Influence of seasonal temperature fluctuations on two different partial nitritation-anammox reactors treating mainstream municipal wastewater

Susanne Lackner, Samuel Welker, Eva M. Gilbert and Harald Horn

ABSTRACT

Partial nitritation-anammox (PN-A) has gained increasing interest for municipal wastewater treatment in recent years due to its high energy-saving potential. Moving the PN-A technology from side- to mainstream exhibited a set of challenges. Conditions are quite different, with much lower ammonium concentrations and temperatures. Biomass retention becomes highly important due to the even lower growth rates. This study compared two laboratory-scale reactors, a sequencing batch reactor (SBR) and a moving bed biofilm reactor (MBBR), employing realistic seasonal temperature variations over a 1-year period. The results revealed that both systems had to face decreasing ammonium conversion rates and nitrite accumulation at temperatures lower than 12 °C. The SBR did not recover from the loss in anammox activity even when the temperature increased again. The MBBR only showed a short nitrite peak and recovered its initial ammonium turnover when the temperature rose back to >15 °C. The SBR had higher biomass specific rates, indicating that suspended sludge is less diffusion-limited but also more susceptible to biomass wash-out. However, the MBBR showed the more stable performance also at low temperatures and managed to recover. Ex situ batch activity tests supported reactor operation data by providing additional insight with respect to specific biomass activities.

Key words | anammox, biofilm, deammonification, mainstream, sequencing batch reactor

INTRODUCTION

The discovery of anaerobic ammonium oxidation (anammox) in the mid-1990s has resulted in the rapid development of new treatment technologies implementing autotrophic nitrogen removal in wastewater treatment. The combination of the anammox process with partial nitritation (PN-A) allows for complete nitrogen removal, especially from wastewaters with low C:N ratios. The main application of PN-A in municipal wastewater treatment has been N-removal from digestor supernatant (side-stream). There are already more than 100 PN-A reactors in operation worldwide, the majority in side-stream applications (Lackner et al. 2014). Characteristic features of these wastewaters are their high temperatures (>30 °C), the low organic carbon content and high ammonium concentrations (>400 mg/L).

Due to the achievable energy savings (Jetten et al. 1997; Siegrist et al. 2008; Lackner et al. 2015), PN-A processes might also be an asset for mainstream nitrogen removal. However, for mainstream applications, PN-A has to cope with much lower temperatures of <20 °C (moderate climates), and lower ammonium concentrations in combination with easily degradable organic carbon. These conditions might result in competition between autotrophic and heterotrophic bacteria. Several studies have focused on PN-A under mainstream conditions using both synthetic and real wastewaters (de Clippeleir et al. 2013; Hu et al. 2015; Persson et al. 2014), however, these studies reduce the temperature step-wise which is not a realistic scenario for a real application.

The first study investigating a seasonal temperature decrease in a moving bed biofilm reactor (MBBR) was conducted by Gilbert et al. (2014), inducing a steady temperature reduction from 20 °C down to 10 °C over a period of 20 weeks. This study, however, has not included the subsequent temperature increase and potential recovery of reactor performance after severe loss of activity at 10 °C.
The present work aims to provide additional new insight into the behavior of PN-A reactors during one complete annual temperature cycle. The research conducted compared two laboratory-scale PN-A reactors, one sequencing batch reactor (SBR) with suspended biomass and one MBBR, and their performance as affected by a seasonal temperature gradient using real municipal wastewater.

**MATERIALS AND METHODS**

**Reactor operation**

The two laboratory-scale reactors had a working volume of 10 L and were fed with real municipal wastewater originating from the effluent of a high rate activated sludge process (no primary clarification) at the wastewater treatment plant Karlsruhe, Germany. Influent characteristics are provided in Table 1. The SBR was inoculated with suspended biomass from the DEMON® plant in Heidelberg, Germany and operated on 4–8 h cycles with a single feeding phase of 1 min at the beginning of each cycle, a reaction phase of 348–468 min (dependent on biomass activity), a settling time of 10 min and effluent withdrawal for 1–2 min. The reaction phase operated with an initial stirring phase of maximum 200 min followed by intermittent aeration with aeration intervals of maximum 2 min followed by 15 min anoxic phases.

The MBBR was operated continuously and was filled with 480 carriers (filling degree 30%) of the type BiofilmChip™ M (AnoxKaldnes, Lund, Sweden), originating from the side-stream reactors at Sjölunda wastewater treatment plant in Malmö, Sweden. Both reactors were operated and controlled by a programmable logic controller (Wago 750-841, Minden, Germany) for NH₄-N and NO₃-N (ISE-max CAS40), oxidation-reduction potential (Orbisint CPS12D Redox), dissolved oxygen (Oxymax H COS22D), pH (Orbisint CPS11 pH), electrical conductivity (Indumax CLS50D) and temperature (CTS1). Influent was controlled by the effluent ammonium concentration which was kept at 6–8 mg-NH₄-N/L, by adjusting the influent pump speed in the SBR and by an NH₄-N set-point (PLC, on/off) for the influent pump of the MBBR. Average operational parameters are summarized in Table 2.

Both reactors had been in operation at the laboratory for more than 300 days prior to the start of these experiments. During the presented operation phase, after keeping a temperature of 20 °C for the first 40 days, the temperature was gradually reduced by 0.5 °C per week from 20 °C to 10 °C (days 40–175), then kept at 10 °C for 25 days (days 175–200), and afterwards the temperature was increased again by 0.5 °C per week from 10 °C back to 20 °C (days 200–335), where it remained for another 40 days.

**Ex situ activity measurements**

Biomass was taken regularly from the laboratory-scale reactors to measure the specific rates for nitritation, nitratation, anammox and heterotrophic activity in *ex situ* batch experiments as described in Gilbert *et al.* (2014). Three carriers were chosen randomly from the MBBR and exactly 200 mL mixed liquor from the SBR were taken for the batch experiments. Residual substrate was removed by washing the biomass. All batch experiments were conducted at a temperature of 20 °C.

**Analyses**

Influent and effluent samples were taken twice per week. The nitrogen species (NH₄-N, NO₂-N, NO₃-N) were analysed by ion chromatography (Metrohm 790 Personal

Table 1 | Characteristics of the influent to the two laboratory-scale reactors. NH₄-N was augmented to reach values of approximately 50 mg-N/L (VSS, volatile suspended solids)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄-N [mg/L]</td>
<td>10</td>
<td>96</td>
<td>49 ± 11</td>
</tr>
<tr>
<td>COD [mg/L]</td>
<td>20</td>
<td>117</td>
<td>55 ± 27</td>
</tr>
<tr>
<td>sCOD [mg/L]</td>
<td>14</td>
<td>58</td>
<td>29 ± 7</td>
</tr>
<tr>
<td>BOD₅ [mg/L]</td>
<td>0</td>
<td>52</td>
<td>17 ± 13</td>
</tr>
<tr>
<td>TSS [mg/L]</td>
<td>10</td>
<td>26</td>
<td>15 ± 10</td>
</tr>
<tr>
<td>VSS [mg/L]</td>
<td>1</td>
<td>17</td>
<td>9 ± 6</td>
</tr>
</tbody>
</table>

*The average values incl. standard deviations are calculated from data collected during all 375 days of operation.*

Table 2 | Operational parameters for the SBR and the MBBR given as average values over the 375 days of operation (HRT, hydraulic retention time; DO, dissolved oxygen)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SBR</th>
<th>MBBR</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH [-]</td>
<td>8.0 ± 0.4</td>
<td>7.5 ± 0.22</td>
</tr>
<tr>
<td>HRT [d]</td>
<td>4.3 ± 0.4</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td>DO [mg/L]</td>
<td>0.18 ± 0.39</td>
<td>0.44 ± 0.15</td>
</tr>
<tr>
<td>TSS [g/L]</td>
<td>1.4 ± 0.5</td>
<td>4.2 ± 0.7</td>
</tr>
<tr>
<td>VSS [%]</td>
<td>73.6 ± 8.0</td>
<td>74.6 ± 5.3</td>
</tr>
</tbody>
</table>

*DO set-points were chosen based on reactor type, the pH was not controlled. The TSS/VSS measurements represent average values (n – 2–5) over the entire operational period.*
IC, Filderstadt, Germany). Chemical oxygen demand (COD) was measured using cuvette tests (Hach Lange, Germany). Biochemical oxygen demand (BOD) was measured manometrically using the WTW OxiTop apparatus (WTW, Germany). Total suspended solids (TSS) of the suspended biomass (SBR) was measured according to standard methods. For the carriers, TSS was measured by drying 15 carriers at 105 °C. After weighing and completely removing the biomass from the carriers, the empty carriers were dried again to determine the weight of only the biomass.

RESULTS AND DISCUSSION

Reactor performance

The operation parameters of the two laboratory-scale reactors illustrate the differences in configuration of these two systems. The TSS concentration was significantly higher in the MBBR than in the SBR with 4.2 vs 1.4 g/L. This difference in volumetric biomass content was reflected in a higher HRT in the SBR of, on average, 4.3 d compared to the 0.8 d in the MBBR. The MBBR also operated at a higher average DO concentration, which was possible due to the structure of the biomass (Table 2). The 2 mm thick carrier material was almost completely filled and thus accommodated an almost 2 mm thick biofilm. The biomass in the SBR had a partly floccular structure with small granules (<1 mm).

Figure 1 presents reactor performances over time given in percentages of nitrogen removal and nitrite/nitrate production related to ammonium turnover. Both reactors exhibited total nitrogen conversions of 70–75% over the first 150 days of operation (down to a temperature of 12 °C) (Figure 1(a)). At temperatures below 12 °C nitrite started to accumulate in both reactors (Figure 1(b)), reaching even 100% (of the converted ammonium) which corresponded to maximum nitrite concentrations of 31 mg-N/L in the SBR and 40 mg-N/L in the MBBR, respectively (at around day 185, temperature 10 °C). Similar nitrite accumulation has also been observed by several other researchers starting at temperatures of 15 °C (Dosta et al. 2012; de Clippeleir et al. 2013) or lower at 10–12 °C (Hu et al. 2013). The MBBR recovered fast from this nitrite accumulation reaching values below 2 mg-N/L from day 202 (11 °C) onwards. Gilbert et al. (2014) made similar observations, having an even lower nitrite peak also in a MBBR system with a thicker biofilm. On the contrary, the SBR did not recover from this nitrite accumulation until reactor operation was back at 19 °C (day 320), and after addition of 1 L fresh DEMON sludge (at 18.5 °C). Other studies showed that using an enriched anammox culture as inoculum (Hu et al. 2013) delayed nitrite accumulation, compared to biomass from PN-A systems treating real wastewater (de Clippeleir et al. 2013; Persson et al. 2014). This indicates that a high amount of anammox bacteria in the biomass mitigates the effect of decreasing anammox activity at low temperatures.

Nitrate production (Figure 1(c)) (as percentage of the ammonium converted) in the MBBR lay between 10 and 20% until the temperature reached 10 °C (after day 188). With the increase in temperature nitrate production spiked at days 220–240 (temperature 12–13 °C), coming back to values far below 20% from 13.5 °C onwards (days 250–375). The SBR only showed significant nitrate production during the temperature decrease from 20 °C down to 12.5 °C. Afterwards, only nitrite production occurred.

Specific biomass activity

The ex situ rates of AnAOB support this observation (Figure 2(a)) revealing a significant drop in anammox...
activity over the course of the experiment and with decreasing temperature. Overall, the specific AnAOB activity was higher in the SBR than in the MBBR, which is probably due to diffusion limitations in the biofilm. Additional batch experiments detaching and homogenizing biomass from the carriers would have been necessary, but were not conducted due to loss of biofilm from the MBBR.

Both systems suffered from a severe decrease in AnAOB activity, down to values below 10 g-N/kg-VSS/d at temperatures of 10°C (Figure 2(a)). These values are within the lower end of other observations of biomass-specific anammox rates at such low temperatures with 30–44 mg-N/gVSS/d at 10°C for enriched anammox biomass (Hendrickx et al. 2014) or <20 mg-N/gVSS/d at less than 15°C (Dosta et al. 2008). The activity loss in the SBR was more severe and only recovered less than a third of its original rate. The anammox rates in the MBBR also remained lower than the initial values at 20°C.

The higher initial ex situ anammox activity of the SBR (Figure 2(a)) was also reflected in the in situ biomass specific NH₄-N turnover (Figure 3(a)). At the start of the experiment, the SBR showed superior NH₄-N turnover with maximum values of 80 g-N/kgVSS/d, compared to 33 g-N/kgVSS/d in the MBBR. However, at 16°C the NH₄-N turnover in the SBR fell drastically below 20 g-N/kgVSS/d, whereas the values in the MBBR remained rather constant. The average NH₄-N conversion over the 10°C period (15 days) was 10.4 ± 3.9 g-N/kgVSS/d in the SBR and 13.6 ± 4.9 g-N/kgVSS/d in the MBBR, respectively.
The following temperature increase (Figure 3(b)) saw some recovery of the NH4-N turnover in the MBBR starting around 16 °C while the rates in the SBR continued to decrease to almost zero activity. These values are slightly lower than NH4-N conversions reported by Lotti et al. (2014) for a granular SBR system with 42 g-N/kgVSS/d at 10 °C. However, in their case NH4-N effluent concentrations were much higher.

The accumulations of nitrite and nitrate detected in the reactors (Figure 1(b) and (c)) were probably caused by a combined effect of reduction in anammox activity as well as a decrease in nitratation rates. It seems, however, that at lower temperatures, reduction in anammox activity was more prominent than nitratation. The ex situ nitrification rates, given as ratios of AOB/NOB activities at 20 °C (Figure 2(b)), support this hypothesis. Values <1 represent higher NOB activity whereas ratios >1 mean higher AOB activity. Only for temperatures >17 °C were AOB activities above 1 and consistently only in the MBBR. The SBR always exhibited more activity for NOB than AOB. However, the ex situ rates were measured at 20 °C and a shift in activities at lower temperatures might have happened.

The turnover of organic carbon was very low in both reactors and could have only contributed a maximum of 14% to the nitrogen removal if considered as denitrification only (based on influent COD). Activities of heterotrophic bacteria were low for both, aerobic and anoxic batch tests (5–25 g/kg-VSS/d). However, the tests were conducted with acetate as carbon source, which might not have been the ideal substrate for these systems. Additional batch tests using, for example, cell decay products as substrate, should be performed in the future to unravel the potential contribution of heterotrophic bacteria (Kindaichi et al. 2004; Gilbert et al. 2014). This might also add an explanation for the nitrite accumulation under conditions that would favour nitrate production (AOB/NOB activities <1). The organic carbon residual from the high rate activated sludge tank did not seem to have any inhibitory influence on reactor performance.

**CONCLUSION**

Two different types of laboratory-scale reactor, an SBR and a MBBR, operated for partial nitritation-anammox under mainstream conditions and fed with municipal wastewater, were exposed to a typical annual temperature cycle (moderate climates).

Both systems showed a decrease in in situ and ex situ activities when the temperature decreased. The MBBR recovered its initial NH4-N turnover, whereas the SBR saw a severe drop in activity at 10 °C that did not re-establish when the temperature increased again. Both systems suffered from nitrite accumulation at lower temperatures, transient in the MBBR, persisting in the SBR.

The results of this study showed that PN-A is possible with real wastewater after a high rate activated sludge process, with the limitation of nitrite accumulations at lower temperatures. The biofilm system, although lower in biomass specific rates, proved to be superior when it comes to process stability and recovery.

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**REFERENCES**


