Partial nitrification in MBBRs for mainstream deammonification with thin biofilms and alternating feed supply

M. Piculell, M. Christensson, K. Jönsson and T. Welander

ABSTRACT

A new principle for mainstream nitrogen removal through nitritation followed by anammox was studied in a two-stage moving bed biofilm reactor (MBBR) configuration. The first stage was optimized for nitritation by using thin biofilms and a feed alternating between synthetic mainstream wastewater at 15 °C and, for shorter periods, synthetic reject water at 30 °C. The exposure of the biofilm to reject water conditions aimed to improve the growth conditions for ammonia oxidizing bacteria, while inhibiting nitrite oxidizing bacteria. The biofilm thickness was maintained below 200 μm to ensure high exposure of the total biomass to the bulk reactor conditions. Nitritation was successfully achieved in the configuration, with a nitrite accumulation ratio above 75% during the majority of the study, and ammonia removal rates between 0.25 and 0.50 g NH4-N/L.d. The anoxic second stage, optimized for anammox, was fed with the effluent from the nitritation reactor, reaching nitrogen removal rates above 0.20 g TN/L.d.

INTRODUCTION

Today, an increasing number of process solutions are being developed to improve energy recovery in wastewater treatment by utilizing the majority of incoming organic matter for biogas production, and energy requirements must be minimized in the aim of reaching energy neutrality. By applying partial nitrification and anammox (PNA) in municipal mainstream wastewater treatment, rather than conventional nitrogen removal (nitrification–denitrification), energy usage for aeration could be significantly reduced and nitrogen removal would not depend on the consumption of organic matter (Daigger 2014). But although this concept shows great promise, few have succeeded in achieving stable PNA at ambient temperatures (<20 °C) and low feed concentrations (<100 mg NH4-N/L) (De Clippeleir et al. 2013; Gilbert et al. 2014; Gustavsson et al. 2014; Lemaire et al. 2014; Lotti et al. 2015).

The three major challenges concerning mainstream PNA can be defined as (i) denitrifiers outcompeting anammox bacteria (AnAOB) at high C/N ratios, (ii) maintaining stable partial nitrification without the accumulation of nitrite oxidizing bacteria (NOB) and (iii) sufficient anammox retention at low temperatures (Xu et al. 2015). While (i) and (iii) can be controlled by minimizing the organic load and enable long sludge retention times, the key to achieving successful mainstream PNA lays with the suppression of NOB (ii). NOB are unfavorable to the PNA process as they compete with ammonia oxidizing bacteria (AOB) for oxygen and with AnAOB for nitrite. At temperatures below 20 °C, the growth rate of NOB generally exceeds that of AOB (Hellinga et al. 1998), enabling NOB establishment in the biomass which can be very challenging to wash out (Kouba et al. 2014). Two common approaches to suppress NOB in systems for partial nitrification (nitrification) are (1) oxygen limitation and (2) inhibition by free ammonia (FA) and/or free nitrous acid (FNA).

Oxygen limitation is by many defined as the key mechanism for suppressing NOB. Although there have been observations where the oxygen affinity of NOB exceeds that of AOB (Wett et al. 2013), most studies have shown that AOB have the higher affinity, meaning that the oxidation rate by AOB exceeds that of NOB at low dissolved oxygen (DO) concentrations (Blackburne et al. 2008). But, due to oxygen mass transfer resistance, a DO gradient will develop in the biomass, especially in biofilms, and different
parts of the biomass will be exposed to different DO. Therefore, the apparent oxygen affinity of AOB and NOB in a specific process will depend on floc size or biofilm thickness, as well as on the thickness of the liquid boundary layer surrounding the biomass, meaning that the ideal DO concentration for successful suppression of NOB can vary significantly (Blackburne et al. 2008; Brockmann & Morganroth 2010). Although oxygen limitation by different means of aeration control has shown to be a successful tool for achieving stable nitritation (Guo et al. 2015; Zhou et al. 2017), it does not guarantee high nitritation efficiency (Lackner & Smets 2012).

Another means of suppressing NOB is by exposing the biomass to inhibiting concentrations of FA or FNA. Studies have shown that AOB and NOB respond differently to different concentrations of FA and FNA, with NOB generally being more sensitive than AOB (Anthonisen et al. 1976; Zhou et al. 2011; Wang et al. 2014). The observed concentration ranges for inhibiting NOB, without significantly affecting AOB activity, varies in different studies, with values from 1.5 to 90 mg NH₃-N/L for FA (Anthonisen et al. 1976; Kim et al. 2008) and 20–1,350 μg HNO₂-N/L for FNA (Zhou et al. 2011; Wang et al. 2014). Just as for DO, the concentration in the bulk will give different responses in different processes, as a result of mass transfer resistance and gradients in the biomass. Consequently, it can be expected that the biomass in thin biofilms is more exposed to close to bulk concentrations and conditions, than is the biomass in deeper layers of thick biofilms. Previous studies have indicated that NOB are disfavored in biofilms thinner than 500 μm, possibly due to the more exposed nature of thin biofilms (Piculell et al. 2015). Bacteria are also known to acclimatize to high concentrations of FA and FNA, and there can be a significant difference between the concentrations required for inhibiting initial establishment of NOB and wash out of already established NOB clusters in the biomass. In general, high FA and FNA are not achievable in mainstream waters without significantly altering the pH, but it has been shown that mainstream NOB suppression can be achieved by exposing the biomass to high concentrations of FNA in a separate stage (Wang et al. 2014). Since AnAOB are also sensitive to FA and FNA inhibition (Zhou et al. 2011; Jin et al. 2012), applications using aggressive exposure to FA and FNA should be limited to two-stage PNA processes, where inhibition of NOB occurs in the nitritation stage only.

Biofilm systems such as the moving bed biofilm reactor (MBBR) are generally advantageous for PNA, since the long biomass retention time in biofilms enables the slow growth of AnAOB, and the stratification of biofilms enables co-existence of aerobic and anaerobic bacteria in one stage. Most full-scale MBBR applications for PNA are designed to treat reject water in one-stage (Christensson et al. 2013), although multiple stage MBBR PNA configurations also exist (Rosenwinkel & Cornelius 2005). One-stage MBBRs have been used to achieve PNA under mainstream conditions (Gilbert et al. 2014; Gustavsson et al. 2014; Lemaire et al. 2014), although the challenges of inhibiting NOB remains for MBBRs as well as for other processes. In order to prevent NOB establishment and avoid oxygen inhibition of AnAOB, one-stage PNA MBBRs must operate at low DO, which limits AOB activity, and in turn limits nitrite availability for AnAOB. In addition, if established in the biofilm, NOB can be hard to inhibit without damaging the AnAOB population.

This study aimed at achieving PNA in a continuously operated MBBR system, fed with low-strength, low-temperature wastewater, by applying a specific operational scheme (Figure 1), consisting of three key concepts:

1. The PNA system was divided into two stages with (1) one or more continuously aerated MBBR(s) for nitritation and (2) an anoxic MBBR for anammox, in order to enable efficient NOB inhibition and ensure high AOB activity in the aerated stage, without significantly affecting AnAOB.
2. The biofilm thickness in (1) was maintained below 200 μm, hence, exposing a large fraction of the biomass to close to bulk liquid FA and FNA concentrations. By doing this, it was expected that NOB establishment in the deeper biofilm layers would be prevented, and the chances of washing out NOB from the biofilm would, hence, increase. In addition, the thin biofilm would

![Figure 1](https://www.water-science-technology.org/sites/default/files/images/73.6_1254_1.png)
ensure high oxygen availability, which was expected to improve nitrification rates.

3. The feed to (1) was switched periodically from low-strength, low-temperature mainstream wastewater to reject water at high temperatures and concentrations. This sudden exposure to high substrate concentrations and temperatures was expected to inhibit NOB growth in the thin biofilm, and possibly also boost AOB activity.

The feasibility of the scheme was studied in laboratory scale, but a schematic of the full-scale two-stage process can be seen in Figure 1. Removal of chemical oxygen demand (COD) upstream of the nitritation reactor will be required in the full-scale system, but was not included in this study. Due to the periodic feed alteration, the final process will require more than one nitritation stage to ensure a stable load to the anammox reactor. This study focused on one single nitritation reactor (1), with a consecutive anammox stage (2) during mainstream feeding, aiming to evaluate the feasibility of the full process.

**MATERIAL AND METHODS**

The feasibility of the nitritation-scheme was studied for 252 days in a 1 L bench-scale MBBR nitritation reactor (R-N), fed with a synthetic low-strength substrate and operated at a target temperature of 15°C. The feed was switched periodically to a phase with synthetic high-strength reject water, during which the temperature was increased to 50°C. The frequency and duration of the reject phases varied in order to test the sensitivity of the system, with a total of nine reject phases during the whole study.

R-N contained approximately 20% of AnoxKaldnes Z-200 carriers (Figure 2) with an approximate area of 0.20 m². As seen in Figure 2, the saddle-shaped Z-200 differs significantly from conventional MBBR carriers, as the biofilm grows on the outside of the carrier and not inside voids. An external grid on the Z-200 protects the biofilm from scouring and controls the biofilm thickness to the grid wall height (200 μm), as the carriers collide in the reactor. The carriers used in this study were already colonized with a nitrifying biofilm from a previous study.

Reactor temperature was adjusted using a thermostat bath, and air was supplied continuously through the reactor bottom. DO was controlled by adjusting the air flow using a Dwyer rotameter. Buffer was added to the feed, as NaHCO₃, but there were no other pH-control in the system. Aside from buffer, the feed contained ammonia (as NH₄Cl), phosphorous (as KH₂PO₄), peptone and trace minerals. No COD-source was added to the feed. The same feed base, containing 3.05 g NH₄Cl/L and 12 g NaHCO₃/L, was used throughout the study, and was supplied in two different dilutions (1:16 and 1:1) to simulate reject and mainstream wastewaters, respectively. The average conditions in R-N during mainstream and reject feeding can be seen in Table 1.

The load to R-N was adjusted by alternating the feeding pump frequency. Successful nitritation was obtained after 20 days of operation, and the load was doubled to increase the effluent ammonia to nitrite ratio during mainstream operation. Some additional smaller adjustments were made during the following weeks, after which the mainstream-feed flow was kept stable from day 53 and onwards, resulting in an average loading rate of 0.7 g NH₄-N/L.d. During reject feeding the flow was significantly reduced to compensate for the stronger influent concentrations, and the load was approximately 1.0 g NH₄-N/L.d during all reject phases, except phase 1 where the load was 0.55 g NH₄-N/L.d.

After 25 days of operation, a subsequent anoxic anammox stage (R-A) was added to the process to test the suitability of R-N effluent as anammox feed. The air-tight, 0.8 L, anammox reactor contained approximately 50% of AnoxKaldnes K5 carriers (pre-colonized from a full-scale ANITA Mox plant treating side-stream wastewater) with an area of approximately 0.20 m², which were kept in suspension by a magnetic stirrer. During mainstream operation, a fraction of the nitritation effluent was fed to R-A, at a varying load depending on nitritation performance. R-A was

![Figure 2](https://example.com/figure2.png)

*Figure 2* | A drawing of the Z-200 together with an enlargement showing the grids that prevent biofilm growth beyond the height of the grid walls (200 μm), giving the biofilm a fixed maximum thickness.

<table>
<thead>
<tr>
<th></th>
<th>Mainstream</th>
<th>Reject</th>
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<tbody>
<tr>
<td>pH</td>
<td>7.8 ± 0.3</td>
<td>8.6 ± 0.3</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>6.6 ± 1.0</td>
<td>4.8 ± 1.2</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>15.6 ± 0.8</td>
<td>29.1 ± 3.6</td>
</tr>
<tr>
<td>Influent NH₄-N (mg/L)</td>
<td>51.6 ± 5.2</td>
<td>813 ± 86.7</td>
</tr>
</tbody>
</table>
operated at the same temperature as R-N, but neither DO nor pH were measured or monitored in the reactor. R-A was idle during reject operation to prevent inhibition of the AnAOB by the high concentrations of ammonia and/or nitrite. During the idle phases, the mixing was switched off, and R-A was kept in starvation at room temperature.

Analyses and calculations

Samples were taken regularly at three points in the treatment line, (1) the feed tank, (2) inside R-N and (3) in R-A effluent, and filtered through 1.6 μm pore size Munktell MG/A glass fibre filters. Analyses were made for ammonia (1–3), nitrate (2–3), nitrite (2–3) and total nitrogen (TN) (1–3) using standard Hach Lange kits (LCK 303, 339, 342 and 238, respectively). DO was measured with a Hach HQ40d multi DO probe, while pH and temperature were measured with a HANNA H1991001 pH-meter. The biofilm was photographed using an OLYMPUS SZ-ET stereomicroscope. Removal and production rates were calculated as mass balances over each reactor, based on ammonia, nitrate, nitrite and TN measurements, respectively, assuming complete mixing and steady state conditions. Nitrite accumulation ratio (NAR) was calculated as nitrite over the sum of nitrite and nitrate. FA and FNA were calculated according to Anthonisen et al. (1976).

It is known that high nitrite concentrations can disturb nitrate measurements, and when studying the difference between the calculated TN (sum of measured ammonia, nitrite and nitrate) and the measured TN in R-N and R-A, the mass balance did not completely add up, especially not in R-N where nitrite was high. The difference in TN showed a linear relation to nitrite concentration, increasing with approximately 0.3 mgTN per mg NO₂-N. It was suspected, although not proven, that this difference was mainly due to errors in nitrate measurements, which should be considered when evaluating NAR in R-N and nitrate production in R-A.

RESULTS

Nitritation reactor

As seen in Figure 3, mainstream nitritation was successfully achieved in R-N, with a NAR of 75–85% during the majority of the study. NAR decreased towards the end of each phase, as a result of increasing nitrite oxidation. For most of the phases, the feed was switched from mainstream to reject as soon as the decline in NAR was observed, but phases 3 to 5 were operated under mainstream conditions for a longer time at a declining accumulation ratio, in order to study the system sensitivity to NOB establishment. As seen in the figure, NAR always recovered after a reject phase, but the duration of stable nitritation in R-N varied in the different phases, from over 30 days in phase 1 down to 10 days in phase 4.

The ammonia removal varied significantly during the study (Figure 3). During the first 21 days the activity in R-N was limited due to the lower load, resulting in almost 100% ammonia removal, after which the load was increased to avoid substrate limitation. When substrate availability was increased, removal rates ranged between 0.25 and 0.35 g NH₄-N/L.d during mainstream feed in phase 2 and 3, and later increased up to 0.50 g NH₄-N/L.d during phase 4. No correlation was observed between ammonia removal

![Figure 3](https://iwaponline.com/wst/article-pdf/73/6/1253/462824/wst073061253.pdf)
activity and NAR, but ammonia removal often dropped after reject feeding and continuously increased during mainstream operation (Figure 3). This behavior is most noticeable in phase 7. The composition of the R-N effluent during mainstream operation varied in the different phases, with average concentrations of 18.0–28.2 mg NH₄-N/L, 5.2–9.4 mg NO₃-N/L and 19.1–34.0 mg NO₂-N/L.

During reject operation, the load to R-N was increased to approximately 1.0 g NH₄-N/L.d (with the exception of reject phase 1, see Material and methods section), and influent concentrations increased significantly. Since there was no pH control in the system, pH increased as a result of the increased load during reject operation (Table 1). As a result of the elevated pH, ammonia concentrations and temperature, FA increased significantly during reject operation, with average concentrations ranging from 7.1 mg NH₃-N/L in phase 8 to 495 mg NH₃-N/L in phase 7 (Figure 4). FNA also increased during reject operation, as a result of increased nitrite concentrations, with an average of 0.2 μg HNO₂-N/L in phase 7, up to 7.0 μg HNO₂-N/L in phase 1. The variation in FA and FNA between the different phases mainly depended on the varying ammonia and nitrite concentrations in the reactors, which were a result of varying AOB and NOB activity. DO also varied in the different phases, as a result of varying activity in relation to aeration intensity.

**Anammox reactor**

The performance of R-A is displayed in Figure 5. Anammox activity, measured as total nitrogen removal, varied over time as a function of load as well as activity in R-N. Due to the variation in feed concentration (R-N effluent), nitrogen removal was sometimes ammonia limited and sometimes nitrite limited. The initial anammox activity, with pre-colonized carriers, was approximately 0.15 g N/L.d, but it decreased gradually during phases 2 and 3. The load was decreased in phase 4 to better match the removal rates, and effluent quality improved as a result, with total nitrogen reaching below 15 mg TN/L and nitrate being the main

**Figure 4** | Average FNA, DO and FA in R-N during each reject phase, including standard deviation.

**Figure 5** | Nitrogen load and removal, as well as effluent concentration in R-A (bottom), and the fractions of ammonia, nitrate and nitrite in the R-A effluent (top). Gaps in data correspond to reject feeding when R-A was idle.
remaining nitrogen fraction. In phase 6 the load to R-A was again increased, to prevent substrate limitation, and removal rates increased up to an average of 0.20 g TN/L.d in phases 7 to 9. With increased substrate availability, the effluent nitrogen concentration increased, especially for nitrite. During phases 7 to 9, when rates and load remained rather stable, average nitrite to ammonia removal in R-A was 1.33 and average nitrate production per ammonia removed was 13%.

**Biofilm properties**

Pictures taken of Z-200 with stereomicroscopy on day 252 showed that the biofilm from R-N remained below the grid wall height, i.e. below 200 μm (Figure 6). The biofilm grew thicker along the edges of each grid, and remained very thin in each centre. The anammox biofilm in R-A, photographed on day 242, was approximately 600 μm thick and dense with characteristic red pigments from anammox.

**DISCUSSION**

**Nitritation performance**

A high NAR (75–85%) throughout the study showed that nitritation was achievable at high DO, when MBBRs with thin biofilm were operated according to a scheme of switching between low and high strength wastewater. Ammonia removal in the nitritation reactor varied between 0.25 and 0.50 g NH4-N/L.d (Figure 3), which corresponds well with previously established nitrification rates under similar conditions (Hem et al. 1994). The increased ammonia removal rates in phase 4 and onwards could indicate a microbial adaptation to the system, where the bacteria became more resilient to the reject feeding over time.

Switching the feed from mainstream to reject always resulted in elevated NAR, due to a temporary competitive advantage for AOB over NOB in the thin biofilms (Figure 3). However, AOB were sometimes also affected negatively by the switch, as ammonia removal often dropped after a reject phase. Both FA and FNA increased during reject feeding, with FA concentrations always exceeding the established limits for NOB inhibition (see introduction), but varied significantly between the different phases. No significant correlation could be observed between the specific FA and/or FNA concentrations at reject operation (Figure 4) and the corresponding ammonia removal activity and/or the NAR during the following mainstream feeding (Figure 3), indicating that the response in AOB and NOB activity to the reject feeding was more complex than a result of direct FA and/or FNA inhibition. In addition, conditions inside the biofilm are likely to differ from bulk conditions. Long term effects of FA and FNA, as well as adaption of the biomass must be studied further, preferably in combination with microbial quantification.

Some NOB were always present in the nitritation reactor (observed as nitrate production), although clearly inhibited by the regular reject feeding. It is likely that a significant amount of NOB grew and recovered in the biofilms during mainstream phase 3, since the NAR dropped to 20%. The fast recovery of NOB activity during mainstream phases 4 to 6 compared to phases 1 to 3 (Figure 3), indicates that once NOB had been established in the biofilm, they were only temporarily inhibited by the reject feedings. It is possible to increase FA and/or FNA concentrations in R-N even more (by varying the load during reject operation) in order to further increase the NOB inhibition, but there is a fine balance between maximal NOB inhibition and maintaining high AOB activity. The high FA concentrations during reject feeding in phase 7 resulted in a significant drop in ammonia removal, indicating that both AOB and
NOB were almost completely inhibited. As the system recovered during mainstream operation, NAR remained high for approximately 30 days. This indicates that high NOB establishment can be prevented, by ensuring that the feed is switched to reject as soon as NAR starts to decline, and that a more complete inhibition can be achieved, although risking AOB inhibition, by significantly increasing FA during reject operation. However, further improvements of the operational scheme are necessary to manage the recovery of the system when NOB has mistakenly been enriched to high concentrations in the biofilm.

Anammox activity

The nitrogen removal in R-A varied significantly (Figure 5) depending on load as well as on the nitrite to ammonia ratio in the R-N effluent. During low loading, the removal efficiency in R-A was 88%, and during phases 7 to 9, when conditions were stable, the activity reached 0.20 g TN/L,d on average. Considering the low filling degree (50%), this rate corresponds well with other studies on anammox under mainstream conditions (De Clippeleir et al. 2013; Gilbert et al. 2014; Lotti et al. 2014). During the same period, the average nitrite to ammonia removal was 1.33 and average nitrate production per ammonia removed was 15%, which corresponds well with the stoichiometry for anammox (Strous et al. 1998). This indicates that nitrogen removal in R-A was mainly a result of AnAOB activity, and that the effluent quality from R-N was well suited as anammox feed. The removal rates also indicate that AnAOB were not significantly inhibited by the high DO in the feed. The initial decline in anammox activity indicates that the fraction of AnAOB in the over-all biomass slowly decreased due to decreased growth rates at the lower temperatures (carriers were sourced from a side-stream process operating at temperatures around 30 °C). However, the recovery of anammox activity in phases 7–9 could be a result of the adaptation of the AnAOB to the lower temperatures.

The fractions of ammonia, nitrate and nitrite in the R-N effluent varied as a result of varying activity in each mainstream phase, which also affected the anammox performance. This should be addressed in full-scale operation, by operating several nitritation stages in parallel, alternating the reject feed between the different reactors, to maintain a stable anammox feed at ideal nitrite to ammonia ratio at all times. An additional control measure could be to bypass influent wastewater with high ammonia content directly to the anammox reactor when nitrite concentrations are high. At this stage of the study, however, focus has been to achieve stable nitritation, and optimization of the effluent quality is addressed in following studies.

This study proves the feasibility of operating a two-stage MBBR PNA system using an alternating feed-scheme and thin biofilms to prevent NOB establishment. The process is, however, still in its cradle, and operational issues – such as reactor configuration, N₂O emissions, and the effect of seasonal variations in load, temperature and mainstream wastewater quality – are still to be evaluated.

CONCLUSIONS

This study shows that stable nitritation and anammox is achievable in a two-stage MBBR configuration at 15 °C treating low-strength wastewaters, by utilizing thin biofilms and a feed alternating between mainstream wastewater and reject water. The results of the study clearly showed periodic exposure of the biomass to high concentration reject water to favor AOB activity and suppress NOB growth. In the nitritation stage, ammonia removal rates ranged between 0.25 and 0.50 g NH₄-N/L,d, with a NAR above 75%, and the effluent was fed to the second-stage anammox reactor, with nitrogen removal rates reaching 0.20 g TN/L,d. Further studies are underway to optimize the scheme and evaluate the fate of the NOB, but once fully applied this concept is expected to enable new solutions for mainstream anammox applications.

REFERENCES

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First received 4 August 2015; accepted in revised form 11 November 2015. Available online 26 November 2015.