Modelling ammonium-oxidizing population shifts in a biofilm reactor
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ABSTRACT
The dynamic reactor behaviour of a nitrifying inverse turbulent bed reactor, operated at varying loading rate, was described with a one-dimensional two-step nitrification biofilm model. In contrast with conventional biofilm models, this model includes the competition between two genetically different populations of ammonia-oxidizing bacteria (AOB), besides nitrite-oxidizing bacteria (NOB). Previously gathered experimental evidence showed that different loading rates in the reactor resulted in a change in the composition of the AOB community, besides a different nitrifying performance. The dissolved oxygen concentration in the bulk liquid was put forward as the key variable governing the experimentally observed shift from *Nitrosomonas europaea* (AOB1) to *Nitrosomonas* sp. (AOB2), which was confirmed by the developed one-dimensional biofilm model.

Both steady state and dynamic analysis showed that the influence of microbial growth and endogenous respiration parameters as well as external mass transfer limitation have a clear effect on the competition dynamics. Overall, it was shown that the biomass distribution profiles of the coexisting AOB reflected the ecological niches created by substrate gradients.

Key words | biofilm reactors, mathematical modelling, nitrification, population dynamics, wastewater treatment

INTRODUCTION
Biological nitrogen removal from wastewater can be considered as a proven technology and has been widely implemented. The most common pathway is the combination of two sequential processes: autotrophic nitrification and heterotrophic denitrification. During nitrification, ammonia-oxidizing bacteria (AOB) convert ammonia to nitrite, which is further oxidized to nitrate by nitrite-oxidizing bacteria (NOB).

While in the conventional treatment systems bacteria are grown in flocs, which are more prone to washout events, biofilm reactors display distinct advantages for the cultivation of slow-growing nitrifiers, due to their specific biomass retention characteristics (Nicolella et al. 2000). Within biofilms, diffusional substrate concentration gradients result in a growth rate gradient. In multi-species biofilm systems, this will lead to a biofilm with a layered structure, giving species with different ecophysiological characteristics the opportunity to survive. Besides different types of organisms (e.g. AOB or NOB), also different species of the same type can coexist. Schramm et al. (1998) identified in bacterial aggregates from a fluidized bed two genetically and morphologically different populations of NOB affiliated with the nitrite oxidizer *Nitrospira moscovitensis*. Another example of the coexistence of two NOB species is given by Downing & Nerenberg (2008). In a nitrifying, membrane-aerated biofilm reactor (MABR), they observed a shift in NOB species with decreasing oxygen concentrations. Also different types of AOB have been reported to coexist in this reactor type (Terada et al. 2013). Lydmark et al. (2006) found in a full-scale nitrifying trickling filter four AOB populations, of which two *Nitrosomonas oligotropha* populations dominated at all depths. These two populations showed different distribution patterns within the biofilm, indicating different ecophysiological niches, even though they belong to the same AOB lineage. In a recent study the niche differentiation between two dominant *Nitrosomonas oligotropha* populations in pilot-scale moving bed biofilm reactors and trickling filters was confirmed experimentally based on their different reaction on changes in ammonium loading (Almstrand et al. 2013). Bernet et al.
Breitholtz et al. (2004) reported that, for a nitrifying inverse turbulent bed reactor (ITBR), the amount of carrier material affects both the macroscopic and microscopic (different AOB types) reactor behaviour. Different solid hold-ups of the reactors resulted in different liquid volumes, leading to different hydraulic retention times (HRTs) and consequently different ammonium loading rates. Upon lowering the ammonium loading rate in the most heavily loaded reactor by lowering its feeding rate, nitrate started to accumulate due to the presence of Nitrospira. Furthermore, nitrate accumulation was accompanied by the appearance of a different ammonium oxidizer population, Nitrosomonas sp. (AOB2), growing at the expense of N. europaea (AOB1) (Volcke et al. 2008). It was postulated that this population shift was due to a selection pressure driven by the different dissolved oxygen concentration in both reactors after the change in ammonium loading rate.

Models provide an adequate tool for understanding phenomena involved in biofilm processes, e.g. Wik & Breitholtz (1996) and Picireanu et al. (1997). However, present mathematical models mostly neglect microbial diversity. Conceptual and predictive mathematical models describing microbial community information should be developed to obtain a deeper understanding of ecosystems and possible ways to manipulate them (Nielsen et al. 2010). From an engineering perspective, it is of interest to include microbial community structure information in mathematical models. Extending an activated sludge model using two AOB populations (Wett et al. 2011) and the Anaerobic Digestion Model No. 1 to describe microbial diversity within functional groups (Ramirez et al. 2009) allowed a more accurate prediction of nitrification and aerobic digestion, respectively, upon changing process conditions. A recent study showed the influence of biomass detachment and microbial growth in the bulk liquid on the microbial community distribution in a heterotrophic biofilm using a highly discretized multi-species biofilm model (Brockmann et al. 2013).

In present nitrifying biofilm models, there is mostly only a distinction between ammonium oxidizers and nitrite oxidizers. Nevertheless, a biofilm model including one type of AOB and two types of NOB was set up by Downing & Nerenberg (2008), to determine the importance of both nitrite and oxygen affinity in the selection of Nitrospira spp. over Nitrobacter spp. in a MABR. Volcke et al. (2008) successfully described the observed microbial population shifts upon the lowering of the loading rate in an ITBR reactor through a zero-dimensional (neglecting spatial variations) nitrification model considering the growth of two types of AOB and one type of NOB. Even though this simplified model was useful in predicting the simulation outcome, it clearly neglects substrate gradients and biomass distribution profiles within the biofilm. To overcome this limitation, in this contribution a one-dimensional biofilm model was developed as an alternative to describe the experimental data of Volcke et al. (2008). As the biofilm structures under study were not characterized by a highly irregular surface, higher dimensional descriptions (two- or three-dimensional, see e.g. Picireanu et al. (2004)), making the biofilm modelling much more complex, were judged unnecessary. The advantages of this one-dimensional model compared to the zero-dimensional model for accurately describing the experimental data of Volcke et al. (2008), in terms of the macroscopic reactor behaviour as well as the underlying microbial dynamics, were evaluated. Particular attention was paid to the influence of microbial growth and endogenous respiration parameters as well as external mass transfer limitation on the competition outcome, through both steady state and dynamic analysis.

**MATERIAL AND METHODS**

**Experimental data**

In the ITBR (reactor volume = 1.35 litre), biomass was grown on low-density inert particles (particle diameter = 147 µm) which are fluidized by an upward current of gas (Bernet et al. 2004). The solid hold-up ratio, i.e. the ratio of static to expanded bed height, of the ITBR considered in this study was 0.3. The porosity of the bed was 0.41, which resulted in an active reactor volume fixed at 1.11 litre. The total amount of particulate material (viable biomass and inerts) was 10 g chemical oxygen demand (COD), corresponding to a biofilm thickness of 9.6 µm. The synthetic influent was supplied at a constant flow rate of 0.0072 m³.d⁻¹ and contained 250 g NH₄⁺-N.m⁻³ as ammonium sulphate. After four months, the ammonium loading rate of the reactor was lowered from 1,622 g NH₄⁺-N.m⁻³.d⁻¹ to 1,164 g NH₄⁺-N.m⁻³.d⁻¹, by decreasing the influent flow rate to 0.0053 m³.d⁻¹ and thus increasing the HRT (from 3.66 to 5 h). Oxygen measurements were occasionally performed (no on-line measurements) but it has been verified that the initial oxygen level in the reactor was limiting (<1 g O₂.m⁻³) for nitrite oxidation and that this was no longer the case after lowering the influent flow rate. After lowering the influent flow rate, the dissolved oxygen concentration of the bulk liquid was observed to be sufficiently high to allow complete nitrite oxidation by Nitrospira (NOB).
The operation shift was further characterized by the growth of Nitrosomonas sp. (AOB2) at the expense of Nitrosomonas europaea (AOB1). Temperature was maintained at 30 °C by a water jacket and pH was controlled at 7.5 by base addition. The airflow rate was kept constant at 0.72 m$^3$. d$^{-1}$. A detailed description of the reactor set-up and operation, besides the analytical and microbiological methods applied, is given by Bernet et al. (2004) and Volcke et al. (2008).

Reactor model

A one-dimensional two-step nitrification biofilm model, including the competition between two different species of AOB, besides NOB, was implemented in the Aquasim software (Reichert 1994). Nitrosomonas europaea (AOB1) is represented as a K-strategist, with a relatively low growth rate but a high affinity for oxygen, and Nitrosomonas sp. (AOB2) is represented as an r-strategist, with a relatively high growth rate and low affinity for oxygen, according to the r- and K-selection theory (Andrews & Harris 1986). Growth of AOB and NOB was described based on Hao et al. (2002). Inhibition of AOB and NOB by NH$_3$ and HNO$_2$ was not considered, to simplify interpretation of the results. As the influent did not contain organic carbon, heterotrophic growth was neglected as well. This was shown to be a valid assumption for co-diffusion systems (Lackner et al. 2008). Mozumder et al. (in press) also reported that heterotrophic growth on biomass decay products could be neglected. To describe biomass decay, endogenous respiration, a state in which microorganisms oxidize cellular storage compounds instead of organic matter from their environment (Van Loosdrecht & Henze 1999), was implemented in the model, considering oxygen, nitrite and nitrate as possible electron acceptors. The overall model stoichiometry and kinetics for the one-dimensional biofilm model are summarized in the Appendix available online (Tables S1 and S2, respectively; http://www.iwaponline.com/wst/069/701.pdf), as are the corresponding parameter values (Table S3; http://www.iwaponline.com/wst/069/701.pdf). Note that the parameter values were based on those from the calibrated and validated model of Volcke et al. (2008). A detailed sensitivity analysis was beyond the scope of this study.

The one-dimensional model developed in this study assumes that the variation of the state variables is restricted to a single direction perpendicular to the surface of the solid carrier. This is a valid simplification when vertical gradients are orders of magnitude higher than those in the directions parallel to the carrier surface (Wanner & Gujer 1986). Since this applies to most biofilm systems, dynamic multispecies one-dimensional biofilm models are sufficient for the majority of practical purposes. As the modelling of biofilm structures with highly irregular surface was not the focus of this study, higher dimensional descriptions (two- or three-dimensional), making the biofilm modelling much more complex, were judged unnecessary. The biofilm, which is autotrophic, was assumed to be quite dense with very small pores, in which no relevant motion of suspended solids takes place. The biofilm was moreover assumed to be rigid, meaning that particulate components are displaced only by the expansion or shrinkage of the biofilm solid matrix. In addition, the biofilm porosity has been assumed constant ($\varepsilon_W = 0.8$). An initial active biomass fractioning of 75% AOB and 25% NOB was assumed, according to the number of electrons exchanged by the oxidation of NH$_4^+$ to NO$_2$ and from NO$_2$ to NO$_3$, respectively. The biomass concentration in the biofilm was set to 93 kg COD m$^{-3}$ (Volcke et al. 2010).

Biofilm growth on the spherical particles was associated with a decrease in bulk liquid volume, to 1 litre. The biofilm growth has been limited by detachment. The equilibrium biofilm thickness was set at 9.6 μm, corresponding with the experimentally determined total particulate matter mass of 10 g COD. The reactor temperature (30 °C) and pH (7.5) were assumed constant. The oxygen level in the bulk liquid was controlled to a fixed value. Constant bulk liquid oxygen concentrations within a range of 0–7 g O$_2$.m$^{-3}$ were considered for steady state simulation. For the dynamic simulations, the bulk liquid oxygen concentration was assumed to be 0.5 g O$_2$.m$^{-3}$ before the reduction in ammonium loading rate and 2 g O$_2$.m$^{-3}$ after. During the experiments, the measured oxygen concentration was checked to be in accordance with these values, although the exact values were not recorded on-line. The bulk liquid was assumed to be homogeneous (Sanchez et al. 2005). At first, external mass transfer limitation was neglected to allow straightforward evaluation of the simulation results. Next, a boundary layer, resulting from external mass transfer limitation, was considered in this one-dimensional model, using an external mass transfer coefficient of 0.91 m.d$^{-1}$ (Bernet et al. 2005).

The initial concentration of ammonium in the bulk liquid has been assumed to be equal to the influent concentration (250 g NH$_4^+$.N.m$^{-3}$). Negligible amounts of nitrite and nitrate (1 g N.m$^{-3}$ each) were assumed to be present in the bulk liquid initially, to avoid numerical errors arising from zero concentrations in the kinetic expressions for endogenous respiration on nitrite and nitrate.
Simulation set-up

The simulation set-up is summarized in Table 1. Firstly, steady state simulations were performed to assess the influence of microbial growth and endogenous respiration parameters on microbial competition dynamics. All steady state simulations were performed over several years of operation to ensure that steady state conditions were achieved. In a first series of steady state simulations (Model 1), endogenous respiration was neglected to allow direct comparison with the zero-dimensional model. Secondly, the endogenous respiration rate of AOB1, AOB2 and NOB was defined as 5% of the maximum growth rate of the species (Model 2). The resulting values for the respiration rates ($b_{AOB1} = 0.068 \text{ d}^{-1}$, $b_{AOB2} = 0.121 \text{ d}^{-1}$, $b_{NOB} = 0.040 \text{ d}^{-1}$) are in the same range as those considered by Hao et al. (2002).

To be able to describe the experimental data from Volcke et al. (2008), some modifications of the model were necessary (Model 3). The endogenous respiration rate of both AOBs was set equal to 0.1 d$^{-1}$, while keeping the endogenous respiration rate of NOB at $b_{NOB} = 0.040 \text{ d}^{-1}$. External mass transfer was also included. Firstly, some steady state simulations were performed with Model 3. The influent flow rate ($Q_{in}$) amounted to 0.0072 m$^3\cdot$d$^{-1}$. Next, dynamic simulations were run for 123 days, preceded by a start-up period of 23 days at a low loading rate ($Q_{in} = 0.0072 \text{ m}^3\cdot\text{d}^{-1}$), and followed by a decrease in loading rate (63 days, $Q_{in} = 0.0053 \text{ m}^3\cdot\text{d}^{-1}$), according to the experimental conditions.

Definition of criteria to determine the competition outcome

For zero-dimensional models, straightforward criteria for the outcome of microbial competition can be defined and applied to AOB (Volcke et al. 2008):

$$S_{AOBi} = K_{NH}^AOBi \cdot \frac{1}{\mu_{max}^AOBi \cdot S_{O2}/(K_{Q2}^AOBi + S_{O2}) \cdot SRT - 1} \text{ for } i = 1, 2 \quad (1)$$

The species with the smallest non-zero value of $S_{AOBi}$ will win the competition, while the other species will be washed out of the reactor. In this study, it was examined whether this criterion can also be applied to determine the competition outcome of one-dimensional models. For the calculation of $S_{AOBi}$ with Equation (1), the solid retention time (SRT) needs to be known. The definition of SRT in biofilms is ambiguous. Either an overall SRT for all species in the biofilm or a species-specific SRT can be used. The overall SRT was calculated as the ratio between the biofilm thickness, $L_F$ (m), and the detachment rate, $u_d$ (m$\cdot$d$^{-1}$):

$$SRT_{overall} = \frac{L_F}{u_d} \quad (2)$$

The SRT of an individual species depends on its position in the biofilm and was calculated as:

$$SRT_{AOBi} = \frac{m_{tot}^{AOBi} / Q \cdot X_{cell}^{AOBi}}{m_{particle}^{AOBi} \cdot n_p} = \frac{m_{particle}^{AOBi} / Q \cdot X_{cell}^{AOBi}}{n_p} \quad (3)$$

In Equation (3), $m_{tot}^{AOBi}$ represents total biomass of AOBi (g COD) in the reactor, $m_{particle}^{AOBi}$ total biomass of AOBi (g COD) on one particle, $n_p$ total number of spherical particles, $Q$ flow rate (m$^3\cdot$d$^{-1}$) and $X_{cell}^{AOBi}$ biomass concentration (g COD$\cdot$m$^{-3}$) present in the effluent due to detachment.

The criteria to determine the competition outcome, as obtained by steady state simulations, were applied to the one-dimensional biofilm model in which both endogenous respiration and external mass transfer were neglected (Model 1). Firstly, the $S_{AOBi}$ was determined using the overall SRT (Equation (2)). Next, the $S_{AOBi}$ was also determined using the mean SRT of each AOB separately (Equation (3)).

Table 1 | Overview of the simulation set-up in terms of parameter values ($\phi$: decay rate; $K_i$: external mass transfer coefficient) and type of simulations (SS: steady state; D: dynamic) performed

<table>
<thead>
<tr>
<th>$b$ (d$^{-1}$)</th>
<th>$K_i$ (m$\cdot$d$^{-1}$)</th>
<th>SS</th>
<th>D</th>
<th>Comments</th>
</tr>
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<tbody>
<tr>
<td>Model 1</td>
<td>$b_{AOB1} = b_{AOB2} = b_{NOB} = 0$</td>
<td>-</td>
<td>✓</td>
<td>Based on zero-dimensional model (Volcke et al. 2008)</td>
</tr>
</tbody>
</table>
| Model 2       | $b_{AOB1} = 0.068$  
$b_{AOB2} = 0.121$ 
$b_{NOB} = 0.040$ | -  | ✓ | Endogenous respiration rate is 5% of maximum growth rate |
| Model 3       | $b_{AOB1} = b_{AOB2} = 0.1$ 
$b_{NOB} = 0.040$ | 0.91 | ✓ | ✓ | Model used for reproduction of experimental data |
RESULTS AND DISCUSSION

Steady state analysis – without endogenous respiration (Model 1)

To compare the one-dimensional model with the zero-dimensional model, both endogenous respiration and external mass transfer were neglected in Model 1. For bulk liquid oxygen concentrations higher than 0.1 g O2·m−3, almost all influent ammonium was converted to nitrite in the reactor for bulk liquid oxygen concentrations lower than 0.2 g O2·m−3. For higher oxygen concentrations, almost all ammonium was converted to nitrate, nitrite accumulation being very low (less than 1.5% of the influent ammonium).

With respect to the AOB population, a microbial population shift occurred around a bulk liquid oxygen concentration of 0.62 g O2·m−3 (Figure 1(b)). For bulk liquid oxygen concentrations lower than 0.616 g O2·m−3, the K-strategist AOB1 won the competition, and for bulk liquid oxygen concentrations higher than 0.622 g O2·m−3, the r-strategist AOB2 completely outcompeted AOB1. In the very narrow oxygen concentration range between these values, AOB1 and AOB2 coexisted at steady state (detail plot in Figure 1(b)). It is important to stress that coexistence of AOB1 and AOB2 was not obtained with the zero-dimensional model of Volcke et al. (2008). In general, coexistence of species performing the same function cannot be obtained with zero-dimensional models. On the other hand, the oxygen concentration at which the population shift between AOB1 and AOB2 occurred was about the same (0.6 g O2·m−3) as for the zero-dimensional model. This could have been expected, since the considered biofilm was very thin (9.6 μm) and the same microbial parameters were used in both studies.

It is important to note that the microbial population shift was not reflected in the macroscopic reactor performance. It seems that, at high oxygen concentrations, AOB2 took over completely the function of AOB1, converting ammonium to nitrite at the same rate. This is in agreement with the observations of Siripong & Rittmann (2007) and Wittebolle et al. (2008), based on the diversity data of nitrifying bacterial communities. They concluded that, by providing functional redundancy, coexistence of different species of one functional type can maintain the stability of the system for nitrification when operation conditions change.

Subsequently, the criterion given by Equation (1) was applied to determine the competition outcome. The overall SRT (Equation (2)) amounted to 25 days for oxygen concentrations higher than 0.2 g O2·m−3. The calculated $S^{*\text{AOB}}$ (Equation (1)) of the AOBs predicted which species was dominant (smallest non-zero value of $S^{*\text{AOB}}$) in a similar way as the simulation results for Model 1 (Figure 1(b)). Furthermore, the bulk liquid oxygen concentration at which the ‘competition switch’ occurred was predicted correctly. However, coexistence of both AOB (Figure 1(b)) could not be predicted. In general, coexistence can never be predicted with a criterion as given by Equation (1), since one $S^{*\text{AOB}}$ will always be smaller than the other, implying one species is dominant and the other one is washed out of the biofilm.

Next, the criterion for the outcome of interspecies competition (Equation (1)) was applied based on the mean SRT for each AOB separately (Equation (3)). In the oxygen concentration range in which a species was dominant, its SRT was about 21 days. However, the SRT of the outcompeted species increased to infinitely large numbers. The concentrations of the outcompeted species are small, both in the biofilm as well as in the effluent. Therefore, their SRT can become very large – even infinitely large for very small concentrations. This implied that the value of $S^{*\text{AOB}}$ was the smallest for the outcompeted species, leading to a wrong prediction of the competition outcome. Furthermore, it should be noted that the simulations with the one-dimensional model first need to be performed to calculate the SRT regardless of the SRT value applied. It can thus be concluded that the criteria are not very useful in combination with one-dimensional models.

Steady state analysis – endogenous respiration rate as a fraction of maximum growth rate (Model 2)

Taking into account the endogenous respiration at a rate equal to 5% of the maximum growth rate, the macroscopic reactor behaviour did not show large differences to the case in which endogenous respiration was neglected (Figure 1(c) versus 1(a)). However, the microbial population shift occurred at a higher oxygen concentration and coexistence was observed in a larger range of oxygen concentrations (4.18–5.5 g O2·m−3) considering endogenous respiration (Figure 1(d) versus 1(b)). Also, AOB1 outcompetes AOB2 up to higher oxygen concentrations compared to the case in which endogenous respiration was not considered (up to about 5 g O2·m−3). The reason for this lies in the fact that the endogenous respiration rate of the species...
with the lowest growth rate (AOB1) has a significantly lower absolute value than the species with the highest growth rate ($b_{AOB1} = 0.068 \text{ d}^{-1}$ versus $b_{AOB2} = 0.121 \text{ d}^{-1}$), which provides an additional competitive advantage for AOB1, on top of its high affinity for oxygen. After adjusting the endogenous respiration of both AOBs to $b_{AOB1} = b_{AOB2} = 0.1 \text{ d}^{-1}$ (data not shown), coexistence of both AOBs at an oxygen concentration of 0.36 g O$_2$.m$^{-3}$ was observed at steady state in a small range comparable to the simulation series in which no endogenous respiration was considered.

Figure 1 | Influence of dissolved oxygen concentration in the bulk liquid on steady state bulk liquid concentrations of nitrogen components (left) and on steady state biomass and particulate fractions in the biofilm (right). Simulation results are plotted for the model without endogenous respiration (top, (a), (b)), defining endogenous respiration rate as 0.05 $\mu_{\text{max}}$ (middle, (c), (d)) and defining $b_{AOB1} = b_{AOB2} = 0.1 \text{ d}^{-1}$ with inclusion of a boundary layer (bottom, (e), (f)). Note the different scale of the x-axis for Figure 1(d).
A clear advantage of using one-dimensional biofilm models instead of zero-dimensional ones is the possibility to study both biomass and substrate concentration profiles in the biofilm. Figure 2 displays the biomass profiles and substrate profiles at steady state for a bulk liquid oxygen concentration of $4.84 \text{ g O}_2 \text{m}^{-3}$. This bulk liquid oxygen concentration was chosen because it corresponds with the coexistence of AOB1 and AOB2 in about equal fractions (making up 17% and 15% of the particulate matter, respectively, see Figure 1(d)). At the surface of the biofilm distance from carrier ($z = 9.6 \mu \text{m}$), where the oxygen concentration was the highest, the species with the highest growth rate (AOB2) was present in a higher concentration than the species (AOB1) with a lower growth rate. On the other hand, close to the sub-stratum ($z = 0$), the oxygen concentration was lower so the species with the highest oxygen affinity (AOB1) had a competitive advantage. The differences were not very pronounced in this case because the biofilm under study was very thin (9.6 \mu m). A thin biofilm is characterized by small substrate concentration gradients (little variation in ecological niches), which result in relatively flat biomass concentration profiles. For such flat profiles, choosing a simple and straightforward zero-dimensional model, as proposed by Volcke et al. (2008), is acceptable. An advantage of the latter models is that they allow straightforward prediction of the competition outcome (Volcke et al. 2008). However, the added value of applying one-dimensional models will become larger for thicker biofilms, showing more pronounced concentration gradients and thus comprising more ecological niches.

**Simulation of experimental data (Model 3)**

The insights gained from the steady state analysis were used to describe the experimental data of Bernet et al. (2004) and Volcke et al. (2008). Using Model 2, dynamic simulation of the observed microbial population shift from AOB1 to AOB2 was not possible, as the chosen endogenous respiration rate resulted in a clear competitive advantage for AOB1 (Figure 1(d)). Therefore, the endogenous respiration rate of AOB1 and AOB2 was changed to a fixed value of $0.1 \text{ d}^{-1}$, resulting in the dominance of AOB2 for dissolved oxygen concentrations higher than $1.04 \text{ g O}_2 \text{m}^{-3}$ at steady state (Figure 1(f)).

However, the resulting model still did not reflect the observed macroscopic reactor behaviour, namely nitrite accumulation before the operation shift. This was remedied by considering external mass transfer ($K_L = 0.91 \text{ m.d}^{-1}$ from Bernet et al. (2005)). Steady state analysis of the resulting model (Figure 1(e)) showed that nitrite accumulation took place up to oxygen concentrations of $1 \text{ g O}_2 \text{m}^{-3}$ and nitrate was formed only if the oxygen concentration was larger than $0.6 \text{ g O}_2 \text{m}^{-3}$.

When Model 3 was used for dynamic simulations, the dynamic simulation results showed a good resemblance with the available experimental data (Figure 3). The results supported the hypothesis that the higher oxygen concentration in phase II allowed complete nitrite oxidation to nitrate (Figure 3(a)) and gave AOB2 the possibility to grow at the expense of AOB1 (Figure 3(b)). The difference in maximum growth rate and affinity for oxygen of the two AOBs thus explained the population shift after lowering...
the loading rate in the ITBR observed during the experiments.

CONCLUSIONS

- Microbial competition in a nitrifying biofilm (ITBR) reactor, operated at a varying loading rate, was described through a one-dimensional nitrification biofilm model, which includes the competition between two genetically different populations of AOB and one population of NOB.
- The biomass distribution profiles in the biofilm reflect the ecological niches created by substrate gradients.
- Microbial competition between different types of AOB is affected by the endogenous respiration rate, while external mass transfer limitations affect the competition between AOB and NOB and thus the macroscopic reactor behaviour in terms of nitrite and/or nitrate production.
- Straightforward criteria for the competition outcome predicted by zero-dimensional models (neglecting spatial variations) are not applicable to one-dimensional biofilm models.

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