Influence of temperature on the activity of anammox granular biomass

D. Sobotka, K. Czerwionka and J. Makinia

ABSTRACT

The aim of this study was to determine a short-term and long-term effect of temperature on the anammox rate and determination of temperature coefficients in the Arrhenius and Ratkowsky equations. The short-term effects of temperature on the anammox granular biomass were investigated in batch tests at ten different temperatures in the range of 10–55 °C. The maximum overall nitrogen removal rate of 1.3 gN gVSS\(^{-1}\) d\(^{-1}\) was observed at 40 °C (VSS: volatile suspended solids). The minimum rate, close to 0 gN gVSS\(^{-1}\) d\(^{-1}\), was observed for the limits of the analyzed temperature range (10 and 55 °C). The activity tests carried out at 55 °C showed an irreversible loss of the activity due to the observed biomass lysis. Subsequently to the batch tests, a sequencing batch reactor (SBR) was operated at different temperatures (from 30 to 11 °C) to determine the long-term effects of temperature. The system was successfully operated at 15 °C, but when temperature was decreased to 11 °C, nitrite started to accumulate and the system lost its stability. The temperature coefficient (\(\theta\)) was 1.07 for the batch tests carried out in the temperature range of 10–40 °C. In contrast, during the long-term SBR operation, substantially different \(\theta\) had to be estimated for two temperature ranges, 1.07 (T = 15–30 °C) and 1.65 (T = 11–15 °C).

Key words | Arrhenius equation, biogranulation, Candidatus Brocadia, Ratkowsky equation, theta factor

INTRODUCTION

In the case of specific wastewater (e.g. reject water from sludge digestion) containing significantly higher concentrations of nitrogen compared to the typical municipal wastewater, the alternative technology to the conventional nitrification–denitrification is the anaerobic ammonia oxidation (anammox) process. Anammox bacteria convert ammonium and nitrite directly to dinitrogen gas (N\(_2\)) under anoxic conditions. Initially, the doubling time of anammox bacteria was reported as 30 days (Van de Graaff et al. 1996), but subsequently that time was decreased to 11 days (Strous et al. 1998) and even 1.8 days (Isaka et al. 2006). Due to the very slow growth rate of anammox bacteria, compared to nitrifiers and denitrifiers, wastewater treatment plants (WWTPs) should be operated at optimum conditions to support the maximum growth rate of anammox bacteria. Temperature is one of the most critical parameters for the microbial growth and efficient biological wastewater treatment. Maintaining the optimum temperature for the anammox process would not be cost effective in a WTTP.

It is essential to find out the optimum way to deal with this problem for wider applications of the low-temperature anammox technology in mainstream biological reactors (Hendrickx et al. 2012; Daverey et al. 2015).

The anammox bacteria have their maximum activity between 30 and 35 °C with the optimum at 43 °C (Ward et al. 2011). Therefore, in most cases, the anammox process applications have been focused on the treatment of wastewater with temperatures around 30 °C in order to operate under optimum conditions. Although several studies showed the ability of nitrogen removal at a lower temperature by the anammox process (Cema et al. 2007; Dosta et al. 2008; Lotti et al. 2015), an important operational objective is to maintain a stable nitritation–anammox process at temperatures lower than 20 °C. Although the anammox process has been found in the arctic ice at extremely low temperatures, i.e. 12 °C (Rysgaard & Glud 2004), maintaining the stable process at such temperatures is not feasible in engineered systems. Dosta et al. (2008) and Isaka et al.
(2008) reported that the activities of anammox bacteria and ammonia oxidizing bacteria decreased at the temperature lower than 20 °C.

The aim of this study was to determine short-term and long-term effects of temperature on the anammox process rate and estimate temperature coefficients in the Arrhenius and Ratkowsky equations. First, the short-term effects of temperature (between 10 and 55 °C) were examined in batch tests with the anammox granular biomass cultivated in a sequencing batch reactor (SBR) at a constant temperature of 30 °C. Subsequently, the SBR was operated at gradually decreasing temperatures to determine the long-term effects.

**MATERIALS AND METHODS**

**Origin of the anammox biomass and feed composition**

The reactor was inoculated with anammox biomass originated from a full-scale sidestream treatment system in Zurich (Switzerland). The long-term granulation of anammox biomass was performed in a SBR (V = 10 L), equipped with temperature and pH control systems and thermostatic jackets. During the granulation studies, the SBR was operated in cycles (2–12 h) divided into four phases: mixed fill (30 min), reaction (120–660 min), settle (20 min) and draw (10 min). The process was carried out at 30 °C and pH was controlled by addition of 1M HCL in the range of 7.5–7.8. The hydraulic retention time (HRT) was set at 12 days at the beginning of the study and the HRT was decreased to 0.5 d from day 271 of the study. The SBR was fed with a synthetic autotrophic medium as recommended by Dapena-Mora *et al.* (2004). The nitrite to ammonium molar ratio in the feeding media varied in the range 1.0–1.3 depending on the actual process temperature (Table 1).

More information about the biomass characteristics and composition can be found elsewhere (Sobotka *et al.* 2016). The phylogenetic analysis revealed that anammox bacteria (*Planctomycetes*) were exclusively represented by *Candidatus Brocadia*. The contribution of these bacteria in the granular biomass was 43%.

**Short-term activity experiments**

In order to determine the short-term effects of temperature on the activity of anammox granular biomass, batch tests were carried out in two parallel batch reactors with the maximum working volume of 4 L each. The reactors were equipped with electrodes for on-line measurement of pH and temperature (Figure 1). The automated control systems for heating/cooling allowed to keep the process temperature at the selected set point. The tests were carried out at ten different temperatures: including 10, 15, 20, 25, 30, 35, 40, 45, 50 and 55 °C under non-aerated conditions. Before each experiment, biomass was sampled from the SBR and the biomass concentration in the batch reactor was maintained at approximately 1.5 g VSS L\(^{-1}\). The initial NH\(_4\)-N and NO\(_2\)-N concentrations in the activity tests were approximately 30 mg NH\(_4\)-N L\(^{-1}\) and 40 mg NO\(_2\)-N L\(^{-1}\). During the tests, samples of the mixed liquor were collected from the reactor with the frequency of 30 minutes and analyzed for NH\(_4\)-N, NO\(_3\)-N and NO\(_2\)-N. Based on the measured

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Feeding and trace solution composition</th>
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<tbody>
<tr>
<td><strong>Feeding composition</strong></td>
<td><strong>Trace solution composition</strong></td>
</tr>
<tr>
<td>Compounds</td>
<td>Concentration, mg L(^{-1})</td>
</tr>
<tr>
<td>NH(_4)-N</td>
<td>100–500</td>
</tr>
<tr>
<td>NO(_2)-N</td>
<td>100–650</td>
</tr>
<tr>
<td>KHCO(_3)</td>
<td>1.25</td>
</tr>
<tr>
<td>CaCl(_2)</td>
<td>1.41</td>
</tr>
<tr>
<td>KH(_2)PO(_4)</td>
<td>50.0</td>
</tr>
<tr>
<td>MgSO(_4)</td>
<td>58.6</td>
</tr>
<tr>
<td>FeSO(_4)·7H(_2)O</td>
<td>9.08</td>
</tr>
<tr>
<td>EDTA</td>
<td>6.25</td>
</tr>
<tr>
<td>Trace solution</td>
<td>1.25 ml L(^{-1})</td>
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</table>
NH₄-N and NO₂-N concentrations over time, specific process rates were estimated for each examined temperature.

**Long-term activity experiments**

The long-term effects of temperature were investigated in the SBR. The total nitrogen load (TNL) applied was in the range of 0.1–1.5 gN L⁻¹ d⁻¹ depending on the actual activity and process temperature in the reactor. The initial biomass concentration in the SBR was 2.2 gVSS L⁻¹. During the long-term experiment, the anammox-enriched granular biomass was gradually adapted to lower process temperatures. Due to a very slow growth rate of anammox bacteria, the excess biomass was not removed from the studied SBR. The long-term reactor operation was divided into seven phases, during which the temperature in the SBR was gradually reduced (Table 2).

**Phase I:** This phase consisted of the SBR start-up period at 30 °C (1–308 d of the operation). With the increasing anammox activity, the TNL supplied to the SBR was rising from 0.1 to 1.5 gN L⁻¹ d⁻¹.

**Phase II:** After stabilization of the anammox process at 30 °C, the process temperature was reduced to 20 °C. Phase II lasted 21 days (from 309 to 330 d of the operation).

**Phase III:** The SBR was operated at 15 °C for 43 days (331–374 d of the operation). Based on the batch test results, the lowest possible temperature for the activity of anammox-enriched granular biomass was expected between 10 and 15 °C. Therefore, the process temperature was reduced by 1 degree in the subsequent phases.

**Phase IV:** After acclimation and achieving the stable SBR operation at 15 °C, the temperature was decreased to 14 °C. In that case, the acclimation and stabilization were achieved during 21 days (375–396 d of the operation). In comparison with the previous phase, a shorter stabilization period was required due to a small difference in the temperature between Phase III and Phase IV. A significant decrease in the bacterial activity was observed in Phase IV. Due to the change in the average NO₂-N/ NH₄-N removal ratio, the NO₂-N/NH₄-N ratio in the feed was modified to 1.2.

**Phase V:** The process temperature was reduced to 13 °C and the SBR was operated for 43 days (397–440 d of the operation). Despite the reduction in the anammox process rates, no operational problems were encountered. The NO₂-N/NH₄-N ratio in the feed was modified to 1.1.

**Phase VI:** The reactor was operated at 12 °C for 19 days (441–460 d of the operation). The NO₂-N/NH₄-N ratio in the feed was modified to 1.0.

**Phase VII:** The last phase also lasted 19 days (461–480 d of the operation). The process temperature in the SBR was reduced to 11 °C. The NO₂-N/NH₄-N ratio in the feed was 1.0. Since hardly any anammox activity was observed, the experiment was ultimately terminated.

**Analytical methods**

Samples of the mixed liquor were filtered through 1.2 μm pore-size nitrocellulose membrane filters (Whatman, Kent, UK). Nitrate, nitrite and ammonia concentrations were determined spectrophotometrically by cuvette tests (Hach Lange GmbH). The concentrations of suspended solids, determined as total suspended solids (TSS) and volatile suspended solids (VSS) were determined according to *Standard Methods* (APHA 2005).
Temperature coefficients ($\theta$)

The effect of temperature on the maximum specific growth rate constant of anammox-enriched granular biomass at the actual process temperature ($\mu_{AM,T}$) was expressed by the Arrhenius equation:

$$\mu_{AM,T} = \mu_{AM,30} \cdot \theta^{(T-30)}$$

(1)

where $\mu_{AM,30}$ is the maximum specific growth rate constant of anammox biomass at 30 °C and $\theta$ is the Arrhenius constant (temperature correction factor). Seven experimental data sets obtained from the batch tests in the different temperatures (between 10 and 40 °C) were used to estimate the Arrhenius temperature correction factor. In the temperature range of 11–15 °C (for the SBR operation), Equation (1) was modified by taking 15 °C (instead of 30 °C) as the reference temperature.

In order to take into account the inhibiting effect of high temperatures (above 40 °C) on the anammox process, the modified Ratkowsky equation was also used:

$$r(T) = [b \cdot (T - T_{min})]^b \cdot (1 - \exp(c(T - T_{max}))$$

(2)

where $T_{min}$ and $T_{max}$ are, respectively, the minimum and maximum temperatures at which the anammox activity was observed, and b and c are model constants obtained by minimizing the sum of squared errors between the model outputs and experimental data for the batch test results.

Temperature activation energy

The temperature activation energy reflects the dependence of a reaction on the temperature and can be determined graphically by taking the natural logarithm of the Arrhenius equation:

$$\ln r = \frac{-E_a}{RT} + \ln A$$

(3)

where r is the specific anammox process rate in kg m$^{-3}$ d$^{-1}$; $E_a$ is the apparent activation energy in kJ mol$^{-1}$; R is the universal gas constant, 8.314 J kg$^{-1}$ K$^{-1}$; T is the temperature given in K, and A is the frequency factor for the reaction.

RESULTS AND DISCUSSION

Short-term effects of temperature on the anammox activity

The short-term effects of temperature on the anammox activity were determined in the batch experiments in the temperature range between 10 and 55 °C (Figure 2). The overall specific anammox activity (SAA) reached its maximum (1.3 gN gVSS$^{-1}$ d$^{-1}$) at 40 °C. The activity tests carried out at 55 °C showed an irreversible loss of the activity due to the observed lysis of anammox biomass. The negative effect of high temperatures (above 37 °C) was found by Isaka et al. (2008). Toh et al. (2002) also observed the anammox activity at a mesophilic range, but thermophilic anammox organisms could not be selected at 55 °C.

The estimated parameters in the Ratkowsky equation were $b = 0.29$ and $c = 0.001$, where $T_{min}$ and $T_{max}$ were set at 11 °C and 54 °C, respectively. The coefficient of determination ($R^2$) for the Ratkowsky equation was 0.91.

Long-term effects of temperature on the anammox activity

The anammox biomass in the SBR was gradually acclimated to lower process temperatures in order to avoid an immediate failure of the SBR operation. The operational conditions and results of the long-term SBR operation are presented in Table 2 and Figure 3, respectively. During the SBR operation at the decreasing temperatures: 30 °C, 20 °C, 15 °C, 14 °C, 13 °C, 12 °C and 11 °C, the influent TNL were set, respectively, at 1.5 gN L$^{-1}$ d$^{-1}$, 1.2 gN L$^{-1}$ d$^{-1}$, 1.0 gN L$^{-1}$ d$^{-1}$, 0.7 gN L$^{-1}$ d$^{-1}$, 0.2 gN L$^{-1}$ d$^{-1}$, 0.2 gN L$^{-1}$ d$^{-1}$ and 0.2 gN L$^{-1}$ d$^{-1}$. Despite a decline in the process efficiency, the SBR was operated stably even at 12 °C. Nitrite was completely depleted in the reactor until the process temperature decreased from 30 to 12 °C. In order to prevent the...
accumulation of nitrite in the reactor and subsequent inhibition of the anammox process by too high nitrite concentrations, the molar ratio of NO$_2$-N/NH$_4$-N was reduced from 1.3 to 1.0. Regardless of this change, the system was not able to remove the entire applied nitrite load and the nitrogen removal efficiency decreased to an extremely low value (only 6%). Consequently, nitrite was accumulated in the reactor up to almost 70 mgN L$^{-1}$ (Figure 3). Strous et al. (1999) and Lotti et al. (2012) reported that high nitrite concentrations (60–400 mg NO$_2$-N L$^{-1}$) could directly inhibit the anammox process. When compared to those results, it is unlikely that the rapidly increasing nitrite concentrations in Phase VII were a significant contributor to the anammox inhibition in the low temperatures.

During the entire study, the molar ratio of NO$_2$-N/NH$_4$-N in the synthetic feed decreased from 1.3 (phases I, II, III) to 1.2 (phase IV), 1.1 (phase V) and ultimately 1.0 (phases VI, VII). Those ratios were adopted in response to the observed conversion ratios of NO$_2$-N/NH$_4$-N which were 1.44, 1.30, 1.28, 1.21, 1.10, 0.99, 0.87, respectively, for the examined temperatures, i.e. 30°C, 20°C, 15°C, 14°C, 13°C, 12°C, 11°C. In the temperature range of 15–30°C, the observed anammox stoichiometry was close to that one (NO$_2$-N/NH$_4$-N = 1.32) presented by Strous et al. (1999), whereas at the lower temperatures (11–15°C), the conversion ratio changed significantly. Similar changes in the anammox stoichiometry were also observed by Dalsgaard & Thamdrup (2002) and Dosta et al. (2008). However, the NO$_2$-N/NH$_4$-N ratio decreased to approximately 1 at higher temperatures in comparison with the present study, i.e. 18°C and 15°C, respectively.

The obtained results revealed that the SAA decreased gradually from 0.81 to 0.41 gN gVSS$^{-1}$ d$^{-1}$ when the process temperature was reduced from 30 to 20°C (Table 2, phase I and II). The decrease in the activity by approximately 50% was also observed by Vazquez-Padin et al. (2011) and Dosta et al. (2008) in the same temperature ranges (Table 3). In the studies of Vazquez-Padin et al. (2011), the SAA decreased from 0.28 to 0.13 gN gVSS$^{-1}$ d$^{-1}$, whereas the corresponding SAAs observed by Dosta et al. (2008) were approximately 0.18 and 0.09 gN gVSS$^{-1}$ d$^{-1}$. The closest results to those obtained in the present study at 20°C (nitrogen removal rate, NRR = 1.03 gN L$^{-1}$ d$^{-1}$) were reported by Winkler et al. (2012), i.e. NRR = 0.9 gN L$^{-1}$ d$^{-1}$ at 18 ± 3°C. This may be due to the occurrence of the same type of anammox bacteria (Candidatus Brocadia) in the granular sludge in the present study. The temperature 15°C (phase III) proved to be sufficient for maintaining the anammox activity at a stable level (0.27 gN gVSS$^{-1}$ d$^{-1}$) without causing any operational problems in the studied SBR. Nearly two times higher SAA, 0.46 gN gVSS$^{-1}$ d$^{-1}$ at 16°C, was reported by Ma et al. (2015). In contrast, in the study of Dosta et al. (2008), the system was not able to remove completely the applied nitrite load at 15°C and irreversibly lost its stability. In the present study, further reductions of the process temperatures (from 15 to 11°C) resulted in a gradual decrease in the SAA until almost complete loss of the anammox activity at 11°C (0.02 gVSS$^{-1}$ d$^{-1}$). The active anammox process at extremely low temperatures (below 10°C) was reported by Isaka et al. (2008), Hendrickx et al. (2012) and Xing et al. (2015). Those studies were conducted in a reactor with gel carrier, gas-lift reactor and upflow anaerobic sludge blanket (UASB), respectively, which may suggest that the type of the reactor may be an important factor for achieving the efficient anammox process at low temperatures. Table 3 contains more details on the literature data for low-temperature anammox systems.

**Activation energy and temperature coefficients for the anammox process**

Results obtained in both batch tests and long-term SBR operation are very similar. In the two cases, the temperature dependency of the anammox activity, expressed as Ea, increased at the lowest range of temperatures (10–15°C) (Figure 4). Isaka et al. (2008) and Lotti et al. (2015) also found similar behavior of the activation energy. In the first case, Isaka et al. (2008) estimated the Ea values of 93–94 and 35 kJ mol$^{-1}$ for the temperature ranges of 6–28°C and 28–37°C, respectively. In the latter case, Lotti et al. (2015)
analyzed $E_a$ in four temperature ranges as 30–25 °C, 25–20 °C, 20–15 °C, 15–10 °C, for which the estimated $E_a$ was 46.6 ± 2.7 kJ mol$^{-1}$, 67.6 ± 7.1 kJ mol$^{-1}$, 104.9 ± 13.3 kJ mol$^{-1}$, 230.5 ± 8.3 kJ mol$^{-1}$, respectively. In the present study, the estimated activation energy was 436.8 kJ mol$^{-1}$ and 50.5 kJ mol$^{-1}$ in the temperature range of 10–15 °C and 15–40 °C, respectively. Results from the typical temperature range are in accordance with those presented by Lotti et al. (2013) and are higher than the results presented by Isaka et al. (2008). The $E_a$ value, estimated in the present study, is also slightly lower in comparison with the value reported by Strous et al. (1999), i.e. 70 kJ mol$^{-1}$ for the temperatures between 20 and 43 °C.

Table 3 | Overview of the literature data on the TNL, NRR and SAA in anammox systems at low temperatures

<table>
<thead>
<tr>
<th>Reactor type</th>
<th>Feeding medium</th>
<th>Temp. °C</th>
<th>TNL gN L$^{-1}$ d$^{-1}$</th>
<th>NRR gN L$^{-1}$ d$^{-1}$</th>
<th>SAA gN gVSS$^{-1}$ d$^{-1}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBR</td>
<td>Synthetic</td>
<td>18</td>
<td>0.3</td>
<td>–</td>
<td>0.08</td>
<td>Dosta et al. (2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>0.05</td>
<td>–</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Reactor with gel carrier</td>
<td>Synthetic</td>
<td>18</td>
<td>3.2</td>
<td>1.20</td>
<td>–</td>
<td>Isaka et al. (2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>1.2</td>
<td>0.60</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.3</td>
<td>0.6</td>
<td>0.36</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>SBR</td>
<td>Synthetic</td>
<td>20</td>
<td>0.08</td>
<td>–</td>
<td>0.13</td>
<td>Vazquez-Padín et al. (2011)</td>
</tr>
<tr>
<td>UASB</td>
<td>Synthetic</td>
<td>9</td>
<td>–</td>
<td>1.37</td>
<td>–</td>
<td>Xing et al. (2015)</td>
</tr>
<tr>
<td>Gas-lift</td>
<td>Synthetic</td>
<td>20</td>
<td>0.31</td>
<td>0.28</td>
<td>–</td>
<td>Hendrickx et al. (2012)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>(max)</td>
<td>0.096</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>0.062</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Bubble column</td>
<td>Synthetic</td>
<td>18 ± 3</td>
<td>–</td>
<td>0.90</td>
<td>–</td>
<td>Winkler et al. (2012)</td>
</tr>
<tr>
<td>UASB-like</td>
<td>Effluent from secondary clarifier + NH$_4$Cl + NaNO$_2$</td>
<td>16</td>
<td>–</td>
<td>2.28</td>
<td>0.46</td>
<td>Ma et al. (2013)</td>
</tr>
<tr>
<td>SBAR</td>
<td>Synthetic</td>
<td>30</td>
<td>–</td>
<td>–</td>
<td>0.16</td>
<td>Lotti et al. (2013)</td>
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<tr>
<td></td>
<td></td>
<td>25</td>
<td>–</td>
<td>–</td>
<td>0.14</td>
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<td></td>
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<td>20</td>
<td>–</td>
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<td></td>
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<td></td>
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<td>10</td>
<td>–</td>
<td>–</td>
<td>0.03</td>
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</table>

SBAR: sequencing batch airlift reactor.

Figure 4 | Arrhenius plot for the anammox conversion by anammox-enriched granular biomass with the $E_a$ values for different temperature ranges in the SBR ($E_{a\text{SBR}}$) and in batch-tests ($E_{a\text{BT}}$).

Figure 5 | The temperature influence on the anammox process expressed by the Arrhenius equation based on the short-term experiments (a) and long-term SBR operation (b).
The results of the batch tests, obtained for the temperatures in the range of 10–40 °C, show nearly a linear influence of temperature on the anammox process rate (Figure 5). High temperatures resulted in the increase in the Arrhenius coefficient (Equation (1)) and consequently in the production of molecular nitrogen. Those results revealed that the temperature dependency of the anammox process cannot be accurately modelled using a single temperature coefficient, as commonly applied for other biological processes. In order to better justify the results, two separate temperature coefficients should be determined for the temperatures above and below 15 °C. The temperature coefficients for the batch tests in the temperature range of 10–40 °C and long-term SBR operation in the temperature range of 15–30 °C were identical (1.07) (Figure 5). In order to compare the short-term and long-term effects of temperature on the anammox process rates, the additional temperature coefficients were determined for the same temperature range (15–30 °C). The temperature coefficients for the batch tests and the SBR operation were 1.08 (standard error (SE) = 0.004) and 1.07 (SE = 0.002), respectively. Changing the temperature range resulted in only a minor increase in the temperature coefficient for the batch tests (from 1.065 to 1.08). However, the temperature coefficient for the lower temperature range in the SBR was much higher (1.65, SE = 0.008).

**CONCLUSION**

The maximum activity of non-acclimated anammox-enriched granular biomass was observed at 40 °C, while the process temperature of 55 °C resulted in an irreversible decrease of the anammox activity due to biomass lysis. The granular biomass acclimation to low temperatures allowed for the successful (efficient) operation of the SBR at 15 °C. The stable operation was still observed at 12 °C, but the nitrogen removal efficiency was very low (<10%).

Furthermore, the temperature influence on the anammox process can be accurately predicted by the Arrhenius and Ratkowsky equations in the temperature ranges of 10–40 °C and 10–55 °C, respectively.

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