

Identification of the adverse effect of nitrate on the phosphate release rate and improvement of EBPR process models

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Abstract The adverse effect of nitrate on the phosphate release rate in the anaerobic phase was observed and was hardly explainable with conventional EBPR process models. Four possible mechanisms were proposed including substrate competition, reduced fermentation, parallel reaction and sequential reaction. Batch experiments were designed and conducted to identify the dominant mechanism. Results showed that the sequential reaction was the only possible mechanism where only denitrification occurred if any nitrate existed in the anaerobic phase. Then the phosphate release following after the nitrate was completely removed. Nitrate inhibition effect was added into the PHA storage rate to incorporate the sequential reaction in the conventional ASM3 plus EAWAG bio-P module (ASM3 + P). Nitrate inhibition coefficient, $K_{i,NO_3,PAO}$ was found to be as low as 0.05 mg/L. This correlated well with experimental observation where no phosphate release occurred with the existence of a little nitrate even though there was sufficient SCOD. It also meant that the anaerobic compartment of a continuous flow reactor could be seriously affected by the residual nitrate contained in the sludge recycle flow. This phenomenon caused overestimation of the phosphate uptake rate and consequently underestimation of PO_4^{3-} -P concentration. This problem was resolved by incorporation of a nitrate inhibition term in the ASM3 + P for more accurate simulation of the EBPR process.

Keywords ASM3; EAWAG bio-P module; EBPR; nitrate inhibition; P release

Introduction

In the anaerobic phase of the enhanced biological phosphorus removal (EBPR) process it is well-known that the phosphate is released into the liquid and corresponding polyhydroxy-alkanoates (PHA) are stored in the biomass. These reactions were included in the activated sludge model No. 2d (ASM2d) (Henze *et al.*, 2000) and the EAWAG bio-P module (Rieger *et al.*, 2001) as occurring even under aerobic and anoxic conditions with relatively lower rates. Nitrate addition to the anaerobic reactor could cause the phosphate removal efficiency to deteriorate by reducing phosphate release. It was reported that the rapid release of phosphate was primarily dependent on the substrate rather than the existence of nitrate (Barker and Dold, 1996). According to Gerber *et al.* (1986, 1987) the phosphate release, even with the existence of nitrate, occurred upon addition of specific substrates such as formate, acetate and propionate among 12 others tested. A fermentation product (S_A) was regarded as the only substrate utilised for phosphate release by phosphate accumulating organisms (PAOs). The S_A content in typical domestic sewage was very low in general as Henze *et al.* (2000) reported that S_A and fermentable readily biodegradable organic substrate (S_F) concentration in the primary effluent was 20 and 30 mg/L, respectively, with a total COD of 260 mg/L. Moreover, not every S_A can induce phosphate release in the existence of nitrate. In the activated sludge model No. 3 (ASM3)

(Henze *et al.*, 2000), the soluble easily biodegradable organics (S_S) represented S_A , S_F and part of hydrolysable organics (Ko *et al.*, 2004). Then all S_S were considered utilisable to phosphate release in the EBPR process for ASM3 plus EAWAG bio-P module (ASM3 + P). Consequently, this might cause overestimating the content of substrate that can induce phosphate release, thus potentially cause an overestimation of the phosphate release rate and consequent phosphate uptake rate.

The researchers' experiences with ASM3 + P revealed that it could successfully predict effluent nutrient concentrations in general even at high variations of operating conditions such as the sludge recycle flow-rate, the nitrate recycle flow-rate and the SRT. However, it overestimated the PO_4^{3-} -P concentration of anoxic reactor where the denitrification by PAOs was one of the dominant reactions (Lee *et al.*, 2005a, b). Moreover, a large estimation error of PO_4^{3-} -P concentration was found during simulation of batch experiments conducted to identify denitrifying PAOs. In this research four possible mechanisms for inhibition of phosphate release by nitrate in the anaerobic phase were examined. In addition, by considering this adverse effect in the ASM3 + P, the prediction capability of the model was improved significantly.

Materials and methods

Possible mechanisms for reduced phosphate release due to nitrate in anaerobic phase

Four possible mechanisms, which were based on Henze *et al.* (2000), were proposed

- (1) Substrate competition: Between the PAOs and the denitrifying fraction of heterotrophs, simple competition for S_A occurred. Therefore, less S_A will be stored as PHA by the PAOs which will result in less phosphate release.
- (2) Reduced fermentation: Denitrifying heterotrophs do not ferment S_F to S_A . If so, less S_A is available for PAOs in the anaerobic reactor which will result in reduced phosphate release.
- (3) Parallel reaction of denitrification and phosphate release: With nitrate input to the anaerobic reactor, some of the PAOs denitrify to detain energy instead of utilising poly-phosphate. These microorganisms do not need to accumulate poly-phosphate and consequently the phosphate removal process may deteriorate.
- (4) Sequential reaction of denitrification and phosphate release: Phosphate release is presumed to occur after complete denitrification. The nitrate might have an inhibitory effect on phosphate release. This can be realised only with the absence of acetate, formate and propionate. It is a quite faithful assumption for the influent of domestic sewage treatment plants.

ASM2d and ASM3 + P included the first three mechanisms in their model already; thus, fine tuning of model parameters should be sufficient to reflect these effects. Sequential reaction was suggested by the researchers based on experimental observations. If it was revealed as the dominant mechanism for reduced phosphate release the model should be modified.

Design of batch experiments

Basis. For design of batch experiments to identify the dominant mechanism, the expected effects of VFA and NO_3^- -N on the phosphate release rate according to each mechanism were investigated. If the substrate competition was dominant, the phosphate release rate would be proportional to VFA concentration and would not be affected by low concentration of NO_3^- -N in the existence of excess VFA. If the reduced fermentation was dominant, the phosphate release rate would be proportional to VFA concentration and the NO_3^- -N had no effect on it if VFA was sufficient. If the denitrification and the phosphate release were parallel reactions, the phosphate release rate would be

proportional to VFA concentration and inversely proportional to NO_3^- -N concentration. If the last mechanism, the sequential reaction of denitrification and phosphate release were real, the phosphate release would be observed only after denitrification. These explanations are summarised in Table 1 and serve as the basis for the design of batch experiments.

Design. Three sets of batch experiments were designed. Experimental conditions are shown in Table 2. Ethanol was used as the sole VFA source and measured as SCOD. At the experimental Set 1, in order to observe the effect of SCOD on NO_3^- -N inhibition, the initial SCOD in mixed liquor was increased from 0 to 60 mg/L, while the initial NO_3^- -N and PO_4^{3-} -P were kept constant as 10 and 25 mg/L, respectively. At the experimental Set 2, it was demonstrated that the NO_3^- -N inhibition effect occurred even with sufficient substrate present condition. Therefore, the initial SCOD was 220 mg/L and the initial NO_3^- -N was increased from 0 to 50 mg/L. At the experimental Set 3, in order to make sure of the inhibitory concentration of NO_3^- -N, a similar experiment was repeated with only small concentrations of NO_3^- -N from 1 to 10 mg/L. At the experimental Set 1 and 2 in order to examine the NO_3^- -N inhibition effect on the following anoxic and aerobic phases, these phases were maintained as 3.5 and 1.5 h, respectively. Additional nitrate was injected at the starting point of the anoxic period to induce denitrification by PAOs with the stored PHA. The denitrification rate would be proportional to the stored mass of PHA, because the carbon source was added in the anoxic phase. Sludge for these experiments was obtained from a laboratory-scale five-stage step-feed EBPR process which had 15 days of SRT. pH was regulated as 7.2 ± 0.2 .



















Model modification and implementation

ASM3 + P served as the base model in this research and was modified for more precise simulation based on experimental evidence. Details are described in Results and discussion. Model parameters were optimised and verified with PO_4^{3-} -P, NO_3^- -N and SCOD concentrations obtained during Set 3 experiments and then validated with those obtained during Set 1 and Set 2 experiments. The Nelder-Mead simplex method was applied for parameter optimisation. The objective function was to minimise the sum of normalised squared error of PO_4^{3-} -P, NO_3^- -N, SCOD and MLSS concentrations as follows:

$$P_{mat}^{\min} J(P_{mat}) = \sum_{t_0}^{t_f} \sum_{i=1}^n \left[\frac{C_{i,t,measured} - C_{i,t,predicted}}{C_{i,t,measured}} \right]^2 \quad (1)$$

where, $C_{i,t}$ is concentration of i th components at time t , i is PO_4^{3-} -P, NO_3^- -N, SCOD and MLSS.

Table 1 Expected variation of the phosphate release rate according to the variation of initial SCOD and NO_3^- -N concentration and possible dominant mechanisms

		Set 1	Set 2	Set 3*
Variation of initial concentration of	SCOD			
	NO_3^- -N			
Possible dominant mechanisms	Substrate competition			
	Reduced fermentation			
	Parallel reaction			
	Sequential reaction			

* Low initial NO_3^- -N concentration range, 1–10 mg/L in this research

Table 2 Experimental conditions of batch experiments

Exp. No.	MLVSS, mg/L		Initial concentration, mg/L						Period of Phase**, h	NO ₃ ⁻ -N add, mg/L***	
			Run 1	Run 2	Run 3	Run 4	Run 5	Run 6			
Set 1	1800 ± 100	SCOD*	0/45	15/60	25/70	60/105	–	–	3.5/3.5/1.0	50	
		PO ₄ ³⁻ -P			25			–			–
		NO ₃ ⁻ -N			10			–			–
Set 2	1800 ± 100	SCOD*			220/270				3.0/3.5/1.0	20	
		PO ₄ ³⁻ -P			30			–			–
		NO ₃ ⁻ -N	0	5	20	50		–			–
Set 3	1600 ± 100	SCOD*				220/260			3.0/–/–	–	
		PO ₄ ³⁻ -P				10					
		NO ₃ ⁻ -N	1	1.5	3	5	7.5	10			

* (Injected as ethanol)/(measured after injection, non-biodegradable COD included); ** anaerobic/anoxic/aerobic; *** NO₃⁻-N added during anoxic phase

Results and discussion

Identification of the dominant mechanism

During the first 120 min of anaerobic phases in the Set 1 experiment the $\text{PO}_4^{3-}\text{-P}$ concentrations were reduced with identical amounts regardless of the initial SCOD concentrations as shown in box 1) in Figure 1(a). This could be regarded as evidence that the phosphate release did not occur during this period. If it occurred, the gradient of $\text{PO}_4^{3-}\text{-P}$ concentration would be higher at the higher initial SCOD case than that at the lower initial SCOD case. In this period, the $\text{NO}_3^- \text{-N}$ was reduced, indicating denitrification occurred but with different rates that depended on the initial SCOD. Where the initial SCOD was as low as 50 mg/L, denitrification was not completed. Only when the initial SCOD was higher than 70 mg/L was denitrification completed. However, only when sufficient biodegradable COD was left as shown in box 2) in Figure 1(a) did the $\text{PO}_4^{3-}\text{-P}$ release occur, which only occurred in the 105 mg/L SCOD case. Similar phenomena can be found in box 4) in Figure 1(b). The $\text{PO}_4^{3-}\text{-P}$ release rate was dependent on the initial SCOD concentration. Therefore, one may summarise that in the beginning of the experiments only denitrification occurred. Once the $\text{NO}_3^- \text{-N}$ was removed completely, the $\text{PO}_4^{3-}\text{-P}$ release was possible, which was an indication of the sequential reaction mechanism as proposed in this work. In the anoxic phase of the Set 1 experiment, the $\text{PO}_4^{3-}\text{-P}$ uptake rate and the denitrification rate with initial SCOD of 105 mg/L were higher than in the other experiments. This phenomenon may be successfully explained by the sequential reaction since the PHA would, in that case, be stored with 105 mg/L SCOD.

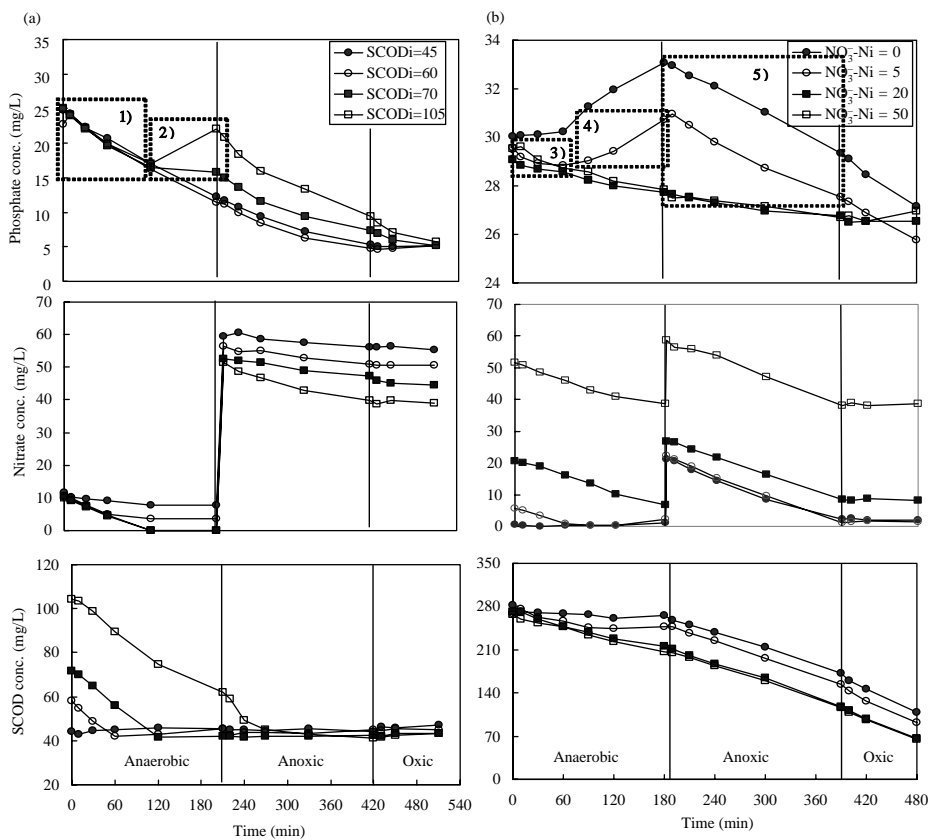


Figure 1 Variation of $\text{PO}_4^{3-}\text{-P}$, $\text{NO}_3^- \text{-N}$ and SCOD concentration during (a) Set 1 and (b) Set 2

In the anaerobic phase of the Set 2 experiments, the phosphate release was not observed as long as the nitrate existed even though SCOD was excessively supplied as shown in box 3) in Figure 1(b). When the initial nitrate concentration was 0 and 5 mg/L, the phosphate release occurred after complete nitrate consumption during the anaerobic phase. Therefore, the phosphate uptake rates in these conditions were higher than those at initial NO_3^- -N concentrations of 20 and 50 mg/L, which are shown in box 5) in Figure 1(b). This was due to the fact that very little PHA would be stored at the initial NO_3^- -N concentrations of 20 and 50 mg/L during the anaerobic phase even though there was sufficient SCOD. This phenomenon proved again that the substrate competition and the reduced fermentation could not be considered as the dominant mechanism. However, both mechanisms play their role in continuous flow reactors even though their effects are not significant. Prominent experimental observations and the validity of each possible mechanism are summarised in Table 3. Only the sequential reaction mechanism could explain and validate every important experimental observation.

Model improvement

Inhibition kinetics. It has been reported that most PAOs have denitrification capacity in nitrate existence (Seviour *et al.*, 2003). The ASM3 + P and ASM2d do not differentiate PAOs according to denitrifying capacity. The sequential reaction of denitrifying PAOs can be easily expressed by adding an inhibition term for nitrate into the PHA storage rate as Eq. 2. Added nitrate inhibition terms work as non-competitive inhibition kinetics on the PHA storage rate corresponding with the phosphate release rate.

$$r_{\text{PHA storage}} = q_{\text{PHA}} \cdot \frac{S_S}{K_{\text{SS,PAO}} + S_S} \cdot \frac{S_{\text{HCO}}}{K_{\text{HCO,PAO}} + S_{\text{HCO}}} \cdot \frac{X_{\text{PP}}/X_{\text{PAO}}}{K_{\text{PP,PAO}} + X_{\text{PP}}/X_{\text{PAO}}} \cdot \frac{K_{\text{I,NO,PAO}}}{K_{\text{I,NO,PAO}} + S_{\text{NO}}} \cdot X_{\text{PAO}}, \quad (2)$$

where $K_{\text{I,NO,PAO}}$ is inhibition coefficient of S_{NO} on phosphate release.

The parallel reaction mechanism originally embedded in the ASM3 + P was changed for implementing the sequential reaction mechanism by the above modification.

Table 3 Prominent experimental observations and validity of each possible mechanism

Conditions as shown in box	Observations	Validity			
		SC*	RF*	PR*	SR*
1) Set 1/every SCODi	Identical PO_4^{3-} -P concentration regardless of SCOD concentration for first 120 min	×	×	×	○
2) Set 1/SCODi = 105	Steep increase of PO_4^{3-} -P concentration after complete denitrification (at 120 min)	△	△	○	○
3) Set 2/ NO_3^- -Ni = 5, 20, 50	Identical PO_4^{3-} -P concentration at excessive SCOD concentration, for first 60 min	×	×	△	○
4) Set 2/ NO_3^- -Ni = 5	Increase of PO_4^{3-} -P concentration after complete denitrification (at 60 min)	×	×	○	○
5) Set 2/ NO_3^- -Ni = 0, 5	Higher phosphate uptake during anoxic phase	×	×	○	○

Substrate competition (SC), reduced fermentation (RF), parallel reaction (PR) and sequential reaction (SR). ○, valid; △, partially valid; ×, invalid

Regardless of this modification, the substrate competition and the reduced fermentation were inherently considered as they were in the model, which can occur at the reactors for treating domestic sewage.

Parameter optimization and model verification. Parameter optimisation was performed with the measured $\text{PO}_4^{3-}\text{-P}$, $\text{NO}_3^- \text{-N}$ and SCOD concentrations when the initial $\text{NO}_3^- \text{-N}$ concentrations were 1, 3 and 10 mg/L at the Set 3 experiment. Data from other initial $\text{NO}_3^- \text{-N}$ concentrations (2, 5 and 7.5 mg/L) were utilised for verification. Data fitting was attempted with the ASM3 + P considering sequential reaction as shown in Figure 2(a) and without sequential reaction in Figure 2(b). SCOD concentration also fit well but is not shown here. The Nelder-Mead simplex method was applied for estimating $K_{I,\text{NO},\text{PAO}}$ and other related parameters. The inhibition coefficient was usually estimated with the Lineweaver-Burk method (Ko *et al.*, 2001). However, in this case the value of $K_{I,\text{NO},\text{PAO}}$ was too small, so mathematical optimisation was implemented. The results are summarised in Table 4. The estimated value of $K_{I,\text{NO},\text{PAO}}$ was 0.05 mg/L, which indicated that the nitrate concentration for 50% inhibition (IC_{50}) of the phosphate release rate was only 0.05 mg/L. Therefore, no phosphate would be released if any nitrate existed. This also meant that the anaerobic compartment of a continuous flow reactor would be seriously affected by residual nitrate if contained in a sludge recycle flow.

The estimated value of rate constant for storage of poly-phosphate (q_{PP}) was two times greater than when the sequential reaction was not considered, while those of other parameters were identical. Without the sequential reaction, simultaneous phosphate release was simulated with phosphate uptake with the existence of nitrate; thus, the phosphate uptake rate should be increased to fit observed phosphate concentration. This fact results in overestimation of the phosphate uptake rate and consequent underestimation of $\text{PO}_4^{3-}\text{-P}$ concentration.

Model validation

Both ASM3 + P models were applied for data fitting for validation with data obtained from Set 1 and Set 2 experiments. Estimated $\text{PO}_4^{3-}\text{-P}$ concentrations were fitted with the measured values as shown in Figure 3. The modified model could approximate measured data very closely in every case. When the sequential reaction was not considered, the $\text{PO}_4^{3-}\text{-P}$ concentration was underestimated meaningfully, due to the overestimated phosphate uptake rate. Especially in Set 1, the different phosphate release rates were estimated according to the initial SCOD concentration; consequently, it could not fit $\text{PO}_4^{3-}\text{-P}$ concentration in the anoxic phase at all. Only the sequential reaction model could explain the identical $\text{PO}_4^{3-}\text{-P}$ concentration regardless of the initial SCOD concentration, as mentioned before. The conventional ASM3 + P underestimated SCOD concentration because it regarded SCOD as being continuously stored as PHA with phosphate release even with the existence of nitrate. However, both models showed a relatively good fit to the $\text{NO}_3^- \text{-N}$ concentration that affected the phosphate release but was not affected by it. With these results, it was confirmed that the sequential reaction of denitrification and

Table 4 Optimised parameters' values for modified ASM3 + P

	ASM3 + P	$K_{I,\text{NO},\text{PAO}}$	$\mu_{\text{max,H}}$	q_{PHA}	q_{PP}	Y_{PO_4}
Optimized	w/o sequential reaction	–	5	2	4	0.34
	w/ sequential reaction	0.05	5	2	2	0.34

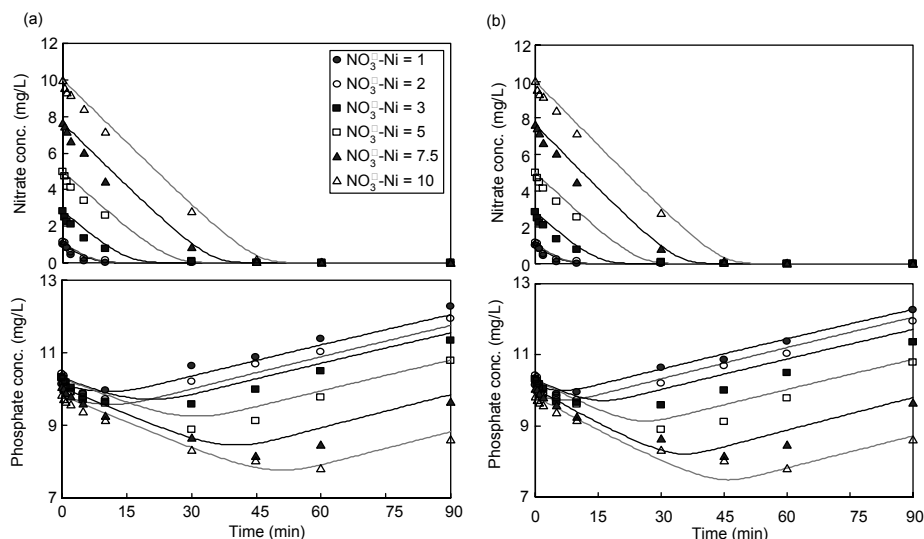


Figure 2 Parameter optimization and model verification results with data from Set 3: (a) using modified ASM3 + P (with sequential reaction); (b) using conventional ASM3 + P (without sequential reaction)

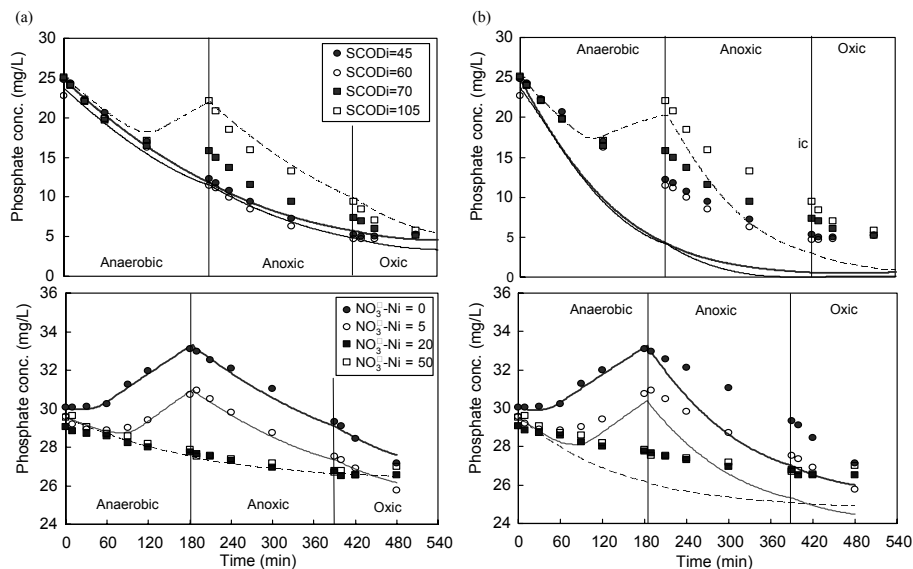


Figure 3 Model validation results with data from Set 1 (upper plots) and Set 2 (lower plots): (a) using modified ASM3 + P (with sequential reaction); (b) using conventional ASM3 + P (without sequential reaction)

phosphate release should be incorporated into ASM3 + P for more precise estimation of PO_4^{3-} -P concentration.

Conclusions

Four possible mechanisms including substrate competition, reduced fermentation, parallel reaction and sequential reaction were tested to determine whether one can explain reduced phosphate release due to nitrate in an anaerobic reactor of the EBPR process. To identify the dominant mechanism, several batch experiments were conducted. Results

showed that sequential reaction was the only mechanism to correspond with every prominent experimental observation. To incorporate the sequential reaction into the conventional ASM3 + P a nitrate inhibition term was added into the PHA storage rate. The substrate competition and the reduced fermentation were inherently considered, but the parallel reaction was replaced with the sequential reaction. Estimated value of $K_{I,NO,PAO}$ was as low as 0.05 mg/L. It corresponded with experimental observations where no significant phosphate release occurred with the existence of nitrate even though SCOD was sufficient. It also meant that the anaerobic compartment of a continuous flow reactor could be seriously affected by residual nitrate contained in the sludge recycle flow. When the sequential reaction was not considered the estimated value of rate constant for storage of poly-phosphate (q_{pp}) was two times greater than that when the sequential reaction was considered, while those of other parameters were identical. This fact resulted in overestimation of the phosphate uptake rate and consequent underestimation of PO_4^{3-} -P concentration in the validation step. A modified model could approximate measured data very closely in every case. Consequently, incorporation of a nitrate inhibition term into ASM3 + P was regarded as essential for more accurate simulation of the EBPR process.

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