Development of the modified activated sludge model
describing nitrite inhibition of aerobic phosphate uptake
Y. Yoshida, Y. Kim, T. Saito and K. Tanaka

ABSTRACT

Since metabolic pathway and enzymatic clarification of poly-phosphate accumulating organisms (PAOs) are still unclear, biological phosphorus removal (BPR) sometimes become unstable. We have focused on nitrite as one of unknown factors deteriorating BPR performance. And we obtained some findings from previous studies, namely 1) nitrite inhibits phosphate uptake and growth of PAOs, 2) nitrite inhibition persists even after nitrite disappearance, 3) PAOs with the higher relative anoxic activity are less sensitive to nitrite exposure. This study provides the modified Activated Sludge Model No.2d (ASM2d) to express nitrite inhibition properly by incorporating the assumed intracellular reaction product (reaction complex) of nitrite. The model also considers the tolerance mechanism of nitrite inhibition by the denitrifying activity of PAOs.

From the results of previous experiments and relevant references, the modified model called "Nitrite-Complex model" incorporates new components, namely nitrite ($S_{NO_2}$) and reaction complex ($X_{complex}$), to ASM2d. The model incorporation only nitrite inhibition was able to fit with measured phosphate concentration while nitrite exists. Whereas, this model couldn't describe the persistence of nitrite inhibition after nitrite disappeared. However, result of nitrite-complex model incorporating nitrite and reaction complex inhibition, it fits well with measured phosphate concentration not only before nitrite disappeared but also after nitrite disappeared.

Key words | activated sludge model, aerobic phosphate uptake, biological phosphorus removal, denitrifying Poly-phosphate accumulating organisms, inhibition, nitrite, sequencing batch reactor

INTRODUCTION

Since metabolic pathway and enzymatic clarification of poly-phosphate accumulating organisms (PAOs) are still unclear, biological phosphorus removal (BPR) sometimes become unstable. Several researchers have proposed some mechanisms, namely 1) competition with glycogen-accumulating organisms (GAOs) (Liu et al. 1997), 2) low influent organic loading (Kuba et al. 1997), 3) introduction of nitrate into anaerobic tank (Hascoet & Florentz 1985), and 4) excessive aeration (Brdjianovic et al. 1998), however, it is widely accepted that there are still unknown factors inhibiting BPR. Also, there are several reports insisting that nitrite inhibits anoxic phosphate uptake (Kuba et al. 1996a; Meinhold et al. 1999). Moreover, recently there are few reports informed that nitrite inhibits aerobic phosphate uptake (Saito et al. 2004; Yoshida et al. 2006). We have focused on nitrite which is known as an inhibitor of microbial metabolism in order to investigate the effect of nitrite as one of unknown factor deteriorating BPR performance. From the results of previous experiments using synthetic and municipal wastewater, the significant decrease of the aerobic phosphate uptake activity (Aerobic PUA) and the increase of the anoxic phosphate uptake activity (Anoxic PUA) by nitrite exposure were observed (Saito et al. 2005). Hence, batch tests of aerobic phosphate uptake were conducted.
uptake with oxygen uptake rate (OUR) measurements in the presence and absence of nitrite were conducted using the enriched PAOs with different relative anoxic activities (Anoxic PUA/Aerobic PUA). From these tests, the following findings were obtained (Saito et al. 2005; Yoshida et al. 2006). 1) In spite of aerobic condition, nitrite was consumed by the reaction which is different from nitrification. 2) In the case of nitrite addition, OUR and phosphate uptake rate (PUR) continuously lower from the start of experiment. Then, both of these rates increased after nitrite disappearance. Therefore, nitrite inhibits not only phosphate uptake but also growth of PAOs. This is because the energy produced by oxygen respiration is used for several activities of PAOs, namely poly-phosphate storage, glycogen restore, cell growth and maintenance. 3) The PUR after nitrite disappearance was lower than the PUR in the case of nitrite absence. Therefore nitrite inhibition continues after nitrite disappearance. This indicated that nitrite inhibition is occurred by not only nitrite but also intracellular reaction product. 4) PAOs with the higher relative anoxic activity were less sensitive to nitrite exposure, and also had higher nitrite consumption rate. 5) From calculation results of utilization efficiency of oxygen and nitrite as the electron accepter in these batch tests, it was indicated that some of PAOs with higher anoxic activity can utilize nitrite as the electron accepter under aerobic condition.

This paper proposes the development of the modified Activated Sludge Model No.2d (ASM2d) to express nitrite inhibition by incorporating the intracellular reaction product (reaction complex) of PAOs. Also, using this model, the mechanisms of high tolerability of the denitrifying activity of PAOs to nitrite were examined.

MODEL DEVELOPMENT

Concept of nitrite inhibition on aerobic phosphate uptake

The “concept of inhibition” is quite important to construct the inhibition model. Generally, oxygen inhibits reduction of nitrogen oxide e.g. nitrate and nitrite. However, some of gram-negative facultative anaerobic heterotrophic bacteria can reduce nitrite even under aerobic condition (Kucera et al. 1987). This is because nitrite reductase (NIR) exists on the surface of periplasmic side of cytoplasmic membrane. Also, it is known that nitrate can not be reduced under aerobic condition because catalytic site of nitrate reductase (NAR) exists on cytoplasmic side of the membrane (Alefounder & Ferguson 1980). One of the inhibition mechanisms by nitrite is the inhibition caused by nitric oxide produced from aerobic nitrite reduction (denitrification). Nitric oxide reacts with the oxygen respiration reductase and its reaction product inhibits oxygen respiration (Carr & Ferguson 1990; Casey et al. 1999). About PAOs, the details of their metabolism are not clear, because PAOs has never been isolated yet. Candidatus Accumulibacter phosphatis that is a gram-negative organism is confirmed as one of PAOs by molecular biological approach (Hesselmann et al. 1999; Crocetti et al. 2000). It is confirmed that they exist in the wastewater treatment plant. Moreover, Zeng et al. (2005a) reported that this species are 40% of dominance in the anaerobic/anoxic SBR (A/A-SBR) using synthetic wastewater. Also, several researchers reported that PAOs which has denitrifying activity can utilize nitrite as the electron accepter under anoxic condition (Meinhold et al. 1999; Ahn et al. 2001; Lee et al. 2001).

From the knowledge above, if PAOs also has nitrite reductase on the surface of their periplasmic side as shown in Figure 1, it is possible to assume that PAOs can utilize nitrite under aerobic condition and produce nitric oxide. Although whether only nitric oxide or both of nitrite and nitric oxide inhibits oxygen respiration is unclear, any of them reacts with the oxygen respiration reductase and its reaction product inhibits oxygen respiration. Consequently, activities of PAOs are inhibited by the reduced oxygen.

![Figure 1](https://iwaponline.com/wst/article-pdf/59/4/621/436886/621.pdf)
respiration as shown in Figure 2. This is the assumed mechanism of nitrite inhibition of aerobic phosphate uptake.

Construction of inhibition model

According to the previous experiments and relevant references as above, the inhibition model was developed on the basis of IWA Activated Sludge Model No.2d (ASM2d) (Henze et al. 1999). Characteristics of the proposed model are as follows; 1) New components e.g. nitrite ($S_{NO_2}$) and reaction complex ($X_{complex}$) are incorporated. 2) Persistence of inhibition after nitrite disappearance is expressed by incorporating the inhibition caused by $X_{complex}$, which is assumed to be formed through nitrite denitrification inside of PAOs. 3) Recovery from $X_{complex}$ inhibition is expressed by denitrification of $X_{complex}$. Table 1 shows the newly-incorporated components, kinetic constants and parameters. Unit of these are based on ASM2d. $K_{NO_2,PN}$ is the saturation constant. $K_{NO_2,PAO}$, $q_{DN,NO_2}$, $K_{complex}$ and $h_{complex}$ are variables which vary in each case of batch tests according to anoxic activity of PAOs especially. Table 2 shows the newly-incorporated process descriptions of the inhibition model. They are based on the reaction processes relating to PAOs ($X_{PAO}$) in ASM2d. Inhibitions by $S_{NO_2}$ ([A] in the table) and $X_{complex}$ ([B] in the table) are expressed, respectively in Process [2] and [3], as the noncompetitive inhibition by using Monod-type inhibition terms to the default equation of ASM2d. And these were expressed by not absolute concentration but specific concentration (mg$S_{NO_2}$/g$X_{PAO}$ and mg$X_{complex}$/g$X_{PAO}$). Because Meinhold et al. (1999) reported that using specific concentration is much better than using absolute one to express the effect of nitrite on anoxic phosphate uptake. Moreover, Saito et al. (2004) also recommended that specific one is probably better, because nitrite effect is rather toxic than inhibitory. Process [4] was added to depict the production of $X_{complex}$ which accompanies aerobic $S_{NO_2}$ consumption (aerobic denitrification) ([C] in the table). Process [5] and [6] are the processes describing anoxic storage of $X_{PP}$ and anoxic growth of $X_{PAO}$ with $X_{complex}$ respectively. They are the modified kinetic equation which describe anoxic storage of $X_{PP}$ and anoxic growth of $X_{PAO}$ in ASM2d, respectively. These processes do not include the effect of nitrate and the adverse effect of oxygen at the moment, because we do not confirm yet the behavior of $X_{complex}$ under anoxic nor low dissolved oxygen condition.

Summary of the proposed inhibition model

To summarize the above-mentioned inhibition model simply, as shown Figure 3, this model progresses in 4 steps, namely (1) nitrite inhibits aerobic activities of PAOs, and the size of inhibition is expressed by the equation

---

**Table 1 | Independently installed component and kinetic parameter**

<table>
<thead>
<tr>
<th>Added component</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_{NO_2}$</td>
<td>gN/m$^3$</td>
</tr>
<tr>
<td>$X_{complex}$</td>
<td>gN/m$^3$</td>
</tr>
<tr>
<td>Added kinetic constant/parameter</td>
<td>Unit</td>
</tr>
<tr>
<td>$K_{NO_2,PAO}$</td>
<td>mg$S_{NO_2}$/g$X_{PAO}$</td>
</tr>
<tr>
<td>$q_{DN,NO_2}$</td>
<td>g$S_{NO_2}$/g$X_{PAO}$/d</td>
</tr>
<tr>
<td>$K_{NO_2,PN}$</td>
<td>GN/m$^3$</td>
</tr>
<tr>
<td>$K_{complex}$</td>
<td>mg$X_{complex}$/g$X_{PAO}$</td>
</tr>
<tr>
<td>$h_{complex}$</td>
<td>Reduction factor for $X_{complex}$ utilization</td>
</tr>
</tbody>
</table>
Table 2 | Process rate equation for ‘Nitrite model’ and ‘Nitrite-Complex model’

<table>
<thead>
<tr>
<th>Process</th>
<th>Kinetic equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Storage of $X_{PHA}$</td>
<td>( q_{PHA} \cdot \frac{S_{O_2}}{K_{O_2} + S_{O_2}} \cdot \frac{S_{PO_4}}{K_{PO_4} + S_{PO_4}} \cdot \frac{S_{ALK}}{K_{ALK} + S_{ALK}} \cdot \frac{X_{PHA}}{K_{PHA} + X_{PHA}} \cdot X_{PAO} \cdot K_{MAX} - \frac{X_{PAO}}{K_{MAX}} \cdot X_{PAO} ).</td>
</tr>
<tr>
<td>2 Aerobic storage of $X_{PP}$</td>
<td>( q_{PP} \cdot \frac{S_{O_2}}{K_{O_2} + S_{O_2}} \cdot \frac{S_{PO_4}}{K_{PO_4} + S_{PO_4}} \cdot \frac{S_{ALK}}{K_{ALK} + S_{ALK}} \cdot \frac{X_{PHA}}{K_{PHA} + X_{PHA}} \cdot X_{PAO} \cdot K_{MAX} - \frac{X_{PAO}}{K_{MAX}} \cdot X_{PAO} ).</td>
</tr>
<tr>
<td>3 Aerobic growth of $X_{PAO}$</td>
<td>( \mu_{PAO} \cdot \frac{S_{O_2}}{K_{O_2} + S_{O_2}} \cdot \frac{S_{NH_4}}{K_{NH_4} + S_{NH_4}} \cdot \frac{S_{PO_4}}{K_{PO_4} + S_{PO_4}} \cdot \frac{S_{ALK}}{K_{ALK} + S_{ALK}} \cdot \frac{X_{PHA}}{K_{PHA} + X_{PHA}} \cdot X_{PAO} \cdot K_{MAX} - \frac{X_{PAO}}{K_{MAX}} \cdot X_{PAO} ).</td>
</tr>
<tr>
<td>4 Aerobic convert (denitrification) from $S_{NO_2}$ to $X_{complex}$</td>
<td>( q_{DN,NO_2} \cdot \frac{S_{NO_2}}{K_{NO_2,NO_2} + S_{NO_2}} \cdot \frac{S_{O_2}}{K_{O_2} + S_{O_2}} \cdot \frac{S_{NH_4}}{K_{NH_4} + S_{NH_4}} \cdot \frac{S_{PO_4}}{K_{PO_4} + S_{PO_4}} \cdot \frac{S_{ALK}}{K_{ALK} + S_{ALK}} \cdot \frac{X_{PHA}}{K_{PHA} + X_{PHA}} \cdot X_{PAO} \cdot K_{MAX} - \frac{X_{PAO}}{K_{MAX}} \cdot X_{PAO} ).</td>
</tr>
<tr>
<td>5 Storage of $X_{PP}$ using $X_{complex}$</td>
<td>( q_{PP} \cdot \frac{X_{complex}}{K_{complex} + X_{complex}} \cdot \frac{S_{PO_4}}{K_{PO_4} + S_{PO_4}} \cdot \frac{S_{ALK}}{K_{ALK} + S_{ALK}} \cdot \frac{X_{PHA}}{K_{PHA} + X_{PHA}} \cdot X_{PAO} \cdot K_{MAX} - \frac{X_{PAO}}{K_{MAX}} \cdot X_{PAO} ).</td>
</tr>
<tr>
<td>6 Growth of $X_{PAO}$ using $X_{complex}$</td>
<td>( \mu_{PAO} \cdot \frac{X_{complex}}{K_{complex} + X_{complex}} \cdot \frac{S_{NH_4}}{K_{NH_4} + S_{NH_4}} \cdot \frac{S_{PO_4}}{K_{PO_4} + S_{PO_4}} \cdot \frac{S_{ALK}}{K_{ALK} + S_{ALK}} \cdot \frac{X_{PHA}}{K_{PHA} + X_{PHA}} \cdot X_{PAO} \cdot K_{MAX} - \frac{X_{PAO}}{K_{MAX}} \cdot X_{PAO} ).</td>
</tr>
<tr>
<td>7 Lysis of $X_{PAO}$</td>
<td>( b_{PAO} \cdot \frac{S_{ALK}}{K_{ALK} + S_{ALK}} \cdot X_{PAO} ).</td>
</tr>
<tr>
<td>8 Lysis of $X_{PP}$</td>
<td>( b_{PP} \cdot \frac{S_{ALK}}{K_{ALK} + S_{ALK}} \cdot X_{PP} ).</td>
</tr>
<tr>
<td>9 Lysis of $X_{PHA}$</td>
<td>( b_{PHA} \cdot \frac{S_{ALK}}{K_{ALK} + S_{ALK}} \cdot X_{PHA} ).</td>
</tr>
</tbody>
</table>

$K_{NO_2,PAO}/(K_{NO_2,PAO} + S_{NO_2}/(X_{PAO}/10^3))$. (2) The apparent denitrification of $S_{NO_2}$, namely conversion of $S_{NO_2}$ to $X_{complex}$ under aerobic condition, is not accompanied with energy production and the maximum denitrification rate of $S_{NO_2}$ is given by the coefficient of $q_{DN,NO_2}$. (3) Produced $X_{complex}$ also inhibits aerobic activities of PAOs and the size of inhibition is given by the term $K_{complex}/(K_{complex} + X_{complex}/(X_{PAO}/10^3))$. (4) The real denitrification which is accompanied with energy production is expressed by denitrification of $X_{complex}$ within the cell under aerobic condition, and the size of denitrification rate is given by the coefficient of $\eta_{complex}$.

**SIMULATION PROCEDURES**

The model was developed in 5 steps. 1) At first, the model was calibrated with the experimental results in the absence of both $S_{NO_2}$ and $X_{complex}$. 2) ‘Nitrite model’ which include only the inhibition caused by $S_{NO_2}$ was calibrated in vain.
with the experimental results in the presence of nitrite and 3) ‘Nitrite-Complex model’ which include the simulation caused by both of $S_{NO_2}$ and $X_{complex}$ was fitted with the experimental results in the presence of nitrite. This study used the results of ten sets of aerobic phosphate uptake batch tests for simulations. The sludge for these batch tests were cultivated by anaerobic/aerobic SBR (A/O-SBR) and A/A-SBR with synthetic wastewater containing acetate as a sole carbon source, and these sludge have different relative anoxic activity which was calculated by batch tests as shown in Table 3. Additionally, in these simulations, DO was consistently given as 5 mg/l by aeration equation using $K_{La}$, and all of simulations were performed with AQUASIM version 2.0.

**Setup of initial conditions for the proposed inhibition model**

In this study, it was assumed that only PAOs exist in the sludge. This is because ATU was added for A/O-SBR to prevent nitrification, and the obtained apparent P/e$^{-}$ ratio (phosphate uptake rate/electron utilization rate using O$_2$ or NO$_3$) was the same level as that of Kuba et al. (1993, 1996) which used the enriched PAOs cultivated with A/O and A/A-SBR using synthetic wastewater. Therefore, initial values of $X_{PAO}$ (gCOD/m$^3$), $X_{PP}/X_{PAO}$ (gP/gCOD), $X_{PHA}/X_{PAO}$ (gCOD/gCOD) were calculated by the experimental results of the removed phosphate (gP/d), the measured MLVSS (mg/l) and the amount of excess sludge (g/d) in each day of A/O and A/A-SBR. Initially, initial $X_{PHAO}/X_{PAO}$ was calculated by using the ASM2d default of $Y_{PO4}$ and the measured phosphate release at the end of anaerobic phase in each day of A/O and A/A-SBR (gP/m$^3$). However it could not represent the measured phosphate behavior in the aerobic phosphate uptake batch tests. Therefore, initial $X_{PHAO}/X_{PAO}$ was re-calibrated until the optimal value was obtained to represent the measured phosphate uptake in each batch test without nitrite. In these calculations, the compositions of biomass and PHA were assumed as $CH_{1.8}O_{0.5}N_{0.2}$ and $CH_{1.5}O_{0.5}$, respectively.

### Table 3 | Sludge used for simulations

<table>
<thead>
<tr>
<th>Cultivation date (days)</th>
<th>A/O-sludge</th>
<th>A/A-sludge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>35 83 97 114 135 142 264 299 61 232</td>
<td></td>
</tr>
<tr>
<td>Relative anoxic activity (%)</td>
<td>0 2 7 14 17 24 25 0 55 70</td>
<td></td>
</tr>
</tbody>
</table>

**SIMULATION RESULTS**

**Stoichiometry of independently added parameters**

ASM2d does not accurately describe existence of GAOs which is concerned competition with PAOs and the consumption of electron acceptor due to maintenance of PAOs. The amount of oxygen consumption compared with the amount of phosphate uptake is set by just adjusting the balance of COD by the calculated amount of $X_{PHA}$ consumption according to $Y_{PHA}$ of default. Therefore, in the simulation case of the short-time batch test like this study, it was easily thought that the simulation result can not represent the measured experimental result. Actually, it occurred. Although there is a reference which investigated the net P/O ratio ($P/e^{-}=0.84$) (Smolders et al. 1994), it was thought that it is not pertinent this case because the model itself include the confusion. Meanwhile, Kuba et al. (1993) evaluated the apparent P/O ratio ($P/e^{-}=0.23$) using enriched PAOs in A/O-SBR. Hence, in this study, $Y_{PHA}$ was calibrated until optimal value was found out to represent the apparent P/e$^{-}$ ratio of 0.23 by separately simulation which assumed aerobic phosphate uptake batch test. From this result, $Y_{PHA}$ of 0.6 (gCOD/gP) was obtained and it was used for simulation in this study (hereinafter called $Y_{PHA,OX}$). Also stoichiometry (amount of reduction) of nitrate for anoxic phosphate uptake is just calculated by divided COD equivalent weight of nitrate (40/14) into $Y_{PHA}$ in order to adjust the balance of COD. Therefore, amounts of nitrate consumption/amounts of phosphate uptake could not be represented the measured phosphate behavior in anoxic phosphate uptake batch test because the amount of nitrate reduction is too small. Consequently, it was thought that the independently another $Y_{PHA}$ (hereinafter called...
Y_{\text{PHA,AX}}) was needed for creation the inhibition model developed in this study. Hence, Y_{\text{PHA,AX}} was calibrated until optimal value was found out to represent the apparent P/N ratio (P/e_{2}^{2}=0.11–0.13) which was obtained from previous experiment in this study by separately simulation which assumed anoxic phosphate uptake batch test. From this simulation, Y_{\text{PHA,AX}} of 1.4 (gCOD/gP) was obtained and it was used for simulation in this study.

Table 4 shows stoichiometry of independently added parameters. All of other stoichiometry except this table used the default value of ASM2d. As shown in the table, since Process [4] describe the apparent denitrification does not include the energy production, defined same value of S_{NO2} reduction and X_{\text{complex}} product. Process [5] referred to anoxic storage of X_{PP} of ASM2d. And it was defined that PAOs use X_{\text{complex}} instead of nitrate. Process [6] also referred to anoxic growth on X_{PAO} of ASM2d. And it was defined that PAOs use X_{\text{complex}} instead of nitrate. About Process [5] and [6], the electron utilization efficiency of X_{\text{complex}} defined the same value as nitrite. Additionally, in consideration of the difference of quantity of electrons, it defined that amounts of X_{\text{complex}} (S_{NO2}) consumption = amounts of nitrite + 5/3. Besides, S_{NH4}, S_{PO4}, S_{ALK} and X_{TSS} calculated by the conservation Equation (Henze et al. 1999).

Simulation results in the case of nitrite presence

From the results of the previous batch tests, it has been confirmed that the phosphate uptake rate was increased according to nitrite disappearance (Saito et al. 2005). Hence, it was easily expected that the calculation of nitrite behavior affects the calculation of phosphate behavior. Therefore, the calculation of phosphate behavior was done after the calculation of nitrite behavior.

Simulation of nitrite behavior

Figure 5 shows the simulation results of nitrite behavior. It could be represented when K_{NO2,DN} which was defined as the same value in all cases of batch tests was given as 0.7 gN/m³. Meanwhile, q_{DN,NO2} was estimated different value in each cases according to the relative anoxic activity of PAOs. About this result discuss later.

Simulation of both of nitrite and phosphate behavior

The measured and simulated nitrite and phosphate behavior at each batch test in aerobic condition are shown in Figure 6. As shown in the figure, although Nitrite model could represent the measured phosphate behavior in case of PAOs with the higher relative anoxic activity, it is not enough. Moreover, in case of lower relative anoxic activity, simulated phosphate uptake according to nitrite disappear-
ance starts faster than the measured phosphate uptake. Hence, it could not represent the continuous inhibition after nitrite disappearance. These results suggested that the phenomenon of nitrite inhibition on aerobic phosphate uptake is considerable and also suggested importance of the inhibition by reaction complex. Meanwhile, Nitrite-Complex model could represent measured phosphate behavior in almost all cases. The difference of accuracy was clear compared to Nitrite model, especially in case of PAOs with lower relative anoxic activity. These results indicated the validity of the considered concept of nitrite inhibition in this study which the reaction product is made by nitrite and oxygen reductase inside of PAOs, and its reaction complex ($X_{\text{complex}}$) cause inhibition of respiration.

**Relationship between relative anoxic activity of PAOs and tolerance for inhibition**

Now the parameter which is related to the anoxic activity of PAOs was calibrated. The calibration results of these parameters namely $K_{\text{NO}_2,PAO}$, $K_{\text{complex}}$, $q_{\text{DN},\text{NO}_2}$, $\eta_{\text{complex}}$ were shown in Figure 7. As shown in the figure, all of four...
parameters increased correspond to the order of the relative anoxic activity of PAOs. $K_{NO_2,PAO}$ and $K_{complex}$ are defined as inhibition coefficients for inhibition substrate ($S_{NO_2}$ and $X_{complex}$). Meanwhile, $q_{DN,NO_2}$ and $\eta_{complex}$ are defined as rate coefficients for $S_{NO_2}$ and $X_{complex}$ respectively. Therefore, this result indicates that PAOs with the higher relative anoxic activity were less sensitive to nitrite exposure, and also had higher inhibition substrate removal rate (in other words, dinitrification rate).

Note, however, that the calibrated result of some case of PAOs with the lower relative anoxic activity is widely. Hence, it is not clearly but these results are describing same

Figure 6 | Simulation results of nitrite and phosphate behavior in the case of nitrite presence.
as the previous experimental result mentioned in introduction. Since thus it described not only representing measured result but also the meaning of each parameter is the same as the experimental result, the validity of the Nitrite-Complex model developed in this study is indicated. Also, it is suggest that the alleviation mechanism for nitrite inhibition by the denitrifying activity is because PAOs with the higher relative anoxic activity are less sensitive (has tolerance) to nitrite in solution and have excellent degradation (denitrification) ability of the inhibitory substance.

CONCLUSION

In our previous studies, we have examined the effect of nitrite on aerobic phosphate uptake with a bench-scale reactor treating municipal wastewater and with nitrite inhibition batch tests using the enriched PAOs cultivated with acetate as a sole carbon source. The results strongly demonstrated that 1) nitrite inhibits aerobic phosphate uptake, 2) the size of inhibition decreases with the increased denitrifying activities of PAOs and 3) inhibition persists even after nitrite disappeared. Based on the previous experimental results, in this study, we proposed the modified ASM2d describing nitrite inhibition of aerobic phosphate uptake called ‘Nitrite-Complex model’. The model was also used to verify the proposed tolerance mechanism of nitrite inhibition by the denitrifying activity of PAOs. The following findings were obtained:

Simulation results suggested that introduction of the reaction complex of nitrite is necessary to express the experimental results properly. ‘Nitrite model’ which incorporates the inhibition by nitrite only never represent the phosphate behavior whereas ‘Nitrite-Complex model’ which incorporates inhibition by reaction complex well fit the represent phosphate behavior.

Although parameters that describe tolerability to nitrite inhibition or removal rate of inhibitor were independently obtained, the obtained values clearly correspond to the order of the relative anoxic activity of PAOs. Therefore, these results strongly support the assumed secondary inhibition by the reaction complex produced inside of PAOs and the validity of ‘Nitrite-Complex model’ developed in this study. Moreover, these results strongly demonstrate the two mechanisms of alleviating nitrite inhibition by denitrifying activity, namely 1) less sensitivity (high tolerability) of PAOs with the higher relative anoxic activity to nitrite in solution and 2) high decomposition (denitrification) ability of inhibitors, namely nitrite and the reaction complex.
REFERENCES


