A benchmark simulation to verify an inhibition model on decay stage for nitrification

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ABSTRACT

Activated Sludge Models (ASMs) are widely used for biological wastewater treatment plant design, optimisation and operation. In commonly used ASMs, the nitrification process is modelled as a one-step process. However, in some process configurations, it is desirable to model the concentration of nitrite nitrogen through a two-step nitrification process. In this study, the benchmark datasets published by the Water Environment Research Foundation (WERF) were used to develop a two-step nitrification model considering the kinetics of Ammonium Oxidising Bacteria (AOB) and Nitrite Oxidising Bacteria (NOB). The WERF datasets were collected from a chemostat reactor fed about 1,000 mg-NH3-N/L synthetic influent with at different sludge retention times of 20, 10 and 5-d, whereas the pH in the reactor varied in the range of 5.8 and 8.8. Supplemental laboratory batch experiments were conducted to assess the toxicity of nitrite-N on nitrifying bacteria. These tests suggested that 500 mg-N/L of nitrite at pH 7.3 was toxic to NOB and resulted in continuous decrease in bulk oxygen uptake rate. To model this phenomenon, a poisoning model was used instead of the traditional Haldane-type inhibition model. The poisoning model for NOB and AOB with different threshold poisonings for unionised NO2-N and NH3-N concentrations could successfully reproduce the three WERF datasets.

INTRODUCTION

In Activated Sludge Models (ASMs) the nitrification process is simplified as a one-step process that assumes ammonia to nitrite oxidation to be the sole rate-limiting step throughout the oxidation sequence (Henze et al. 2000; Ben-Youssef et al. 2009). However, due to successful development of nitritation processes (e.g. SHARON, Hellinga et al. (1998)), modelling of two-step nitrification (nitritation followed by nitratation) is widely focused on to improve the process performance as well as a development of direct coupling of nitritation and denitrification (Moussa et al. 2005; Iacopozzi et al. 2007; Kaelin et al. 2009; Ostace et al. 2011).

Since the early work of Anthonisen et al. (1976) indicating that the high free nitrous acid (FNA) and free ammonia (FA) inhibit the biological reactions for Ammonium Oxidising Bacteria (AOB) and Nitrite Oxidising Bacteria (NOB), various kinds of kinetic inhibition models have been proposed (Wett & Rauch 2003; Jubany et al. 2005, 2009; Park & Bae 2009; Kaelin et al. 2009; Munz et al. 2011). Most commonly the traditional Haldane-type switching function (Equation (1)) or its modification (Equation (2)) have been employed to model the substrate inhibition. One of the characteristics of these expressions is that the peak biological reaction rate (\(r\)) depends on the values of the inhibition coefficient (\(K_I\)) and the concentration of inhibitory substance (\(S\)) like FNA and FA. These empirical expressions apparently resemble enzymatic uncompetitive inhibition and non-competitive inhibition reactions respectively. At present, these kinetic expressions are employed to model the inhibition responses of AOB and NOB by FNA and FA (Makinia 2010):

\[
r_{\text{Growth}} = \frac{r_{\text{max}} S}{K_S + S} \frac{K_I}{K_I + S}
\]

(1)

\[
r_{\text{Growth}} = r_{\text{max}} S \frac{K_I}{K_S + S} \frac{1}{1 + \frac{S}{K_I}}
\]

(2)

On the other hand, unlike instantaneous enzymatic reactions, microorganisms in biological wastewater treatment...
systems adapt to shock loading, probably due to changes in the biochemical reaction in the cells (Speece 1996). In fact, Liu et al. (2011) observed that $K_1$ of NOB quickly increased along with time and finally the inhibition disappeared after several hours in the 104 batch experiments. This recovery was observed across the experiments with the initial nitrite concentration ranging from 125 to 2,000 mg-N/L at pH 7.3. Another interesting phenomenon observed here was that the bulk oxygen uptake rate decreased consistently under high nitrite concentration in spite of a corresponding decrease of FNA by nitratation, indicating that poisoning took place. These points suggest that use of traditional Haldane-type inhibition functions are not appropriate to express the biological reaction and may give potential technical problems for plant simulation. First, the phenomenon for the irreversible biomass inactivation have to be addressed rather than the reversible biomass inactivation expressed by Equation (1) and/or Equation (2). Second, if the $K_1$ changes along with time, time-dependent adaptation phenomena have to be incorporated in the equations. This means a residence time distribution (RTD) has to be considered for the individual cell particles in the wastewater treatment plant. Accordingly, the calculation procedure becomes significantly complicated. Unless occurring in a perfect plug-flow system all particles have to be tracked to solve the model. For instance, kinetically recovered biomass in the secondary clarifier returning to the aeration tank where inhibition effect is experienced. Third, even if the plant response can be simulated based on the traditional approach using the Haldane-type inhibition functions as mentioned in the above, the obtained $K_1$ would be solely apparent, which is the mixed output of plant hydraulic conditions and inherent $K_1$ of the microorganisms:

$$\begin{align*}
  r_{\text{Decay}} &= b + k
  \quad \text{for} \quad k = \frac{k_{\text{max}} P}{K_1 + P}
\end{align*} \quad (3)$$

To cope with the technical difficulty a modified NOB inhibition model including poisoning threshold was recently presented (Liu et al. 2011). In the model the Haldane-type inhibition on the growth stage was neglected since it lasted only a short time. Instead of the inhibition, a poisoning effect ($k$) was added to the bacterial inherent decay ($b$) as shown in Equation (3). To initiate the irreversible loss of biological activity under high inhibitory substances (FNA and FA), thresholds were newly defined in the rate expression using a switching function with a power coefficient ($n \geq 1$) to enhance the effect. In case of high $n$ values, Equation (3) may be rewritten as: if $S > K_1$, then $k = k_{\text{max}}$, else $k = 0$.

Since the model was developed through batch experiments, it was necessary to justify the model structure under continuous operation. For this purpose, the datasets collected by Zimmerman et al. (2004) in a Water Environment Research Foundation (WERF) benchmark project were used. These datasets corresponded to different sludge retention times (SRTs) and pHs in the reactor. The corresponding effluent qualities were simulated after individual evaluation of the response of NOB and AOB. In addition to model verification, a possible reason for the sudden unexpected decrease in NOB biomass during one of the experiments was explored.

**MATERIALS AND METHODS**

**Poisoning model structure**

The structure of the inhibition model evaluated in this study is shown in Table 1 using NOB reactions as an example. The main difference between the proposed model and the conventional inhibition models was that in the proposed model the inhibition reactions lead to irreversible biocidal effect (poisoning) while in conventional inhibition models the effect was biostatic and reversible. In other words, in the proposed model the inhibitory concentrations lead to

![Table 1: NOB inhibition model including toxicity threshold](https://iwaponline.com/wst/article-pdf/68/6/1242/472720/1242.pdf)
net reduction in active biomass amount (Equation (3)), whereas in conventional models, the biomass amount might still increase during inhibition depending on the growth rate (Equations (1) and (2)).

Data extraction from benchmark datasets

The three datasets published by Zimmerman et al. (2004) obtained from chemostat operations with SRTs of 20, 10 and 5-d feeding a synthetic inorganic wastewater ((NH4)2SO4 996–1,449 mg-N/L; Na2HPO4 2,481 mg/L; KH2PO4 1,215 mg/L; NaHCO3 2,453 mg/L; trace elements of Ca, Mg, Mn and Fe) were used as a benchmark for model verification. In the three operations the bioreactors were seeded with activated sludge containing nitrifiers.

The bulk pH in the reactor was dynamically changed using the alkalinity in the influent during operation to observe its effect on nitritation and nitratation. The 20, 10 and 5-d SRT continuous tests were operated for 260, 252 and 76 d respectively. The temperature and dissolved oxygen (DO) in the reactors were maintained at 20 ± 2 °C and 8.0 ± 2 mg-DO/L, whereas the pHs in the reactors were 5.8–8.2, 6.1–8.8 and 6.9–8.8 respectively. Influent and effluent total volatile solids (TVS) concentration, and effluent alkalinity were measured at regular intervals in each operation. The datasets were electronically scanned and imported into a Microsoft Excel spreadsheet.

The effluent concentrations of nitrogen compounds, biomass (AOB and NOB) and corresponding kinetics were calculated according to the influent load and the effluent ammonia and nitrate concentrations from the datasets using Equations (4) and (5). The schematic representation of the system is illustrated in Figure 1:

\[
\begin{align*}
\frac{dS_{\text{NH4}}}{dt} & = -r_{\text{AOB}} + D \cdot (S_{\text{NH4}\text{-inf}} - S_{\text{NH4}\text{-eff}}) \\
\frac{dS_{\text{NO3}}}{dt} & = +r_{\text{NOB}} - D \cdot S_{\text{NO3}\text{-eff}}
\end{align*}
\]

Dynamic simulation

The corresponding experimental conditions of the datasets were input into GPS-X (Hydromantis Environmental Software Solutions, Inc., Canada) simulation software and the response of effluent qualities were compared to the data plots. Unionised nitrous acid (FNA) and ammonia (FA) were calculated from Equations (6) and (7) (Anthonisen et al. 1976):

\[
\begin{align*}
\text{FNA} &= \frac{S_{\text{NO2}}}{K_a \times 10^{pH}} \\
K_a &= \exp(-2300/(273 + T))
\end{align*}
\]

\[
\begin{align*}
\text{FA} &= \frac{S_{\text{NH4}} \times 10^{pH}}{K_b/K_w + 10^{pH}} \\
K_b &= \exp(6344/(273 + T))
\end{align*}
\]

where \(r_{\text{AOB}}\): volumetric AOB reaction rate (mg-N/L/d), \(D\): dilution rate (d \(^{-1}\)), \(S_{\text{NH4}\text{-inf}}\): influent total ammonium concentration (mg-N/L), \(S_{\text{NH4}\text{-eff}}\): effluent total ammonium concentration (mg-N/L), \(r_{\text{NOB}}\): volumetric NOB reaction rate (mg-N/L/d), \(S_{\text{NO3}\text{-eff}}\): effluent total nitrate concentration (mg-N/L).

Based on the inhibition model mentioned in Table 1, the volumetric reaction rates for AOB and NOB were individually calculated using the bulk nitrogenous concentrations (\(S_{\text{NH4}}\) and \(S_{\text{NO2}}\)). These rates were compared to those extracted from the datasets. After calibrating the individual response of each microorganisms, dynamic simulations were conducted using the reactions of both AOB and NOB. The model calibration was conducted manually by mainly focusing on the periods when the reaction rates/effluent concentrations changed remarkably. It was likely that the estimated parameters through this manual procedure could be further improved using statistical methods of parameters estimation. The initial biomass concentration of AOB and NOB were estimated based on the initial response of OUR (oxygen uptake rate, mg-O2/L/d). In preliminary simulations, it was observed that for all the three datasets, the initial NOB concentration of more than 5 mg-COD/L resulted in initial OUR responses substantially different than the measured OUR. On the other hand, the initial concentration of AOB did not affect the initial response as much. Based on these results and considering that the initial measured volatile suspended solids (VSS) concentrations in the reactor were about...
10–20 mg/L, it was decided to use 5 mg-COD/L as the initial concentration for the both the microorganisms.

For the 5-d experimental dataset, as the effluent ammonia and nitrate were only monitored in the initial phase and nitrate was not produced, the produced nitrite in the period was calculated from the decrement of ammonia concentration between the influent and effluent. To calibrate the nitrifier biomass yield coefficient, a typical ratio of COD to activated sludge VSS was used at a conversion factor of 1.42 \((\text{Speece \& McCarty 1964})\). Until reasonable matching of the calculated curves and data plots, individual kinetic coefficients were calibrated. Since preliminary calibration revealed that inhibition for AOB was rather limited in the operating conditions, NOB was mainly focused on the model verification unless otherwise specified.

In addition to the model verification, with respect to the dataset for 10-d SRT, an unexpected partial loss of nitrification after 170 d was reported in the publication. In the period nitrite oxidation suddenly deteriorated although the bulk concentration for FNA, FA and pH were certainly at acceptable levels that has given sufficient nitrite conversion until that day. Based on this, it was deduced that the activity loss was not attributed to the inhibition of the non-ionised compounds. Accordingly an inhibition-independent microbial lysis (e.g. due to infection by Bacteriophage and/or Bdellovibrio spp.) was assumed to be the third type of decay \((=k_3 \times X_{\text{NOB}})\) in order to conduct the simulation adopted from Starr \& Baigent (1966), van Loosdrecht \& Henze (1999) and Moussa et al. (2005).

**RESULTS AND DISCUSSION**

**Individual evaluation for the benchmark data simulation**

For the 20-d SRT experiment (Figure 2), the \(r_{\text{AOB}}\) consistently increased until day 30 after start-up, and peaked at days 40–50. The bulk concentration of ammonia decreased from 1,000 mgN/L to about 200 mgN/L during this period. The decrease in both the bulk ammonia concentration and the pH resulted in significant decrease in FA concentration. The nitrite nitrogen correspondingly increased up to 700 mg-N/L while the conversion of nitrite to nitrate was limited to less than 100 mg-N/L. Since the accumulation of FA lasted for only 20–30 d, AOB growth could be simulated without considering the inhibition effect of FA. On the other hand, the low values of \(r_{\text{NOB}}\) were mainly attributed to NOB poisoning by FA that reduced its overall specific growth rate. Consequently the nitrite produced by the AOB did not get oxidised by NOB. Since the \(r_{\text{NOB}}\) was inhibited even after FA inhibition disappeared, this inhibition was attributed to FNA. After the disappearance of both the FA and FNA inhibitory conditions (after day 100), the \(r_{\text{NOB}}\) increased exponentially leading to production of nitrate.
After day 100, the FA concentration in the bulk liquid was about 10 mgN/L while almost no nitrite-N was detected. After day 180, complete conversion of both bulk ammonia and nitrite-N were observed. The effluent nitrate concentration was reasonably simulated using the model. Without incorporating the poisoning phenomena, the $r_{\text{NOB}}$ was remarkably overestimated in the initial phase.

For the 10-d SRT experiment (Figure 3), a consistent increase of $r_{\text{AOB}}$ was observed for the initial 50 d after an initial lag. Correspondingly, the bulk ammonia concentration reduced from 1,000 mg-N/L to about 100–200 mg-N/L. The observed high pH during the initial phase resulted in significantly high FA concentration affecting $r_{\text{NOB}}$. After 50 d, the bulk pH reduced from 9 to 6 due to nitrification leading to drop in FA concentration. The accumulation of nitrite-N along with a reduced pH resulted in an increase in the FNA concentration. The accumulation of FNA significantly inhibited the reaction of NOB as well as AOB in this period and as a result the nitrite concentration in the reactor was observed to increase up to 600–700 mg-N/L. Again without the poisoning phenomenon, the delay in nitratation could not be reproduced. From day 50 to day 100, the $r_{\text{AOB}}$ decreased consistently due to reduced influent ammonium load. Because of the reduced $r_{\text{AOB}}$, the FNA concentration in the reactor reduced leading to minimisation of FNA inhibition on NOB. This resulted in an observed increase in the $r_{\text{NOB}}$ during this period. Between day 150 and day 140, accumulation of FA was observed. This accumulation was attributed to the imbalance between the influent ammonium load and $r_{\text{AOB}}$. The observed FA accumulation caused NOB inhibition and led to temporary pause in the increasing trend of $r_{\text{NOB}}$. As both the FA and FNA concentrations stabilised below inhibitory concentrations, complete nitrite conversion to nitrate was achieved after day 140.

From day 170 an unexpected $r_{\text{NOB}}$ drop occurred and lasted until day 220 as indicated by the dashed line. During this period, FNA and FA were sufficiently low and comparable to those of day 150. In fact, the other periods operated under much higher FNA and FA showed reasonable nitratation. Since the causative reason for this sudden decrease in $r_{\text{NOB}}$ could not be explained through available kinetic inhibition concepts for FNA, FA and pH, it was speculated that cell lysis suddenly appeared due to infection by Bacteriophage and/or Bdellovibrio spp. contaminating the reactor biomass. Such phenomena occasionally occur in wastewater treatment systems and specified biomass activity was lost accordingly (Dias & Bhat 1965; Ewert & Paynter 1980; Hantula et al. 1991; Khan et al. 2002). In particular Bdellovibrio spp. are known parasites that reduce activity of Gram-negative bacteria, like NOB (Stolp & Starr 1965). Based on this assumption, a simplified lysis rate expression (specific lysis rate = $k_{\text{l}}X_{\text{NOB}}$) was added to model the event during this period. Inclusion of additional expression of lysis for NOB could simulate the response.

![Figure 3](https://iwaponline.com/wst/article-pdf/68/6/1242/472720/1242.pdf)
for the period of 170–230 d, however, stabilisation period after day 230 could not be simulated well. Considering these results, it was not possible to explain the observed data after day 170 with the modified model. It is likely that the modelling of predation/infection lysis requires a more sophisticated model including the population of predator. This topic would be of importance to design robust wastewater treatment processes where specific microbial species and cultures grow (van Loosdrecht & Henze 1999; Moussa et al. 2005).

With respect to the 5-d SRT experiment (Figure 4), the initial AOB activities were significantly inhibited due to the presence of high FA. This period lasted until day 30 when the FA concentration reduced due to the lowering of pH in the bulk. Based on this period, the FA inhibition kinetics for AOB was calibrated. Through further intentional pH control, the FA concentration reached an acceptable level for AOB and significant production of nitrite started from day 30. Even after the improvement of AOB activity, the bulk ammonia concentration remained over 900 mg-N/L throughout the operational period. With respect to NOB, because of high FA and FNA, NOB could not grow in the system and were ultimately washed out. After day 30, the response of $r_{A Oxygen}$ corresponded to the influent ammonium load and could be reproduced without considering FNA inhibition during this period.

**Model verification in the nitrification process**

To complete the model verification, simulations using the reactions from both AOB and NOB were conducted as shown in Figure 5. The effluent ammonium, nitrite and nitrate were reasonably reproduced from the model for the three datasets. The coefficients of the model used in the study are summarised in Table 2. For biomass yield coefficients of AOB the maximum specific growth rates were close to the literature values ($Y_{AOB} = 0.25$, $Y_{NOB} = 0.03$, $Y_{Nitrifier} = 0.80$ d$^{-1}$) (Stensel & Barnard 1992; Henze et al. 2000). However the TVS concentrations were underestimated in the period after 120 d for the 20-d SRT experiment. As biomass in the system during the period was relatively stable, since complete nitrification was achieved, the discrepancy was considered to be attributed to the influent TVS fraction in the synthetic inorganic wastewater having high calcium salts used by Zimmerman et al. (2004).

Calibration of the half-saturation coefficients was needed to meet the dynamic responses and the values could be used across the three datasets. The specific decay rates ($b_{AOB}$ and $b_{NOB}$) were not calibrated and literature values were used (Henze et al. 2000; Liu et al. 2011). With respect to the poisoning coefficients, in the case that the non-ionised concentration of ammonia is the same as that of nitrite, it seemed that the poisoning impact of FA became dominant when it exceeded about 10 mg-N/L.
Figure 5 | Benchmark simulation results for 20-d SRT (left), 10-d SRT (middle) and 5-d SRT (right) (+: ammonium, △: nitrite, ○: nitrate).

Table 2 | Coefficient list used in the inhibition model

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Value</th>
<th>20-d SRT</th>
<th>10-d SRT</th>
<th>5-d SRT</th>
<th>Unit</th>
<th>References</th>
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<td>AOB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>$Y_{AOB}$</td>
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<td>←</td>
<td>←</td>
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<tr>
<td>$\mu_{\text{max},AOB}$</td>
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<td>←</td>
<td>←</td>
<td>←</td>
<td>d$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$K_{S,NH3,AOB}$</td>
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<td>←</td>
<td>←</td>
<td>mg-N/L</td>
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<tr>
<td>$b_{AOB}$</td>
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<td>←</td>
<td>←</td>
<td>←</td>
<td>d$^{-1}$</td>
<td></td>
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<tr>
<td>FNA poisoning for AOB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{\text{max},FNA,AOB}$</td>
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<td>0.8</td>
<td>Not used</td>
<td>←</td>
<td>d$^{-1}$</td>
<td></td>
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<td>0.1</td>
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<td>←</td>
<td>mg-N/L</td>
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<td>$n_{FNA, AOB}$</td>
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<td>ditto</td>
<td>←</td>
<td>←</td>
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<tr>
<td>FA poisoning for AOB</td>
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<tr>
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<td>5</td>
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<td>←</td>
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<td>NOB</td>
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<td>←</td>
<td>←</td>
<td>←</td>
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<tr>
<td>$K_{S,NO2,NOB}$</td>
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<td>←</td>
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<tr>
<td>$k_{\text{max},FNA,NOB}$</td>
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<td>←</td>
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<td>←</td>
<td>←</td>
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<tr>
<td>$n_{FA, NOB}$</td>
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<td>ditto</td>
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<td>Additional lysis (?)</td>
<td>$k_3$</td>
<td>←</td>
<td>0.37</td>
<td>←</td>
<td>d$^{-1}$</td>
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</table>

ditto: same as the above; ←←: same as the left.

References:
- Henze et al. (2000) (as dominant nitrifier biomass)
- Liu et al. (2011)
whereas the impact of FNA initiated from about 1 mg-N/L as shown in Figure 6.

Since different sets of kinetic parameters were required to simulate the datasets of 5, 10 and 20 d, it may be questioned whether the model structure was adequately validated. It is fair to assume that the model structure may need further improvement to be applicable to various operational conditions with a single parameter set. It is however worth noting that need for change of parameters also often arises in simulating with the well established activated sludge and anaerobic digestion models. One of the notable causes for requirement for different parameter sets lies in the fact that all models use simplifying assumptions and do not capture all the complexities of the multiple species biological processes. In the case of nitrifiers, the observed variation in the value of kinetic coefficients is quite significant depending on the bacterial species. For example, the reported variation in the maximum specific reaction rate for AOB: 0.24–9,100 μM/g-cell/h and half-saturation coefficient for AOB: 34–3,970 μM/L (Urakawa et al. 2010) and maximum tolerated nitrite concentration for NOB 6–25 μM/L (Daims et al. 2010) are common. Therefore, the observed difference in the kinetics parameters may very well be due to the differences in the participating species of NOB at different SRT.

CONCLUSIONS

This research presented an alternative model using poisoning concept to simulate the inhibition effects of FA and FNA on nitrifiers. Although Haldane-type inhibition is traditionally used to model the inhibition of ammonia oxidation and nitrite oxidation processes, it appeared that the alternative poisoning model with poisoning concentration threshold could also express the nitrifier’s response reasonably well. Since the model assumed an irreversible inactivation of the microorganisms, further experimental validation involving characterisation of active/inactive cells could strengthen the concept. Apart from substrate inhibition of nitrifiers, additional mechanisms for microbial lysis which might be due to predator infection needs further investigation. The results obtained in the study are the first step in modelling the phenomenon of irreversible inhibition and will encourage more research and advancement of the knowledge.

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