Modelling and simulation revealing mechanisms likely responsible for achieving the nitrite pathway through aeration control

A. Guisasola, M. Marcelino, R. Lemaire, J. A. Baeza and Z. Yuan

ABSTRACT

Nitrogen removal via nitrite has recently gained a lot of interest because it results in significant savings in both aeration costs and COD (chemical oxygen demand) requirements for denitrification, when compared to the conventional biological nitrogen removal via nitrate. The effectiveness of two different control strategies to achieve the nitrite pathway in systems with sludge retention has been experimentally demonstrated: (i) control of aerobic phase length, with which aeration is terminated as soon as ammonia is completely oxidised; (ii) operation at low DO setpoints in the aerobic phase. These strategies have been extensively studied in nitrifying reactors and are currently applied in real systems achieving biological carbon, nitrogen and phosphorus removal. In this work, we aim to demonstrate, through modelling and simulation, that the competition between nitrite reducers and nitrite oxidisers for nitrite, rather than kinetic selection plays a major role in NOB washout. Moreover, the results show that the occurrence of simultaneous nitrification and denitrification under "aerobic" conditions is very helpful for the nitrite pathway obtainment and for a more efficient COD utilisation.

Key words | aeration, COD/N, control, modelling, nitrite pathway, NOB

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INTRODUCTION

Nitrogen removal via the nitrite pathway involves partial oxidation of ammonia to nitrite and denitrification from nitrite using organic matter as electron donors. It has a number of advantages versus conventional biological nitrogen removal via nitrate (i.e. nitrification of ammonium to nitrate followed by denitrification) including savings in both aeration costs and COD requirements for denitrification. The first step of the nitrite pathway is the achievement of partial nitrification, i.e. the partial oxidation of ammonia to nitrite. The control strategies for achieving partial nitrification ultimately aim at suppressing nitrite oxidising bacteria (NOB) by modifying the apparent growth rates of ammonia oxidising bacteria (AOB) and NOB so that $\mu_{NOB} < 1/SRT < \mu_{AOB}$. For example, in the well-known SHARON process (Hellinga *et al.* 1998), this is achieved through reducing SRT to very low levels (<2 days), taking advantage of the fact that $\mu_{AOB}^{MAX} > \mu_{NOB}^{MAX}$ at 35°C.

Two different strategies have been experimentally demonstrated to achieve the nitrite pathway in systems with sludge retention:

(i) control of *aerobic phase length* with which aeration is terminated as soon as ammonia is completely oxidised (Peng *et al.* 2004; Fux *et al.* 2006; Blackburne *et al.* 2008a; Lemaire *et al.* 2008). The ammonia depletion point can be easily determined using DO or pH signals. This strategy eliminates the fraction of the aerobic phase where ammonia depletes but oxygen and nitrite coexist and thus, NOB growth is significantly disadvantaged.

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(ii) operation at *low DO levels* (Munch *et al.* 1996; Ruiz *et al.* 2003). As AOB and NOB are aerobic autotrophs, their growth rate is affected by oxygen limitation. Many researchers have experimentally demonstrated that NOB have lower oxygen affinity in comparison to AOB (Guisasola *et al.* 2005; Blackburne *et al.* 2008*b*). Consequently, an optimum range of DO can be found for a certain SRT where AOB can grow but NOB growth is not sustained. For example, Ma *et al.* (2009) achieved nitrogen removal via nitrite in a 300 L continuous pilot plant with a low DO set-point (around 0.5 mg/L); ammonia was nearly completely converted to nitrite with nitrate being almost non-detectable.

These strategies have been mainly applied in nitrifying reactors for the achievement of partial nitrification or for the treatment of wastewaters containing low COD:N ratios (e.g. anaerobic digester supernatant, landfill leachate), whereas denitrification was achieved in a post anoxic period with the dosage of external carbon sources (Hellinga *et al.* 1998; Peng *et al.* 2004; Mace *et al.* 2006). Recently, these strategies have been proven to be effective in systems for simultaneous biological C/N/P removal (Lemaire *et al.* 2008), however the exact mechanisms involved are still not clearly understood. In this work, we aim to demonstrate, through modelling and simulation, that, in these systems, the competition of heterotrophic biomass with NOB for nitrite, rather than kinetic selection, plays the key role in the NOB suppression. In addition to revealing the mechanisms, we also aim to study the impact of various factors on the establishment of the nitrite pathway, and provide guidelines for the application of the above two strategies.

MATERIALS AND METHODS

The reactor simulated was an SBR used in the experimental work by Lemaire *et al.* (2008), which aimed at treating abattoir wastewater using a single sludge SBR. In short, it was a lab-scale SBR with a working volume of 7 L, operating with a cycle time of 6 h. In each cycle, 1 L of wastewater was pumped into the reactor over the three filling periods with a volume distribution of 0.5 L, 0.3 L and 0.2 L respectively. Each filling period was followed by non-aerated (either anoxic or anaerobic depending on when the oxidised nitrogen was completely consumed) and aerated periods. After the settling period, 1 L supernatant was removed from the reactor resulting in a HRT of 42 h. 115 mL of mixed liquor was wasted every cycle to keep a constant SRT of 15 days. Table 1 summarises the cycle configuration.

The biological part of the model was an extension of the well known ASM2d (Henze *et al.* 1999). The autotrophic fraction of ASM2d was divided into AOB and NOB for a proper description of partial nitrification. Hence, nitrite, AOB and NOB were added as new state variables. Moreover, denitrification was modelled as nitrate reduction and nitrite reduction. Details of the model can be found in Lemaire *et al.* (2008). The settling phase was modelled as a reactive settler for a better description of the nutrient profiles. The wastewater composition simulated that of an abattoir wastewater used in Lemaire *et al.* (2008), with different COD and N fractions according to its biodegradability. In total the influent contained 1,350 mg COD/L, 160 mg N-NH_4^+ and 40 mg P/L, resulting in COD/N and COD/P ratios of 8 and 34, respectively (Lemaire *et al.* 2008). This ratio was changed in some of the simulations by

Table 1 | SBR cycle configuration

Phase	Length (min)	Phase	Length (min)
(1) First feeding (0.5 L)	5	(7) Third feeding (0.2 L)	2
(2) Anaerobic	30	(8) Anoxic/anaerobic	55
(3) Aerobic	50	(9) Aerobic	20
(4) Second feeding (0.3 L)	3	(10) Waste	2
(5) Anoxic/anaerobic	65	(11) Settling	90
(6) Aerobic	30	(12) Extraction	8

a proportional reduction/increase of all the COD fractions of the influent.

The model was developed in MATLAB® platform. In each simulation, the model was first run for 100 days without any control strategy to reach steady state. Then, the appropriate control strategy was applied for 300 days (1,200 cycles).

Three different sets of simulations were run:

a. applying aerobic phase length control: the hydraulic model was modified so that the aerobic phase finished as soon as ammonium depleted. The DO setpoint under aerobic conditions was set to 4 mg DO/L to avoid any effect of oxygen limitation.
b. operating at low DO concentrations: the length of the three aerobic phases was kept as described in Table 1 and the aerobic DO setpoint was modified, as will be detailed below.
c. combining the two strategies.

RESULTS AND DISCUSSION

Two different parameters were studied in each of the simulations:

1. Time to reach the nitrite pathway (Time for NP). This was defined as the time necessary to obtain $NO_2/(NO_2 + NO_3) \geq 95\%$ at the end of the first aerobic phase in a cycle. The first cycle with an $NO_2/(NO_2 + NO_3)$ ratio higher than 0.95 is considered as the time for NP.
2. Percentage of simultaneous nitrification and denitrification (%SND). The utilisation of low DO values under aerobic conditions enables denitrification using either nitrate or nitrite. The parameter %SND was defined as the amount of nitrogen gas formed in the first aerobic phase with respect to the initial ammonium. According to this definition, ammonium assimilation for bacterial growth was neglected because it represented around 1% of the total ammonium consumption. This value was calculated in the cycle where nitrite pathway is reached.

Figure 1 shows an example of the evolution of the nitrogenous compounds in a typical cycle for a better understanding of these two parameters. In this case, the ratio $NO_2/(NO_2 + NO_3)$ was 0.81 and the % SND was 27%.

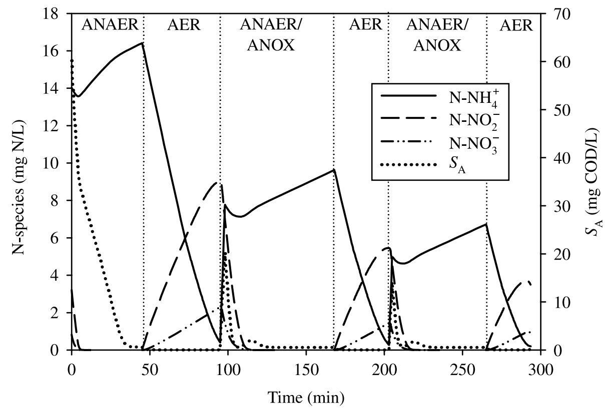

Figure 1 | Ammonium, nitrite, nitrate and RBCOD (S_A) profiles in a typical SBR cycle.

It should be noted that the obtained values of these parameters (Time for NP or %SND) are obviously specific to the simulated reactor and cannot be generalised. However, the objective of this paper is not to optimise the operation of our particular reactor but to use the simulation results for a better understanding of the underlying mechanisms responsible for the nitrite pathway achievement and to obtain guidelines for its application.

Control of the aerobic phase length

Figure 2 summarize the results obtained with the set of simulations applying the aerobic phase length control strategy. Figure 2(a) shows the Time for NP and the %SND at different influent COD/N ratios. By changing the COD/N ratio, the potential effect of this ratio on the achievement of the nitrite pathway using the aeration phase length control was studied. Figure 2(a) shows that the %SND was negligible (lower than 5%) at the DO setpoint used (4 mg DO/L). For COD/N ratios lower than 5.5, an $NO_2/(NO_2 + NO_3)$ ratio greater than 95% could not be achieved. The amount of nitrite/nitrate aerobically produced could not be completely reduced under anoxic conditions. Then, nitrate accumulates because nitrite was again oxidized to nitrate under the subsequent aerobic phase. This was the worst scenario possible for NP achievement because NOB were even favoured as nitrite was entering to the aerobic phase. When the COD/N ratio was increased above 5.5, denitrifying biomass could reduce all the aerobically produced nitrite and nitrate.

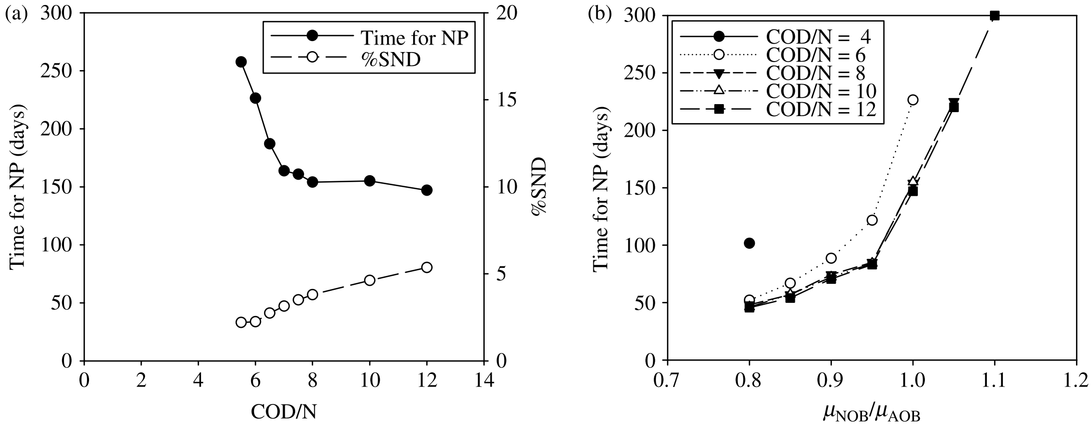

Figure 2 | Summary of the results applying the control of the aerobic phase length strategy: (a) at different COD/N values for $\mu NOB_{MAX} = \mu AOB_{MAX} = 1\,d^{-1}$, (b) at different COD/N and $\mu NOB_{MAX}/\mu AOB_{MAX}$ ratios.

For a COD/N ratio between 5 and 8, the higher the COD/N ratio, the easier (i.e. the shorter) to reach NP. Finally, COD/N ratios higher than 8 did not further reduce the Time for NP.

Figure 2(b) shows the dependency of the Time for NP on the ratio of $\mu NOB_{MAX}/\mu AOB_{MAX}$ at different COD/N values. The ASM2d model considers a maximum growth rate for autotrophic biomass of $1\,d^{-1}$. The simulations performed in this work assumed $\mu NOB_{MAX} = \mu AOB_{MAX} = 1\,d^{-1}$ if the contrary is not stated. In this particular set of simulations, the value of μNOB_{MAX} was modified in each simulation to obtain varied $\mu NOB_{MAX}/\mu AOB_{MAX}$ ratios. Figure 2(b) shows that NOB could be washed out of the system even when μNOB_{MAX} was higher than μAOB_{MAX}, at COD/N ratios higher than 8. However, the higher the $\mu NOB_{MAX}/\mu AOB_{MAX}$ ratio was, the more difficult it was to achieve the nitrite pathway. The results again demonstrated that the COD/N ratio was important. NP at COD/N = 4 could be achieved only when μNOB_{MAX} was 0.8 times μAOB_{MAX}

Overall, the simulation results show that aeration phase length control is an effective strategy for achieving the nitrite pathway. The nitrite pathway can be achieved even when NOB possess superior growth kinetics than AOB, although the faster the NOB grow (relative to AOB) the more difficult it is to achieve the nitrite pathway. The simulation results also show that the COD/N ratio is an important factor for the nitrite pathway. A minimum COD/N ratio of 5–6 is required for the strategy to be

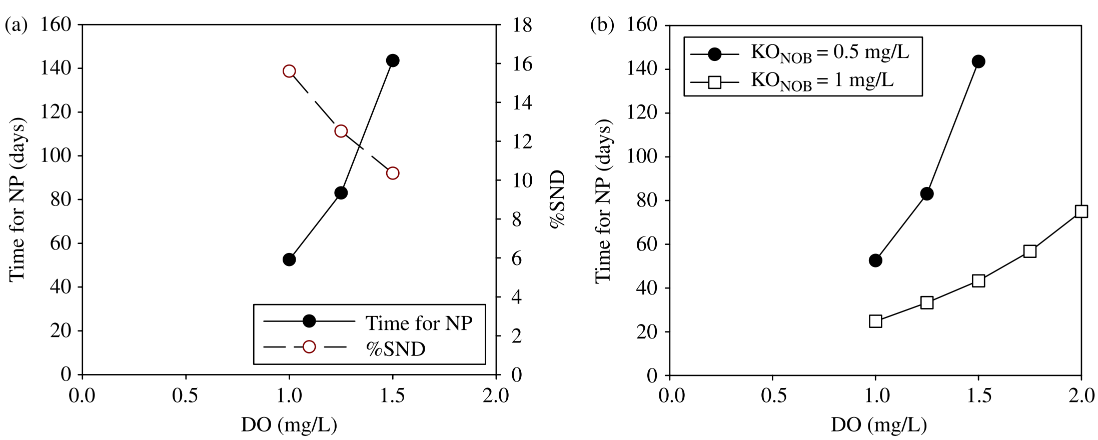

Figure 3 | Summary of the results applying the low DO setpoint strategy: (a) Time for NP and %SND at different DO setpoints; (b) Impact of KO_{NOB} on the achievement of the nitrite pathway.

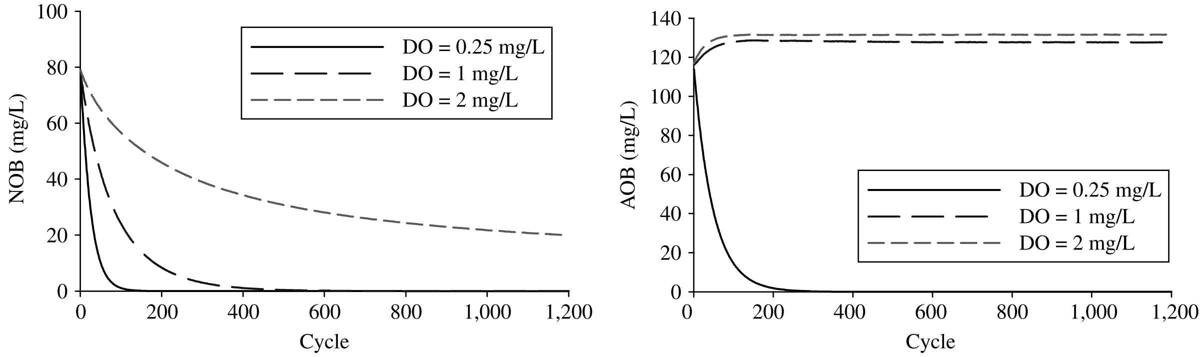

Figure 4 | Evolution of AOB and NOB at different DO values ($KO_{AOB} = KO_{NOB} = 0.5$ mg/L).

effective. The effectiveness of aeration length phase control to obtain nitrite pathway is enhanced with increased activities of nitrite-based denitrifying bacteria.

Control of DO setpoint under aerobic conditions

Figure 3(a) shows the Time for NP and the %SND for different DO setpoints for the aerobic phase. As expected, the activity of heterotrophic denitrifiers (%SND) increased as DO decreased. The results show that the nitrite pathway could only be achieved in a relatively narrow range of DO, between 1–1.5 mg/L for the particular wastewater composition, reactor configuration and kinetics used in this simulation study. For low DO values (< 1 mg DO/L in this study), neither AOB nor NOB could survive because of oxygen limitation. When high DO setpoints (> 1.5 mg/L in this case), neither AOB nor NOB could be washed out. Figure 4 shows example AOB and NOB profiles at different DO levels illustrating the washout of NOB and AOB.

In the DO range of 1 to 1.5 mg/L, the lower the DO setpoint was, the easier (i.e. the shorter) the achievement of the nitrite pathway was, mainly due to the competition between NOB and nitrite-based denitrifiers. AOB form nitrite at low rate due to oxygen limiting conditions and, under these conditions, nitrite utilisation for denitrification was not negligible when compared to nitration. The higher the heterotrophic activity, the faster the NOB would be washed out. Also, the less affinity NOB for O_2 and nitrite, the less competitive NOB would be in competing with nitrite reducers for nitrite (more easily to achieve the nitrite pathway). Figure 3(b) confirms that the Time for NP was reduced substantially when the affinity constant (KO_{NOB}) was increased from 0.5 to 1 mg/L. Also, the nitrite pathway could be achieved at a DO concentration of 2 mg/L, which was not possible when $KO_{NOB} = 0.5$ mg/L. Another important parameter is the affinity of heterotrophic bacteria with oxygen (KO_{HET}), with a lower KO_{HET} value favoring "aerobic" denitrification thus enhancing the

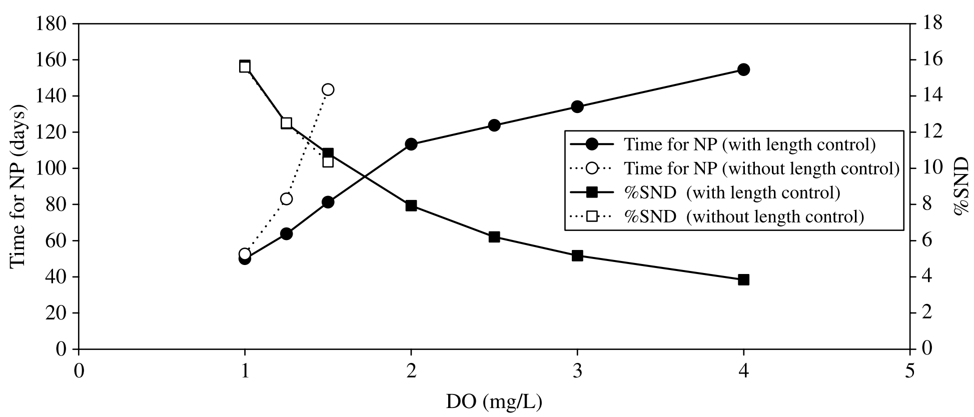

Figure 5 | Comparison of the Time for NP and %SND as functions of DO setpoint, with and without aeration phase length control.

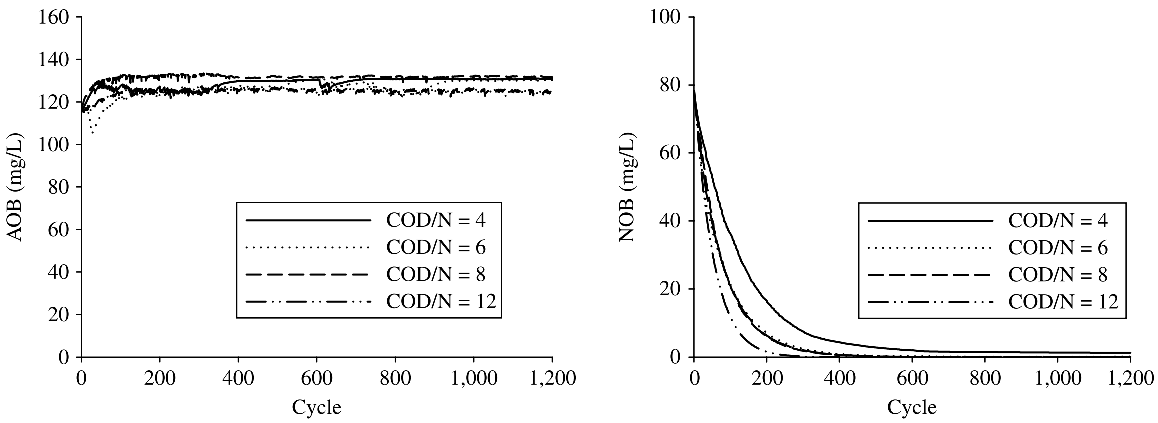

Figure 6 | Evolution of AOB and NOB with different COD/N ratios, DO = 1 mg/L.

competitiveness of nitrite reducers. The affinity of AOB with oxygen (KO_{AOB}) does not have a direct impact on the competitiveness of NOB with nitrite reducers. However, a smaller KO_{AOB} would allow the application of a lower DO (without having AOB washed out), and hence facilitate the achievement of the nitrite pathway.

The simulation results support several reports in literature that SND sludge could not perform nitrite oxidation when exposed to nitrite under aerobic conditions (Zeng *et al.* 2003; Yilmaz *et al.* 2008). The low DO condition limits NOB growth while at the same time facilitates nitrite reduction by nitrite reducers during the "aerobic" period. The competition for nitrite by nitrite reducers gradually reduces the availability of nitrite (produced by ammonia oxidation) to NOB, resulting in an apparent μ_{NOB} that is smaller than 1/SRT. This eventually will lead to the washout of NOB.

Combination of both control strategies

The final set of simulations consisted of combining both control strategies. Figure 5 compares the Time for NP and the %SND at different DO values with and without the aeration phase control strategy. The results show that adding this control strategy enabled the NP achievement in 300 days even at DO setpoints higher than 1.5 mg/L because aerobic periods with nitrite and without ammonium were suppressed, favouring the utilisation of nitrite for denitrification instead of nitratation. The Time for NP in the range of DO = 1–2 mg/L, where NP was already achieved at fixed aerobic phase length, was significantly decreased. Finally, Figure 5 shows that aeration phase length control was useless at very low DO setpoints, as ammonium oxidation rate was very low, and most of the aeration phase was needed to oxidise the ammonium of the feed. With respect to the %SND, it can be observed that the degree of SND was independent of implementing the aerobic phase length control strategy. %SND was only a function of the DO setpoint.

Figure 6 shows the AOB and NOB profiles obtained for a fixed DO setpoint of 1 mg/L and different COD/N ratios. This figure should be compared to Figure 2(a) for a better understanding of the effect of low DO levels. For a fixed setpoint of 4 mg DO/L, the minimum COD/N ratio required for the NP achievement was 5.5 whereas Figure 6 shows that NOB could be washed out from the system even at a COD/N ratio of 4. The occurrence of SND not only enables a faster achievement of the NP but also allows a more efficient COD utilisation and thus, the treatment of wastewaters with lower COD/N ratios. The reason is that the requirements of COD to reduce 1 mg N-NO_3 and 1 mg N-NO_2 are 2.86 and 1.71, respectively. Hence, if ammonium is partially oxidised to nitrite instead of fully oxidised to nitrate, the COD requirements for a complete biological nitrogen removal would be significantly reduced.

CONCLUSIONS

The nitrite pathway can be achieved in a single sludge SBR using either the aerobic phase length control strategy or the low DO setpoint strategy in a certain range of operational conditions. In both cases, the nitrite-based denitrifying activity underpins the nitrite pathway. The competition between nitrite reducers and nitrite oxidisers for nitrite, rather than kinetic selection, is the main reason for NOB washout. When the aerobic phase length control strategy is applied, the reduction of nitrite when the aerobic phase is terminated prevents the nitrite from being available for nitrite oxidisers and nitrite oxidisers would be washed out gradually. With low DO setpoint, the competition of nitrite reducers and nitrite oxidisers under aerobic conditions eventually leads to the washout of nitrite oxidisers. Stable simultaneous nitrification and denitrification (SND) occurring under "aerobic" conditions should always be via nitrite rather than nitrate. This observation explains literature finding that SND sludge can not perform nitrite oxidation when exposed to nitrite under aerobic conditions. The combination of both control strategies results in the most efficient way to achieve the nitrite pathway.

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