Quantitative estimation of the role of denitrifying phosphate accumulating organisms in nutrient removal

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Abstract It has been reported that a proportion of polyphosphate-accumulating organisms (PAOs) can denitrify or utilize nitrate as an electron acceptor. The usage of denitrifying-PAO (DN-PAO) can relieve the competition for COD between denitrification and phosphorus removal because they can treat nitrate and phosphate by using the same carbon source. To effectively use DN-PAO for biological nutrient removal (BNR), a new system was proposed in which an anaerobic phase is placed at the influent end, followed by the anoxic and external nitrification phase. In this study, the lab-scale proposed system (A2N system) was operated with a municipal wastewater 1) to confirm stable settlement of DN-PAO in the proposed system, 2) to quantitatively estimate the fraction of different groups of organisms like denitrifiers without polyphosphate accumulating capability, aerobic-PAO and DN-PAO and 3) to estimate the advantages of DN-PAO's presence in the system. Moreover, batch experiments in which anoxic and aerobic phosphate uptake rates (PUR) were measured were also carried out. The activity of DN-PAO was observed throughout the experimental period by the batch experiment. From the results of the calculation of COD utilized by each group of organisms, it was concluded that the proposed system could accumulate much more PAO (as DN-PAO) than conventional BNR systems. Moreover, they were responsible for both EBPR and denitrification.
Keywords Biological nutrient removal; denitrifiers; denitrifying polyphosphate-accumulating organisms (DN-PAO); hydrolysis; polyphosphate-accumulating organisms (PAO)

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Introduction

In conventional biological nutrient removal (BNR) systems, denitrifiers play the main role in nitrogen removal and polyphosphate-accumulating organisms (PAO) are responsible for enhanced biological phosphorus removal (EBPR). Both of them require carbon sources (or COD) independently to carry out their roles in BNR systems. Thus, the availability of COD is often an essential limiting factor when simultaneous removal of nitrogen and phosphorus is attempted in BNR systems.

On the other hand, anoxic or denitrifying phosphate uptake has been reported since the late 1980s (Gerber *et al.*, 1987; Comeau *et al.*, 1987). This phenomenon implies that a proportion of PAOs can denitrify or utilize nitrate as an electron acceptor. Thus, PAOs are divided into two groups, aerobic PAO and denitrifying PAO (DN-PAO) (Kerrn-Jespersen and Henze, 1993). From the comparison of their stoichiometric and kinetic properties, Kuba *et al.* (1993) suggested that DN-PAO had as high EBPR potential as aerobic PAO had. Moreover, the usage of DN-PAO can relieve the competition for COD between denitrification and EBPR because they can treat nitrate and phosphate by using the same carbon source (Mino *et al.*, 1998).

To effectively use DN-PAO for BNR, a new system was proposed in which an anaerobic phase is placed at the influent end, followed by anoxic and external nitrification phases. This system is referred to as the "A2N" system hereafter. In the system, DN-PAO are maintained in the anaerobic-anoxic stream and nitrifiers are maintained in the external aerobic tank respectively. It was demonstrated that the A2N system could achieve good BNR efficiencies when they treated synthetic (Kuba *et al*., 1996), piggery (Bortone *et al*., 1994) and municipal (Sorm *et al*., 1996; Hu *et al*., 2000) wastewaters.

Wachtmeister *et al*. (1997) and Meinhold *et al*. (1999) established a batch test to quantitatively measure the portion of DN-PAO among all PAO, in which anoxic and aerobic phosphate uptake rates (PUR) were measured. They concluded that the ratio of anoxic PUR to aerobic PUR reflected the ratio of DN-PAO to total PAO.

In this study, a lab-scale A2N system was operated with a municipal wastewater 1) to confirm stable settlement of DN-PAO in the proposed process, and 2) to investigate the BNR performance of the proposed system. It was also attempted 3) to quantitatively estimate the fraction of COD used by different groups of organisms like denitrifiers without polyphosphate accumulating capability, PAO and DN-PAO, and 4) to estimate the advantages of DN-PAO's presence in the system.

Materials and method

Process set up

Two lab-scale A2N systems were operated with the same configuration shown in Figure 1. A municipal wastewater is introduced into the anaerobic tank. The internal settler separates the sludge from the supernatant. Most of readily biodegradable COD must have been taken up by the sludge and stored by PAO or DN-PAO as intracellular storage substrates like polyhydroxy alkanoates (PHA). The substrate-storing sludge is sent to the anoxic tank while the supernatant goes to the external nitrification tank, where nitrifiers immobilized in PEG media (Emori *et al*., 1994) convert ammonia into nitrate. The remaining COD can be also oxidized in this tank. Then nitrate-rich supernatant is sent to the anoxic tank through screen and mixed with the sludge. DN-PAO can take up phosphate by using the nitrate as electron acceptor and the stored PHA as electron donor.

The design and operational parameters are shown in Table 2. The major characteristics of the influent (settled municipal wastewater) are shown in Table 3.

Batch experiment

Mixed liquor samples were taken from the end of the anoxic tank. After the sludge was settled, the supernatant (80% of mixed liquor) was removed and replaced with a mineral

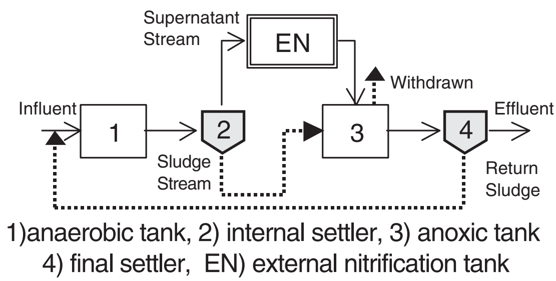

Figure 1 Configuration of A2N system

Table 1 Operational condition

	Reactor 1	Reactor 2
Term 1	Run-B: +Acetate* to anaerobic tank	Run-C: +Acetate* to anoxic tank
Term 2	Run-D: Longer SRT	Run-A: Control
Term 3	Run-E: Shorter HRT of anaerobic tank	Run-F: Longer HRT of anaerobic tank

* 10 mgC per 1 L influent

Table 2 Design and operational parameters

Operational conditions	-	Reactor volume (liter)	
SRT for Run-D (day)	12~16	Anaerobic for Run-E	3.3
SRT for others (day)	6~8	Anaerobic for Run-F	9.9
		Anaerobic for others	6.6
Temp (°C)	20	Nitrification	7.0
Influent (L/day)	72	Anoxic	13.6
Return sludge (L/day)	12	Total	27.2±3.3
Sludge bypass (L/day)	12	Internal settler	10

Table 3 Characteristics of influent (average)

Total COD (mgCOD/L)	215
Soluble COD (mgCOD/L)	109
NH_4-N (mgN/L)	26.7
NO_3-N (mgN/L)	0.2
NO_2-N (mgN/L)	0.0
Total-P (mgP/L)	3.8
PO_4-P (mgP/L)	2.8

solution (150 mg/L $CaCl_2$, 70 mg/L KCl and 22 mg/L $MgCl_2$) so that the activated sludge should be diluted to be approximately 1,000 mgMLSS/L. All experiments were conducted in a 1 L glass bottle at 20°C. pH was controlled around 7.2 by adding 0.05 mol l^{-1} sulfuric acid.

Two identical aliquots of sludge were incubated for 60 minutes under anaerobic conditions after sodium acetate addition (6–7 mgC/L in the bottle). Then one was exposed to aerobic conditions. As dissolved phosphate concentration decreased linearly, the aerobic PUR was calculated by the slope of the concentration decrease. On the other hand, the other one was exposed to anoxic conditions where potassium nitrate was added at a concentration of 15 mgN/L. The anoxic PUR was also measured in the same way as the aerobic PUR. The ratio of the anoxic PUR to the aerobic PUR (hereafter referred to as the PUR ratio) was calculated.

Analytical methods

The analysis of phosphorus, nitrate, MLSS and MLVSS were performed in accordance with *Japanese Standard Methods for Examinations of Wastewater*. Ammonia, acetate and nitrite were measured by Capillary Electrophoresis (Hewlett Packard HP^{3D}CE). COD (Chromium) was measured by HACH COD kit. Glass fiber filters (Whatman GF/F) were used for filtration to separate dissolved samples.

Simulation

Simulations were carried out to evaluate COD consumption by different functional groups of organisms. The assumption about classification of organisms and their functions in the content of COD consumption are shown in Table 4.

To calculate the biomass distribution of the A2N system, the assumptions in the IWA model (ASM 2 and ASM 2d) were included. The calculations employed in this study are based on the following assumptions.

1. *Characterization of COD in the calculation*
   - The sum of inert particulate organics (X_I) and heterotrophic biomass (X_H) is equal to total COD times 55/260 (the typical wastewater composition proposed in ASM 2/2d assumed). Production of X_I and X_H is neglected in the calculation.

Table 4 Classification of organisms and their functions

	Anaerobic tank		Anoxic tank		Nitrification tank	
	COD uptake	Denitrification	COD uptake	Denitrification	COD uptake	Denitrification
Aerobic PAO	+	–	–	–	*(Not present)*	
DN-PAO	+	–	–	+	*(Not present)*	
Denitrifiers	–	–	+	+	*(Not present)*	
Heterotrophs (Attaching on media in nitrification tank)	*(Not present)*		*(Not present)*		+	–

- Slowly biodegradable substrate (X_S) : Initial X_S is equal to the influent particulate-COD minus X_I and X_H. This component can be changed into readily biodegradable substrate (S_S) by hydrolysis.
- Inert dissolved organics (S_I) is equal to the effluent dissolved-COD. This component neither decreases nor increases.
- Readily biodegradable substrate (S_S) is equal to observed dissolved-COD minus S_I. This component is supplied by influent or hydrolysis of X_S.

2. *Simulation of the conversion of X_S into S_S by hydrolysis*
   - Simulation was performed in accordance with the parameters of the hydrolysis process of ASM 2 or ASM 2d. In ASM 2d, hydrolysis rate under anaerobic conditions is much faster than that in ASM 2. From the HRT of each tank, S_S supplied by hydrolysis is calculated in each tank.
3. *Balance of S_S in each tank*
   S_S is supplied from the influent and through hydrolysis of X_S. Then it is consumed by organisms or goes out in the effluent. From the balance of S_S, consumed COD can be calculated in the tank.
4. *Denitrification*
   It is assumed that denitrifiers or DN-PAO require 2.86 gCOD for denitrification of 1 gNO_3-N.

From the function of each group of organisms (see Table 5) and results of calculation, the fraction of COD consumption by each group of organisms can be estimated as the ratio of consumed COD by the group to total consumed COD.

Results and discussion

In Run-D, concentration of nitrogen and phosphorus after the final settler were about 6 mgN/L and 1 mgP/L, respectively. Run-A also kept good performance. On the other

Table 5 Effluent characteristics and P content in the anoxic tank by each Run

Run		Ammonia (mgN/L)	Nitrite+nitrate (mgN/L)	Phosphate (mgP/L)	COD Cr (mgCOD/L)	P content average, (%)
A: Control	range	1.8~4.7	0.8~13.7	0.1~1.8	12~33	5.8
	avg.	3.3	5.6	0.7	21	
B: +Ac to anaerobic	range	2.9~6.0	4.0~12.0	1.4~2.8	22~30	2.6
	avg.	4.2	6.9	2.3	28	
C: +Ac to anoxic	range	2.5~3.8	0.1~2.9	0.7~3.4	20~26	2.5
	avg.	3.3	1.5	2.0	24	
D: Longer SRT	range	2.0~4.5	0.3~7.2	0.1~2.1	11~27	5.2
	avg.	3.5	2.4	1.0	21	
E: Shorter anaerobic	range	6.8~12.6	0.1~4.7	0.4~4.7	30~36	4.6
	avg.	9.7	0.6	2.4	32	
F: Longer anaerobic	range	8.3~8.8	0.1~0.3	1.0~5.8	25~32	4.5
	avg.	8.5	0.1	2.9	28	

hand, EBPR was not stable in the other runs and nitrification was not enough in Run-E or Run-F.

Table 6 shows the results of batch experiment. Sufficient activity of DN-PAO is observed throughout the experimental period. Moreover, both the anoxic PUR and the PUR ratio are much higher than the results of conventional BNR systems such as A2O process (Shoji *et al.*, 2001).

Table 7 shows the fraction of COD consumption by each group of organisms. The result by ASM 2 and ASM 2d are different in the fraction of denitrifiers and DN-PAO. In ASM 2 base calculation where anaerobic hydrolysis rate is assumed 0.3 d^{-1}, most hydrolysis occurs in the anoxic tank due to slow hydrolysis in the anaerobic tank. On the other hand, most of X_S is degraded in the anaerobic tank before it reaches the anoxic tank in ASM 2d base calculation where the rate is assumed 1.2 d^{-1}. The rate of anaerobic hydrolysis of X_S is critical for this calculation because X_S is one of the main components of influent COD. Thus, the modeling of hydrolysis process must be studied further to accurately estimate the balance of COD.

In Run-D, DN-PAO used 37% (ASM 2 base) or 55% (ASM 2d base) of total COD for simultaneous removal of nitrogen and phosphorus. In a conventional BNR system like the A2O process, the fractions of PAO are calculated as 12.2% (Chuang and Ouyang, 2000), about 20% (Bortone *et al.*, 1999) and less than 10% (Amano *et al.*, 2002), respectively. In the proposed system, it is concluded that much more PAO (as DN-PAO) could be accumulated than the conventional BNR system.

When EBPR was not stable (Run-B and Run-C) in spite of enough nitrification, PAO appeared to consume significant amounts of COD in the calculation. In reality, a kind of competitor like glycogen-accumulating organisms (GAO) grew significantly as indicated by the observed low phosphate release in the anaerobic tank (see Table 8). Moreover, as shown in Figure 2, the fractions of COD consumption by DN-PAO and phosphorus content have a good correlation. Thus, DN-PAO should be responsible for the main part of EBPR in the system.

Hu *et al.* (2002) reported that DN-PAO contributes little (maximum 20%) to the denitrification in the system because the specific denitrification rate of the PAO on stored

Table 6 Results of batch experiment

Run	Aerobic PUR (mgP/gVSS/hour)	Anoxic PUR (mgP/gVSS/hour)	PUR ratio
A	22.4	11.1	0.50
B	18.1	9.8	0.55
C	8.2	5.4	0.66
D	20.4	9.6	0.47
E	12.0	7.5	0.63
F	24.6	13.3	0.57

Table 7 Fraction of COD consumption by each group of organisms

Run	Fraction of COD consumption (%)							
	ASM 2 base calculation				ASM 2d base calculation			
	Denitrifiers	PAO	DN-PAO	*Heterotrophs*	Denitrifiers	PAO	DN-PAO	*Heterotrophs*
A	41	(−2)*	30	31	22	(−4)*	49	33
B	38	23	(−2)*	41	22	20	13	44
C	52	33	(−12)*	28	35	30	4	31
D	35	(−3)*	37	30	16	(−3)*	55	31
E	35	10	5	49	11	9	29	51
F	30	17	11	42	11	14	30	45

*These negative data imply that the groups consumed little COD.

Table 8 Ratio of released phosphate to COD uptake

Run	Released-P/COD uptake (gP/gCOD)
A	0.42
B	0.15
C	0.18
D	0.48
E	0.39
F	0.42

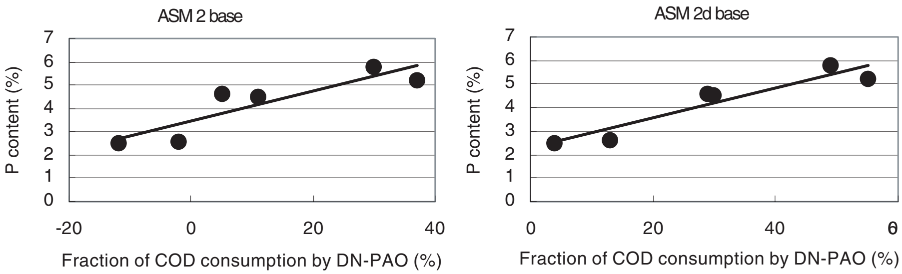

Figure 2 Correlation between P content and COD consumption by DN-PAO

substrate was about 20% of that of the denitrifiers on S_S under anoxic conditions. In this study, however, DN-PAO contributes about 50% (by ASM 2) or 70% (by ASM 2d) to the denitrification in Run-D. Therefore, from the calculation of COD balance, it is concluded that DN-PAO can play a significant part in both EBPR and denitrification.

Conclusions

A lab-scale A2N system was operated with municipal wastewater. The following main conclusions may be drawn from the operation of the system, from the performed batch tests and from the calculation of fraction of utilized COD.

1. The rate of anaerobic hydrolysis of X_S (slowly biodegradable substrate) is critical for the calculation of COD balance.
2. The proposed A2N system could accumulate much more PAO (as DN-PAO) than the conventional BNR system.
3. DN-PAO could play a significant part in both EBPR and denitrification in the system.

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