Start-up of a full-scale partial nitritation-anammox MBBR without inoculum at Klagshamn WWTP

Ivelina Dimitrova, Agnieszka Dabrowska and Sara Ekström

ABSTRACT

Partial nitritation and anaerobic ammonium oxidation (PNA) is a useful process for the treatment of nitrogen-rich centrate from the dewatering of anaerobically digested sludge. A one-stage PNA moving bed biofilm reactor (MBBR) was started up without inoculum at Klagshamn wastewater treatment plant, southern Sweden. The reactor was designed to treat up to 200 kgN d⁻¹, and heated dilution water was used during start-up. The nitrogen removal was >80% after 111 days of operation, and the nitrogen removal rate reached 1.8 gN m⁻² d⁻¹ at 35 °C. The start-up period of the reactor was comparable to that of inoculated full-scale systems. The operating conditions of the system were found to be important, and online control of the free ammonia concentration played a crucial role. Ex situ batch activity tests were performed to evaluate process performance.

Key words | anammox, MBBR, PNA, side-stream treatment, start-up, without inoculum

INTRODUCTION

The centrate from the dewatering of anaerobically digested sludge accounts for 15–20% of the total nitrogen load in a wastewater treatment plant (WWTP), with a hydraulic load of ~1% of the incoming flow (Fux & Siegrist 2004). Due to the high energy demand for conventional nitrification-denitrification (~50% of the total energy required at a WWTP), dedicated side-stream treatment processes are designed to reduce this nitrogen load in the main wastewater treatment line (Gao et al. 2014). Partial nitritation anammox (PNA) of nitrogen-rich centrate with a low soluble carbon content (sCOD/NH₄⁺–N < 2) has evolved as a cost-effective and efficient alternative for side-stream nitrogen removal. This increase in popularity has led to over 100 full-scale applications worldwide (Lackner et al. 2014).

PNA is achieved through fully autotrophic nitrogen removal by combining partial nitritation and anammox (Lackner et al. 2014). Aerobic ammonium-oxidizing bacteria (AOB) oxidize 57% of the influent NH₄⁺–N into NO₂⁻–N under aerobic oxidation. Anaerobic ammonium-oxidizing bacteria (AMX) oxidize the remaining NH₄⁺–N into N₂, with NO₃⁻–N as an electron acceptor. According to anammox stoichiometry, ~11% of the oxidized nitrogen is produced as NO₃⁻–N (Strous et al. 1998). Hence, the oxygen demand and the need for an organic carbon source are reduced by 57% and 86% (Fux & Siegrist 2004; Wett 2007).

The PNA process can take place in suspended-growth or biofilm reactors, configured as one- or two-stage systems (Lackner et al. 2014). In a moving bed biofilm reactor (MBBR), the ammonium-oxidizing PNA biofilm is attached to plastic carriers. Operation at dissolved oxygen (DO) concentrations between 0.5 and 1.5 mg L⁻¹ allows partial nitritation and anammox to co-occur in a one-stage reactor. The AOB are found in the outer aerobic biofilm layer, while the AMX thrive in the deeper anoxic layer (Christensson et al. 2015). The AMX compete with the co-existing NOB and fast-growing heterotrophic bacteria (HB) for NO₂⁻–N.

The start-up of a PNA system is challenging, and is influenced by several factors: (i) the slow growth rate of AMX, (ii) the choice of a suitable inoculum, (iii) effective AMX retention and NOB repression, and (iv) the inhibition of microbial groups as an effect of operational conditions (Rükkmann et al. 2018; Cai et al. 2020). Reported doubling times vary from 2–4 days (Lotti et al. 2015; Zhang et al. 2017) to 11 days (Strous et al. 1998), depending on the process configuration and operating conditions. Long start-up periods of 8–10 months are common when the influent...
centrate is the only source of AMX (Plaza et al. 2011; Rikkemann et al. 2018). Hence, researchers have focused on developing start-up strategies that shorten this period. It has been reported that the start-up period can be reduced to 5 months by using activated sludge (Mehrdad et al. 2014) and 2–4 months when using AMX sludge or pre-colonized carriers (Wett 2007; Christensson et al. 2013; Klaus et al. 2017). Start-up with AMX-inoculated carriers require considerable quantities of seeding carriers, between 3 and 15% of the design media fill (Christensson et al. 2013), which might not always be available. Kanders et al. (2016) recently reported the un-inoculated start-up of a one-stage PNA MBBR, with a pre-colonized nitritation biofilm on the carriers, in 120 days. Rapid establishment of an initial nitritation biofilm on the virgin carriers, prior to inoculation with AMX, indicated the theoretical potential to reduce the start-up period of a one-stage MBBR system to 56 days (Kowalski et al. 2019a). In addition, Kanders et al. (2018) claimed that the centrate from mesophilic digesters fed with high-sludge-age nitrifying bacteria is a reliable and adequate sole source of AMX for rapid PNA start-up. As the development of a PNA biofilm depends on the reactor conditions and a sufficient source of bacteria, the MBBR technique, with high and efficient biomass retention, should be suitable for rapid start-up with indigenous AMX.

Key and process parameters that should be controlled are the pH, temperature, and concentrations of DO, NO2-N and FA, especially during process initiation when conversion and growth rates are slow (Cai et al. 2020). Anthonisien et al. (1976) have described the dependence of the inhibitory effect of FA on pH, temperature, and NH4-N concentration. In general, AOB and AMX tolerate higher concentrations of FA (10–150 mg L–1 and 2–150 mg L–1, respectively) than NOB (0.1–1 mg L–1) (Aktan et al. 2012; Jaroszynski et al. 2012). Furthermore, AMX are sensitive to NO2-N concentrations between 5 and 400 mg L–1 (Wett 2007; Lotti et al. 2012). Levels of FA <10 mg L–1 and NO2-N <40 mg L–1 have been found to be critical concentrations for successful start-up (Klaus et al. 2017; Rikkmann et al. 2018). The DO affinity of NOB is lower than that of AOB, thus low DO concentration can be used to suppress NOB activity while favoring the growth of AMX in the inner layer of the biofilm (Strous et al. 1997; Wett et al. 2013). Intermittent aeration with a low DO setpoint has been reported to be effective in achieving a suitable oxygen gradient in the biofilm during start-up (Lackner et al. 2014).

The rapid start-up of pilot or lab reactors without AMX inoculum has been widely discussed in the literature (Mehrdad et al. 2014; Kowalski et al. 2019a; Cai et al. 2020), but to the best of our knowledge, only Kanders et al. (2016) have reported such a start-up in a full-scale PNA MBBR. The objectives of this study were therefore, to demonstrate the rapid start-up of a full-scale PNA MBBR using only existing resources at the WWTP, and to present a detailed description of the start-up strategy.

**METHODS**

**Klagshamn WWTP**

Klagshamn WWTP is located in Malmö, southern Sweden, and is designed for 90,000 population equivalents, with an influent flow of 22,000 m3 d–1. Phosphorus is mainly removed by pre-precipitation using iron chloride as precipitant. Biological nitrogen removal is accomplished in an activated sludge process with simultaneous carbon removal and nitrification, followed by an MBBR process for post-denitrification with external carbon dosage. The final polishing step takes place in a dual-media filter. Primary and secondary sludge are mixed and co-digested in two mesophilic digesters operated in series. The centrate from the dewatering facility constitutes 2% of the total influent flow and 17–25% of the NH4-N load in the WWTP. The ammonium mass load from the centrate varies between 100 and 150 kgNH4-N d–1.

**The PNA reactor**

The centrate is pumped to the PNA reactor via a 400 m long pipe to a storage tank with a volume of 10 m3. This pipe is made of polypropylene to avoid a decrease in temperature and struvite precipitation. The insulated and covered PNA reactor is filled to 40% with carrier material, and a top-mounted mechanical mixer keeps the carriers in motion. The reactor design parameters are given in Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (m3)</td>
<td>256</td>
</tr>
<tr>
<td>Area × depth (m2 × m)</td>
<td>61 × 4.2</td>
</tr>
<tr>
<td>Max load (kgN d–1)</td>
<td>200</td>
</tr>
<tr>
<td>Type of carriers</td>
<td>Anox™ K5</td>
</tr>
<tr>
<td>Protected surface area (m2 m–3)</td>
<td>800</td>
</tr>
<tr>
<td>Filling degree (%)</td>
<td>40</td>
</tr>
<tr>
<td>Average flow (m3 h–1)</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 1 | Main design parameters of the full-scale reactor
airflow is controlled and modulated by motor-actuated control valves using fixed DO control. After treatment, the effluent from the reactor is discharged to the inlet of the activated sludge process.

Pre-precipitated and pre-settled dilution water (PSW) was used during start-up to avoid AMX inhibition due to FA and NO\textsubscript{2}-N. A heat exchanger was installed to pre-heat the dilution water to a temperature of \(\sim 30^\circ\text{C}\) to improve the AMX growth rate. Two feed pumps, one for the centrate and one for the PSW, were controlled by variable frequency drives. The setpoint for the two flows varied depending on the process performance.

Temperature, pH, NH\textsubscript{4}-N, NO\textsubscript{2}-N, NO\textsubscript{3}-N, sCOD, and DO were monitored with online sensors (WTW IQ SENSOR NET system from Xylem, Sweden). The FA concentration was monitored in the supervisory control and data acquisition system and used for manual control of the ammonium load. The characteristics of the influent to the PNA reactor are given in Table 2.

Free ammonia was calculated as described by Anthonisen et al. (1976):

\[
FA = \frac{17}{14} \frac{NH_4^- - N \cdot 10^{PH}}{e^{0.134 + 10^{PH}}} \left( \text{mg NH}_3 - N \text{ L}^{-1} \right)
\]  

(1)

**Start-up strategy**

The start-up of the PNA process was divided into two phases, which were followed by a continuous operation phase.

**Phase I**

The first objective during start-up was to establish a biofilm on the virgin carriers. PSW (~2 sCOD/NH\textsubscript{4}-N) was available at the plant and proved to be a reliable source of HB for the rapid establishment of an initial biofilm. This biofilm matrix favors the attachment of AOB and AMX on the carrier material (Kowalski et al. 2019b). The second objective was to achieve stable nitritation by AOB, while avoiding AMX inhibition by restricting the NO\textsubscript{2}-N concentration to the range 30–50 mg L\textsuperscript{-1}. The NOB activity was suppressed through intermittent aeration (DO setpoint of 0.6–0.8 mgO\textsubscript{2} L\textsuperscript{-1}) and by operation at inhibitory FA concentrations between 5 and 20 mg L\textsuperscript{-1}. The supplies of PSW and centrate were controlled to ensure suitable NO\textsubscript{2}-N and FA concentrations, respectively.

**Phase II**

The first signs of AMX activity; that is, a discrepancy in the nitrogen balance over the reactor system and/or evidence of AMX activity during batch measurements, indicated the commencement of start-up phase II. The objective of phase II was further enrichment of the AMX bacteria culture by applying an NH\textsubscript{4}-N load slightly exceeding the actual reduction capacity of the reactor. Care was taken not to overload the system. As the AOB community increased, the intermittent aeration became continuous to meet the increased oxygen demand.

**Continuous operation**

Transition to continuous operation was defined by treatment of all available centrate with a nitrogen reduction >80%.

**Analytical procedures**

Grab samples were collected from the reactor and analyzed to evaluate the accuracy of the online sensors and to monitor the reactor nitrogen compounds. In addition, 24-h flow-proportional samples were collected three times a week from the reactor inlet and outlet, to calculate the surface specific load and mass balances, and the reduction rates of inorganic nitrogen (Inorg N) and FA. All samples were analyzed according to Swedish and ISO standard methods for the following parameters: NH\textsubscript{4}-N, NO\textsubscript{2}-N, NO\textsubscript{3}-N (ISO 15923-1:2013), and chemical oxygen demand (COD) (SS 028142-2). Samples were filtered with an MGA filter (1.6 \(\mu\text{m}\), Ahlstrom Munksjö) for the evaluation of sCOD.

**Biofilm growth**

The dry solids (DS) on two samples of carriers (five pieces each) was measured once a week, to evaluate biofilm growth. The carriers were dried at 105 °C for 2 h and weighed. The biomass was then removed by treating the carriers with 5M NaOH and washing them thoroughly with

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Centrate (mg L\textsuperscript{-1})</th>
<th>Dilution water (mg L\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH\textsubscript{4}-N</td>
<td>580 ± 90</td>
<td>25 ± 5.0</td>
</tr>
<tr>
<td>sCOD</td>
<td>450 ± 60</td>
<td>50 ± 10</td>
</tr>
<tr>
<td>Alkalinity HCO\textsubscript{3}</td>
<td>3,700 ± 400</td>
<td>320 ± 50</td>
</tr>
<tr>
<td>sCOD/NH\textsubscript{4}-N</td>
<td>0.8 ± 0.32</td>
<td>2.3 ± 0.15</td>
</tr>
<tr>
<td>TSS</td>
<td>480 ± 390</td>
<td>50 ± 10</td>
</tr>
</tbody>
</table>
distilled water. The process of drying and weighing was then repeated, and the biomass on the carriers was determined as the difference between the initial and the final weight. The biomass was converted from gDS to gDS m\(^{-2}\) using a factor of 0.00242 m\(^{2}\)/K5 carrier.

**Activity tests**

Endogenous respiration, and AOB and NOB activity were measured through the oxygen uptake rate (OUR), while the specific anammox activity (SAA) and the specific denitrification activity (SDA) were measured manometrically, as described below.

**The OUR test**

Eighty carriers, corresponding to a filling degree of 40%, were used to perform the OUR measurements. The activity of aerobic bacteria was determined by measuring the depletion of DO in the bulk solution over a limited time when different substrates were added, as initially described by Hagman & la Cour Jansen (2007).

When no substrates or inhibitors are added to the solution, the DO depletion is equal to the endogenous respiration. The combined OUR of AOB + NOB is measured by adding NH\(_4\)-N. Allylthiourea is then added to inhibit the AOB activity (Ginestet et al. 1998), and NO\(_2\)-N, a substrate for the NOB is then added. The oxygen uptake rate is then defined as in Equation (2):

\[
\text{OUR} = -60 \times \frac{\Delta C}{X \cdot A_e} \cdot \frac{dt}{\Delta t} \cdot \frac{\Delta V}{\Delta t} \cdot M_{N_2} \cdot gN \cdot m^{-2}h^{-1}
\]  

where \(\frac{\Delta C}{\Delta t} \cdot [gL^{-1} min^{-1}]\) is the time derivative of the oxygen concentration in the solution, \(\Delta V [L]\) is the volume of the liquid phase, \(X\) is the number of carriers, and \(A_e [m^2]\) is the effective area of one carrier.

The measurements were started when the solution was well saturated (7–8 mgO\(_2\) L\(^{-1}\)). DO was measured (HACH LDO101) and recorded (HACH HQ40d) and the temperature was maintained at 28 °C.

**Manometric tests**

The SDA and SAA were determined by quantifying the amount of nitrogen gas produced. The measurements were performed by measuring the pressure increase in a closed reactor vessel, as described by Dapena-Mora et al. (2007).

SDA and SAA are expressed as the nitrogen produced per unit time per unit biofilm area, as in Equation (3):

\[
\text{SDA or SAA} = 60 \times 24 \times \frac{\Delta n_{\text{tot}}}{X \cdot A_e} \cdot M_{N_2} \cdot gN \cdot m^{-2}d^{-1}
\]  

where \(\frac{\Delta n_{\text{tot}}}{\Delta t} \cdot [mol \cdot min^{-1}]\) is the production rate of nitrogen gas, \(M_{N_2} [g mol^{-1}]\) is the molar weight of nitrogen gas, \(X\) is the number of carriers used in the test, and \(A_e [m^2]\) is the effective area of each carrier. The factor of 60 \(\times\) 24 was use to convert days to minutes.

NH\(_4\)-N and NO\(_2\)-N were used as substrates to determine SAA, and NO\(_3\)-N and acetic acid to determine SDA.

The overpressure resulting from the production of nitrogen gas was logged using a GMH 5150 pressure meter, with a GMSD 350MR sensor and GSOFT3050 software (Greisinger electronic GmbH, Germany).

**RESULTS AND DISCUSSION**

**Start-up and continuous operation**

The one-stage PNA process was started at the end of December 2018, and the results from the first 200 days of operation are presented in this study (Figure 1). The vertical dashed line on day 79 indicates the transition from start-up phase I to phase II. After 111 days, all the available centrate could be treated and the nitrogen removal was >80%, which was the definition of continuous operation. The second vertical dashed line in Figure 1 indicates the transition to continuous operation mode.

**Start-up phase I**

To enhance biofilm development on the virgin carriers and washout of the suspended biomass, the hydraulic retention time was kept low (12 h) during the first 20 days of operation, and then continuously increased as the biofilm developed (Mehrdad et al. 2014).

Intermittent aeration cycles of 30 min were applied with aeration times varying between 20 and 25 min, depending on the influent load. The DO setpoint was ~0.8–1.0 mg L\(^{-1}\), to promote the growth of AOB and to repress the growth of NOB during the early stage of start-up (Christensson et al. 2015; Wett et al. 2013). The stirring speed was adjusted to ensure low turbulence, encouraging the growth of a thicker
biofilm. The pH was monitored and used in the determination of the FA concentration.

The average pH during the whole period (200 days) was relatively high, fluctuating between 7.5 and 8.5. Figure 2 shows the nitrogen fractions in the reactor, the inorganic nitrogen load, and the FA, obtained from the analysis of grab samples. At the beginning of start-up, when the biological activity is low, there is a risk of inhibition of AOB by FA if the ammonia loading exceeds the conversion rate (Lackner et al. 2017). The FA was therefore kept below 15 mg L\(^{-1}\) by the addition of dilution water at ratios between 1:10 and 1:15.

During this start-up, the COD-rich PWS used as dilution water served two purposes. Firstly, it prevented inhibitory conditions arising from high levels of FA and NO\(_2\)\(-\)N (Mehrdad et al. 2014; Kanders et al. 2016). Secondly, the establishment of a biofilm containing HB on the carrier material will enhance the enrichment of AMX. A layer of HB biofilm used prior to inoculation with AMX biomass acts as an extracellular polymeric matrix that facilitates the rapid development of AMX (Kowalski et al. 2019b). In contrast to our study, Kanders et al. (2016) and Mehrdad et al. (2014) used dilution water (effluent and washing water, respectively) only to avoid substrate inhibition. Due to the strong dependence of the bacterial growth rate on temperature, the PSW was preheated to 30 °C.

The biomass on the carriers was examined for the first time on day 2. A thin biofilm, equivalent to 0.9 gDS m\(^{-2}\) (Figure 3) was detected. This biofilm was probably developed during the 7-day period before the reactor was
brought into operation, when the centrate pump was brought into operation. The reactor was filled with PSW under 7 days and the aeration was on in 3 days, to soak and distribute the carriers. This procedure was recommended by the carrier supplier due to the hydrophobic characteristics of the carrier material. The first batch

![Figure 3](http://iwaponline.com/wst/article-pdf/81/9/2033/712055/wst081092033.pdf)
measurement, when the amount of biomass was insignificant, showed that bacterial activity was dominated by fast-growing endogenous HB. Biofilm development was rapid from the beginning of phase I, reaching 1.9 gDS m\textsuperscript{−2} on day 17, which is similar to 2.1 gDS m\textsuperscript{−2} previously reported after 22 days of operation (Kowalski et al. 2019b). Ammonium conversion activity was first observed on day 20, as an increase in NO\textsubscript{3}−N concentration in the reactor, to ∼40 mg L\textsuperscript{−1}. Similar progress has been reported by Kowalski et al. (2019b), who also detected increased nitrification after two weeks.

High aerobic HB activity and NO\textsubscript{3}−N production, presumably by NOB, indicated that the aeration time could be reduced, which led to a daily average DO concentration of ∼0.1 mg L\textsuperscript{−1}. The load to the reactor was maintained between 50 and 60 kgN d\textsuperscript{−1}, corresponding to 0.5–0.7 gN m\textsuperscript{−2} d\textsuperscript{−1}. As a result of the low bacterial activity, the reduction rate was low, 5–15 kgN d\textsuperscript{−1} or 0.1–0.2 gN m\textsuperscript{−2} d\textsuperscript{−1}, resulting in a nitrogen removal rate of 5–10% (Figure 4).

Operational problems with the dilution water pump (day 23) led to elevated NO\textsubscript{2}−N concentrations in the reactor (170 mg L\textsuperscript{−1}), exceeding the optimal concentration (<40 mg L\textsuperscript{−1}) for the promotion of AMX growth (Klaus et al. 2017). The biofilm began to grow rapidly after 23 days, increasing from 1.9 to 12 gDS m\textsuperscript{−2}. Kowalski et al. (2018) observed rapid biofilm growth simultaneously with high substrate load. The low removal rate in our study indicates also overloading. No significant change was observed

Figure 4. (a) Inorganic nitrogen load and removal rate (kgN d\textsuperscript{−1}), and effluent NO\textsubscript{3}−N (kgN d\textsuperscript{−1}), NH\textsubscript{4}+−N, and inorganic nitrogen removal efficiencies (%). (b) Inorganic nitrogen load and removal rate (gN m\textsuperscript{−2} d\textsuperscript{−1}).
in the sCOD/NH₄⁺-N ratio, which varied between 1.1 ± 0.3 (at dilution ratios of 1:10–1:15) and 1.5 ± 0.3 (at dilution ratios of 1:2–1:3). The sCOD reduction and the ratio sCOD/ NH₄⁺-N under the start-up period are available as supplementary material (Figures S1 and S2).

On day 50, the FA reached >20 mg L⁻¹, which is higher than the setpoint in the start-up strategy. The reported ranges in which the FA concentration becomes inhibitory for AOB and AMX vary considerably (Aktan et al. 2012; Jaroszyński et al. 2012), and it was therefore necessary to determine the FA concentration tolerated in this particular system.

During sampling of the carriers for the determination of biomass on day 51, it was observed that the fluffy biofilm had become detached, leading to a significant loss of biomass, from 12 to 1 gDS m⁻². A possible explanation of this sudden loss of biomass was a malfunctioning DO sensor, which caused an increased airflow rate; that is, high turbulence and shear stress. Ødegaard et al. (1994) have described various cases of biomass loss; that is, shear stress, predation by higher organisms, or changes in the reactor conditions. Kowalski et al. (2018) also reported that a loosely attached biofilm was easily detached from the carrier media when the mixing was intensified. It is not clear whether the increased FA (~20 mg L⁻¹), the slow increase in the sCOD/ NH₄⁺-N ratio, or the turbulence caused by the air flow alone, or all together caused the loss of biomass in this study.

The reactor was loaded with 60–80 kgN d⁻¹ (0.5–1.0 gN m² d⁻¹) until day 60, and no significant reduction was observed (Figure 4); 5–10% of the nitrogen probably disappeared due to nitrogen assimilation by the biomass and some denitrification. After stabilizing the NO₂⁻-N concentration below 20 mg L⁻¹ and the FA concentration in the range of 5–10 mg L⁻¹, the inlet flow was reduced in order to reduce the load. This reduction led to a decreased NO₂⁻-N concentration <20 mg L⁻¹ and NH₄⁺-N converted/NO₃⁻-N converted increased to ~12% (day 78). Although a discrepancy was observed in the nitrogen balance (22 kgN d⁻¹), the activity measurements did not show any AMX activity. A new batch test was performed on day 81, when the first sign of AMX activity was confirmed (Figure 3). Kanders et al. (2018) reported reaching full capacity within 120 d of start-up without seeding, which confirms that AMX activity should appear about 3 months after start-up. In the present study, AMX activity was not observed or detected earlier, probably due to FA inhibition and high nitrogen load, but under favorable conditions the AMX started to consume NO₂⁻-N and the levels in the reactor decreased rapidly after day 79.

The first phase of start-up, when a few important events occur simultaneously; that is, the formation of the biofilm, the initiation of the nitritation and later the anammox at the same time as inhibition should be avoided, is the most critical for successful and rapid start-up.

**Start-up phase II**

As the activity of AMX increased (Figure 3), the load was gradually increased from 0.4 gN m⁻² d⁻¹ to 1.5 gN m⁻² d⁻¹, resulting in an increase in N reduction rate, from 0.3 gN m⁻² d⁻¹ to 1.2 gN m⁻² d⁻¹ (Figure 4(b)). On day 98, all the available centrate could be treated, which was one of the criteria for transition to continuous operation. However, due to operational problems with the centrate pump, this phase was extended to day 111 in an attempt to ensure stable operation. During phase II, the maximum nitrogen reduction was 1.64 gN m⁻² d⁻¹, corresponding to a reduction of 77%. The activity measurements showed a slight increase in NOB activity (Figure 5), which could explain the finding that the NO₃⁻-N produced corresponded to 10–16%, which is slightly higher than the theoretical value of 11%. The NOB could be suppressed by reducing the airflow. During this phase, the NH₄⁺-N load was slightly higher than the actual capacity, in order to promote the growth of AMX and increase the SAA activity (Figure 3).

**Continuous operation**

The development of the biomass proceeded further, and the SAA activity increased from 1.7 to 6.4 gN m⁻² d⁻¹. A mass balance showed that the NO₃⁻-N produced was 5.57 ± 1.2%, which is only about half of the theoretical value of 11%. Heterotrophic denitrification was confirmed by SDA measurements, showing a peak value of 1.1 gN m⁻² d⁻¹ for SDA (Figure 3). Although the sCOD /NH₄⁺- Nᵢᵣᵣ ratio was low (0.4 ± 0.2), the TSS mass balance indicated that some particulate COD was converted into sCOD, which could probably be utilized as a carbon source by the HB. As long as the denitrifying HB do not outcompete the AMX for NO₂⁻-N as substrate and the SAA increases, denitrification is not competitive and contributes to increased nitrogen removal in the reactor.

As the biofilm grew thicker with time, the DO setpoint in the bulk was increased to 1–1.5 mg L⁻¹. New operational problems with the NH₄⁺-N online sensor controlling the centrate flow to the reactor occurred on day 185. This led to a rapid increase in FA, to 55 mg L⁻¹, and reversible inhibition.
of AMX and AOB was observed and confirmed by the batch activity measurements (Figure 3).

CONCLUSIONS

The start-up of a full-scale PNA MBBR process was successfully accomplished from virgin carriers without seeding within 111 days. Heated dilution water was a prerequisite for the rapid start-up during this study. The first sign of AMX activity was observed 79 days after the reactor was brought into operation. The centrate was found to be a reliable source for the seeding of AMX. If the process is started up without inoculum, it is of great importance to have access to dilution water to avoid inhibitory conditions during the initial phase. The maximum N removal rate achieved corresponded to 1.8 gN m\(^{-2}\) d\(^{-1}\), and >80% nitrogen reduction was achieved during continuous operation.

The results of this study show that FA concentrations >10 mg L\(^{-1}\) can disturb the process due to reversible inhibition. As the operation of the PNA process requires more attention than regular processes at a WWTP, an online control system for the FA concentration is recommended to ensure full capacity and stable operation.

This study highlights the importance of a stable biofilm development. Other parameters, including DO, FA, and temperature, must be balanced to ensure favorable conditions for the AMX bacteria. The retention of AMX is critical for the PNA process, and the MBBR is an effective solution.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this paper is available online at https://dx.doi.org/10.2166/wst.2020.271.

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