Microbial population dynamics in nitrifying reactors: Experimental evidence explained by a simple model including interspecies competition

Eveline I.P. Volcke a,b, Omar Sanchez c, Jean-Philippe Steyer a, Patrick Dabert d, Nicolas Bernet a,*

a INRA, URS50, Laboratoire de Biotechnologie de l’Environnement, Avenue des Etangs, F-11100 Narbonne, France
b Department of Applied Mathematics, Bionetrics and Process Control, Ghent University, Coupure links 653, 9000 Gent, Belgium
c Departamento de Ingenierı´a Quı´mica, Universidad Catı´lica del Norte, Av. Angamos 0610, Antofagasta, Chile
d CEMAGREF, GERE, 17 avenue de Cucillé - CS 64427, 35044 Rennes cedex, France

1. Introduction

Biological nitrogen removal from wastewater can be considered as a proven technology and has been widely implemented. Processes based on nitrification–denitrification over nitrate have been most commonly applied, although significant cost savings in terms of aeration and carbon source addition are realized when ammonium is oxidized to nitrite and further oxidation to nitrate is prevented [1]. This is particularly true when dealing with wastewater streams containing high ammonium concentrations and relatively little organic carbon.

Biofilm reactors display distinct advantages for the cultivation of slow growing nitrifiers [2], allowing high loading rates while requiring only a small footprint. Nitrite accumulation in these reactors can be established acting on the difference in oxygen affinity between ammonium oxidizers and nitrite oxidizers, the latter being more sensitive to oxygen limitation [3–5].

Where it is well-known that at low oxygen concentrations, nitrite oxidizers are outcompeted because of their relatively low affinity for oxygen, experimental evidence is available suggesting that different reactor conditions also favour the presence of different nitrifying species belonging to the same functional group. When studying the influence of the different solid hold-ups in two inverse turbulent bed reactors (ITBRs) [6] discovered not only a difference in macroscopic performance in terms of nitrate and/or nitrite production and an associated presence or absence of nitrite oxidizers, but their results also indicated that the major ammonium oxidizing species present differs according to the reactor operating conditions. Additional experimental data presented in this contribution demonstrate that, in a single reactor, a shift in the macroscopic performance upon changing the operating conditions (influent load) is accompanied by a shift in the microbial population, with respect to the presence of nitrite oxidizers as well as in terms of the dominant ammonium oxidizing species. This indicates a reversibility of the observed phenomena.

Present mathematical models describing the nitrification process mostly neglect microbial diversity. At the most, they distinguish between ammonium oxidizers and nitrite oxidizers, assuming the same properties for all bacteria of each group. The model presented in this contribution includes interspecies competition between different organisms of the same functional group - in this case two different types of ammonium oxidizers - to
describe the experimental data. The model is capable to describe the competition outcome and to predict microbial population shifts upon changes in operating conditions. The effect of microbial growth parameters on the outcome of interspecies competition is assessed in calibrating the model to the available experimental data.

2. Materials and methods

2.1. Reactor set-up

The biofilm reactors mentioned in this study are inverse turbulent bed reactors consisting of a tubular PVC pipe of 0.06 m internal diameter and 0.7 m height with an active fluidized volume of 1.35 l. In this type of reactors, biomass is grown on low density particles which are fluidized by an upward current of gas. The reactors have been filled with Extendosphere™ particles as solid carrier material. This natural residue (spherical particles of \( d_p = 147 \mu m, \rho = 690 \text{ kg m}^{-3} \)) is a mineral granular material composed mainly of silica and alumina. The only difference between the two reactors in this study is the solid hold-up ratio, i.e. the ratio of static to expanded bed height: 0.1 (reactor R10) and 0.3 (reactor R30). These two values correspond to extreme conditions in terms of particle-to-particle collision frequency and therefore detachment force [7], with high collision frequency at 0.3 and low collision frequency at 0.1. Fig. 1 displays the reactor set-up.

2.2. Operating conditions

The reactors have been inoculated with activated sludge from the municipal wastewater treatment plant of Coursan (Aude, France) prior to adaptation to the synthetic wastewater used as a feeding medium. The reactors have been operated in batch mode during 5 days, then in continuous mode with biomass recycling during 18 days, and finally in continuous mode without biomass recycling. The synthetic influent, supplied at a constant flow rate of 0.3 l h\(^{-1}\), contained per liter 250 mg \( \text{NH}_4^+ \) as ammonium sulphate, besides 100 mg \( \text{KH}_2\text{PO}_4 \), 10 g \( \text{KHCO}_3 \) and 0.65 ml of a trace element solution. Temperature was maintained at 30°C by a water jacket, pH was controlled at 7.5 by automatic addition of an alkaline solution (0.5 M NaOH, 0.25 M \( \text{Na}_2\text{CO}_3 \)). The airflow rate was kept constant at 30 l h\(^{-1}\). After 4 months, the influent flow rate in the R30 reactor has been lowered to obtain a hydraulic retention time of 5 h.

2.3. Analytical and molecular methods

Ammonium, nitrite and nitrate were analyzed by ion chromatography [DIONEX 100] using conductivity detection (APHA 1992, section 4110). Separation and elution of the anions were carried out on IonPac AS12A analytical column, utilizing a carbonate/bicarbonate eluant and AutoSuppression technology. Integration was done using a PC with the Peaknet Software.

Identification of the dominant bacterial peaks revealed on the SSCP profiles was performed by sequencing. Cloned DNA of the identified peaks were finally sequenced to obtain the bacterial primers W91 (ACGGTCCAGACTCCTACGGG)–W94 (HEX-TTACCCGCCGCTGCGGCCAC), E. coli positions F330 and R533 respectively as described in [10]. The resulting PCR products were separated by SSCP electrophoresis using GENESCAN 5.58-Glyceral 10% polymer and an ABI 310 Genetic Analyser (Applied Biosystems) equipped with a capillary tube (47 cm × 50 mm) as described in [8]. Reliable comparison between sample profiles was obtained by careful alignment of the GS-400 Rox internal standard using the GeneScan 3.1 software (Applied Biosystems). Areas of all distinguishable peaks were recorded (GeneScan 3.1 software) to be able to calculate the relative area of each peak in its respective well profile.

2.4. Reactor model

A simple two-step nitrification model has been set up to describe the behaviour of soluble components (ammonium, nitrite and nitrate, with respective concentrations \( S_{\text{NH}_4} \), \( S_{\text{NO}_2} \), and \( S_{\text{NO}_3} \)), and nitrifying biomass. The nitrifying population considered consists of two ammonium oxidizing species and one nitrite oxidizing species, with respective concentrations \( X_{\text{AOB1}}, X_{\text{AOB2}} \) and \( X_{\text{NOB}} \). In this way, the model contains the same number of nitrifying species as observed experimentally. Note however that its extension to more than two different types of ammonium oxidizers and/or different types of nitrite oxidizers can be done straightforwardly.

Both reactors are operated under the same conditions of constant temperature and pH (\( T = 30^\circ \text{C}, \text{pH 7.5} \)). The synthetic influent does not contain nitrite, nitrate, nor biomass. The oxygen concentration in \( g \text{ O}_2 \text{ m}^{-3} \) is assumed constant in each of the reactors. Biomass retention in the reactor has been modelled in the simplified way suggested in the ADM1 report [11], distinguishing only between hydraulic retention time (i.e. HRT = 1/D, with D the dilution rate) and solid retention time (SRT). This results in the following mass balances for soluble and overall biomass (suspended + biofilm) components, respectively:

\[
\frac{ds_j}{dt} = -D \left( \frac{S_j}{C_0} - S_j \right) + \sum_{i=1}^{3} a_{ij} \rho_j i = 1, 2, 3; S_1 = S_{\text{NOB}}; S_2 = S_{\text{AOB1}}; S_3 = S_{\text{AOB2}}; S_4 = S_{\text{NOB}};
\]

\[
\frac{dx_j}{dt} = \frac{-1}{\text{SRT}} x_j + \sum_{i=1}^{3} a_{ij} \rho_j i = 1, 2, 3; x_1 = x_{\text{AOB1}}; x_2 = x_{\text{AOB2}}; x_3 = x_{\text{NOB}};
\]

This model can be seen as ‘0-dimensional’ with respect to space (the time dimension is included), indicating that a homogeneous distribution of soluble and overall biomass throughout the reactor has been assumed. Biomass decay has not been considered explicitly, but is typically proportional to the biomass concentration. Neglecting its effect on the ammonium concentrations, decay can be seen as lumped into the parameter SRT.

Table 1 displays the process stoichiometry and kinetics. No inhibition effects have been considered, a reasonable assumption given the relatively low influent ammonium concentration. Table 2 lists the values of stoichiometric and kinetic parameters applied in this study.

2.5. Criteria for interspecies competition

For the above simple 0-dimensional model, conditions for the survival of AOB1, AOB2 and/or NOB can be rigorously defined and are summarized below. These results are independent of the initial biomass concentrations in the reactor.

\[
S_{\text{NH}_4} \text{ max} = \frac{1}{\mu_{\text{AOB1}} (S_{\text{AOB1}}/R_{\text{AOB1}} + S_{\text{AOB2}}) - \text{SRT}} \quad \text{for } i = 1, 2, 3
\]
In order for an ammonium oxidizing species to have at least a chance to survive, the following condition should be fulfilled:

\[ 0 < S_{\text{AOB}} - S_{\text{NOB}} < \frac{c_{\text{AOB}}}{Y_{\text{NOB}}} \]

(4)

Typically only one ammonium oxidizing species (say AOB1) survives, while the other one (AOB2) will eventually be washed out. The condition for AOB1 to outcompete AOB2 is the following:

\[ 0 < S_{\text{AOB1}} - S_{\text{AOB2}} < \frac{c_{\text{AOB1}}}{Y_{\text{AOB2}}} \]

(5)

In case \( S_{\text{AOB1}} = S_{\text{AOB2}} \) both species survive (at least if also fulfilling Eq. (4)). From Eqs. (3) and (5), it is clear that the following species characteristics lead to a competitive advantage: high growth rate \( \mu_{\text{max}} \), high ammonium and/or oxygen affinity (i.e. small \( K_{\text{NH}} \) and/or \( K_{\text{O2}} \), respectively). The influential ammonium concentration does not affect the competition between the two species, although it does determine whether the strongest species of the two actually survives (Eq. (4)). The competition between the two types of ammonium oxidizers is further independent of their yield on substrate (even if different for the two species).

### 3. Results and discussion

#### 3.1. Experimental observations

The influence of the different solid hold-up in both ITBRs on biofilm growth and nitrifying performance was studied from a macroscopic (i.e. nitrate and/or nitrite production) and microbiological point of view [6]. The reactor R30 (highest support concentration) accumulated nitrite (95% of oxidized ammonium) whereas R10 produced only nitrate as a final nitrification product. The comparison of microbial communities in both reactors (Fig. 2) was in agreement with this result: the same population of nitrite-oxidizing Nitrosospira sp. 12 (accession number Y14639 with 98% similarity) was present in both reactors but in very low proportion in R30 compared with R10. The major ammonium-oxidizer was different in both reactors, Nitrosomonas europaea (AOB1; accession number AF353106 with 100% similarity) in R30 and Nitrosomonas sp. (AOB2; accession number AF386753 with 100% similarity) in R10. The third prominent peak in R10 (peak 2) belonged to the Cytophaga–Flexibacter–Bacteroides group (92% similarity with an uncultured clone, accession number Y14639) and was thus determined with certainty not to be a nitrifier. No clone was identified as corresponding to peaks B and C in R30.

The question arises how the reactors’ solid hold-up, being the only operating parameter different between both reactors, can act

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### Table 1

Stoichiometric matrix \( A_i \) and kinetics for a two-step nitrification model with two ammonium oxidizing species and one nitrite oxidizing species

<table>
<thead>
<tr>
<th>( A_i )</th>
<th>Process rate ( \rho_j )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Ammonium oxidation by ( X_{\text{AOB1}} )</td>
<td>( \frac{\mu_{\text{max}}}{\text{AOB1}} \frac{S_{\text{NH}}}{K_{\text{NH}}} + \frac{S_{\text{NO2}}}{K_{\text{NO2}}} + \frac{S_{\text{O2}}}{K_{\text{O2}}} )</td>
</tr>
<tr>
<td>2 Ammonium oxidation by ( X_{\text{AOB2}} )</td>
<td>( \frac{\mu_{\text{max}}}{\text{AOB2}} \frac{S_{\text{NH}}}{K_{\text{NH}}} + \frac{S_{\text{NO2}}}{K_{\text{NO2}}} + \frac{S_{\text{O2}}}{K_{\text{O2}}} )</td>
</tr>
<tr>
<td>3 Nitrite oxidation</td>
<td>( \frac{\mu_{\text{max}}}{\text{NOB}} \frac{S_{\text{NO2}}}{K_{\text{NO2}}} )</td>
</tr>
</tbody>
</table>

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### Table 2

Stoichiometric and kinetic (at pH 7.5 and \( T = 30^\circ \text{C} \)) parameter values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
<th>Unit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \gamma_{\text{AOB1}} )</td>
<td>Yield coefficient of AOB1</td>
<td>0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>g COD g N&lt;sup&gt;−1&lt;/sup&gt; d&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>[12]</td>
</tr>
<tr>
<td>( \gamma_{\text{AOB2}} )</td>
<td>Yield coefficient of AOB2</td>
<td>( = \gamma_{\text{AOB1}} )</td>
<td>g COD g N&lt;sup&gt;−1&lt;/sup&gt; d&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>Assumed</td>
</tr>
<tr>
<td>( \gamma_{\text{NOB}} )</td>
<td>Yield coefficient of NOB</td>
<td>0.057&lt;sup&gt;a&lt;/sup&gt;</td>
<td>g N g COD&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>[12]</td>
</tr>
<tr>
<td>( h_{\text{NOB}} )</td>
<td>Nitrogen fraction of biomass</td>
<td>0.086&lt;sup&gt;a&lt;/sup&gt;</td>
<td>g N g COD&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>ASM1 [13]</td>
</tr>
<tr>
<td>( \mu_{\text{max}} )</td>
<td>Maximum growth rate of AOB1</td>
<td>1.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>d&lt;sup&gt;−1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.51–2.71</td>
<td>d&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>Arbitrary range</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>d&lt;sup&gt;−1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( K_{\text{NH}} )</td>
<td>Oxygen half-saturation coefficient of AOB1</td>
<td>0.3</td>
<td>g O&lt;sub&gt;2&lt;/sub&gt; m&lt;sup&gt;−3&lt;/sup&gt;</td>
<td>[12]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.4–1.2</td>
<td>g O&lt;sub&gt;2&lt;/sub&gt; m&lt;sup&gt;−3&lt;/sup&gt;</td>
<td>Arbitrary range</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.2</td>
<td>g O&lt;sub&gt;2&lt;/sub&gt; m&lt;sup&gt;−3&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( K_{\text{O2}} )</td>
<td>Oxygen half-saturation coefficient of NOB</td>
<td>0.25</td>
<td>g N m&lt;sup&gt;−3&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( K_{\text{NO2}} )</td>
<td>Ammonium half-saturation coefficient of AOB1</td>
<td>( = K_{\text{NH}} )</td>
<td>g N m&lt;sup&gt;−3&lt;/sup&gt;</td>
<td>Assumed</td>
</tr>
<tr>
<td>( K_{\text{NO2}} )</td>
<td>Ammonium half-saturation coefficient of AOB2</td>
<td>1.6</td>
<td>g N m&lt;sup&gt;−3&lt;/sup&gt;</td>
<td>[16]</td>
</tr>
</tbody>
</table>

<sup>a</sup> After unit conversion, considering typical textbook value of 1.37 g COD g<sup>−1</sup> dry organic matter.

<sup>b</sup> Value recalculated at 30 °C, according to general T-relationship. Note: no pH-correction has been applied, since pH 7.5 is very close to optimal value of 7.23 determined by [17].
upon nitrifying activity and on the major ammonium oxidizer present? Note that the gas–liquid mass transfer coefficient in the turbulent bed reactor has been shown to be independent of the solid concentration[21]. Nevertheless, the different solid hold-ups of the reactors R10 and R30 result in different liquid volumes of 1.27 and 1.1 l, respectively, leading to different hydraulic retention times of 4.23 and 3.66 h respectively and consequently different ammonium loading rates, 1420 and 1640 g NH₄⁺-N m⁻³ d⁻¹ for R10 and R30, respectively. The 15% higher loading rate in R30 compared to R10, for the same aeration flow rate, results in oxygen depletion in R30, on its turn causing nitrite accumulation. It is postulated that the difference in the major ammonium oxidizer is also due to a selection pressure driven by the different dissolved oxygen concentration in both reactors.

In order to confirm this hypothesis, the ammonium loading rate of the R30 reactor has been decreased to 1200 g NH₄-N m⁻³ d⁻¹, by lowering its feeding rate to obtain a hydraulic retention time of 5 h. The airflow rate was kept constant, in order not to change the reactor fluidization constraints. Within 24 h, oxidation of nitrite was observed and complete conversion of nitrite to nitrate was achieved after 5–10 days (Fig. 3a). During the experiments, the measured oxygen concentration was checked to be in accordance with these results (increased value compared to initial operation, when oxygen concentration was limiting), although the exact values were not recorded on-line. The corresponding bacterial SSCP profiles show an increasing proportion of the NOB population (Nitrospira sp. 12, peak A/1) within the community (Fig. 3b). These observations validate our hypothesis of an oxygen limitation of nitrite-oxidizers in the reactor. Particularly noteworthy is the appearance of a different ammonium oxidizer population of Nitrosomonas sp. (AOB2) at the expense of N. europaea (AOB1), along with the presence of the NOB Nitrospira. This new couple of ammonium and nitrite oxidizers present in R30 after 63 days (Fig. 3c) is the same as the one observed in reactor R10 (Fig. 3d) suggesting a better fit of this nitrifying community structure to the reactor conditions.

The simultaneous occurrence of different nitrifying species in biofilm reactors, related to the environmental conditions, has also been reported by other authors: [22] observed the concurrent
presence of *N. europaea* (AOB) and *Nitrobacter* sp. (NOB) at relatively high substrate and oxygen concentrations, outcompeting *Nitrosospira* (AOB) and *Nitrospira* spp. (NOB). The shift observed in our reactors appears even more subtle, as the ammonium oxidizers involved belong to the same *Nitrosomonas* genus.

It is interesting to note that the different phenomena observed (Fig. 3) take place at clearly different timescales. After the decrease of the ammonium loading rate in R30, the nitrate production attains its steady state after about 10 days. An additional lag of about 10 days was observed before a convincing increase of the Nitrosospira peak in the bacterial SSCP profile could be detected. The population shift between the two types of ammonium oxidizers occurs at an even slower timescale. From the SSCP profiles, it is clear that AOB1 (*N. europaea*) concentration decreases and the AOB2 (*Nitrosomonas* sp.) concentration increases upon influent load reduction.

### 3.2. Analysis of microbial competition through simulation

The presented model is used to study the observed behaviour, both on a macroscopic (concentration of nitrogen compounds) and microscopic level (microbial population dynamics). Postulating the reactor oxygen concentration as the determining factor for survival or outcompetition of nitrite oxidizers, it will be examined whether different oxygen levels can also explain the selection of different types of ammonium oxidizers in both reactors as well as the population shift in reactor R30.

#### 3.2.1. Survival of nitrifying species in terms of operating conditions

Fig. 4 depicts the survival of AOB1 (*N. europaea*), AOB2 (*Nitrosomonas* sp.) and/or NOB (*Nitrobacter*) in the reactor according to its operating conditions in terms of the influent ammonium concentration and the prevailing oxygen concentration in the reactor, for two different values of the biomass retention time. The values for the parameters $\mu_{\text{AOB}1}^\text{max}$ and $K_{\text{AOB}2}^\text{O2}$ applied in Fig. 4 are the ones obtained after model calibration (see further).

For very low oxygen concentrations in the reactor, Eq. (4) is not fulfilled for any of the two AOB species, resulting in complete biomass wash-out (zone W). In case of moderately low oxygen concentrations and/or very low influent ammonium concentrations (Eq. (4) is fulfilled for at least one AOB species but Eqs. (7) and (8) are not fulfilled at the same time, zones AOB1 and AOB2), nitrite oxidizers cannot maintain themselves in the reactor, but at least one type of ammonium oxidizers can. The operating zones W and AOB1 are separated by the $S_{\text{AOB}1}^\text{lim}$ curve. Its vertical asymptote corresponds with $S_{\text{AOB}1}^\text{lim}$, the oxygen lower limit above which the sign of $S_{\text{AOB}1}$ becomes positive ($i$ indicating the surviving species at the given oxygen concentration, in this case $i = 1$). $S_{\text{AOB}1}^\text{lim}$ is directly calculated from Eq. (3) as

$$S_{\text{AOB}1}^\text{lim} = \frac{K_{\text{AOB}1}^\text{O2}}{\mu_{\text{AOB}1}^\text{max} \times \text{SRT} - 1}$$  \hspace{1cm} (11)

The individual $S_{\text{AOB}1}$-curves are not depicted as they are lying too close to each other to allow clear differentiation between the two. At low oxygen concentrations, AOB1 wins the competition as it has the lowest oxygen affinity constant ($K_{\text{AOB}1}^\text{O2} = 0.3 \text{ g O}_2 \text{ m}^{-3}$; $K_{\text{AOB}2}^\text{O2} = 1 \text{ g O}_2 \text{ m}^{-3}$); while at higher oxygen concentrations AOB2 becomes dominant because of its higher maximum growth rate ($\mu_{\text{AOB}1}^\text{max} = 1.36 \text{ day}^{-1}$; $\mu_{\text{AOB}2}^\text{max} = 2.41 \text{ day}^{-1}$). AOB1 can be seen as a $K$-strategist, while AOB2 is a $r$-strategist. In case both AOB species have the same ammonium affinity constant $K_{\text{NH}_3}^\text{AOB}$ and are subject to the same sludge retention time SRT, as assumed further, the oxygen concentration in the reactor at which the ‘competition switch’ occurs, $S_{\text{comp}}^\text{O2}$, is straightforwardly calculated from Eq. (3) as

$$S_{\text{comp}}^\text{O2} = \frac{K_{\text{AOB}1}^\text{O2} / \mu_{\text{AOB}1}^\text{max} - K_{\text{AOB}2}^\text{O2} / \mu_{\text{AOB}2}^\text{max}}{1 / \mu_{\text{AOB}1}^\text{max} - 1 / \mu_{\text{AOB}2}^\text{max}}$$  \hspace{1cm} (12)

and is marked as the ‘AOB competition boundary’ in Fig. 4, which separates the zones AOB1 and AOB2. At oxygen concentrations below $S_{\text{comp}}^\text{O2}$, $S_{\text{AOB}1}^\text{lim}$ equals $S_{\text{AOB}1}^\text{max}$ while at higher oxygen concentrations $S_{\text{AOB}2}^\text{lim}$ equals $S_{\text{AOB}2}^\text{comp}$ holds. In case the oxygen concentration in the reactor strictly equals $S_{\text{comp}}^\text{O2}$, both AOB1 and AOB2 coexist, although it is unlikely that this condition will be exactly fulfilled in practice.

For relatively high reactor oxygen concentrations and not too low influent ammonium concentrations (Eqs. (7) and (8) fulfilled), besides ammonium oxidizers also nitrite oxidizers survive in the reactor, resulting in nitrate formation (operating zones AOB1 + NOB and AOB2 + NOB). The curve that separates the regions with and without ingrowth of NOB is defined by $S_{\text{AOB}1}^\text{comp} + (1 - i_{\text{NOB}}) \times S_{\text{NOB}}^\text{lim}$. Its vertical asymptote corresponds with the oxygen lower limit above which the sign of $S_{\text{NOB}}^\text{lim}$...
becomes positive, \( S_{O2}^{NOB lim} \), which follows directly from Eq. (6) as:

\[
S_{O2}^{NOB lim} = \frac{K_{O2}^{NOB}}{\mu_{NOB max} \cdot SRT - 1}
\]

(13)

For increasing sludge retention times, ingrowth of AOB and NOB respectively occurs at lower oxygen concentrations. The oxygen concentration at which the competition switch occurs, \( S_{O2}^{comp} \), is not affected by the sludge retention time (Eq. (12)). As a result, the operating zones corresponding with only nitrite formation (AOB1 and AOB2) become smaller at the expense of the zones in which also nitrate is formed (zones AOB1 + NOB and AOB2 + NOB).

Note that, for SRT = 12 days, the operating zone AOB2 (as indicated by an arrow in Fig. 4) becomes so small that cannot be reached in practice. Besides, for the given parameter set, at relatively low sludge retention times (SRT = 5 days, Fig. 4) coexistence of AOB1 and NOB is not possible, while at higher sludge retention times (SRT = 12 days, Fig. 4) operating zones exist for coexistence of AOB1 and NOB as well as for AOB2 and NOB. As the sludge retention time increases further, the operating zone at which only AOB1 survives without NOB becomes so small that it is practically impossible to establish. For the extreme case, SRT \( \rightarrow \infty \), both ammonium oxidizers (in this case AOB1) and nitrite oxidizers will survive in the reactor, even for infinitely low oxygen levels. This behaviour is typical for a 0-dimensional model, in which concentrations are the same throughout the biofilm reactor. In reality, an oxygen gradient will occur throughout the biofilm, in such a way that the nitrite oxidizers in the inner part of the biofilm are faced with oxygen depletion, while still sufficient oxygen is provided to the ammonium oxidizers at the outer layers of the biofilm. As a result, only nitrite formation is also possible at high sludge retention times. This could be modelled more accurately with biofilm models of higher dimension, which take into account spatial variations.

On the other hand, an advantage of studying the potential of 0-dimensional models, as is done in this contribution, is that rigorous criteria hold to describe their behaviour. It is expected that the obtained results with respect to biomass competition in distinct biofilm reactors governed by different oxygen levels and in a single reactor displaying a varying oxygen level can later be generalized for biofilm systems in which an oxygen gradient has been measured. Regarding the dualistic character of the distribution of different ammonium and nitrite oxidizing species in time (as in our contribution) or in space (e.g. [21]), which are both influenced by the oxygen level, it is indeed likely that similar methodologies can be applied during the analysis of both systems.

3.2.2. Model calibration to describe the behaviour of the R30 reactor—
influence of microbial growth parameters

The survival of only the ammonium oxidizer AOB1 at low oxygen concentrations can describe the occurrence of Nitrosomonas europa in the R30 reactor (Fig. 4 vs. Fig. 2). For high oxygen concentrations, as realized upon lowering the influent load, both AOB2 (Nitrosomonas sp.) and NOB (Nitrosira) colonize the reactor. Recall that precise oxygen measurements have not been recorded on-line, but it has been verified that the initial oxygen level in the reactor was indeed limited (<1 g O2 m\(^{-3}\)) and that this was not longer the case after lowering the influent flow rate. In the simulations, the oxygen level in the reactor has been set to 0.2 g O2 m\(^{-3}\) initially and to 5 g O2 m\(^{-3}\) after the operation shift, which generates the experimentally observed behaviour. The sludge retention time was set to 12 days, the highest possible value for which the given model still possesses an operating zone in which AOB1 can survive without ingrowth of NOB.

Microbial parameter values for AOB1 and NOB have been based on literature values (Table 1). The yield coefficient and the ammonium affinity constant for AOB2 have been assumed equal to the ones for AOB1. The competition between AOB1 and AOB2 will thus be governed solely by their different oxygen affinity constants and maximum growth rates (see further).

Model calibration has been performed in the following way. Firstly, the nitrite/nitrate concentration dynamics after the shift in operation of reactor R30 has been calibrated to the experimental data by adjusting the initial amount of nitrite oxidizers in the reactor, which on its turn determines the amount of NOB present at the time of the shift (after 4 months, say 120 days). The initial nitrifying population was assumed to consist of 75% ammonium oxidizers and 25% nitrite oxidizers, corresponding with the number of electrons involved in the oxidation of ammonium and nitrite and in this way with biomass yield coefficients. At the same time it was assumed that equal amounts of AOB1 and AOB2 were present. Initial concentrations of 300 g COD m\(^{-3}\) total AOB and 100 g COD m\(^{-3}\) NOB were found to reasonably simulate the desired behaviour (Fig. 5b and c).

The dynamics of interspecies competition, reflected in the concentrations of the individual AOB1 and AOB2 (Fig. 3b) has been assessed subsequently. In a first series of simulations (Fig. 5), the oxygen affinity constant and the maximum growth rate of AOB2 have been varied in such a way that the oxygen concentration in the reactor at which the competition switch occurs, \( S_{O2}^{comp} \), stays constant (at 0.6 g O2 m\(^{-3}\)). An increasing \( K_{O2}^{AOB2} \) therefore also implies an increasing \( \mu_{AOB2 max} \), which means that at low oxygen concentrations, AOB2 further loses competitive advantage compared to AOB1, but gains competitive power at higher oxygen concentrations. During initial operation, the total AOB concentration is still so low compared to the amount of ammonium that needs to be converted, that both AOB1 and AOB2 have a chance to grow (increasing concentrations \( X_{AOB2} \), see Fig. 5f and g). Afterwards, the concentration of AOB2 starts to decrease, although this decrease occurs later and proceeds more slowly as the microbial parameters of AOB2 are closer to the ones of AOB1 (less pronounced competitive advantage). This results in a decreasing AOB2:AOB1 ratio at the end of the first operation period (day 120) for increasing \( K_{O2}^{AOB2} \) (Fig. 5h). After the operation shift to higher oxygen concentrations, the concentration of AOB2 increases at the expense of AOB1. This increase is more pronounced (steeper slope) as AOB2 has a higher growth rate \( \mu_{AOB2 max} \) (increasing competitive advantage of AOB2 at high oxygen concentrations). Only in case \( K_{O2}^{AOB2} = 0.4 g O2 m^{-3} \), where the parameters of AOB2 are very close to the ones of AOB1, an initial decrease of AOB2 after the shift is noted along with the fact that the total AOB concentration at this point is higher than its steady state concentrations corresponding with the new operating conditions (lower influent load).

It is very interesting to see that the different AOB2 parameters do not affect the reactor behaviour on the level of concentrations of nitrogen compounds, nor the total biomass concentrations. The latter should however be put in the right context: in case a different yield coefficient is attributed to the individual AOB species, the total AOB concentration will not fully attain steady state as long as the individual AOB1 and AOB2 concentrations vary. It is further remarkable that relatively small parameter changes can cause largely different results in terms of interspecies distributions, in this case AOB1 and AOB2.

The effect of the oxygen concentration for competition switch, \( S_{O2}^{comp} \), is assessed in Fig. 6. \( K_{O2}^{AOB2} \) was kept constant. An increasing \( S_{O2}^{comp} \) corresponds with a AOB1–AOB2 competition shift at higher oxygen concentrations, in this case because of a lower maximum growth rate \( \mu_{AOB2 max} \). The operating region in which AOB1 wins the
competition becomes larger for increasing $S_{\text{comp}}$; the higher competitive advantage of AOB1 compared to AOB2, essentially results in lower AOB2:AOB1 ratios at all times. Note that the concentrations of soluble components and total biomass concentrations are the same as in Fig. 5.

The experimental observations, in terms of nitrite and nitrate concentrations, as well as the AOB2:AOB1 ratio, are also displayed in Figs. 5 and 6. The simulation results show a good agreement with the experimental data, not only in terms of macroscopic variables (nitrite and nitrate concentrations) but also regarding the microbial community composition, more specifically the relative amount of the two types of ammonium oxidizers. It is important to note here that SSCP profiles represent relative abundances and hence do not allow to draw conclusions on absolute values of biomass concentrations. Comparison of the experimental data with the simulation results in Figs. 5f, g and 6a and b are therefore not possible. The AOB2:AOB1 ratio is reproduced very well by the simulations for $S_{\text{comp}} = 0.6 \text{ gO}_2 \text{ m}^{-3}$, $K_{\text{AOB2}} = 1 \text{ gO}_2 \text{ m}^{-3}$ and $\mu_{\text{max, AOB2}} = 2.41 \text{ d}^{-1}$ (Figs. 5h and 6c). The applied parameter values for AOB2 are in the same range from the ones applied to AOB1 and other literature values (e.g. $K_{\text{AOB2}} = 0.6 \text{ gO}_2 \text{ m}^{-3}$ and $\mu_{\text{max, AOB2}} = 2.05 \text{ d}^{-1}$ used by [15]). Note however that the model parameters are not necessarily uniquely identifiable.

### 3.2.3. Model validation to the R10 reactor

It is now examined to which extent the model, calibrated for R30, is able to simulate the behaviour of the R10 reactor, operated at a loading rate of $1420 \text{ g NH}_4^+ \text{ N m}^{-3} \text{ d}^{-1}$, which is in between...
the loading rates applied in the R30 reactor before and after the shift. Therefore, an intermediate oxygen level of 3 g O₂ m⁻³ has been assumed to prevail in the R10 reactor (exact measurements have not been recorded but were verified to be in a range corresponding with what is expected for complete ammonium oxidation to nitrate). The same initial ammonium, nitrate, nitrite and biomass concentrations have been assumed as for the R30 reactor. Fig. 7 displays the simulation results for the R10 reactor. The results for the R30 reactor during its initial operation (before decreasing the influent loading rate) have been discussed previously and are taken up for comparison. The conditions at day 120 correspond to the experimental data in Fig. 2. At this moment, in reactor R10 almost all ammonium (97%) is converted to nitrate, hardly any nitrite (<1%) is built up in the reactor (the remaining ammonium is used for incorporation in biomass). This corresponds to the experimentally observed macroscopic behaviour in terms of these components, as well as to the fact that nitrite oxidizers (NOB) were present in this reactor (Fig. 2). The total ammonium oxidizing biomass in R10 is lower than in R30 because of the lower loading rate applied (less ammonium converted per unit of time). Regarding the type of ammonium oxidizers present after the start-up period of 4 months (120 days), AOB2 is the dominating species in reactor R10, which corresponds to the observed presence of Nitrospira sp. (Fig. 2). At this moment, the amount of AOB1 (Nitrosomonas europaea) in reactor R10 is very small: the ratio AOB1:AOB2 is less than 2%. So also in terms of microbial populations, the simulated behaviour is in accordance with the experimental observations.

4. Conclusions and perspectives

This contribution deals with microbial competition in two nitrifying reactors of the ITBR (inverse turbulent bed reactor) type, which configuration only differs in the applied solid hold-up. Experimental evidence is presented, showing that different process conditions favour the selection of different types of bacteria. The most densely packed reactor (R30), which was observed to accumulate nitrite, contained the ammonium oxidizer N. europaea as the only dominant species. In the reactor with a lower solid hold-up (R10), ammonium was almost completely converted to nitrate, so also nitrite oxidizers (Nitrospira) were present. Besides, the major ammonium oxidizer in reactor R10, Nitrospira sp., was different from the one in reactor R30. The lower oxygen concentration in the most densely packed reactor (R30), resulting from a higher loading rate has likely caused these differences. This hypothesis is strengthened by the observation of nitrate accumulation upon lowering the loading rate in the R30 reactor, accompanied by a population shift in the ammonium oxidizers, from N. europaea to Nitrospira sp.

It has been demonstrated how these experimental observations can be explained with a simple 0-dimensional model (neglecting spatial variations), taking into account interspecies competition between two different types of ammonium oxidizers. A relatively lower oxygen affinity (higher oxygen affinity constant) and at the same time a relatively higher maximum growth rate of one AOB species compared to the other one, explain a competitive disadvantage at low oxygen concentrations and a competitive advantage at high oxygen concentrations, respectively. A particular advantage of 0-dimensional models is that straightforward criteria for the outcome of microbial competition can been formulated and analyzed, as demonstrated. A rough model calibration has been performed to simulate the behaviour of the R30 reactor. The different timescales at which changes in the concentrations of soluble components, total biomass and interspecies variations occur, are clearly reproduced by the model. The influence of microbial parameters affecting interspecies competition has been assessed explicitly. It is particularly noteworthy that the same results on a macro-scale (concentrations of soluble components and total biomass) do not imply a unique behaviour on the level of individual populations. The calibrated model was successfully validated to reproduce the behaviour of the R10 reactor.

In future, systems with more than two competing species will be studied. Extension of the presented model to this purpose is straightforward and not limited to nitrification processes. Moreover, where the present study deals with biomass distribution in time, it will further be examined to which extent the principles of interspecies competition hold in the spatial domain. This implies the use of biofilm models taking into account spatial variations (at least 1-dimensional).

Acknowledgement

Eveline Volcke has been supported by the EU through a Marie Curie Intra-European Fellowship (EIF), Proposal 039401–PopCon4–Biofilms, followed by a postdoctoral grant of the Research Foundation – Flanders (Belgium) (FWO).

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