the duration of the experiment. Both reactors were mixed using propeller mixers set at a mixing speed of 150 rpm. Oxygen diffusion into the reactor from the surrounding atmosphere was minimized through maintaining overpressure inside of the reactors by nitrogen gas produced during the reaction, and tight connections. The whole system was placed in a walk-in environmental chamber set at 35 °C.

Biomass for the partial nitrification reactor was taken from a nitrification reactor located in the laboratory at the University of Manitoba. The original anammox biomass was obtained from the pilot scale attached growth anammox reactor located in Stockholm, Sweden, courtesy of Dr. J. Trela from Royal Institute of Technology in Stockholm, Sweden. *Brocadia anammoxidans* was identified by Cema (2009) in that pilot plant as the dominant anammox organism.

### 2.2. Analyses

$\text{NH}_4^+\text{-N}, \text{NO}_2^-\text{-N}, \text{NO}_3^-\text{-N}$ were measured daily using an automatic flow injection analyser (QuichChem8500, Lachat Instruments). The pH was measured at constant sampling points by an Accumet portable AP61 pH-meter with an Ag/AgCl electrode. Samples for nitrogen analysis were filtered through a 0.45 μm filter. Alkalinity, solids and bulk dissolved oxygen were measured occasionally at the beginning of the experimental period (five times), according to Standard Methods (APHA, 1998), and by the Sension 378 HACH DO-meter, respectively. Samples were collected from the feed tank, the equalization tank, and the anammox reactors. Free, un-ionized ammonia $\text{FA}$ was calculated based on Anthonisen et al. (1976):

\[
\text{Free ammonia as NH}_3^-\text{N} (\text{mg N/L}) = \frac{\text{Total ammonia as N} (\text{mg NH}_4^+\text{-N/L}) \cdot 10^{pH}}{e^{0.644 - 21.37/pH} + 10^{pH}}. \tag{2}
\]

### Table 2

Operation of reactors R1 and R2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Low load condition</th>
<th>Medium load condition</th>
<th>High load condition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R1</td>
<td>R2</td>
<td>R1</td>
</tr>
<tr>
<td><strong>Research period</strong></td>
<td>d</td>
<td>34–54</td>
<td>34–54</td>
<td>0–19</td>
</tr>
<tr>
<td><strong>NH$_4$-N</strong></td>
<td>mg N/L</td>
<td>42.0 ± 15.0</td>
<td>26.1 ± 14.0</td>
<td>66.4 ± 17.1</td>
</tr>
<tr>
<td><strong>NO$_2$-N</strong></td>
<td>mg N/L</td>
<td>3.3 ± 0.4</td>
<td>3.1 ± 0.6</td>
<td>9.3 ± 0.8</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td></td>
<td>6.5 ± 0.01</td>
<td>7.46 ± 0.07</td>
<td>6.5 ± 0.01</td>
</tr>
<tr>
<td><strong>NRR</strong></td>
<td>mg N/Ld</td>
<td>1143 ± 135</td>
<td>1090 ± 75</td>
<td>2466 ± 74</td>
</tr>
<tr>
<td><strong>FA</strong></td>
<td>mg N/L</td>
<td>0.2 ± 0.1</td>
<td>0.8 ± 0.4</td>
<td>0.4 ± 0.5</td>
</tr>
<tr>
<td><strong>NO$_2$-N/ NH$_4$-N</strong></td>
<td>mg N/mg N</td>
<td>1.16 ± 0.05</td>
<td>1.10 ± 0.05</td>
<td>1.19 ± 0.09</td>
</tr>
<tr>
<td><strong>NO$_3$-N/ NH$_4$-N</strong></td>
<td>mg N/mg N</td>
<td>0.15 ± 0.04</td>
<td>0.12 ± 0.01</td>
<td>0.16 ± 0.04</td>
</tr>
</tbody>
</table>

Fig. 2. The NRR and nitrite during long-term reactors operation in: (a) R1 at pH 6.5 ± 0.01 and (b) R2 at an ambient pH 7.8 ± 0.24.
Solids accumulated on the moving media were estimated based on randomly chosen rings and subsequent scraping of the biomass from the media using metal wire and de-ionised water. Such prepared samples were used to estimate solids content in reactors according to Standards Methods (APHA, 1998).

### 2.3. Reactors operation

Two parallel reactors were set up; R1 with pH set at 6.5 and R2 naturally maintaining pH between 7.5 and 8.1, without pH control. The nitrogen removal rate (NRR) was calculated as the difference between the nitrogen load to and from the anammox reactor:

\[
NRR = \left( \frac{N_{in} - N_{out}}{V} \right) \text{mg N/Ld},
\]

where: \(N_{in}\), sum of nitrogen coming into the reactor (ammonium, nitrite, nitrate) [mg N/d]; \(N_{out}\), sum of nitrogen coming out of the reactor (ammonium, nitrite, nitrate) [mg N/d]; \(V\), volume of the anammox reactor [L].

The two reactors were operated at the low loading rate maintaining nitrite concentration at about 15 mg N/L for one year before tests were conducted on stability. During these tests, the reactors' response to three (low, medium and high) nitrogen loads and nitrite concentrations under the two different pH conditions was investigated.

### 3. Results and discussion

#### 3.1. Reactors operation and observations

In the reactors operation test, two MBBRs, R1 with pH 6.5 ± 0.01 and R2 with the ambient pH naturally maintaining at about 7.8 ± 0.24 (reported by van Hulle et al. (2010) as the optimum), were compared. In both reactors, similar anammox activities were observed with overall nitrogen balance at a ratio of \([\text{NH}_4^- + \text{N conversion:NO}_2^- + \text{N conversion:NO}_3^- \text{production}]\) of \([1:(1.23 \pm 0.06):(0.21 \pm 0.02)]\) and \([1:(1.15 \pm 0.09):(0.13 \pm 0.03)]\) for R1 and R2, respectively.

Over 99% of the total measured VSS in the MBBR reactors were in attached form. Suspended solids concentration in the effluent of the MBBRs was 0.1 and 0.06 g VSS/L for R1 and R2, respectively, while suspended solids concentration in the influent to the anammox MBBRs was 0.04 g VSS/L. Some of these incoming solids originate from the nitritation reactor with the balance coming from the feed centrate. It is possible, that oxygen utilizing organisms from the nitritation reactor will scavenge any dissolved oxygen present in the anammox reactor. This could assist the anammox process by eliminating the well studied inhibitory effects of dissolved oxygen (Strous et al., 1997a).

Both reactors R1 and R2 had stable nitrite concentration and similar NRR when they were operated under low and medium load and nitrite concentrations (days 0–55 for R1 and R2, Fig. 2, Table 2). Subsequently, under the high load and nitrite concentrations tested (days 100–168 for R1 and days 55–102 for R2), it was observed that R1 had relatively stable nitrite concentration and high volumetric specific nitrogen removal rate (NRR, Fig. 2a, Table 2) while R2 experienced some NRR instability, which was correlated to nitrite concentrations (Fig. 2b). High nitrite loads in reactor R2 at first enhanced the NRR, reaching the highest activity at 3746 mg N/Ld, and subsequently hindered the NRR, which is in agreement with similar observations in literature (van der Star et al., 2007; Szatkowska et al., 2007). However, to authors’ knowledge, such a demonstration of a dynamic adverse effect of elevated nitrite on NRR in reactor operation was not documented.

A significant increase in NRR occurred under high load and nitrite conditions for R1, as compared to R2. Indeed the average NRR obtained in R1 was 61% greater than in R2 (Table 2). To the authors' knowledge, such a high NRR observed in R1 was never reported for anammox MBBR system, although it agrees with the theoretical prediction proposed by van der Star et al. (2007).

#### 3.2. Impact of nitrite concentration on NRR

Low nitrite concentration has been considered critical to preventing anammox bacteria inhibition (Szatkowska et al., 2007; Wett et al., 2007). In this study, the NRR was plotted against nitrite concentration for both reactors (Fig. 3) to evaluate any inhibitory effect of elevated nitrite levels. For R1 and R2, nitrite was not causing inhibition within the range tested, since a clear declining trend for NRR was not observed. For R1, higher nitrite concentrations resulted in higher NRR, until a plateau was reached at 25 mg N/L. For R2, nitrite concentration was linearly correlated with the NRR (similar to R1), but at levels above 10 mg N/L, the NRR reached a scattered plateau. While the two reactors were inoculated from the same source, it was possible that the differences in pH regime for R1 and R2 during the acclimation process, which lasted 1 year, caused the selection of different anammox populations with different pH optima and tolerances to nitrite. These results suggest that anammox reactors can operate at higher rates and higher nitrite levels when the pH is kept low.

To further study the relationship between operating pH and potential nitrite inhibition, R2 was exposed to two different pHs (first pH 8.0 and then to pH 7.0) under constant load conditions (Fig. 4).

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**Fig. 3.** The relation between the nitrite concentration and the NRR in R1 with low pH and R2 with ambient pH for the entire research period.
When the pH was set at 8.0, the NRR gradually decreased causing nitrite accumulation. When the pH was decreased and set at 7.0 on day 7, the NRR sharply increased. At this point, the nitrite concentration was about 250 mg NO2-N/L and at pH 7, a high fraction of nitrite would be in the un-ionized form of nitrite, free nitric acid (HNO2). According to the literature, this should have constituted the worst-case scenario for nitrite inhibition (Fernández et al., 2008), however, the NRR rebounded in less than a day. Therefore, it is unlikely that nitrite was responsible for the NRR deterioration in R2 at nitrite levels exceeding 40 mg NO2-N/L, as observed in Fig. 3. Lack of operational stability, reflected in sudden spikes of nitrite and changes in NRR, was reported by Rosenthal et al. (2009). These authors could not explain sudden NRR deterioration by using the nitrite inhibition concept reported in literature, since their reactor was operated under low nitrite concentration (below 1 mg N/L). They also concluded that there must be another parameter which caused sudden NRR fluctuations.

3.3. The pH-related free ammonia effect on NRR

According to the literature, the reported optimum pH range for anammox organisms is 7–8.5 (Strous et al., 1997b). In this study, low bulk pH provided better performance under high-load and high-substrate concentrations. Because of the potentially toxic nature of FA and its pH dependent fluctuation at constant total ammonia concentration, it is difficult to distinguish pH effects from FA effects. Therefore, FA concentration was investigated as a potential factor, which may have greater impact on the anammox activity than pH alone. During the entire research period, R1 had a pH set at 6.5 with the bulk FA averaging 0.4 ± 0.3 mg N/L, and R2 had naturally occurring pH of 7.81 ± 0.24 with the bulk FA averaging 4.5 ± 3.2 mg N/L, a concentration that is typically observed in full and pilot scale anammox reactors (van der Star et al., 2007; Szatkowska et al., 2007). The lowest FA toxicity threshold concentration provided in literature was 1.7 mg N/L (Jung et al., 2007), which was lower than the values obtained for R2. Therefore, FA was tested as a potential cause for NRR deterioration. When the NRR was plotted versus FA an inverse correlation was observed between NRR and FA ($R^2 = 0.86$, only points referred to nitrite and ammonium concentrations range above 70 and 15–50 mg NO2-N/L, respectively, between day 67 and 102 of the experiment), supporting the contention that FA is the inhibitor. This could explain the phenomenon observed in Fig. 4 where the pH decrease from 8 to 7 caused an immediate NRR improvement, despite high nitrite levels. R1, operating at low pH and FA, outperformed R2, operating at ambient pH and high FA. This trend is also reflected in literature (Table 3). In Table 3, only anammox reactors with synthetic feed were presented as a comparison to this study in order to eliminate the effect of the inert solids coming to the system with the feed and to minimize the influence of other microorganisms. It was observed that high specific anammox activity was associated with low FA. This observation, together with the collected data from the conducted experiment, suggests that FA concentration may be critical to anammox process stability, while nitrite toxicity may be overestimated in some cases in the literature.

### Table 3

<table>
<thead>
<tr>
<th>Reactor type</th>
<th>pH</th>
<th>FA [mg N/L]</th>
<th>NO2–N [mg N/L]</th>
<th>SAA [g N/g VSS d]</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>UASBa</td>
<td>7.9–8.2</td>
<td>1.2–2.0</td>
<td>15–50</td>
<td>1.8</td>
<td>Tang et al. (2010)</td>
</tr>
<tr>
<td>FBRb</td>
<td>8</td>
<td>Around 8</td>
<td>Close to 0</td>
<td>0.15–0.18</td>
<td>Strous et al. (1997b)</td>
</tr>
<tr>
<td>FBRb</td>
<td>7</td>
<td>Below 0.8</td>
<td>Close to 0</td>
<td>1.0</td>
<td>van de Graaf et al. (1996)</td>
</tr>
<tr>
<td>SBR</td>
<td>7–8</td>
<td>1–10</td>
<td>Close to 0</td>
<td>1.9</td>
<td>Strous et al. (1998)</td>
</tr>
<tr>
<td>Attached growth</td>
<td>7.0–7.5</td>
<td>–</td>
<td>224</td>
<td>1.6</td>
<td>Tsushima et al. (2007)</td>
</tr>
<tr>
<td>R1</td>
<td>6.5 ± 0.01</td>
<td>0.5 ± 0.1</td>
<td>30 (120)</td>
<td>0.7(1.1)</td>
<td>This study</td>
</tr>
<tr>
<td>R2</td>
<td>7.8 ± 0.2</td>
<td>8.3 ± 5.1</td>
<td>30</td>
<td>0.3</td>
<td>This study</td>
</tr>
</tbody>
</table>

a Upflow anaerobic sludge blanket.
b Fluidized bed reactor.
c VSS was not monitored; result based on one VSS measurement from two randomly chosen rings.

4. Conclusion

It was observed that the reactor operating under an ambient pH of about 7.5–8.1 exhibited 61% lower NRR than a reactor operating at a constant pH of 6.5. It was found that low nitrite provided stable anammox reactor performance, however high nitrite was not necessarily the cause for the reactor destabilization. Free ammonia was shown to be an important stability parameter in anammox systems, while nitrite toxicity may be overestimated in some literature. Nitrite as high as 170–250 mg NO2-N/L did not cause...
deactivation of the anammox consortium despite 2 days of exposure time.

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References


