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Biomass segregation in hybrid biofilm systems improves NOB control in mainstream partial nitritation and anammox

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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 (Siegrist et al. 2008, van Loosdrecht and Brdjanovic 2014). Successful partial nitritation and anammox (PN/A) relies on the fine balance between anammox (AMX) and ammonia oxidizing (AOB) bacteria, and on a robust suppression of nitrite oxidizers (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 nutrients removal systems, (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 biomass aggregate types remain largely unexplored, especially under mainstream conditions.

Here, we studied the mechanisms underlying the unfavourable long-term persistence and successful control of NOB in hybrid PN/A systems, where biofilms and suspended biomass coexist. A hybrid moving-bed biofilm reactor (MBBR) was run in sequencing batch (SBR) mode on aerobically pre-treated MWW at 15 °C and different dissolved oxygen (DO) setpoints. Spatial segregation of the three main autotrophic guilds of AMX, AOB, and NOB between the two biomass fractions, and their share in N-turnover were assessed by means of microbial activity tests, mass balances, and 16S rRNA gene-based amplicon sequencing analysis. In parallel, a dynamic mathematical model of the hybrid system was developed to elucidate the mechanisms governing the interaction between biofilm and suspended biomasses, and their implications for NOB control. Numerical simulations targeted the impact of operational conditions on NOB washout.

MATERIALS AND METHODS

The 12 L hybrid MBBR was operated as SBR for PN/A on aerobically pre-treated MWW for 565d at 15°C. The hybrid MBBR comprised both attached (2.8 $g_{TS}\cdot L^{-1}$; AnoxKaldnes K5-carriers; 33%

filling-ratio) and suspended (up to 3 $g_{TSS}\cdot L^{-1}$) biomass fractions. The aerobic solids retention time (SRT) of the suspended fraction was in the range 40-50 d. The DO concentration was varied between 0.17 and 1.6 $mg_{O2}\cdot L^{-1}$. The pre-treated MWW (Dübendorf, Switzerland) displayed the following composition: 54 $mg_{CODsol}\cdot L^{-1}$, 23 $mg_{NH4\text{-}N}\cdot L^{-1}$, < 0.3 $mg_{N}\cdot L^{-1}$ of NO_2^- and NO_3^-.

The spatial segregation of the three main guilds of AMX, AOB, and NOB was monitored over time by means of weekly maximum volumetric activity tests (non-limiting substrates concentrations). Maximum anammox activity was estimated anoxically *in situ*. Maximum AOB and NOB activities were estimated *ex situ* under fully aerobic conditions, separately on total and suspended biomass fractions. Volumetric activities of the three guilds under regular operation were estimated during the aerobic phase of an SBR cycle based on mass balances of dissolved nitrogen-species. Biomass samples of both biomass fractions were collected for 16S rRNA gene-based amplicon sequencing analysis. Selected biofilm samples were fixed for cryosectioning and FISH analysis.

The dynamic model of the hybrid MBBR was developed in MatLab according to stoichiometric and kinetic formulations commonly used for PN/A systems (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. Therefore the model did not explicitly track changes in the AMX concentration but instead assumed a maximum AMX activity. To this end, AMX concentration was kept constant during each simulation resulting in constant maximum rates of ammonium and nitrite consumption. Substrate limitations were considered in the AMX rate. Similarly to the experimental reactor operation, each modelled SBR cycle was ended at a residual ammonium concentration of 2 $mg_{N\text{-}NH4}\cdot L^{-1}$.

RESULTS AND DISCUSSION

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 biofilm. AOB and NOB were first successfully enriched in the suspended biomass at high DO (1.6 $mg_{O2}\cdot L^{-1}$). Prolonged operation under limiting DO (0.17 $mg_{O2}\cdot L^{-1}$) resulted in the selective washout of NOB and prevented their re-growth on the long term. Throughout the experiment, NOB were not washed out from the biofilm. Nevertheless, their activity was consistently controlled by the operational DO and completely suppressed at 0.17 $mg_{O2}\cdot L^{-1}$. Short-term operation under non-limiting NO_2^- concentrations confirmed DO rather than NO_2^- as the limiting substrate. AMX activity was stably maintained in the biofilm. This was supported by amplicon sequencing (not shown) and by FISH analysis (Fig. 1).

The system consistently achieved the highest effluent quality reported so far for mainstream PN/A systems with 1.9 $mg_{NH4\text{-}N}\cdot L^{-1}$ (94% removal), 0.3 $mg_{NO2\text{-}N}\cdot L^{-1}$, and 0.5 $mg_{NO3\text{-}N}\cdot L^{-1}$ and that meets with the most stringent water quality standards on nitrogen discharge. A nitrogen removal efficiency of 88% was reached with an average nitrogen removal rate during aeration of 79 $mg_{N}\cdot L^{-1}\cdot d^{-1}$ (HRT of 11 h).

The dynamic model of the hybrid systems was able to qualitatively reproduce the experimental results. Numerical simulations identified *(i)* the DO setpoint, *(ii)* the fraction of suspended activated sludge removed per cycle (WAS), and *(iii)* the available AMX biomass in the biofilm as the main parameters controlling NOB washout. In principle, all three parameters can be controlled in full scale installations. As an example, the pseudo steady-state concentrations of AOB (X_{AOB}) and NOB (X_{NOB}) as a function of different AMX (X_{AMX}) concentrations in the biofilm and of WAS percentages per SBR cycle are presented in Fig. 2. The corresponding concentrations of nitrate in the effluent are also presented in Fig. 2. The simulations refer to a DO setpoint of 0.15 $mg_{O2}\cdot L^{-1}$.

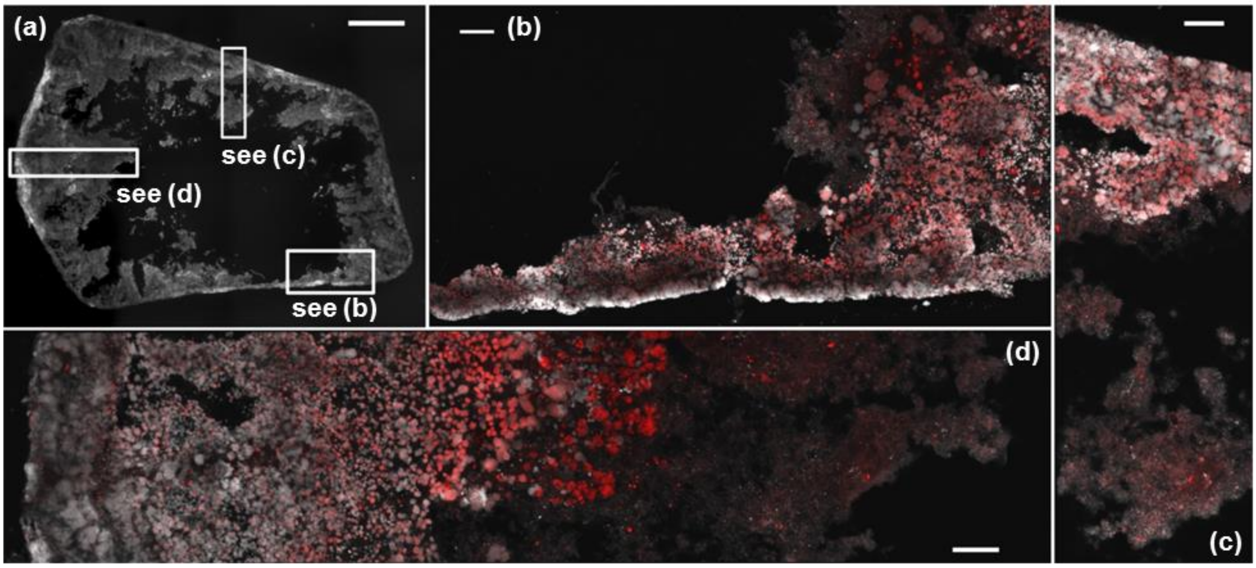

Figure 1. Thin-section FISH-stained micrographs of one biofilm carrier. (**a**) Wide-field epifluorescence micrograph showing DAPI-stained bacteria (white). Scale bar = 500 µm. (**b-d**) Confocal maximum intensity projections showing anammox bacteria (red) and DAPI-stained bacteria (white). Scale bars = 50 µm.

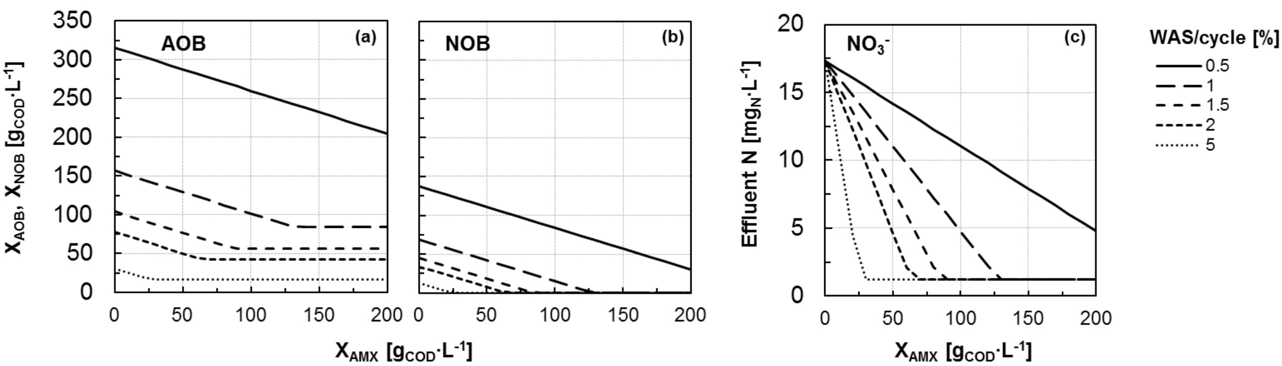

Figure 2. Concentrations of AOB (**a**) and NOB (**b**) in the flocs under pseudo steady-state conditions modelled as a function of the AMX concentration at dissolved oxygen setpoint of 0.15 $mg_{O2}\cdot L^{-1}$. (**c**) Residual concentration of NO_3^- in the effluent at pseudo steady state under the same conditions. Separate lines represent different percentages of sludge removal per cycle (WAS/cycle).

In the absence of AMX, partial or complete nitrification occurs. X_{AOB} decreases in suspension with increasing X_{AMX} in the biofilm. This is explained by a decrease of the ammonium available for AOB growth when the AMX ammonia consumption increases. This effect disappears as soon as the NOB are fully washed out. When present in the system, NOB consume nitrite and thus indirectly favour AOB by decreasing the nitrite available to the AMX. Increasing the WAS reduces the required X_{AMX} for successful NOB washout. However, higher WAS also result in lower X_{AOB} and thus reduced process rates (*i.e.* higher HRT and lower amount of batches of wastewater treated per day). Increasing the DO leads to higher ammonium oxidation rates and lower HRTs. At the same time, higher X_{AMX} or WAS are required to achieve successful NOB washout (data not shown).

Differently from sole biofilm systems, the assumption of a lower oxygen affinity constant of AOB over NOB is not required. The simulation results indicate that NOB can be washed out from the suspension even when the ratio $K_{O2,NOB}/K_{O2,AOB}$ is as low as 0.14.

CONCLUSIONS

The mechanisms leading to long-term persistence and successful control of NOB in hybrid PN/A systems, in which biofilms and suspended biomass coexist, were studied. Experimental evidence and simulation results indicate that NOB can be selectively washed out from the suspended biomass and their activity in the biofilm can be suppressed. The *(i)* DO setpoint, the *(ii)* controlled removal of the suspended sludge, and the *(iii)* AMX activity in the biofilm were identified as the main parameters controlling NOB washout. The activity of the persistent NOB population in the biofilm is consistently controlled by the operational DO setpoint and fully suppressed at low DO. Ultimately, the results strongly support the inherent flexibility offered by hybrid biofilm-floc systems for NOB control towards establishment of robust PN/A under mainstream conditions.

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