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Robust NOB control in hybrid biofilm systems operated for mainstream partial nitritation and anammox: an experimental and modelling study

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Summary

The control of NOB challenges the implementation of partial nitritation/anammox (PN/A) at main line. The mechanisms leading to long-term persistence or successful control of NOB were studied in hybrid PN/A systems, where biofilms and suspended biomass coexist. A hybrid MBBR was run during 565 days in SBR mode on aerobically pre-treated MWW at 15°C and different DO setpoints. Selective NOB washout from the flocs was successfully achieved and their re-growth prevented under prolonged operation at low DO. NOB could not be washed out from the biofilm in the long-term, but their activity was consistently controlled by DO setpoint, and fully suppressed by operation at limiting DO. High nitrogen removal efficiency (88%) and volumetric nitrogen removal rates during aeration (79 mgN L-1 d-1) were achieved along with the best effluent quality reported so far for mainstream PN/A (1.9 mgNH4-N L-1, 0.3 mgNO2-N L-1, and 0.5 mgNO3-N L-1). A dynamic mathematical model of the hybrid system was developed assuming perfect biomass segregation with AOB and NOB in suspension and AMX in the biofilm. Experimental evidence and simulation results identified the DO setpoint, controlled removal of suspended sludge, and anammox activity in the biofilm as the main parameters controlling NOB washout. Differently from sole biofilm systems, a lower oxygen affinity constant of AOB over NOB is not required. NOB washout is in principle possible even when KO2,NOB/KO2,AOB=0.14. This study strongly supports the inherent flexibility of hybrid systems for NOB control towards robust mainstream PN/A.

Mainstream anammox; hybrid system; mathematical modelling

1. Introduction

Direct treatment of municipal wastewater (MWW) based on anaerobic ammonium oxidation (anammox) provides the basis for bringing wastewater treatment plants closer to energy autarky (van Loosdrecht and Brdjanovic 2014). Successful partial nitritation and anammox (PN/A) relies on the fine balance between anammox bacteria (AMX) and ammonium oxidizing bacteria (AOB), and on the robust suppression of nitrite oxidizing bacteria (NOB). Proofs-of-principle for combined PN/A under mainstream conditions have been recently reported, with nitrogen removal rates and effluent qualities comparable to conventional systems for biological nutrient removal (De Clippeleir et al. 2013, Gilbert et al. 2015, Laureni et al. 2016, Lotti et al. 2014). However, the dominant mechanisms controlling microbial interactions and the role of different biomass configurations remain largely unexplored, especially under mainstream conditions. This work aimed to understand the mechanisms that promote long-term persistence or successful control of NOB in hybrid PN/A systems, where biofilms and flocs coexist.

2. Materials and Methods

A hybrid moving-bed biofilm reactor (MBBR) was run during 565 days in sequencing batch mode on aerobically pre-treated municipal wastewater (MWW) at 15 °C. The reactor was operated at different dissolved oxygen (DO) setpoints (*i.e.*, 0.17-1.6 mg_{O2} L^{-1}). In parallel, a dynamic mathematical bioprocess model of the hybrid MBBR was developed to elucidate the mechanisms governing the interaction between biofilm and suspended biomasses, and their implications for NOB control. The model was developed in MATLAB, and stoichiometric and kinetic formulations commonly used for PN/A systems were implemented (Perez et al. 2014). Perfect biomass segregation between biofilms and flocs was assumed. AOB and NOB were assumed to grow in suspension and their abundance and activity to be influenced by growth and washout. AMX were assumed to grow in a deep biofilm and their activity to be mass-transfer rather than biomass limited. The model did not explicitly track changes in AMX concentration but instead assumed a fixed maximum AMX activity. To this end, AMX concentration was kept constant during each simulation resulting in constant maximum volumetric rates of ammonium and nitrite consumption. Substrate limitations were accounted in the AMX rate.

3. Results and Conclusions

The DO was highlighted as a key factor that *(i)* governs NOB growth in or washout from the suspended biomass and that *(ii)* promotes or suppresses their activity in the biofilms. Selective NOB washout from the flocs was successfully achieved and their re-growth prevented under prolonged operation at low DO (0.17 mg_{O2} L^{-1}). This was supported by measurements of *(i)* activity under regular operation, *(ii)* maximum activity under non-limiting substrate availability ($r_{i,max}$; Fig. 3.1), and *(iii)* 16S rRNA gene-based amplicon sequencing analyses of bacterial community compositions and population dynamics. Importantly, NOB could not be washed out from the biofilm in the long-term, but their activity was consistently controlled by DO setpoint, and fully suppressed by operation at limiting DO (0.17 mg_{O2} L^{-1}). DO rather than nitrite was the substrate limiting NOB activity. AMX activity was stably maintained in the biofilms (Fig. 3.1). High nitrogen removal efficiency (88%) and volumetric nitrogen removal rates during aeration (79 mg_N L^{-1} d^{-1}) were consistently achieved, leading to the best effluent quality reported so far for mainstream PN/A (1.9 $mg_{NH4\text{-}N}$ L^{-1}, 0.3 $mg_{NO2\text{-}N}$ L^{-1}, and 0.5 $mg_{NO3\text{-}N}$ L^{-1}).

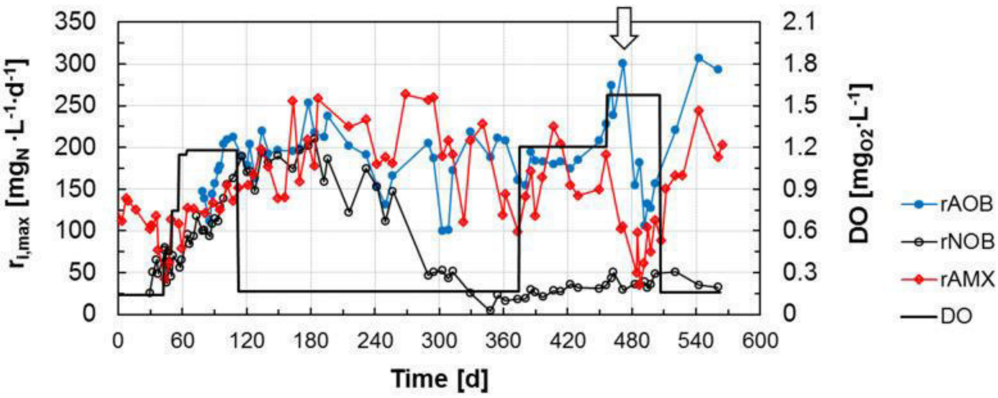

Figure 3.1 Time series of the maximum volumetric activities ($r_{i,max}$) of the three guilds, AOB, NOB, and AMX, measured under non-limiting substrate concentrations. AOB and NOB activities were measured under fully aerobic conditions, and AMX activity under anoxic conditions. The DO pattern refers to the average DO concentration measured during regular operation. The vertical white arrow indicate the time when a dramatic decrease in all activities was recorded after a prolonged rain event.

The mathematical model qualitatively reproduced the experimental results, including the washout of NOB under limiting DO concentrations (Fig. 3.2). By means of numerical simulations, we identified the *(i)* DO setpoint, *(ii)* controlled removal of the flocs (f_{WAS}), and *(iii)* maximum volumetric activity of AMX ($r_{AMX,max}$) in the biofilms as the main variables controlling the washout of NOB from the flocs. In principle, all three variables can be controlled in full scale installations. The pseudo steady-state concentrations of AOB (X_{AOB}) and NOB (X_{NOB}) as a function of different $r_{AMX,max}$ in the biofilms and of f_{WAS} per SBR cycle are presented in Fig. 3.3. The corresponding concentrations of nitrate in the effluent are also presented in Fig. 3.3. The results were obtained with perfect control of the DO at a setpoint of 1.5 mg_{O2} L^{-1}. In the absence of AMX, complete nitrification occurs. X_{AOB} decreases when higher $r_{AMX,max}$ are assumed. This is explained by a decrease of the ammonium available for AOB growth when the ammonium consumption by AMX increases. This effect disappears, and X_{AOB} stabilizes, as soon as NOB are fully washed out. When present in the system, NOB consume nitrite and indirectly favour AOB by decreasing ammonium depletion by AMX. Increasing f_{WAS} reduces the required $r_{AMX,max}$ required for successful NOB washout. However, it also results in lower X_{AOB},and thus lower process rates, *i.e.,* a higher HRT required for nitrogen removal.

The validity of our conclusions does not depend on the values assumed for the oxygen and nitrite affinity constants. In contrast to sole biofilm systems, the assumption of a lower oxygen affinity constant for AOB versus NOB was not required. Simulated results indicate that NOB can be washed out from the flocs even when the ratio $K_{O2,NOB}/K_{O2,AOB}$ is as low as 0.14, provided the right combination of DO, f_{WAS} and $r_{AMX,max}$.

Ultimately, the presented experimental evidence and simulation results strongly support the high operational flexibility offered by hybrid systems for the control of NOB and the establishment of robust PN/A under mainstream conditions.

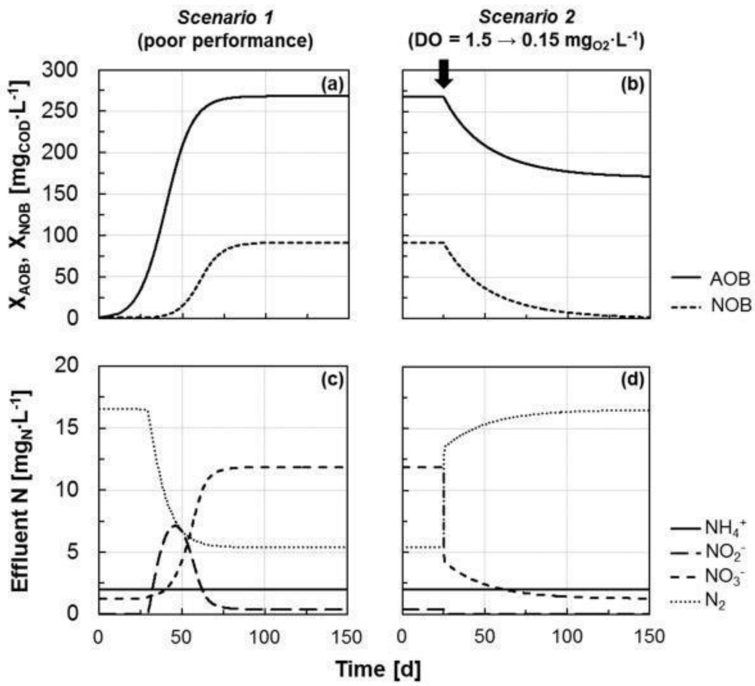

Figure 3.2 Dynamics in the simulated X_{AOB} and X_{NOB}, and effluent N concentrations for two different scenarios (N_2 is modelled as completely dissolved). (**a, c**) AOB and NOB are enriched in the flocs under prolonged operation at high DO of 1.5 $mg_{O2}\ L^{-1}$ (with a $r_{AMX,max}$ of 86 $mg_{(NH4+NO2)\text{-}N}\ L^{-1}\ d^{-1}$ and f_{WAS} of 5 10^{3}). The simulation was started with a low concentration of AOB and NOB, by analogy to the reactor operation during the period of days 70-115 (Figure 3.1). The biomass concentrations at pseudo steady state under *Scenario 1* are taken as initial conditions to assess the effect of lowering the operational DO (from 1.5 to 0.15 $mg_{O2}\ L^{-1}$, *Scenario 2;* **b, d**), by analogy to the reactor operation (days 115-375; Figure 3.1). The vertical black arrow indicates the time when the DO was decreased.

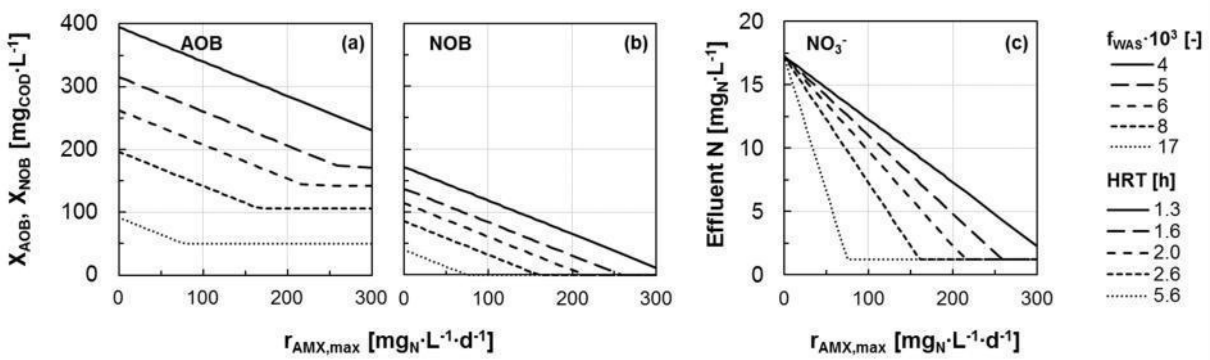

Figure 3.3 Concentrations of AOB (**a**) and NOB (**b**) in the flocs under pseudo steady-state conditions modelled as a function of the maximum voluetric activity of MAX ($r_{AMX,max}$) at a dissolved oxygen setpoint of 1.5 $mg_{O2}\ L^{-1}$. (**c**) Residual concentration of NO_3^- in the effluent at pseudo steady state under the same conditions. Separate lines represent different fractions of flocs removed per cycle (f_{WAS}).

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