Analysing the effects of the aeration pattern and residual ammonium concentration in a partial nitritation-anammox process

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ABSTRACT

A mathematical model was used to evaluate the effect of the aeration pattern and ammonium concentration in a partial nitritation-anammox sequencing batch reactor with granular and flocculent sludge. In the tested conditions, model results indicate that most of the aerobic ammonium oxidation potential would occur in the bulk liquid, with 70% of the ammonium-oxidizing bacteria (AOB) biomass in suspension rather than in granules. The simulated granular sludge consisted predominantly of anammox bacteria with AOB present in the outer layer of the granule (50 μm AOB layer, accounting for 3% of the granule weight). Simulation results indicated that when granules do not contain any AOB, the amount of granular biomass required to achieve the same level of nitrogen removal would strongly increase (in the simulated conditions, by a factor of three) due to anammox inhibition by oxygen. This underlines the importance of a small fraction of AOB present in the granular anammox sludge. The aeration pattern had an important impact on the nitrogen removal: a better performance was suggested for continuous aeration (90% N-removal) than for intermittent aeration (68–84% N-removal). Anammox inhibition during the periods of high oxygen concentration was identified as the main reason for the lower nitrogen removal in the intermittently aerated system. With increasing oxygen concentration, a higher residual (effluent) ammonium concentration was needed to assure nitrite-oxidizing bacteria repression in the system. This study contributes to further understand the complexity of a reactor with both granular and flocculent sludge and the impact of operation conditions on reactor performance.

1. Introduction

Nitrogen removal from wastewater is necessary to prevent the negative impacts of ammonium to the environment (e.g. eutrophication, toxicity). Traditional nitrogen removal is done via the nitrification/denitrification (N/DN) route. This route requires high amounts of oxygen and energy to provide an aerobic environment for bacterial nitrification, and organic carbon to remove nitrate by bacterial denitrification, thus increasing the cost of the process.[1] One of the most innovative developments in biological wastewater treatment is the use of the partial nitritation/anammox (PN/A) route. The first step in this process is performed by ammonium-oxidizing bacteria (AOB) nitrifying a fraction of the ammonium to nitrite (partial nitritation). The second step is the anaerobic oxidation of the remaining ammonium with nitrite for the production of nitrogen gas (N₂) by anammox bacteria. Therefore, the nitrification of nitrate to nitrite has to be repressed by outcompeting the nitrite-oxidizing bacteria (NOB).[2] The application of anammox bacteria in the side-stream of wastewater treatment plants (WWTPs) has several advantages compared to the N/DN technology. The aeration requirements decrease by 60%, sludge production decreases by approximately 90% and there is no need for organic carbon.[3–5] This technology is currently applied to warm and high-strength wastewaters, such as digester effluents and anaerobically treated industrial effluents.[6,7]

Early PN/A implementations for side-stream treatment used two-stage reactor configurations for a better control of the nitritation step or to make use of already existing nitritation systems.[7] However, the majority of current implementations consist of a single-stage configuration (88%), while more than 50% of all PN/A systems apply the sequencing batch reactor (SBR) technology.[8] The SBR configuration can be applied with different operation strategies. Aeration can be intermittent (e.g. DEMON™, see [9,10]) or continuous at very low dissolved oxygen (DO) levels (e.g. full-scale partial nitritation-anammox in one SBR in Zürich, Switzerland, see [11]). Sludge retention is a distinguishing factor in
the operational strategy of anammox-based systems. For example, most DEMON® systems use a cyclone to waste fine particulate and flocculent sludge and retain larger anammox granules in the reactor while AOB biomass is supposed to be present in flocculent form. In the case of the SBR in Ingolstadt, Germany, sludge is wasted with the discharged effluent by applying short settling times.

The complexity of the various processes currently applied in full-scale WWTPs for nitrogen removal in the side-stream make a mathematical approach a desired tool to explore the differences between these operational conditions and further analyse their effect on NOB repression. Numerical approaches have proved useful to gain insight in the most important factors that affect the granular sludge processes. Previous studies have examined the effect of granule size and heterotrophic growth on autotrophic nitrogen removal systems. The sensitivity and the effect of temperature on a completely autotrophic ammonium removal over nitrite system have also been studied. The influence of mass transfer and microbial kinetics was investigated in a sensitivity analysis study of an autotrophic N-removal granular bioreactor.

In the present study, we examine a single-stage PN/A process with granular sludge biomass in an SBR. A mathematical model implemented in the AQUASIM simulation software was used to evaluate the effect of intermittent versus continuous aeration on the SBR performance, the role of AOB in suspension versus AOB in granules and the relation between ammonium concentration and NOB repression.

2. Methodology

The mathematical model describing the performance of a full-scale SBR with granular and flocculent sludge was implemented in the AQUASIM simulation software. To represent the mass transport and conversion processes occurring in the time-dependent SBR system, the bulk liquid of changing volume was implemented in AQUASIM as a well-mixed compartment exchanging liquid with a biofilm compartment that represents the granules.

2.1. Conversion processes

The model consists of seven particulate components accounting for the main biomass types present in the reactor: nitrifiers (ammonium-oxidizers, XAOB and nitrite-oxidizers, XNOB), anammox bacteria (XAMN), heterotrophs (grouped by electron acceptor: oxygen, XO2, nitrite, XNO3, nitrate and nitrile XNO2, HET and nitrate XNO3, HET) and inactive biomass (X). The seven dissolved components taken into account are: ammonia (SNH4), nitrite (SN2), nitrate (SNO3), nitrogen (SN), oxygen (SO2) and soluble organic substrate (SS).

In nitrification, ammonium is oxidized to nitrite by AOB and the produced nitrite is further oxidized to nitrate by NOB. Anammox bacteria perform the anaerobic oxidation of ammonium with nitrite as electron acceptor, producing nitrogen gas. Furthermore, three types of heterotrophic bacteria have been considered, each growing on one of the three electron acceptors: oxygen, nitrate or nitrite. Since there is no real distinction between these heterotrophic groups, the corresponding reaction rates were expressed in terms of the total amount of heterotrophic bacteria (XHET = XAOB + XNOB, HET + XNOD, HET). The production of organic materials from biomass decay was taken into account using the death-regeneration approach, where living cells are turned partly into biodegradable substrate and partly into inert material. In this model, the direct formation of soluble organic substrate (SS) rather than particulate organic substrate assumes that decay and not hydrolysis is the rate-limiting step of soluble substrate formation from decay.

The stoichiometry and kinetics are based on those of Hao et al. and Koch et al., considering the effect of heterotrophic activity according to Mozumder et al. Inhibition of XAOB and XNOB at high nitrite and ammonium concentrations has been included in the model. The stoichiometry, rate expressions and parameters used are presented in the Supplementary Material (Tables S1–S3).

2.2. Granular sludge

Two sets of simulations were carried out in the SBR configuration: [1] multispecies granular sludge, that is, all microbial types can grow both in the biofilm and in suspension; and [2] anammox granular sludge, that is, all microbial types can grow in suspension, but only anammox can grow in the granular biofilm.

The biomass density in granules was set to 80000 gCOD/m³. This represents a concentration in the granules of 60000 gVSS/m³ for a conversion factor of 0.75 gVSS/gCOD. Porosity was assumed constant (80%) throughout the granule. To represent the spherical geometry, a radius-dependent biofilm area was set in AQUASIM. To reach a stable granule size (1.1 mm diameter), the biomass detachment rate was $u_{det} = u_{lf}(L_{f}/L_{mf})^{4}$, with $u_{lf}$ the biofilm growth velocity, $L_{f}$ the current granule radius and $L_{f, max} = 0.55$ mm. Equal initial concentrations for each of the six active biomass species were set. When the maximum granule size was reached, the biomass concentration in the reactor was
2.3 gVSS/m³, consisting of 90% granular sludge and 10% biomass in suspension.

To evaluate the importance of oxygen consumption in the granule by AOB, NOB and HET, a hypothetical granule consisting of only anammox biomass (density 80000 gCOD/m³) was implemented. When a stable granule size was reached, the biomass concentration in the reactor was 7.2 gVSS/m³.

2.3. Biomass in suspension

Conversion rates for the biomass in suspension were functions of the bulk concentrations of dissolved components. Due to the relatively long hydraulic residence times (HRT) used in DEMON® reactors, the ammonium-oxidizers (AOB) are not expected to be washed out easily. In addition, the long settling time applied (ca. 2 h) will retain a rather large fraction of flocculent sludge in the reactor and will not promote the biomass granulation. [20] In the model, the effectiveness of the settling phase was described by recirculating a variable fraction of biomass in suspension such that the sludge retention time (SRT) was close to 30 d. The extent of the recirculation was manipulated to meet the overall SRT reported for the Strass WWTP side-stream reactor (30 ± 5d, see [9]).

2.4. SBR reactor configuration

The DEMON® configuration is the most applied SBR technology.[8] This is why the WWTP in Strass, Austria,[9] was taken as a point of reference for this study (Table 1). The eight-hour SBR cycle is divided in a six-hour fill/aeration phase, followed by a two-hour non-aeration and discharge phase. Because the AQUASIM software does not allow the bulk liquid volume from the biofilm compartment to vary in time, the biofilm compartment was linked with a well-mixed compartment of variable volume to simulate the filling and discharge of the SBR, as described in [19]. A high recycling flow rate between the compartments was set to ensure that both compartments have the same bulk liquid concentrations.

From the total reactor volume (500 m³), the model biofilm compartment had a constant volume (385 m³) consisting of both granule and bulk liquid, and the mixed liquid compartment volume varied between 51 and 115 m³ (64 m³ are fed each cycle). An exchange flow rate between the two compartments of 3500 m³/d maintained the same concentrations in the bulk liquid in each compartment. A constant influent flow rate is set during the six-hour feeding period, while the DO was regulated in either an intermittent or a continuous fashion. The aeration stops after the feeding phase, during the last two hours of the operation cycle.[9,10]

2.5. Continuous stirred-tank reactor configuration

In order to study the effect of residual ammonium concentration on NOB repression, a continuous stirred-tank reactor (CSTR) system was also implemented in AQUASIM as a biofilm compartment with constant volume (480 m³) consisting of both granules and bulk liquid. The minimum ammonium concentration required to repress ammonium was obtained at different oxygen concentrations and for different ratios between half-saturation coefficients of AOB and NOB (Section 2.7). For each of the tested cases, an iterative strategy was used to obtain its minimum residual ammonium concentration to repress NOB, with 5 to 10 simulations required per tested case. Further tests showed only a small difference between the minimum ammonium concentration obtained with the SBR and the CSTR approaches (figure S1, Supplementary Material). Therefore the CSTR model was adopted to assess the effect of DO and ammonium concentration on NOB repression because of much shorter computational times needed.

2.6. Aeration pattern comparison

Four aeration patterns were tested (Figure 1) in the SBR configuration. Three cases (A–C) had intermittent aeration and another one used continuous aeration. Minimum and maximum oxygen concentrations were set, while maintaining the same average oxygen concentration for each case (0.25 gO²/m³). This resulted in different ‘on’ and ‘off’ periods of aeration in each case.

2.7. DO and ammonium concentration effect on NOB repression

The effect of the ammonium concentration on NOB repression was studied at several oxygen concentrations (0.25–2 gO²/m³) and at two temperatures (20°C and 30°C). In these simulations, the CSTR approach was used.
In order to assess the NOB repression, a new soluble component ($S_{NO3,NOB}$) was used, produced by NOB in the same amount as regular $S_{NO3}$, but not consumed in denitrification. NOB repression was considered achieved when there was no production of $S_{NO3,NOB}$ in the reactor. The inflow was modified (i.e. higher or lower flow rate, but the same influent concentrations) to find the minimum concentration of ammonium at each oxygen concentration that results in NOB repression.

Because a recent report suggested NOB to have a much higher oxygen affinity than AOB [28] and the reported affinity coefficients vary widely in literature, the half-saturation coefficients for oxygen, $K_{O2, AOB}$ and $K_{O2, NOB}$, were varied over a wide range to study the effect on NOB repression in the granular system. Let $R_{KO}$ express the ratio between half-saturation coefficients of AOB and NOB, $R_{KO} = K_{O2, AOB}/K_{O2, NOB}$, because relative ratios rather than absolute values are expected to affect the overall process rates.[14] The $R_{KO}$ ratios used were: 0.3/1.75 (0.17), 0.3/1.1 (0.27), 0.16/0.16 (1), 0.18/0.13 (1.38) and 1.16/0.16 (7.25). References for these values can be found in Table S4 from Supplementary Material.

### 3. Results and discussion

#### 3.1. Model evaluation

The model was first evaluated by simulating the standard operation of a single-stage SBR with multispecies granular and flocculent sludge, with continuous aeration. The steady-state operation was reached after about 900 days. The bulk concentrations during two operation cycles at steady-state are shown in figure S3, Supplementary Material. The model results were directly compared with data from the WWTP in Strass, Austria,[9] in terms of N-removal, biomass concentration and solids retention time (Table 2). The model predictions differed less than 5% when compared to the reported WWTP values in terms of N-removal (Table 2), predicting as well NOB repression and a similar biomass concentration value (23% difference).

The model simulations indicated that in the multispecies granular sludge at steady-state the population distribution was 96% w/w AMX, 3% w/w AOB and 1% w/w inert biomass (Table 3 and Figure 2), while NOB and heterotrophs were completely eliminated from the granule. The substrates required for anammox growth (ammonium and nitrite) are consistently available across the granule (figure S5), resulting in constant growth across the granule and a small inert biomass fraction. The small fraction of AOB was located in the outer layer of the biofilm, within 50 μm from the surface. The AOB layer maintained minimal oxygen levels (less than 0.05 gO2/m3) in the inner part of the granule and formed 31% of the total AOB in the reactor (Table 3). Most of the aerobic ammonium oxidation potential was due to biomass in suspension (69% of AOB population in suspension) and the anaerobic ammonium

### Table 2. Comparison between the performance and characteristics of the single-stage SBR reported for the WWTP in Strass, Austria,[9] with those from the model.

<table>
<thead>
<tr>
<th>Nitrogen Removal</th>
<th>NH4-N (%)</th>
<th>Total N (%)</th>
<th>Biomass concentration (gVSS/L)</th>
<th>Solids Retention Time (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Real case: WWTP Strass</td>
<td>90 ± 3</td>
<td>86 ± 5</td>
<td>3.0 ± 0.8</td>
<td>30 ± 5</td>
</tr>
<tr>
<td>Model case (1): Multispecies granule</td>
<td>94 (+4%)</td>
<td>90 (+5%)</td>
<td>2.3 (−23%)</td>
<td>27.4 (−9%)</td>
</tr>
<tr>
<td>Model case (2): Anammox granule</td>
<td>98 (+9%)</td>
<td>97 (+13%)</td>
<td>7.2 (+140%)</td>
<td>64.7 (+116%)</td>
</tr>
</tbody>
</table>

Notes: In case (1) all microbial populations are allowed to grow in the granule, while in case (2) only anammox bacteria exist in the granule. Values within parentheses are percentage differences between model and the average value reported for the WWTP.
oxidation potential was located in the granules (95% of AMX population in granules). NOB were eliminated from both the granule and reactor in spite of an SRT of 30 days and heterotrophs were present only in suspension as a small fraction of the reactor’s biomass (1%), which is expected for the NH4-N/CODbiodegradable ratio of 9.25 in the influent (Table 1). These model results are in accordance with previous experimental results showing that aerobic activity can be mainly located in flocculent biomass and anaerobic activity in AMX-rich granular biomass. [10] In fact, due to the conditions imposed (HRT > 1/μmax,AOB, a long settling phase and an SRT of ca. 30 days), the proliferation of a high fraction of biomass as flocs is expected. The advantage of having a larger fraction of AOB biomass in suspension than in granular sludge reactors is that the biomass in flocs would experience less mass transfer limitation and higher concentration of substrates than in the granules. This is also in accordance with the study of Hubaux et al. [29]; however, the tested HRT in that study was shorter (0.66 d). Nevertheless, the use of flocs requires lower DO levels (to avoid Anammox inhibition by oxygen) that reduce considerably the nitrogen-loading rate that can be applied, resulting in less compact reactors. [8]

In DEMON® reactors, the settling time is not selective enough, since it is too long (ca. 2 hours settling-decanting period, see [30]) to select for fast settling sludge. In addition, free cells or small flocs that may not settle (even with the long settling time applied) are not totally washed out because of a long HRT applied (on average 62 h for 6 different full-scale installations, range 26–114 h, see [8]), and they accumulate in the reactor. These two operating conditions result in AOB growing mainly in flocculent sludge in the DEMON® process, in contrast to the dominant growth of AOB on the anammox granules in other technologies (CANON, for instance).

3.2. Effect of absence of nitrifiers in the sludge granules

To evaluate the role of the aerobic ammonia oxidizers (AOB) in the granules, a process in which the granules consisted entirely of anammox was also implemented in AQUASIM. The same influent and hydraulic characteristics as for the other simulations were used. By using the aeration pattern shown in Figure 1, the nitrogen removal in the reactor was too low, and far from what is reported from full-scale plants. [9] To alleviate to some degree the anammox inhibition by oxygen, a different aeration pattern was implemented, exclusively for this case. The aeration pattern was intermittent (as reported in [9] for the WWTP in Strass) by maintaining a maximum oxygen concentration (0.25 gO2/m3) and lowering the concentration to 0.1 gO2/m3 in the non-aerated periods (see figure S4, Supplementary Material).

The long-term steady-state in bulk concentrations was reached after 1500 simulated days (i.e. the solute concentrations change within one operation cycle did not change anymore from one cycle to the next). A direct comparison between the results of this simulation and the experimental data from the WWTP in Strass, Austria, [9] is presented in Table 3. N-removal was 13% higher than the reported full-scale data in [9], while biomass concentration and SRT were more than doubled.

The required amount of anammox biomass to obtain a similar N-removal in the reactor with anammox-only granular sludge is approximately three times the one with AOB present in the granular biomass (Table 3). This directly relates to the anammox inhibition by oxygen. In a multispecies granule, most of the anammox experienced an O2 concentration lower than 0.05 gO2/m3, while in an anammox-only granule, there

### Table 3. Biomass distribution in granules, biomass distribution in the reactor, and % biomass in granular form from total biomass in the reactor.

<table>
<thead>
<tr>
<th>Mass percentage (%)</th>
<th>AOB</th>
<th>NOB</th>
<th>AMX</th>
<th>Het</th>
<th>Inert</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass distribution in granules</td>
<td>3</td>
<td>0</td>
<td>96</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Biomass distribution in the reactor</td>
<td>7</td>
<td>0</td>
<td>90</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Biomass in granular form</td>
<td>31</td>
<td>–</td>
<td>95</td>
<td>0</td>
<td>69</td>
</tr>
</tbody>
</table>

*NOB were practically washed out from the reactor
was 0.25 gO$_2$/m$^3$ in the whole granule during aerated periods. According to the reaction kinetics used in this model (Table S2, Supplementary Material), anammox bacteria experiencing 0.05 gO$_2$/m$^3$ can grow three times faster than at 0.25 gO$_2$/m$^3$. In reality, excessive shear stress either in the reactor vessel or in the hydrocyclones used in DEMON$^\text{®}$ reactors could expose AMX to oxygen concentrations closer to those maintained in the bulk liquid and thus worsen reactor performance when the AOB protection layer was compromised.

3.3. Influence of the aeration pattern on reactor performance

The average oxygen concentration during the 6 h aeration period was the same for each of the three aeration patterns presented in Figure 1. Therefore, when shorter aeration periods were implemented, the maximum oxygen concentration in the system was increased proportionally, resulting in deeper oxygen penetration in the granule and inhibition of anammox bacteria. The simulation results indicated that longer aeration periods (i.e. % of aeration phase when aeration is on) improved both the ammonium oxidation and the nitrogen removal in the system (Figure 3). The highest nitrogen removal (90%) was observed in the continuous aeration regime.

Anammox inhibition by oxygen is represented by the term $I_{O_2} = K_{I_{O_2, AMX}}/(K_{I_{O_2, AMX}} + S_{O_2})$. The oxygen inhibition $I_{O_2}(t,x)$ averaged over the granule radius was calculated when the oxygen concentration was the highest for each of the aeration patterns, giving $I_{O_2}(t)$. This was used to compare the degree of inhibition experienced during high oxygen concentration periods of each aeration pattern, with the inhibition experienced in the continuous aeration (figure S2, Supplementary Material). The inhibition on anammox conversion rate during periods of high oxygen concentration was therefore identified as the main reason for the worse N-removal in the system, as shown in Figure 4 and figure S2, Supplementary Material.

Joss et al. [11] studied the full-scale nitrogen removal from digester liquid with an SBR through partial nitritation-anammox. That study maintained a target oxygen concentration $\leq 0.8$ gO$_2$/m$^3$ and tested continuous and intermittent aeration. With this maximum oxygen concentration, they were able to obtain similar ammonium removals (with continuous and intermittent aeration) but required longer aeration periods when intermittent aeration was implemented. These results are in line with our calculations. The maximum oxygen level was observed to be an important parameter to control nitrite accumulation and maintain an elevated nitrogen removal. Continuous aeration appears to enable higher nitrogen and ammonium removal with lower oxygen concentrations and possibly shorter aeration times.

3.4. Oxygen and ammonium concentration effects on NOB repression

The effects of residual ammonium and DO concentrations on NOB repression were studied with a simplified CSTR setup instead of an SBR, which permits a quicker evaluation of multiple factors. Preliminary tests showed that the difference in the minimum ammonium concentration for NOB repression required for the SBR and CSTR was 13% in average and no more than 20% for individual cases (figure S1, Supplementary Material). This type of N-removal systems for side-stream treatment are usually not operated at low ammonium concentration because the effluent is typically recirculated.
to the inlet of the WWTP for further treatment before discharge.[8] Therefore, we considered that calculating the SBR operation should be avoided given the relatively higher computation demands required compared with the CSTR.

It was found that for each oxygen concentration in the bulk liquid, there is a minimum ammonium concentration which results in NOB repression (Figure 5). The $R_{K_O}$ (i.e. the ratio $K_{O_2,AOB}/K_{O_2,NOB}$) and temperature affected this value considerably. With higher $R_{K_O}$ higher ammonium concentrations were required to repress NOB. For example, a change of $R_{K_O}$ from 0.17 to 7.25 would increase the required ammonium concentration to repress NOB from 0.31 to 220 gN/m$^3$ (Figure 5). Temperature affects the inhibition by ammonium and nitrite through their ionization constants (Supplementary Material, Temperature and pH effects on ionization constants), while growth and decay rates of all biomass types are exponential functions of temperature (Table S3, Supplementary Material). Lower minimum ammonium concentrations for NOB repression were observed at 30°C than at 20°C. As expected, when the affinity coefficients for O$_2$ are similar for AOB and NOB ($R_{K_O} = 1$ and 1.38) the DO had little effect on the minimum ammonium required for NOB repression. The modelling results show that NOB repression at 20°C should be feasible. However, lower temperature may also promote development of filamentous bacteria, as shown recently by López-Palau et al.[31] which will eventually lead to lower biomass concentrations and less nitrogen removal.

In these simulations, the required ammonium concentration for efficient NOB repression in the case of an $R_{K_O} = 7.25$, as reported in [28], is very high (e.g. at low DO > 150 gNH$_4^+$-N/m$^3$ at 30°C, Figure 5). The results in fact suggest that the oxygen affinities of AOB and NOB in this type of installations are far from those reported by Regmi et al.[28] Efficient NOB repression was reported in many DEMON® installations (see, for instance [8]) where the residual ammonium concentration is often lower than 100 gNH$_4^+$-N/m$^3$.

In both the SBR and CSTR reactor configurations, residual ammonia concentration affects NOB repression despite the high HRT (i.e. HRT > 1/$\mu_{max, AOB}$), long SRT and an important fraction of biomass in suspension. Previous studies with preponderant AOB biofilm activity support these results. Ammonium concentration was observed to change an airlift biofilm reactor operation from full nitritation to complete nitrification.[32,33] Another model-based study [34] of a single-stage autotrophic nitrogen removal system indicated that certain residual ammonium concentrations were required for efficient NOB repression and high system sensitivity to the $R_{K_O}$. All of these means that controlling the ratio DO to ammonium could be an efficient way to repress NOB in partial nitritation-anammox systems for the side-streams of WWTPs. In mainstream applications, however, the low temperature and significantly lower ammonium concentrations would present a challenge for effective NOB repression by this strategy.

4. Conclusions

Modelling results obtained for a single-stage partial nitration-anammox SBR with granular sludge indicate that:

- Aerobic ammonium oxidation occurs mostly in suspension rather than in the granules because there is no driving force for AOB to form granular sludge due to the long settling times, the SRT of ca. 30 days and the long HRT applied.
- The presence of a small fraction of AOB in the outer layer of granules is paramount to avoid oxygen repression of anammox and maintain a high anaerobic ammonium oxidation.
- Continuous aeration may result in better nitrogen and ammonium removal. Maximum oxygen concentration during aeration periods has a major effect in process efficiency. The inhibition on anammox...

Figure 5. Minimum ammonium concentration required to repress NOB as a function of the ratio of oxygen affinities of AOB and NOB ($R_{K_O}$: 0.17, 0.27, 1, 1.38 and 7.25) at different oxygen concentrations (gO$_2$/m$^3$: 0.25 (●), 1.0 (●) and 2.0 (x)) and temperatures (a) 30°C and (b) 20°C.
conversion rate during periods of high oxygen concentration was identified as a main factor reducing nitrogen removal.

- A residual ammonium concentration is required for efficient NOB repression in systems with relatively high HRT, long SRT and an important biomass fraction in suspension.

**Disclosure statement**

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**Supplemental data**

Supplemental data for this article can be accessed at [10.1080/09593330.2015.1077895](10.1080/09593330.2015.1077895)

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