# PHOSPHORUS RELEASE AND UPTAKE ACCORDING TO NITRATE LOADING IN ANOXIC REACTOR OF BNR PROCESS

# Kwang-Soo Kim<sup>†</sup>

Dept. of Construction Environment Research, Korea Institute of Construction Technology, Korea (received July 2005, accepted October 2005)

Abstract: A batch and a continuous type experiments were conducted to test the conditions for simultaneous phosphorus release and uptake, and denitrification, taking place in one process. The bacteria able to denitrify as well as to remove phosphorus were evaluated for the application to biological nutrient removal (BNR) process.

In the batch-type experiment, simultaneous reactions of phosphorus release and uptake, and also denitrification were observed under anoxic condition with high organic and nitrate loading. However the rate and the degree of P release were lower than that occurred under anaerobic condition.

BNR processes composed of anaerobic-anoxic-oxic(AXO), anoxic-anaerobic-oxic(XAO) and anoxic-oxic(XO) were operated in continuous condition. The anoxic reactors in each process received nitrate loading. In the AXO process, P release in anaerobic reactor and the luxury uptake in oxic reactor proceeded actively regardless to nitrate loading. However in XAO and XO processes, P release and luxury uptake occurred only with the nitrate loading less than 0.07 kgNO<sub>3</sub>-N/kgMLSS-d. With higher nitrate load, P release increased and the luxury uptake decreased. Therefore, it appeared that the application of denitrifying phosphorus-removing bacteria (DPB) to BNR process must first resolve the problem with decrease of luxury uptake of phosphorus in oxic reactor.

Key Words: Denitrifying phosphorus-removing bacteria(DPB), Phosphorus release, Luxury uptake, Denitrification

#### INTRODUCTION

Conventional secondary biological treatment systems take up phosphorus from liquid for biomass synthesis during BOD oxidation. Phosphorus, required in intracellular energy transfer becomes an essential cell component. For this reason, phosphorus is taken up in an amount related to the stoichiometric requirement for biosynthesis. Typical phosphorus content of microbial solids is 1.5 to 2% on a dry weight basis. However the content may increase up to 4-12% in biological phosphorus removal process where

sequential anaerobic and aerobic conditions are allowed.

The mechanism of biological phosphorus removal process is well understood; influent organic is converted to acetate or other short-chain fatty acids under anaerobic condition, or it can be accumulated in body cells as polyhydroxy-alkanoates(PHA). The energy for this process comes from ATP hydrolysis, and as a result, orthophosphate is released from cells. Now in aerobic condition, the organism may uptake phosphorus much higher than the amount released in anaerobic condition for the resynthesis of intracellular polyphosphate. Finally phosphorus removal is achieved by obtaining P-rich sludge in the aeration tank. New cells are

Tel: 031-910-0299, Fax: 031-910-0291

<sup>&</sup>lt;sup>†</sup> Corresponding author E-mail: kskim@kict.re.kr

produced then by the PHA stored within cells with oxygen as an electron acceptor, leaving carbon dioxide and water the final products.

Likewise, conventional P treatment concept is understood as the repetition of anaerobic condition without electron acceptor and aerobic condition with oxygen as an electron acceptor. It is known that if nitrate or oxygen is introduced in anaerobic condition, the P release is inhibited so that P uptake is lowered in the following aerobic. This is why the UTC and VIP processes reactor, utilize extra denitrification reactor to receive return sludge before it is introduced to anaerobic reactor.

Recent studies<sup>3-7)</sup> confirmed that phosphorus uptake and denitrification can take place in anoxic conditions with presence of nitrate. DPB may utilize PHB as a carbon source with nitrate as an electron acceptor for the removal of phosphorus and nitrogen. Kerrn-Jepersen, 'et al.,3) and Bortone, et al.,8) also detected in their BNR systems the presence of both denitrifying phosphorus accumulating organisms(DPAO) and phosphorus accumulating organism(PAO). Though, most researches so far have focused on the luxury P uptake and denitrification in anoxic condition, but still little is known about the mechanism and conditions for simultaneous P release/uptake and denitrification, the application to BNR process.

## **METHODOLOGY**

#### **Batch Type Experiment**

A batch-type experiment was undertaken to test whether or not P release and luxury uptake can take place simultaneously in anoxic condition. The influent was synthesized by adjusting a real wastewater to a TCOD level of 700 mg/L

and SCOD level of 550 mg/L with glucose. Soluble phosphate (S-P) and nitrate were also added into the real wastewater to the levels aimed for each phase.

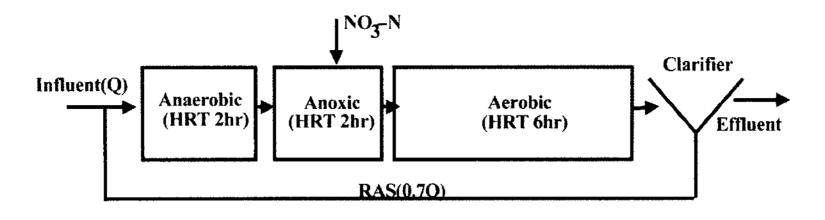
Phase I was set for 550 mg/L SCOD, 10 mg/L S-P and 23 mg/L nitrate-nitrogen in influent levels, and phase II had same SCOD and S-P levels and 5 mg/L nitrate-nitrogen. Two nitrate levels were tested to simulate anoxic and anaerobic conditions. In phase III, influent COD was down to 200 mg/L and nitrate-nitrogen level was made 26 mg/L to examine N and P behavior in low C/N ratio.

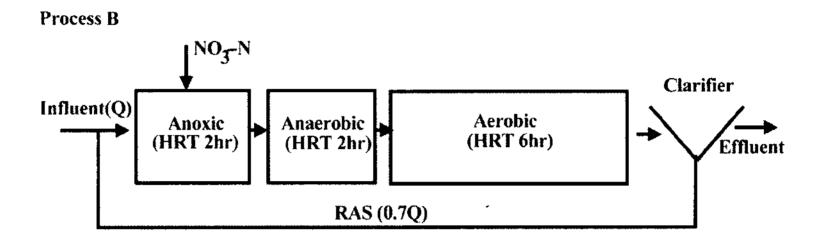
Organic and nitrogen loading were ranged from 0.02-0.06 gCOD/gMLSS and 0.0006-0.003 gNO<sub>3</sub>-N/gMLSS, and an activated sludge from a pilot-scale municipal wastewater treatment plant was used for seeding material.

Continuous feeding experiment. Continuous experiment was undertaken to find out optimum nitrate loading for simultaneous P release/uptake and denitrification under anoxic condition and to examine the applicability of DPB to a BNR process. Figure 1 shows the reactor configuration of each process used in the continuous experiment. Process A is the typical A2/O process including anaerobic-anoxic-oxic reactor and used to examine N and P behaviors in the anaerobicanoxic process. Process B and C consist of anoxic-anaerobic-oxic and anoxic-oxic reactors and were used to test N and P behavior in anoxic and anoxic-anaerobic conditions, respectively. Hydraulic retention times (HRTs) for each process are listed in Figure 1. The treatment loading for individual process was 40L/day, and sludge retention time (SRT) was set to 6 days. Mixed liquor suspended solid (MLSS) concentration of each process was controlled approximately 3,500 mg/L.

Table 1. Operating conditions and characterastics of influent for batch type experiment

Phase	SCOD (mg/L)	PO <sub>4</sub> <sup>3-</sup> -P (mg/L)	$NO_3$ -N $(mg/L)$	COD loading (gCOD/gMLSS)	NO <sub>3</sub> -N loading (gNO <sub>3</sub> -N/gMLSS)
I	550	10	23	0.0652	0.0030
П	550	12	5	0.0621	0.00056
Ш	200	4	26	0.0226	0.0029





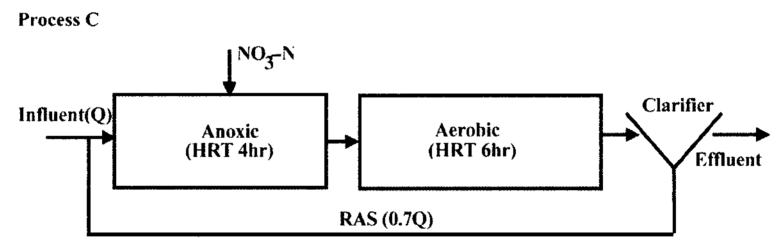


Figure 1. Schematic diagram of various BNR processes.

Table 2. Characteristics of influent for continuous feeding experiment

TCODer	SCODer	TKN	NH4 <sup>+</sup> -N	T-P	S-P	COD/TKN	COD/T-P
(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	ratio	ratio
700	480	55	37.4	12.68	7.3	8.72	37.85

Table 3. NO<sub>3</sub>-N Loading in anoxic basin of BNR processes at different phases

Phase	I	II	III	IV
NO <sub>3</sub> -N(mg/L) in anoxic basin	included NO <sub>3</sub> -N conc. in RAS	10	20	40
NO <sub>3</sub> -N Loading in anoxic basin (kgNO <sub>3</sub> -N/kg.MLSS · d)	0.02	0.065	0.13	0.19

Table 2 lists the influent composition and Table 3 shows nitrate-N loading in anoxic reactors of each process. At phase I, nitrate-N was loaded only with the return sludge from the second settling tank of municipal wastewater treatment plant. In phase II, III, and IV, concentrate KNO<sub>3</sub> solution was loaded by pump, thus to minimize phosphorus dilution effect in anoxic reactor. The final nitrate-N concentration in anoxic reactor ranged from 10 mg/L to 40 mg/L.

#### RESULT AND DISCUSSION

#### **Batch Type Experiment**

Figure 2 shows the changes of S-P and nitrate concentrations by time courses. At phase I with high organic and nitrate-N content, P release occurred from activated sludge resulting in 18 mg/L S-P concentration. As denitrification proceeded nitrate-N concentration began to decrease and so did the P concentration. This indicates that sequential P release and uptake,

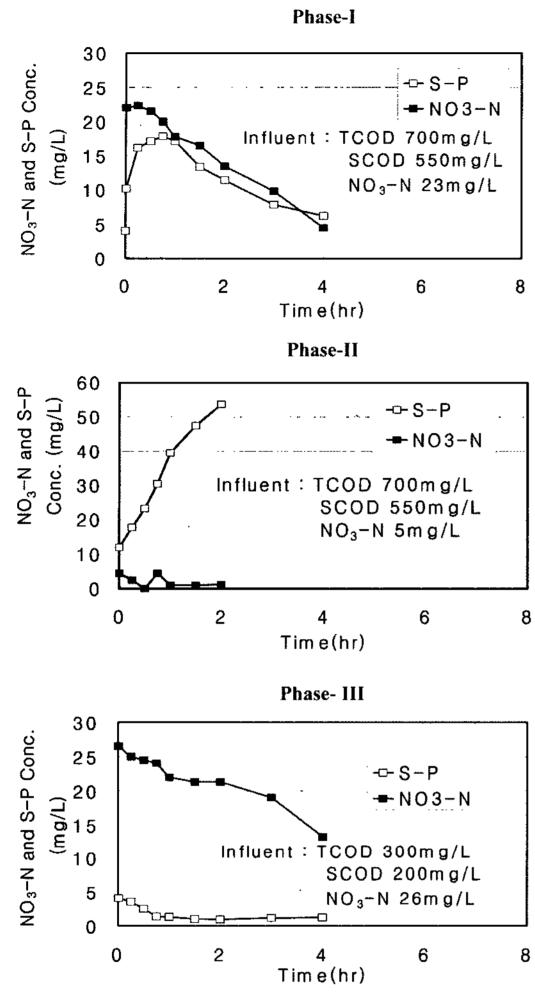


Figure 2. Time course of soluble phosphorus and nitrate in batch test.

and denitrification were undertaken in anoxic condition.

On the other hand, when influent nitrate concentration was as low as 5 mgN/L, only P release was observed but P uptake was not detected. Likewise, in phase III when influent organic was low rather than nitrate, P uptake was undertaken but not P release. As a result, it is possible to obtain simultaneous P release and uptake, and denitrification under anoxic conditions in biological nitrogen and phosphorus removal process, and the most important factors for the reaction appears as the organic and nitrate concentrations in the influent.

Regarding the P release rate, it took 90

minutes for phase I case to release phosphorus to 18 mg/L concentration, whereas it took only 30 minutes for phase II case for the same P concentration released. In the phase I, reactions of P release/uptake and denitrification were undertaken simultaneously, but in phase II, only P release and denitrification took place. This may be explained by the possible competition between the organisms related to phosphorus release and denitrification process.

The experimental results from each phase are applicable to the theories of biological phosphorus removal. In phase II where sufficient organic was provided with no electron acceptor, the organic loading was converted to acetate by fermentation and the acetate was finally accumulated as PHA in cells via acetyl CoA. The energy for the conversion of acetate to acetyl CoA was provided by ATP hydrolysis and soluble phosphate was released as a result.

When acetyl CoA is converted to PHA, there is no energy source required but reduction equivalent such as NADH<sub>2</sub> is used instead. NADH<sub>2</sub> is provided by acetyl CoA during the reactions through TCA (tri carboxylic acid) cycle with oxygen as an electron acceptor. 9,10)

The theory can be applied to the phase I, that was undertaken in anoxic conditions with high organic load. The organic in the phase I case functioned as extracellular carbon source to be converted to acetyl CoA. The part of NADH2 from TCA cycle was used for PHB production, and the other part used nitrate nitrogen as electron acceptor to produce ATP, thus the extra ATP production resulted in the decrease of polyphosphate cleavage. This may explain that why in phase I, denitrification increased but P release decreased. In phase III, the lack of organic source caused the utilization of PHA, the internal carbon source to be consumed for denitrification and ATP production.

As a result, theoretical biological metabolism can properly explain the possibility for simultaneous reactions of denitrification and luxury P uptake under anoxic condition.

#### Continuous Feeding Experiment

Figure 3 describes P concentration changes in the whole systems of each process based on the various nitrate loading in anoxic basin. The reason for low P release in the early experimental period was that influent pH levels were lowered to 5.5 from glucose degradation under the room temperature. The pH levels were then controlled to neutral range after providing temperature control device.

In case of process A, that followed typical A<sup>2</sup>/O process (AXO), influent S-P concentration was 10 mg/L and the level increased to 40-47mg/L in anaerobic reactors of each phase as a result of P release. P levels decreased slightly in anoxic reactors to 36-40 mg/L, then luxury uptake was followed in oxic reactors resulting the final P concentration around 1 mg/L, regardless the nitrate loading.

In process B, P concentration in anoxic reactor for phase I and II ranged 34-36 mg/L as a result of P release, and the concentration remained similar in anaerobic reactor. In the oxic reactor, luxury P uptake removed most P content in the liquid to less than 1 mg/L. The result revealed the fact that simultaneous P release and denitrification take place under anoxic condition. When nitrate loading becomes high however, such as in phase III and IV, S-P concentrations in anoxic reactor were 24 mg/L and 14 mg/L and in oxic reactor were 5 mg/L and 12 mg/L. This indicated that process A with AXO configuration performed better P removal than process B, with XAO configuration.

Likely to process B, process C also showed low P uptake in oxic reactor resulted from high nitrate loading. It is thus resulted that with sufficient carbon source and nitrate-nitrogen used as an electron acceptor, simultaneous P release and denitrification take place by DPB and this may increase nitrogen removal rate in BNR process. However it also affects P uptake in oxic condition causing negative point of this process.

Figure 4 compares the P release rates in the anoxic reactors of process B and C, and the

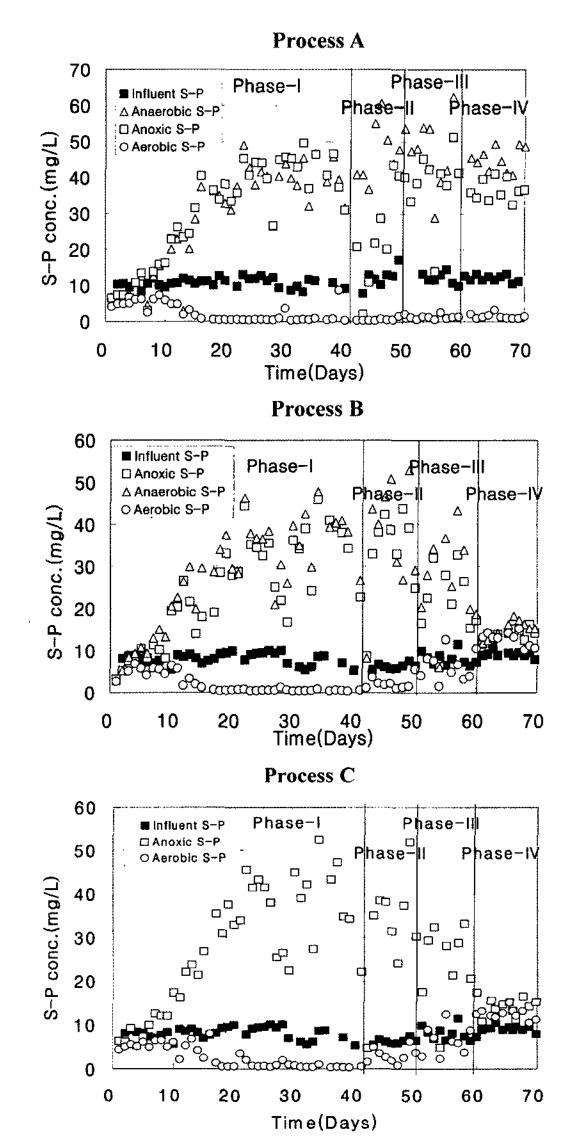


Figure 3. Variation of soluble phosphate concentration in anaerobic, anoxic and oxic reactor of BNR processes.

anaerobic reactor of process A, based on the nitrate loading. Phosphorus release rate in the process B and C followed similar trend with process A within the less than 0.07 kgNO<sub>3</sub>-N/kg-MLSS-d nitrate loading, but decreased to the half level then stopped in the higher nitrate load.

Figure 5 shows the P uptake rate in the oxic reactors of process B and C, and in the anoxic and oxic reactors of process A. The uptake rates in the process B and C are higher than the

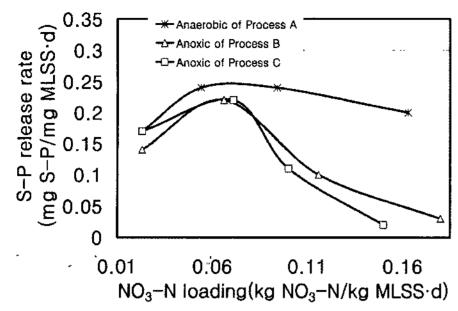


Figure 4. Phophorus release rate according to NO<sub>3</sub>-N loading at different BNR processes.

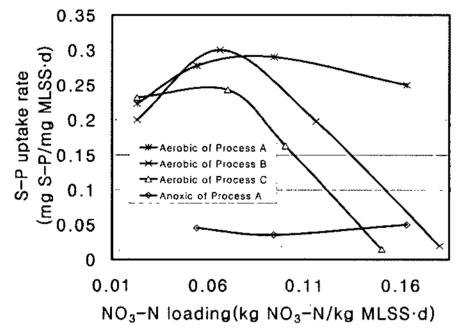


Figure 5. Phophorus uptake rate according to NO<sub>3</sub>-N loading at different BNR processes.

release rate in figure 4, resulting in a good P removal performance. However in process A, the P uptake rate in the anoxic condition appeared as very low but high in the oxic reactor. It may conclude that in BNR process with sufficient organic support, P release can take place regardless to the configuration of reactors (either anoxic-anaerobic, anaerobic-anoxic, or anoxic), but P uptake occurs much faster and actively in the oxic reactor coming after anaerobic reactor, not anoxic reactor. As it was mentioned, in anoxic conditions the competition between the organisms related to P release and to denitrification, and the utilization of nitrate for ATP synthesis resulted in the decrease of P uptake.

Figure 6 describes total-N removal rate for each process. It appears that process B and C performed better than process A. Soluble P concentrations of effluent from each process are plotted in Figure 7, showing denitrification and P release may occur up to the 0.07 kgNO<sub>3</sub><sup>-</sup>-N/kg-MLSS-d nitrate loading under anoxic condition.

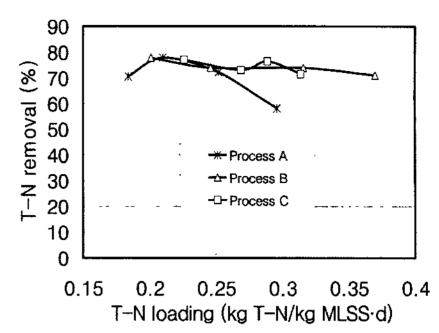


Figure 6. T-N removal efficiency.

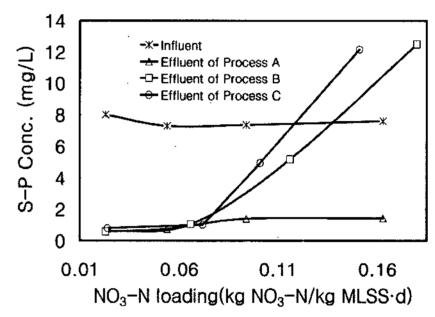


Figure 7. Influent and final effluent phosphorus concentration according to NO<sub>3</sub>-N loading at different BNR processes.

### **CONCLUSION**

The conditions for simultaneous reactions of phosphorus release and uptake, and denitrification were found from the batch-type experiment, that the organic and nitrate loading should be kept as high as 0.0652 KgCOD/KgMLSS and 0.003 KgNO<sub>3</sub>-N/Kg-MLSS, respectively. Under the anoxic condition, P release took place sharply but the total amount of P released was less than that in anaerobic condition. Luxury P uptake was also relatively slow. In continuoustype experiment, nitrate loading did not affect P release and uptake in the anoxic reactor of AXO process. However, in the XAO and AO processes, nitrate loading higher than 0.07 kgNO<sub>3</sub>-N/kg-MLSS-d inhibited P uptake in oxic reactor leaving increase in P release. Therefore it concludes that in BNR process where carbon source is sufficient, P release may take place actively disregarding the process configuration, but optimum P uptake occurs where aerobic condition follows anaerobic condition.

#### REFERENCES

- 1. Comeau, Y., Hall, K. J., Hancock, R. E. W., and Oldham, W. K., "Biochemical model for enhanced biological phosphorus removal," *Wat. Res.*, 20, 1511-1521 (1986).
- 2. Lee, S. E., Kim, K. S., and Ahn, J. W., "Comparison of phosphorus removal characteristics between various BNR processes," Wat. Sci. Tech., 36(12), 61-68 (1997).
- 3. Kerrn-Jesprtsen, J. P. and Henze, M., "Biological phosphorus uptake under anoxic and aerobic conditions," *Wat. Res.*, 27:617-624 (1993).
- 4. Kuba, T., Smolders, G. J. F., van Loosdrecht, M. C. M., and Heijnen, J. J., "Biological phosphorus removal from wastewater by anaerobic-anoxic sequencing batch reactor," *Wat. Sci. Tech.*, 27:241-252 (1993).
- 5. Waner, J., Cech, J. S., and Kos, M., "New process design for biological nutrient removal," *Wat. Sci. Tech.*, 25:445-448.
- 6. Ostgaard, K., Christensson, M., Lie, E., Jonsson, K., and Welander, T., "Anoxic bio-

- logical phosphorus removal in a full-scale UCT process," Wat. Res., 31(11), 2719-2726 (1997).
- 7. Kim, K. S., Cho, K. M., and Choi, H. C., "Enhancement nutrient removal in combined anoxic/anaerobic-aerobic reactor," *1st world water congress of IWA*, Paris, France (2000).
- 8. Bortone, G., Saltarelli, R., Alonso, V., Sorm, R., Wanner, and J., Tilche, "A Biological anoxic phosphorus removal- The Dephanox Process," 18th IAWQ biennial international conference, Singapore, 102-109 (1996).
- 9. Arun, V., Mino, T., and Matsuo, T., "Metabolism of carboxylic acids located in and around the glycolytic pathway and TCA cycle," in the biological phosphorus removal process, *Wat. Sci. Tech.*, 21:363-374 (1989).
- 10. Smolders, G. J. F., van der Meij, J., van Loosdrecht, M. C. M., Heijnen, J. J., "Model of the anaerobic metabolism of the biological phosphorus removal process: stoichiometry and pH influence," *Biotechnol. Bioeng.*, 43:461-470 (1994).