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Outcompeting nitrite-oxidizing bacteria in single-stage nitrogen removal in sewage treatment plants: A model-based study

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ABSTRACT

This model-based study investigated the mechanisms and operational window for efficient repression of nitrite oxidizing bacteria (NOB) in an autotrophic nitrogen removal process. The operation of a continuous single-stage granular sludge process was simulated for nitrogen removal from pretreated sewage at 10 °C. The effects of the residual ammonium concentration were explicitly analyzed with the model. Competition for oxygen between ammonia-oxidizing bacteria (AOB) and NOB was found to be essential for NOB repression even when the suppression of nitrite oxidation is assisted by nitrite reduction by anammox (AMX). The nitrite half-saturation coefficient of NOB and AMX proved non-sensitive for the model output. The maximum specific growth rate of AMX bacteria proved a sensitive process parameter, because higher rates would provide a competitive advantage for AMX.

1. Introduction

In the search for energy neutral (or even energy positive) wastewater treatment plant (WWTP) configurations, the use of anammox for nitrogen removal from pretreated sewage is seen as an imperative (Jetten et al., 1997; Siegrist et al., 2008; Kartal et al., 2010). The pretreatment of sewage would remove most of the organic matter, and the remaining liquid would contain mainly ammonium, that could be treated by anammox-based technologies. In particular, the single-stage nitritation-anammox biofilm (SNAB) process is considered the more convenient way of implementation, since a single reactor devoted to nitrogen removal would decrease both the investment and the operational costs (Kartal et al., 2010; De Clippeleir et al., 2013; Wett et al., 2013). Challenges associated to such a sewage treatment process are related to (i) low wastewater temperatures, (ii) low nitrogen concentration of

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the wastewater, and (iii) the high effluent quality required (Kartal et al., 2010; Winkler et al., 2011; Wett et al., 2013; Hu et al., 2013; De Clippeleir et al., 2013).

Many laboratory studies have investigated the use of a sequencing batch reactor (SBR) for implementation of the SNAB process. SBR operation allows for easy granulation and enables different operational strategies, such as aerated and non-aerated periods, or intermittent aeration or feeding. It furthermore facilitates selective granular sludge removal and easy automation and control (Winkler et al., 2011; Wett, 2007; Joss et al., 2009; Hu et al., 2013). Nevertheless, in full scale installations a continuous mode of operation would be preferred due to simpler and more economic operation and more effective use of the aeration equipment (among others).

The major challenges in selecting the desired microbial community are related to the competition between anammox (AMX) and nitrite-oxidizing bacteria (NOB) for nitrite, and between ammonia-oxidizing bacteria (AOB) and NOB for oxygen (Winkler et al., 2011). Nitrate accumulation due to nitrite oxidation by NOB has become a clear symptom of undesired reactor performance, the repression of NOB in single-stage nitritation-anammox biofilm reactors has been identified as one of the main challenges for successful implementation of the SNAB process for sewage treatment (Volcke et al., 2010; Winkler et al., 2011; Volcke et al., 2011; De Clippeleir et al., 2013; Wett et al., 2013).

Successful implementation of the SNAB process thus relies on the definition of the operational conditions that enable effective enrichment of AOB and AMX dominated biofilms and repression of the NOB population. In the present study a computational biofilm model is used to investigate the development of a microbial community consisting of ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB) and anammox (AMX) in bioreactors operated in continuous mode. The main aim is to understand how these microbial community interactions evolve at low temperatures and low nitrogen concentrations and to identify the most sensitive parameters leading to NOB repression. A second objective is to determine the domain of operating conditions in which a granular sludge based reactor operated in continuous mode achieves stable nitrogen removal, while long term NOB repression is assured.

2. Model description

2.1. *Biofilm model, kinetics and parameters*

A dynamic model was developed to simulate the granular sludge reactor performance, based on the one-dimensional multispecies biofilm model of Wanner and Reichert (1996), implemented in the software package AQUASIM v.2.1d (Reichert, 1998). The reactor volume (including the bulk liquid phase and the granular sludge) was considered constant at an arbitrary volume of 2000 m^3, with a fully-mixed liquid phase. The inflow composition was set to represent pretreated sewage, with a temperature of 10 °C, without soluble nor particulate COD and with an ammonium concentration of 70 g N/m^3.

Four particulate components were considered in the biofilm matrix: AOB, NOB, AMX and inert biomass (Table S1). Initial weight fractions of active biomass in the biofilm were assumed to amount 40% AOB, 40% NOB, and 20% AMX. The initial fractions of the biomass do not impact the results obtained with the model at steady state, which are the results presented in the graphs. The porosity of the biofilm (80%) and the total biomass concentration in the granule (90 kg VSS/m^3) were kept constant during all the simulations. The total biofilm area was defined as a function of granule size and number of granules, calculated from the total biomass concentration in the reactor (3 kg VSS/m^3) and the assumed granule diameter and density. Initial granule size was set to 180 μm in all simulations. A variable detachment rate was used to reach a constant granule size set to 1.5 mm. A single granule size was selected, without including a granule size distribution, to ease the interpretation of results. Although the standard case was investigated for a granule size of 1.5 mm, the effect of granule size was also assessed with the model, by using sizes of 1 and 2 mm in additional simulations. Detached biomass from the biofilm was active in suspension and following the same kinetics as the biomass in the biofilm, but it was assumed to be washed out with the effluent. Attachment of biomass onto the biofilm surface has been neglected.

Four soluble components were considered: oxygen (O_2), ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-). The microbial kinetics and the stoichiometry used are detailed in Tables S1–S3 in the supplementary information. Growth of AMX was inhibited by O_2. Decay of all active biomass types to inert material was included. Lower diffusivity was assumed in the biofilm (Table S4) and external mass transfer resistance has been neglected.

2.2. *Closed process control loops*

To determine the effect of ammonium and oxygen bulk liquid concentrations on the microbial interactions, two different control loops were implemented as proposed by Jemaat et al. (2013). Including the control loops in the mathematical model is very convenient, since it allows for investigating the impact of both ammonium and dissolved oxygen (DO) concentrations independently. For the mathematical description of the DO control loop, aeration was introduced as a dynamic process only active in the bulk liquid phase: $dS_{O_2}/dt = k_La((S_{O_2})_{SP} - S_{O_2})$ with S_{O_2} the dissolved oxygen concentration in the bulk liquid and oxygen solubility $(S_{O_2})_{SP}$ as the set point. A high value for the volumetric gas–liquid oxygen transfer coefficient ($k_La = 10000\ d^{-1}$) was selected.

For the ammonium concentration control loop the wastewater flow-rate (Q_{in}) was used as manipulated variable:

$$Q_{in} = Q_{in,0} + Q_{in,0}\,a\left(\frac{\left(S_{NH_4^+}\right)_{SP} - S_{NH_4^+}}{\left(S_{NH_4^+}\right)_{SP}}\right) \tag{1}$$

where $Q_{in,0}$ is the bias of the control action, i.e. the default value of flow-rate. The controller always acts either increasing or decreasing Q_{in} around $Q_{in,0}$. $S_{NH_4^+}$ is the ammonium concentration in the bulk liquid and $(S_{NH_4^+})_{SP}$ is the set point. The expression (1) is similar to that applied in a conventional proportional control law with gain a (Jemaat et al., 2013).

The effect of the ammonium concentration on the performance of the SNAB process remained systematically unclear in previous studies. Often the reactor loading was directly explored, but only a limited range of residual ammonium concentration was investigated (Hao et al., 2002a,b; Vangsgaard et al., 2012). In addition, since previous research was focused on sidestream treatment, the residual ammonium concentration was not of key importance because the effluent is usually recirculated to the inlet of the WWTP for further treatment before discharge (see typical values in Lackner et al., 2014). With the present approach, the residual ammonium concentration can be explicitly manipulated allowing for a more directed investigation of the microbial interactions and the true mechanisms leading to NOB repression. Since the results are presented for steady state, a constant NLR is achieved, meaning that the results are valid and fully equivalent to those obtained with a reactor operating at a given NLR, as far as it is rather constant.

2.3. Simulation strategy

Several DO and ammonium concentrations in the bulk liquid were explored to determine the threshold concentrations required to obtain long term NOB repression. For each fixed DO concentration value considered, an iterative procedure was used to determine the minimal ammonium concentration in the bulk liquid required to outcompete NOB in the biofilm (i.e. NOB activity repression), noted as $(S_{NH4+})_{min}$. The NOB activity repression has been considered stable when the effluent nitrate concentration –as produced by NOB– was less than 1 g N/m^3 in steady state conditions. Since nitrate is also produced by anammox, the conventional process stoichiometry was modified by labeling the nitrate produced by NOB (see Table S1 in the supplementary information).

Although it could be more precise to present the results by providing the ratio of fluxes of DO and ammonium towards the biofilm (like for instance in Lackner and Smets, 2012) we used instead bulk concentrations, to ease the interpretation of the results. Because the ammonium and oxygen diffusivities are similar, the use of bulk concentrations instead of fluxes is justified.

The specific role of AMX in the microbial population interaction was independently assessed by repeating the determination of $(S_{NH4+})_{min}$ for a granular sludge without anammox, i.e. with a conventional nitrifying granule (noted as AOB-NOB in the graphs).

In addition, to assess the effect of the AMX concentration on NOB repression, the stoichiometry of AMX has been manipulated by artificially creating a fictitious AMX strain with either only ammonium consumption or only nitrite consumption (i.e. the stoichiometric coefficient for nitrite or ammonium, respectively, is set to zero; see Table S1).

Although the standard case was investigated at 10 °C, a wider range of temperatures was also explored with the model (from 8 to 30 °C).

3. Results and discussion

3.1. Mapping the operational conditions resulting in NOB repression

Effective autotrophic nitrogen removal relies on establishing a system with AOB and AMX bacteria and absence of NOB. Practically, relevant control parameters influence the DO concentration and bulk ammonium concentration. The domain of operating conditions in which NOB repression is attained at steady state at 10 °C has been delineated in a phase diagram of bulk ammonium concentration versus bulk DO concentration (Fig. 1A). In this diagram, for a fixed DO concentration, the $(S_{NH4+})_{min}$ curve shows the minimal required concentration of ammonium in the bulk liquid to outcompete NOB. For instance, at 1 g O_2/m^3 NOB repression will be attained when the bulk ammonium concentration is ca. 8.4 g N/m^3 or higher (see Fig. 1A). For bulk ammonium concentrations lower than 8.4 g N/m^3, NOB will proliferate in the granular sludge and a fraction of the nitrite will be oxidized to nitrate.

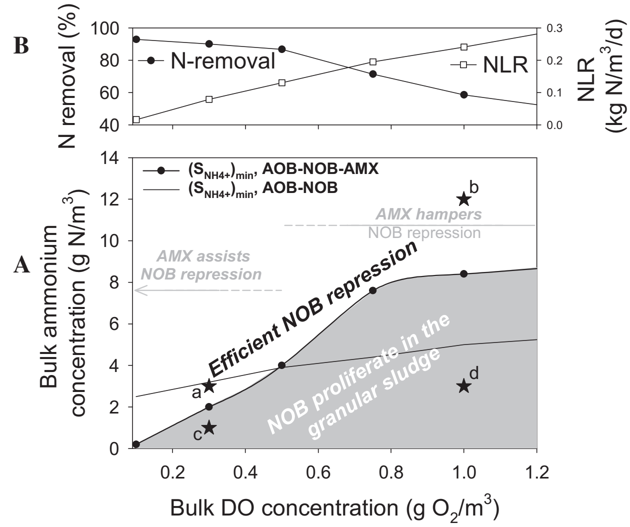

Fig. 1 – A: Phase diagram showing the domain of operating conditions achieving either non-efficient or efficient NOB repression, depending on bulk ammonium and DO concentrations imposed in the granular sludge reactor. Ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB) and anammox (AMX) are present in the granular sludge. The set of points delineating the boundary between both regions at 10 °C correspond to the minimal bulk ammonium concentrations required to repress NOB, noted as $(S_{NH4+})_{min}$. The $(S_{NH4+})_{min}$ curve is also shown for nitrifying granules (noted as AOB-NOB), to assess the effect of AMX in the NOB repression. Stars show particular operating conditions whose substrate and biomass profiles are quantified in Fig. S1. NOB repression is AMX assisted at low bulk DO concentration values. B: Nitrogen removal and the nitrogen loading rate (NLR) for the values of bulk ammonium concentration plotted in 1A (i.e. $(S_{NH4+})_{min}$).

Simulations in which the bulk ammonium concentration was above the $(S_{NH4+})_{min}$ curve led to efficient NOB repression. In these conditions nitrate is only produced by anammox, and NOB are either washed out from the granular sludge or present in very low numbers (white region in Fig. 1A). The substrate and biomass profiles in the sludge granules for two representative operating conditions of this region (marked with stars a&b in Fig. 1A) are presented in Figs. S1A&B. When the reactor is operated in such a way that ammonium concentration in the bulk is lower than $(S_{NH4+})_{min}$, NOB are able to proliferate in the granular sludge and no efficient repression of the nitrite oxidation by NOB is attained in steady state (grey highlighted region in Fig. 1A). The corresponding substrate and biomass profiles are shown in Figs. S1C&D. The major fraction of the nitrate in the effluent is produced by NOB, considerably decreasing the nitrogen removal efficiency (Fig. 1B).

The bulk DO concentration required to obtain $(S_{NH4+})_{min}$ depends mainly on the nitrogen loading rate (see Fig. 1B). Lower bulk DO concentrations are therefore linked to lower volumetric nitrogen loading rates (or larger reactor volumes), because the ammonium oxidation rate is directly correlated to the oxygen mass transfer rate. At high nitrogen loading rate, when the bulk DO concentration is rather high (ca. 1.2 g O_2/m^3), the model predicts a decrease in N removal efficiency even when NOB repression is efficient, mainly because the oxygen penetrates too deep into the granule to maintain an adequate anoxic volume fraction for anammox (see Fig. 1B).

Ammonium conversion rates of conventional activated sludge systems are ca. 0.1 kg N/m^3/d (APHA, 1998). However, for retrofitting of existing WWTPs, i.e. when using SNAB process to replace the B-stage, the current dimensions of the installation will condition the required ammonium oxidation rate. For instance, in the Dokhaven–Sluisjesdijk WWTP (Rotterdam, The Netherlands), at least ca. 0.2 kg N/m^3/d would be required (Kampschreur et al., 2008). The modeling results obtained indicate that this rate can be achieved when the DO set-point is around 0.75 g O_2/m^3 (Fig. 1).

Although with this model-based study we have focussed on the microbial interactions among AOB, NOB and AMX, which are the main players in the granular sludge, heterotrophic biomass will as well compete for oxygen with AOB and for nitrite with AMX when oxygen is not available. The extent of such competitions will be linked to the efficiency of the COD removal prior to the SNAB process. Further research would be desirable to clarify the impacts of the presence of biodegradable COD on NOB repression, since potential challenges may arise, as pointed out in the literature for membrane aerated biofilms (Lackner et al., 2008).

From the simulations it is clear that at decreasing DO concentration, the ammonium concentration required to repress NOB activity is decreased as well. The exact values depend on the actual model parameters, but this is still in line with previous model based studies (Hao et al., 2002a). However, several questions remain: why is NOB activity repressed by increasing ammonium concentration, while ammonium is neither their energy substrate nor their product? To what extent does AMX activity contribute to NOB repression by lowering the nitrite concentration? These questions are investigated in the following sections, and when required, specific simulations were run to test the hypotheses proposed.

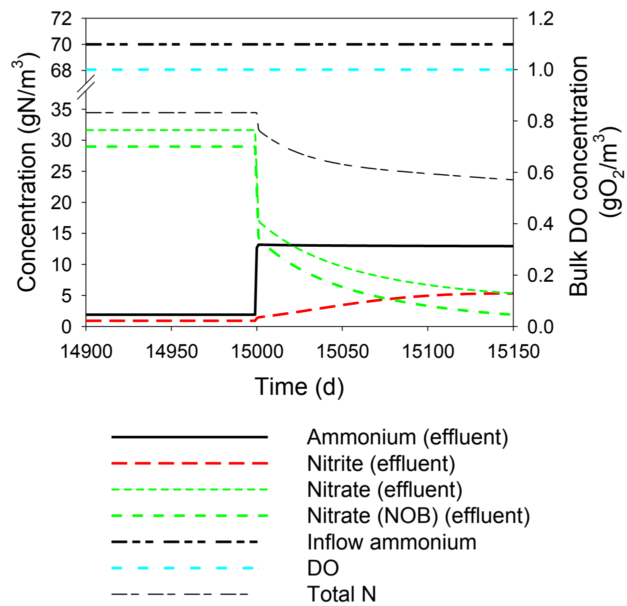

Fig. 2 – Time course impact of a step-up in the bulk ammonium concentration from ca. 2 to 13 gN–NH_4^+/L at 10 °C, meaning a switch from non-efficient to efficient NOB repression. The nitrate produced by NOB has been labeled in the model, and presented here as a different plot (nitrate (NOB)), whereas the nitrate plot accounts for the total nitrate.

3.2. *Repression of NOB due to oxygen limitation: importance of the bulk ammonium concentration*

In a biofilm reactor, ammonium oxidation to nitrate (non-limited by oxygen) requires roughly 4 g O_2 per g N–NH_4^+ in the bulk liquid (Harremoes, 1982; Bartrolí et al., 2010). At a DO concentration of 1 g O_2/m^3 and 12 g N/m^3 ammonium concentration in the reactor liquid (meaning 0.08 g O_2 per g N–NH_4^+ in the bulk liquid), NOB are outcompeted by AOB due to the strong oxygen limiting conditions imposed (see profiles of biomass and substrate in the biofilm in Fig. S1A). In these conditions the affinity constants for oxygen and ammonium of AOB allow for more rapid growth of AOB compared to NOB in the aerobic zone of the granule. At the same DO concentration (1 g O_2/m^3) but operating at a lower ammonium concentration in the bulk liquid, e.g. 2 g N/m^3, the situation in the sludge granule markedly changes (see the biomass and substrate profiles in the biofilm in Fig. S1B). Oxygen is still the limiting compound for ammonium oxidation, since for 2 g N/m^3 at least ca. 8 g O_2/m^3 are required to avoid oxygen limitation. However, the lower ammonium concentrations are not selective enough to outcompete NOB. Consequently, a fraction of the nitrite produced by AOB is oxidized by NOB, instead of being consumed by AMX to produce N_2. As a result, NOB proliferate in the biofilm and a relatively high nitrate concentration is present in the effluent.

To further investigate the effect of ammonium concentration on NOB repression, the system dynamics when switching from non-efficient to efficient NOB repression were explored. A DO concentration in the bulk liquid of 1.0 mg O_2/L has been fixed as an example. The ammonium concentration was increased from ca. 2 to 13 gN$-NH_4^+$/L in the bulk liquid (Fig. 2). These two sets of operating conditions have been highlighted as stars d&b, respectively, in Fig. 1A. The profiles of substrate and biomass in steady state are available in Fig. S1D&B, respectively. The reactor operating at the conditions of point d was at steady state, when the bulk ammonium concentration was stepped up (Fig. 2). Notably, during the first day after the sudden increase in bulk ammonium concentration, a strong decrease in the nitrate concentration produced by NOB was predicted by the model. Later, nitrate production by NOB decreased to almost zero during a rather long transient state (ca. 150 days). Due to the label used in the model on the nitrate produced by NOB, these effects can be easily highlighted in the figure.

In the present study for single-stage nitritation-anammox reactors at low temperatures, the conventional description of the nitritation rate depends on the product of two Monod-saturation terms, one for oxygen and one for ammonium (Table S2, process 1). When comparing two operating conditions in which the bulk DO concentration is the same, but bulk ammonium concentration is different (Fig. 2), the main difference in nitritation rate is therefore linked to the ammonium saturation term. In fact, for an ammonium half-saturation coefficient of ca. 1 gN/m^3, the ammonium saturation term will importantly change if the bulk ammonium concentration is increasing from 2 to 12 gN/m^3. Particularly at low temperatures, when NOB have a higher maximum growth rate than AOB (Hunik, 1993), the possibility to improve the nitritation rate becomes important for NOB repression, as supported by the AOB and NOB growth rate profiles in the granular sludge (Fig. S2).

The repression of nitratation achieved at 12 g N$-NH_4^+$/m^3 is the reason behind the steep decrease of the nitrate concentration during the first hours after applying the disturbance (Fig. 2). The strong oxygen limitation for the biofilm system is effectively inhibiting NOB activity. The second (slower) decrease is related to NOB washout from the biofilm, which is particularly slow at 10 °C since decay rates of the well-developed NOB population are very slow.

The influence of the bulk ammonium concentration on NOB repression when operating at the same bulk DO concentration has been observed for nitrifying granular sludge reactors treating rich ammonium wastewater (~1 g$NH_4^+$$-$N/L) at high and ambient temperatures (Bartrolí et al., 2010; Jemaat et al., 2013) and supported by mathematical modeling of nitrifying biofilm reactors (Pérez et al., 2009; Brockmann and Morgenroth, 2010; Jemaat et al., 2013). For a given bulk DO concentration, when the reactor was operated at a higher bulk ammonium concentration, the nitritation rate increased. Given the oxygen limiting conditions imposed and the better oxygen affinity of AOB, the extra nitritation rate achieved at a higher ammonium concentration is leading to less oxygen available for nitrite oxidation by NOB. The potential effect of residual ammonium has been also recently reported as a possible strategy for NOB repression in activated sludge reactors for sewage treatment (Regmi et al., 2014).

Incorporation of external mass transfer resistance in the calculation would decrease the ammonium concentration required to repress NOB at a given bulk DO concentration, but at the same time, the NLR applied would also decrease because of a reduction of the oxygen flux towards the biofilm. This would agree with the low NLR (ca. 0.01 kg N/m^3/d) recently reported for an SNAB process with efficient NOB repression at low temperatures (10–13 °C) in a moving bed biofilm reactor (Gilbert et al., 2014). The required operational conditions were 6–8 gN$-NH_4^+$/m^3 in the bulk liquid and bulk DO concentrations in the range 0.1–0.3 g O_2/m^3.

Controlling the ammonium concentration with a closed loop would be an efficient way to avoid proliferation of NOB during the start-up of this type of reactors, or when temperature starts to decrease at the end of the summer. Ammonium accumulation could be a good strategy for reactor starting-up, since NOB repression would be efficiently maintained during the granule development, even if anammox proliferation is still incipient, or under inhibitory conditions, like high nitrite or oxygen concentrations.

Nevertheless, relying on ammonium accumulation to maintain NOB repression in SNAB reactors for sewage treatment evidently worsens the effluent quality. However, SBR or plug-flow operation, with high ammonium concentrations during a large fraction of the operational cycle or reactor length, respectively, would be beneficial, without compromising the effluent quality. A very simple approach consisting of two reactors in series, to roughly mimic the hydrodynamics of a plug-flow could be used to illustrate this idea. The first tank (with a higher volume) would operate at rather high DO and a second tank (with a reduced volume) operating at rather low DO. In this way, we have tested with the model how effluent quality is not compromised whereas a rather high nitrogen loading rate is achieved (for details of a sketched process diagram and simulation results see Fig. S3).

3.3. *The role of AMX in the microbial interactions: stimulating or hampering NOB repression?*

Two different effects could be distinguished associated to the AMX activity in the biofilm. First, the occurrence of a flux of ammonium towards the granule core due to anammox activity tends to decrease the ammonium concentrations in the outer layer of the biofilm. In case of lower ammonium concentrations in the biofilm, the nitritation rate will decrease, and therefore this effect is hampering the NOB repression (i.e. it enhances the NOB activity). The effect is very similar to a decrease in the bulk oxygen concentration, as previously discussed (Fig. S1). Second, the flux of nitrite towards the granule core will decrease the nitrite concentrations in the outer layer of the biofilm, thus helping to repress NOB activity by decreasing the nitratation rate.

The decrease in the ammonium concentration in the biofilm (the first effect) is impacting directly the competition for oxygen between AOB and NOB. Since there will be less ammonium excess in the biofilm, NOB repression will be more difficult. To demonstrate this effect, $(S_{NH4+})_{min}$ have been computed at steady state for a fictitious AMX strain consuming only ammonium and not nitrite (Fig. 3). The obtained steady state values for $(S_{NH4+})_{min}$ are higher than those computed with the regular stoichiometry, showing that indeed the lowered ammonium flux is hampering NOB repression.

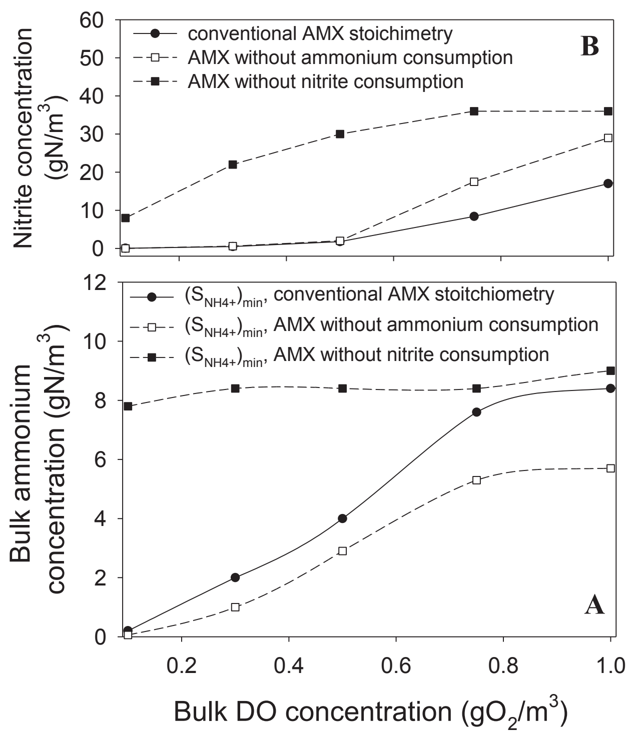

Fig. 3 – Assessment of the effects of AMX activity on NOB repression at 10 °C. The stoichiometry of AMX has been modified to (i) only nitrite consumption, and (ii) only ammonium consumption. A: Minimal bulk ammonium concentrations required for efficient NOB repression ($(S_{NH4+})_{min}$). Note how a lower value of $(S_{NH4+})_{min}$ means that NOB repression requires of less ammonium accumulation in the bulk liquid phase. B: Bulk nitrite concentration for the conditions of each one of the $(S_{NH4+})_{min}$ curves.

The decrease in the nitrite concentration in the biofilm (the second discussed effect) impacts directly the competition for nitrite between NOB and AMX. Since the nitrite availability in the biofilm will be lower, the NOB activity will be decreased, thus helping the NOB repression. To demonstrate this factor, $(S_{NH4+})_{min}$ has been computed for a fictitious AMX strain consuming only nitrite and not ammonium (Fig. 3). The obtained $(S_{NH4+})_{min}$ were lower than those computed with the regular stoichiometry, demonstrating that the lowered nitrite concentration can assist NOB repression. Effluent nitrite concentrations (Fig. 3B) allow for a direct assessment of the required nitrite concentration to assist NOB repression by nitrite unavailability.

Comparing the two new curves for the hypothetical stoichiometries with the curve of $(S_{NH4+})_{min}$ found for the correct AMX stoichiometry (Fig. 3), it is clear that the two effects are opposite, and consequently, there is a (partial) trade-off between both in the regular operation of single stage granular sludge reactors for N-removal. Because the AMX activity may have two opposing effects on the NOB population, it is not straightforward to evaluate in a given system if NOB are repressed mainly by oxygen limitation (outcompeted by AOB) or by nitrite limitation (outcompeted by AMX). By comparing the $(S_{NH4+})_{min}$ values for simulations with or without AMX bacteria present, it is possible to assess if AMX influenced NOB repression (Fig. 1A). Since both $(S_{NH4+})_{min}$ curves are crossing at ca. 0.5 g O_2/m^3, this is defining the boundary between the two sets of operating conditions allowing for efficient NOB repression: (i) on the left hand side of the graph, for low bulk DO concentrations AMX is assisting NOB repression, and (ii) AMX hampers NOB repression, towards the right hand side of the graph, as highlighted in Fig. 1A. At a bulk DO concentration below 0.5 g O_2/m^3, NOB repression is AMX assisted. At very low DO concentrations (effectively microaerophilic conditions), the excess of ammonium is no longer required (see Fig. 1A), because NOB activity is hindered by nitrite limitation. This operating condition becomes interesting for a stable operation assuring NOB repression and low ammonium and nitrite concentrations in the effluent. However, model predictions in the conditions tested show how this region is only attainable at very low DO concentration, and therefore only low NLR (e.g. 0.017 kg N/m^3/d for a DO of 0.1 g O_2/m^3 at 10 °C, see Fig. 1B) can be applied.

Although the reduced nitrite concentrations in the biofilm due to AMX activity can assist NOB repression, a minimal bulk ammonium concentration is required, because the NOB repression is still driven by oxygen limitation. The decrease in the nitratation rate due to low nitrite concentrations leads to more oxygen available for ammonium oxidation by AOB which will dominate the external layer of the granular sludge, repressing NOB activity.

3.4. *Factors and parameter sensitivity for NOB repression*

Among the different parameters used to model the SNAB process, stoichiometry and growth rates are relatively well established (Weismann, 1994; Strous et al., 1998). Additionally, small differences (20–50%) in the values of stoichiometry parameters and growth rates did not significantly impact the competition between AOB and NOB (Hao et al., 2002a). The values of the affinity constants are known with a much lower accuracy. Often a wide range of values is reported for AOB, NOB and AMX (Brockmann et al., 2008). Rather than the impact of the absolute values individually, the ratio between affinity constants is known to govern the SNAB process performance (Hao et al., 2002a). Therefore the impact of $K_{O2,NOB}/K_{O2,AOB}$ and $K_{S,NO2,NOB}/K_{S,NO2,AMx}$ ratios on the range of operating conditions to outcompete NOB (i.e. $(S_{NH4+})_{min}$ curve) has been assessed (Fig. 4). An overview of the particular values used in the simulations is presented in Table 1.

The competition for oxygen between AOB and NOB was found to be the most influential factor, as small variations of the $K_{O2,NOB}/K_{O2,AOB}$ ratio yielded strong variations of the $(S_{NH4+})_{min}$ curve. For equal specific affinity ($\mu_{max,AOB}/K_{O2,AOB} = \mu_{max,NOB}/K_{O2,NOB}$; $K_{O2,NOB}/K_{O2,AOB} = 1.4$; $K_{O2,NOB} = 0.3$ g O_2/m^3) it is still possible to repress NOB in the long term just by using higher bulk ammonium concentrations (Fig. 4A). However, for lower values of the $K_{O2,NOB}/K_{O2,AOB}$ ratio (i.e. for $K_{O2,NOB} < 0.3$) the curve becomes asymptotic (see an example in Fig. 4A), and NOB can develop in the granular sludge even at high bulk ammonium

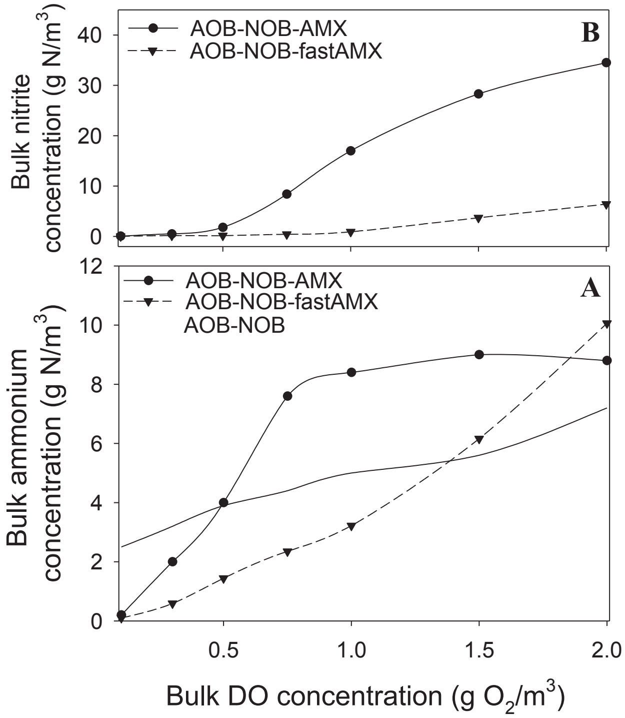

Fig. 4 – A: Minimal bulk ammonium concentrations required for efficient NOB repression ($(S_{NH4+})_{min}$) for: (i) standard case with conventional kinetics (AOB-NOB-AMX) and (ii) a fast-growing anammox population (AOB-NOB-fastAMX), and (iii) nitrifying granular sludge (AOB-NOB). B: Bulk nitrite concentration for the conditions of each one of the $(S_{NH4+})_{min}$ curves.

concentration for DO > 0.3 g O_2/m^3. Interestingly, at low DO concentration NOB repression is still possible because it is AMX assisted.

There are recent reports showing that the oxygen half-saturation coefficient of NOB could be lower than that of AOB, and because of a lower maximum specific growth rate of NOB at low temperatures, it is better to operate at relatively high DO (e.g. 1.5 g O_2/m^3) (Wett et al., 2013). To try to quantitatively assess this potential situation, we have tested the model considering $K_{O2,AOB} > K_{O2,NOB} = 0.16$ (Manser et al., 2005; Wett et al., 2013) and $\mu_{max,AOB} > \mu_{max,NOB} = 0.16\ d^{-1}$, to maintain equivalent oxygen specific affinity to that in the standard case, i.e. $\mu_{max,NOB}/K_{O_2,NOB} = 0.16/0.16 m^3/gO_2/d = (\mu_{max,NOB}/K_{O_2,NOB})_{standard\ case}$. The $(S_{NH4+})_{min}$ curve found with these parameters was similar to that obtained for the standard case (Fig. S4). Therefore it is clear that oxygen affinity drives NOB repression, as also reported for nitritation in biofilm reactors (Pérez et al., 2009).

The impact of the ammonium inflow on NOB repression was also assessed with the model. When a lower inflow ammonium concentration is used (i.e. 40 g N/m^3), only slight differences in the $(S_{NH4+})_{min}$ curve were obtained (Fig. S5). Lower residual nitrite concentrations indicate that AMX assists NOB repression at slightly higher DO values.

The impact of the $K_{S,NO2,NOB}/K_{S,NO2,AMX}$ ratio on NOB repression was found to be very limited even for large variations of the ratio (Fig. S6 & 7). The strong decrease of $(S_{NH4+})_{min}$ in all figures when DO decreases (Figs. 1, 3–5) is because the excess of ammonium required is linked to the particular DO concentration imposed. The ratio DO/ammonium is governing the extent of the DO limitation in nitrifying biofilms (Harremöes, 1982; Çeçen and Gönenç, 1995: Bernet et al., 2005; Bougard et al., 2006; Bartroli et al., 2010; Brockmann and Morgenroth, 2010), and this effect is very relevant for bulk DO values lower than 1 mg O_2/m^3, simply because the stoichiometry of the process and because of similar diffusion coefficients of ammonium and oxygen (Harremöes, 1982; Bartroli et al., 2010; Brockmann and Morgenroth, 2010). To assess the effects of the $K_{S,NO2,NOB}/K_{S,NO2,AMX}$ ratio on NOB repression, it is better to use a slightly different graph, in which the Y axis is the ratio DO/ammonium (noted as R_{max}) (Fig. 6). For the set of curves in Fig. 6, it is now evident what the effect of the $K_{S,NO2,NOB}/K_{S,NO2,AMX}$ ratio is, since there are clear variations in the R_{max} curves. In terms of concentrations (Fig. S2), the decrease of the bulk ammonium concentration required to outcompete NOB ($(S_{NH4+})_{min}$) is linked to the stoichiometry required to keep a similar excess of ammonium, i.e. a similar DO/

Table 1 – Values of affinity constants used in the different simulations. Fig. 1 contains the standard case. When a parameter value remains unchanged with respect to the standard case, the symbol '–' is used. In all cases the temperature is 10 °C.* $\mu_{max,NOB} = 0.16\ d^{-1}$ to maintain equivalent oxygen specific affinity to that in the standard case.

Fig.	$K_{O2,AOB}$	$K_{O2,NOB}$	$K_{S,NH4,AOB}$	$K_{S,NO2,NOB}$	$K_{S,NO2,AMX}$	$K_{O2,NOB}/K_{O2,AOB}$	$K_{S,NO2,NOB}/K_{S,NO2,AMX}$
1	0.2	0.4	1.1	0.5	0.005	2	100
5A	–	0.6	–	–	–	3	–
5A	–	0.3	–	–	–	1.4	–
5A	–	0.1	–	–	–	0.05	–
5B	–	–	0.1	–	–	–	–
5B	–	–	1.1	–	–	–	–
5B	–	–	5.0	–	–	–	–
S4	–	0.16*	–	–	–	0.8	–
S6	–	–	–	0.001	–	–	0.2
S6	–	–	–	0.1	–	–	20
S6	–	–	–	1.5	–	–	300
S7	–	–	–	–	1	–	0.5

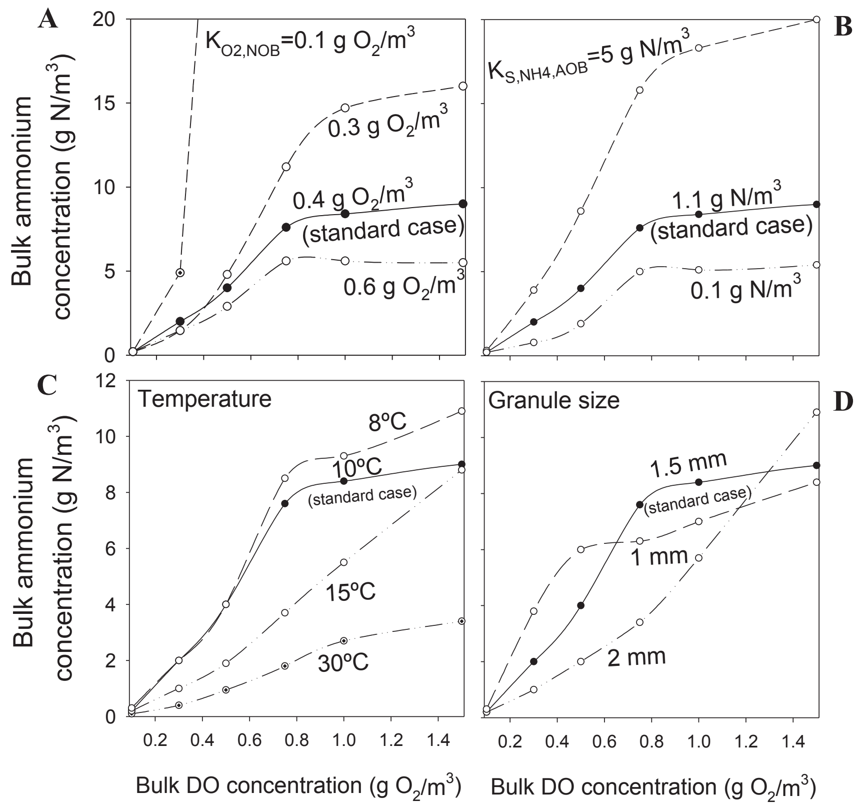

Fig. 5 – Main parameters affecting NOB repression at 10 °C. A: effect of NOB affinity for oxygen ($K_{O2,NOB}$). B: effect of AOB affinity for ammonium ($K_{S,NH4,AOB}$). C: Effect of temperature. D: Effect of granule size.

ammonium ratio. The impact of these concentrations in case of a better AMX affinity for nitrite or a worse NOB affinity for nitrite is rather negligible (Fig. S6&7). The affinity of NOB or AMX for nitrite is therefore not very important. At low DO concentrations, NOB repression is effective even if the affinity for nitrite would be very high.

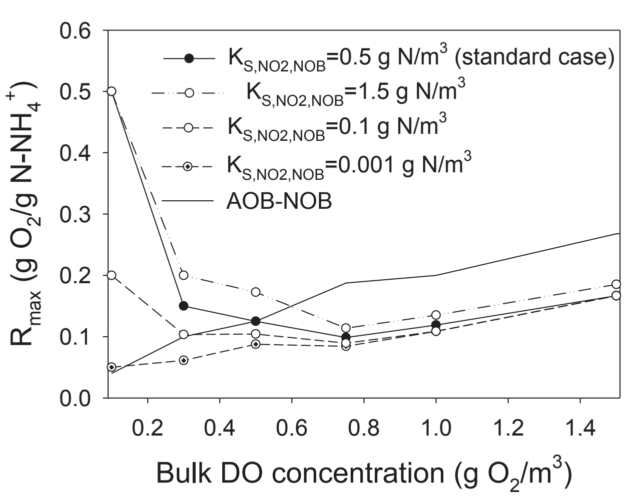

Fig. 6 – Minimal bulk ammonium concentrations required for efficient NOB repression expressed as a ratio of DO/ammonium concentrations (*R_{max}*) at different values of the nitrite affinity constant of NOB ($K_{S,NO2,NOB}$). The values for a nitrifying granular sludge (AOB-NOB) have been plotted for a direct comparison. In all cases the temperature is 10 °C.

The impact of $K_{S,NH4,AOB}$ was also assessed (Fig. 4B). A higher affinity for ammonium yields advantage for AOB to outcompete NOB. These variations in $K_{S,NH4,AOB}$ produce an important variation in the bulk ammonium concentration required to outcompete NOB at DO concentrations higher than 0.5 g O_2/m^3.

In the same way, the effects of temperature and granule size have been assessed to determine their impact on the range of operating conditions required for efficient NOB repression (Fig. 5C and D). Previous research has indicated that at lower temperatures the optimal DO concentration for maximum nitrogen removal considerably decreases (Hao et al., 2002b). In the present study, temperature was also found to strongly affect the $(S_{NH4+})_{min}$ curve (Fig. 5C). At 30 °C the required bulk ammonium concentration to repress NOB is rather low (well below 3 g N/m^3), because AOB have in addition to a higher specific oxygen affinity a higher maximum specific growth rate at 30 °C, because of different temperature dependency. SNAB process applied to sidestream, at warm temperatures, usually operates at elevated residual ammonium concentration (typically 50–100 g N/m^3, Lackner et al., 2014). Since the effluent is recirculated to the inlet of the WWTP for further treatment before discharge (Lackner et al., 2014) there is no emphasis on low effluent ammonium values. However, when the SNAB process is intended for mainstream treatment, the residual ammonium concentration becomes important in relation to NOB repression unless the DO applied is in the microaerophilic range (Fig. 5C).

Granule size was found to have also an influence on NOB repression at low temperatures (Fig. 5D). Overall, the region corresponding to efficient NOB repression is wider for larger granule sizes (Fig. 5D). Therefore, in view of the simulation results at low temperatures, larger granules would also be preferred for efficient NOB repression. The results agree with previous research demonstrating how granule size impacts the SNAB process performance at warm (25–35 °C; Vlaeminck et al., 2010) and moderate temperatures (17–22 °C; Winkler et al., 2011) and correctly described through modeling (Volcke et al., 2010, 2011). However, the $(S_{NH4+})_{min}$ curves are crossing, and therefore, the assumption of the "bigger the better" would not hold for the complete range of DO values tested (Fig. 5D). The DO level at which AMX is assisting NOB repression is decreasing for smaller granule sizes. At lower granule sizes the anoxic volume is reduced, lowering the amount of AMX in the granular sludge. Trying to keep rather high granule size in the reactors might be one of the challenges for efficient NOB repression, as already supported by previous research (Winkler et al., 2011).

3.5. *Faster anammox strains enhance NOB repression by nitrite unavailability*

Typical anammox doubling times in anammox reactors are 15–30 days at mesophilic temperatures (Fux et al., 2004; Strous et al., 1998). Nevertheless, lower doubling times have been also reported, with values as low as 3.3 days in a membrane bioreactor at 30 °C (Lotti et al., 2014). Low doubling times were also reported for enriched AMX cultures (1.8 days at 37 °C, Isaka et al., 2006 and 1.2 days at 35 °C by Bae et al., 2010). Conditions leading to NOB repression were evaluated with the model for a fast-growing anammox population by taking the doubling time of 2 days at 37 °C and calculating a maximum growth rate of 0.03 day^{-1} at 10 °C when using an activation energy of 70 kJ/mol/K (Strous et al., 1999). Results are presented in Fig. 4. The range of reactor operating conditions leading to efficient NOB repression due to competition for nitrite, in which NOB repression is assisted by AMX activity is much wider. The results show that stable NOB repression would provide better nitrogen removal efficiencies at higher nitrogen loading rates if such a fast growing anammox can be used.

By plotting the nitrite concentration in the effluent (Fig. 5A) the domain of operating conditions where NOB repression is assisted by AMX activity can also be associated to effluent nitrite concentration. The influence of bulk nitrite concentration is strongly related to the balance between AOB and AMX activities and the corresponding biofilm structure (Vlaeminck et al., 2010). Note how for nitrite concentrations higher than ca. 8 g N/m^3 achieving NOB repression is uniquely due to oxygen limitation, despite AMX activity negatively contributing to the NOB repression. Conversely, for a bulk nitrite concentration lower than ca. 4 g N/m^3 NOB repression is AMX assisted.

4. Conclusions

The modeling results obtained for single-stage nitritation-anammox granular sludge reactors operating in continuous mode in the conditions tested indicate that:

- At low temperatures, efficient NOB repression can be obtained if bulk ammonium concentrations are above certain level (denoted as $(S_{NH4+})_{min}$).
- Operating the reactor at a bulk ammonium concentration higher than $(S_{NH4+})_{min}$ increases the nitritation rate, which leads to less oxygen available for nitrite oxidation, triggering NOB repression.
- If the bulk DO concentration is not low enough, AMX activity is hampering NOB repression, because it decreases the nitritation rate.
- NOB repression is AMX assisted due to a decrease in the nitratation rate, but still an adequate excess of ammonium is required.
- In the whole range of DO tested, NOB are effectively outcompeted by AOB, and never directly outcompeted by AMX through nitrite unavailability.
- The region of operating conditions in which NOB repression is AMX assisted would be wider if the AMX specific growth rate is faster than currently general assumed.

As an output of the sensitivity analysis, the value of the $K_{O2,NOB}/K_{O2,AOB}$ ratio was found to be the most critical factor, therefore it requires more experimental investigation. Further verification of the maximum specific growth rates of AMX, AOB and NOB at low temperatures would also be desirable.

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