การบำบัดธาตุอาหารโดยใช้ไนไตรต์เป็นตัวรับอิเล็กตรอนในถังปฏิกรณ์ตรึงฟิล์มซีเควนซิ่งแบตช์

ชาญยุทธ กฤตุนันท์กุล[1] และ เฉลิมราช วันทวิน[2]

มหาวิทยาลัยเทคโนโลยีพระจอมเกล้าธนบุรี บางมด ทุ่งครุ กรุงเทพฯ 10140

รับเมื่อ 16 สิงหาคม 2548 ตอบรับเมื่อ 9 พฤศจิกายน 2548

บทคัดย่อ

กระบวนการบำบัดไนโตรเจนสามารถเกิดขึ้นไปพร้อมๆ กับการบำบัดฟอสฟอรัส โดยกลุ่มจุลินทรีย์ Denitrifying polyphosphate accumulating organisms (ดีเอ็นพีเอโอ) ในงานวิจัยนี้ใช้ไนไตรต์เป็นสารรับอิเลคตรอน และใช้กระบวนการตรึงฟิล์มซีเควนซิ่งแบตช์ (เอสบีบีอาร์) ที่ควบคุมเวลากักเก็บน้ำและสลัดจ์เท่ากับ 16 ชั่วโมง และ 12 วัน ตามลำดับ มีการทำงานเป็นแบบ แอนแอโรบิก-แอนนอกซิก-แอโรบิก จำนวน 2 ถังปฏิกรณ์ซึ่งเติมไนไตรต์และส่วนผสมของไนเตรตกับไนไตรต์ ในช่วงแอนนอกซิกตามลำดับ น้ำเสียสังเคราะห์ที่ใช้มีกรดอะซิติกเป็นสารคาร์บอน มีความเข้มข้นซีโอดี 400 มิลลิกรัมต่อลิตร ฟอสฟอรัส 15 มิลลิกรัมต่อลิตร และแอมโมเนียไนโตรเจน 100 มิลลิกรัมต่อลิตร ผลการทดสอบพบว่า ดีเอ็นพีเอโอสามารถใช้ไนไตรต์เป็นตัวรับอิเล็กตรอนในการบำบัดฟอสฟอรัส ภายใต้สภาวะแอนนอกซิกได้ อย่างไรก็ตาม ไนเตรตจะถูกใช้ได้ดีกว่าไนไตรต์ และการบำบัดไนโตรเจนพร้อมกับฟอสฟอรัสสามารถเกิดขึ้นได้ภายใต้สภาวะแอโรบิก กรณีที่เติมไนไตรต์ 60 มิลลิกรัมไนโตรเจนต่อลิตร ของปริมาตรถังปฏิกรณ์ อัตราการบำบัดแอมโมเนียและฟอสฟอรัสในถังปฏิกรณ์เท่ากับ 0.51 กรัมไนโตรเจนต่อตารางเมตรต่อวัน และ 0.048 กรัมฟอสฟอรัสต่อตารางเมตรต่อวัน ตามลำดับ อัตราส่วนของโมลฟอสฟอรัสที่ถูกจับใช้ต่อโมลอิเล็กตรอนที่ถูกใช้ไปของไนไตรต์ มีค่าสูงเท่ากับ 0.11 โมลฟอสฟอรัสต่อโมลอิเล็กตรอนในการทดลองแบบแบตช์ ซึ่งชี้ให้เห็นว่ากลุ่มจุลินทรีย์ส่วนใหญ่ที่ดีไนตริฟายโดยใช้ไนไตรต์เป็นพวกดีเอ็นพีเอโอ

คำสำคัญ : ไนโตรเจน / ฟอสฟอรัส / ไนไตรต์ / ถังปฏิกรณ์ตรึงฟิล์มซีเควนซิ่งแบตช์

---

[1]นักศึกษาระดับบัณฑิตศึกษา บัณฑิตวิทยาลัยร่วมด้านพลังงานและสิ่งแวดล้อม

[2]รองศาสตราจารย์ ภาควิชาวิศวกรรมสิ่งแวดล้อม

Nutrient Remove by Using Nitrite as an Electron Acceptor in Sequencing Batch Biofilm Reactor

Chanyud Kritsunankul [1] and Chalermraj Wantawin[2]
King Mongkut's University of Technology Thonburi, Bangmod, Toongkru, Bangkok 10140

Received 16 August 2005 ; accepted 9 November 2005

Abstract

Simultaneous nitrogen and phosphorus removal by denitrifying polyphosphate accumulating organisms (DNPAOs) can take place in the biofilm system. Nitrite was utilized as an electron acceptor in this study. The lab-scale sequencing batch biofilm reactors (SBBRs) were used to investigate the performance of the system by control hydraulic and solid retention times of 16 hours and 12 days, respectively. Two SBBRs were operated under alternating anaerobic-anoxic-aerobic condition, in which nitrite and mixed nitrate-nitrite were added respectively. The synthetic wastewater with acetic acid as a carbon source was supplied at the concentrations of 400 mg COD/l. The phosphorus and ammonia concentration in the synthetic wastewater were 15 mg P/l and 100 mg N/l, respectively. The results showed that phosphorus could be removed under anoxic condition by DNPAOs utilizing nitrite as an electron acceptor. However nitrate was more favorable than nitrite. The simultaneous nitrogen and phosphorus removal could be also occurred under aerobic condition. Under the condition of nitrite supplying at 60 mg N/l of reactor volume, the ammonia and phosphorus removal rates were 0.51 g N/m^2.d and 0.048 g P/m^2.d, respectively. The high ratio of mole phosphorus uptake to mole electron utilization of nitrite of 0.11 mole P/mole electron in the batch experiment indicated that the major group of denitrifiers which can utilize nitrite was DNPAO.

Keywords : Nitrogen / Phosphorus / Nitrite / Sequencing Batch Biofilm Reactor

---

[1] *Graduate Student, The Joint Graduate School of Energy and Environment.*

[2] *Associate Professor, Department of Environmental Engineering.*

1. Introduction

Several researchers have reported that high removals of nitrogen and phosphorus can be achieved in a single sludge system when a sequencing bath reactor (SBR) is applied. Moreover, the problem of COD limitation can be solved by introducing denitrifying polyphosphate accumulating organisms (DNPAOs) in the system [1-5]. The DNPAOs have capability on utilizing nitrite or nitrate instead of oxygen as an electron acceptor for phosphorus uptake that results to simultaneous nitrogen and phosphorus removal. Nevertheless, it has been reported that the nitrite accumulation in the system can inhibit phosphorus removal process. The phosphorus uptake activity is completely suppressed at nitrite concentration of 8 mg N/l or greater [3]. In comparison to anoxic phosphorus uptake activity, the aerobic one is more sensitive to nitrite. Moreover, the presence of nitrite can be one of the factors affecting to the competition between glycogen accumulating organisms (GAOs) and polyphosphate accumulating organisms (PAOs) [6]. In the batch experiment, Ahn et al. [5] showed that the phosphorus-uptake activity was observed when nitrite concentration of 40 mg N/l was applied, while Hu et al. [7] reported that nitrite concentration less than 115 mg N/l did not inhibit phosphorus uptake. Then, nitrite is an alternative electron acceptor besides oxygen and nitrate, if the concentration is not excess the critical inhibiting one. Therefore, in this study, the effects of nitrite on nitrogen and phosphorus removal in sequencing batch biofilm reactor were investigated.

2. Materials and method

2.1 Reactor operation

The cylindrical vessels with working volume of 8 l and containing commercial ring-lace type fibrous media of 0.6 m (media surface area of 3.4 m^2/m) were applied as sequencing batch biofilm reactors (SBBRs). Two reactors were operated under alternating anaerobic-anoxic-aerobic condition by using Program Logical Controller (PLC). The cycle time and solid retention time were maintained at 16 hours and 12 days, respectively.

The operational conditions of system were summarized in Table 1. The nitrite of 2,460 mg N/l was supplied with the flow rate of 50 ml/h in $SBBRNO_2L$ through the whole period under anoxic condition, while the only nitrite and mixed 1:1 ratio of nitrite and nitrate of 4,100 mg N/l were supplied with the same flow rate in $SBBRNO_2H$ and $SBBRNO_xH$, respectively. In each run, the steady state concentrations of COD, phosphorus and nitrogen in the effluent as well as in the samples collected from SBBRs in a range of time until the end of cycle were measured.

Table 1 The operational conditions of system

Conditions	$SBBRNO_2L$	$SBBRNO_2H$	$SBBRNO_xH$
Anaerobic (h)	4	4	4
Anoxic (h)	4	4	4
NO_x^- loading (mg N/l-cycle)[a]	-	-	NO_2^- = 50[c] NO_3^- = 50[c]
NO_2^- loading (mg N/l-cycle)	NO_2^- = 60[b]	NO_2^- = 100[c]	-
Aerobic (h)	8	8	8

[a] The ratio of mixed nitrite and nitrate was 1:1

[b] $(50\frac{ml}{h})(\frac{1\ l}{1{,}000\ ml})(2{,}460\frac{mg\ N}{l})(4\frac{h}{cycle})/(8\ l\ \text{of reactor volume})$

[c] $(50\frac{ml}{h})(\frac{1\ l}{1{,}000\ ml})(4{,}100\frac{mg\ N}{l})(4\frac{h}{cycle})/(8\ l\ \text{of reactor volume})$

2.2 Batch experiments

The aerobic and anoxic phosphorus uptake rates of $SBBRNO_2L$ sludge were conducted in batch experiments. Sludge at the end of anaerobic condition was transferred to the batch reactor added synthetic wastewater with phosphorus of 30 mg P/l and specific nitrogen but without organic carbon. In an anoxic test, nitrogen gas was purged into the batch reactor (1,000 ml) and nitrate of 50 mg N/l was applied. In case of using nitrite as an electron acceptor, nitrate was replaced with nitrite while other operating parameters were the same. On the other hand, there was no nitrogen in synthetic wastewater in aerobic test and air was supplied continuously to maintain the dissolved oxygen not less than 2.0 mg/l. Samples of mixed liquor were collected in a range of time. The phosphorus uptake rate was calculated from the slope of the tangent line at the initial of phosphorus uptake curve for both anoxic and aerobic conditions.

2.3 Microorganism identification

Only the microorganism of $SBBRNO_2L$ sludge was identified by fluorescence in *situ* hybridization (FISH) technique. The microorganisms which could utilize nitrite as an electron acceptor such as *Nitrobacter,* denitrifier and anammox microbe were identified. The fixation solution and the hybridization method were following to Ahn et al. [8]. Other groups of microorganisms, *Nitrosomonas* which oxidized ammonia to nitrite and PAO which could remove phosphorus have been detected too. The probes of these microorganisms were shown in Table 2.

Table 2 The oligonucleotide probes with used in the experiment

Probes	Sequence (5' to 3')	Target groups	%Formamide	References
Nsm156	tattagcacatctttcgat	*Nitrosomonas*	5	[9]
NIT3	cctgtgctccatgctccg	*Nitrobacter*	40	[10]
PAO846	gttagctacggcactaaaagg	PAO	35	[11]
PAR1457	ctaccgtggtccgctgcc	Denitrifier	35	[12]
Amx820	aaaacccctctacttagtgccc	Anammox	25	[13]

2.4 Synthetic wastewater

Acetic acid was used as carbon source in synthetic wastewater which contained COD of 400 mg/l, phosphorus of 15 mg P/l and ammonia of 100 mg N/l. The fresh synthetic wastewater was used by prepared day by day. The compositions of synthetic wastewater consisted of 0.37 ml/l CH_3COOH, 0.049 g/l K_2HPO_4, 0.028 g/l KH_2PO_4, 0.4 g/l NH_4Cl, 0.6 g/l $MgSO_4.7H_2O$, 0.07 g/l $CaCl_2.2H_2O$, 0.02 g/l yeast extract, 0.01 g/l EDTA and 2 ml/l of trace mineral which contained 1.5 g/l $FeCl_3.6H_2O$, 0.15 g/l H_3BO_3, 0.03 g/l $CuSO_4.5H_2O$, 0.03 g/l KI, 0.12 g/l $MnCl_2.4H_2O$, 0.06 g/l $Na_2MoO_4.2H_2O$, 0.12 g/l $ZnSO_4.7H_2O$ and 0.15 g/l $CoCl_2.6H_2O$. The synthetic influent wastewater was controlled to a pH of 7-7.3 by using $NaHCO_3$.

2.5 Analytical methods

The samples withdrawn from the SBBRs were filtered through 0.45 μm filter paper to remove suspended solids before analysis. Standard methods [14] 5220 C, 4500-NH_3 C, 4500-NO_2^- B, 4500-NO_3^- E, 4500-P C, 4500-H^+ B, 2540 D and 2540 E were used for chemical oxygen demand (COD), ammonia, nitrite, nitrate, phosphorus, pH, suspended solids (SS) and volatile suspended solids (VSS) analyses, respectively.

3. Results and discussion

3.1 Nutrient removal under different nitrite loading

Fig. 1 shows profiles of COD, phosphorus and nitrogen in SBBRNO_2H with high nitrite loading. The results showed that COD was removed and phosphorus was released under the anaerobic condition. During the anoxic period, phosphorus was taken up but not completely. The residual of nitrite after stop feeding was 27 mg N/l lower than supplying load and still gradually decreased under aerobic condition.

For ammonia, it slightly decreased under the anaerobic and anoxic conditions and was removed by being oxidized to nitrite and nitrate under aerobic condition. As the generated nitrite and nitrate were lower than the loss of ammonia, phosphorus was also reduced. This phenomenon implied that nitrite and nitrate might be used as electron acceptors in the process of denitrification and phosphorus uptake. However, nitrite was utilized incompletely, the accumulation of nitrite was higher to 21.5 mg N/l and the effluent concentrations of phosphorus and ammonia were 8.4 mg P/l and 40 mg N/l, respectively. The average removal efficiencies of COD, phosphorus and ammonia in $SBBRNO_2H$ were 93%, 44% and 60%, respectively.

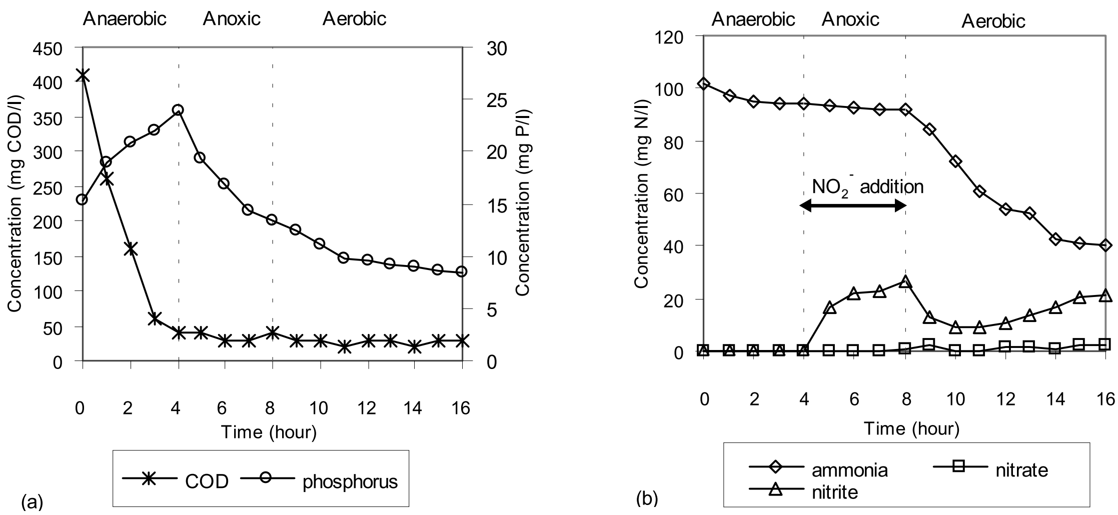

Fig. 1 Profiles of COD, phosphorus (a) and nitrogen (b) in $SBBRNO_2H$ with supplied only nitrite of 100 mg N/l

For $SBBRNO_2L$, the total nitrite supplied in the reactor was 480 mg N (60 mg N/l x 8 l) and added continuously through anoxic period. Although, the profiled of COD, phosphorus and ammonia remaining in $SBBRNO_2L$ were similar to the $SBBRNO_2H$ with high nitrite loading, nitrite was not accumulated as shown in Fig. 2. The results showed that phosphorus could be removed under the anoxic condition when volumetric nitrite loading was 60 g N/m^3. The same as in high loading experiment, the results clearly indicated that nitrite was used as an electron acceptor in denitrification process by denitrifiers and partially by DNPAOs. The average efficiencies of COD, phosphorus and ammonia removal in $SBBRNO_2L$ were 95%, 53% and 84%, respectively. Comparing to the $SBBRNO_2H$ (Fig. 1), phosphorus and ammonia removal efficiencies in $SBBRNO_2H$ in which nitrite was accumulated were lower than in $SBBRNO_2L$. It indicated that low phosphorus and ammonia removal in $SBBRNO_2H$ may be caused by the inhibition of high nitrite concentration on phosphorus uptake and nitrification processes.

The phosphorus and ammonia removal rates of $SBBRNO_2L$ were 0.048 g P/m^2.d and 0.51 g N/m^2.d, respectively. The ratio of COD utilization to phosphorus removal was 46 g COD/g P which was higher than other reports (17 g COD/g P using anaerobic-aerobic system [15] and 26 g COD/g P using anaerobic-anoxic system [1]). It is possible that COD may be competitively used by ordinary denitrifiers under anaerobic condition.

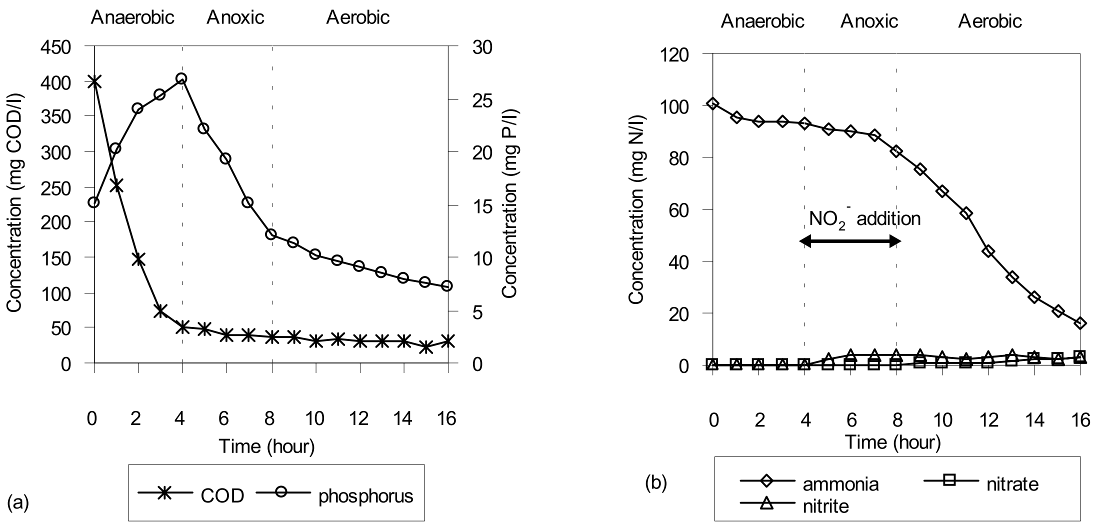

Fig. 2 Profiles of COD, phosphorus (a) and nitrogen (b) in $SBBRNO_2L$ with supplied only nitrite of 60 mg N/l

3.2 Consumption of electron acceptors under anoxic condition

In case of $SBBRNO_xH$, nitrate supplied under anoxic condition was used almost completely as shown in Fig. 3, while nitrite was slightly utilized. The accumulation of nitrite was observed throughout the anoxic period. It was accumulated higher to 34.4 mg N/l at the end of anoxic period. The results indicated that nitrite and nitrate could be utilized as electron acceptors for DNPAOs to uptake phosphorus, but nitrate has been selected first. However, nitrite-acclimatized denitrifiers could utilize nitrite as well as nitrate that was concurrent to the other works [4, 5]. These researches reported that nitrate was preferably consumed while nitrite was consumed at lower rate when supplied the mixture of nitrite and nitrate, but nitrite started to be rapidly utilized as the same rate as nitrate when the nitrate concentration decreased below 1 mg N/l.

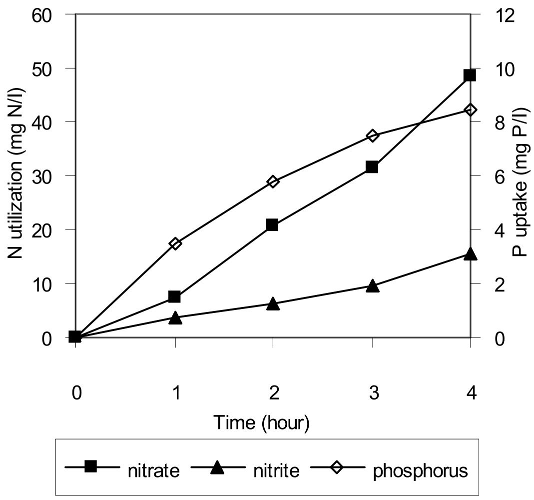

Fig. 3 The phosphorus uptake and the utilizations of nitrite and nitrate under anoxic condition in $SBBRNO_xH$

Although, the amount of nitrogen in nitrate-nitrite mixture supplied in $SBBRNO_xH$ was the same as in nitrite-nitrogen supplied in $SBBRNO_2H$, the amount of mole electron acceptor in mixture was higher. Moreover, nitrite-acclimatized denitrifiers in $SBBRNO_2H$ may utilize nitrite better than denitrifiers in $SBBRNO_xH$. Consequently, more accumulation of nitrite in $SBBRNO_xH$ than in $SBBRNO_2H$ was observed. Fig. 4 shows the utilization of electron acceptor and phosphorus uptake under anoxic condition in $SBBRNO_xH$ and $SBBRNO_2H$. The results showed that the electron acceptor in $SBBRNO_xH$ was consumed higher than in $SBBRNO_2H$, but phosphorus was taken up lower. Low phosphorus removal and high accumulation of nitrite in $SBBRNO_xH$ indicated that the high nitrite concentration may suppress the phosphorus uptake process.

According to the low phosphorus uptake was observed in high nitrogen oxide loading, GAOs in $SBBRNO_xH$ was possible to compete with PAOs on utilizing nitrogen oxide. The pervious research [16] also showed that, the lower phosphorus was uptaken when slug load of nitrate was performed.

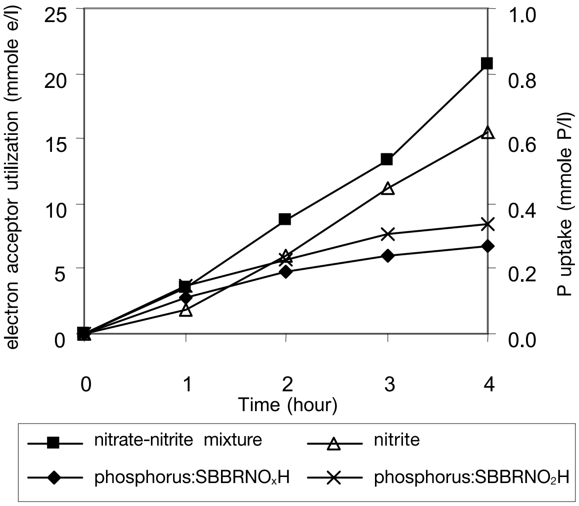

Fig. 4 The utilization of electron acceptor and phosphorus uptake under anoxic condition in $SBBRNO_xH$ and $SBBRNO_2H$

3.3 Microorganisms community

When the steady state was reached, the aerobic and anoxic phosphorus uptake rates of $SBBRNO_2L$ sludge (supplied nitrite of 60 mg N/l of reactor volume) were investigated in the batch experiments. The specific phosphorus uptake rate of $SBBRNO_2L$ sludge when using nitrite as an electron acceptor was higher than those using nitrate and oxygen as shown in Table 3. The results showed that $SBBRO_2L$ sludge could utilize not only nitrate but also nitrite for phosphorus uptake similar to the work of Hu et al. [7]. The specific phosphorus uptake rates under using nitrite and nitrate as electron acceptors were nearly to those reported in the literature (16.8 mg P/g VSS.h for nitrite and 15.4 mg P/g VSS.h for nitrate [7]) when using sludge from anaerobic-anoxic-aerobic SBR. High specific phosphorus uptake rate under anoxic condition suggested that $SBBRNO_2L$ consisted of both facultative and strictly anoxic DNPAOs. Moreover, the proportion of DNPAOs, which strictly utilized nitrite and nitrate, may be more than PAOs, which strictly utilized oxygen. The FISH technique was used to identify the microorganisms in sludge of $SBBRNO_2L$ as shown in Fig. 5, the microorganisms community of PAOs was 16.5% (Table 4).

However, the partial of PAOs could be repeatable counted when identifying denitrifiers due to denitrify capability in some PAOs (PAO846 probe could identify DNPAOs [8]). The proportion of denitrifiers was 19.3%. For specific nitrogen utilization rate, it showed that denitrifiers could utilize nitrate with higher rate than nitrite. However, the ratio of mole phosphorus uptake to mole electron utilization of nitrite was higher than nitrate. Moreover, the ratio was nearly the same as reported in the work of Shoji *et al.* [17] that used enriched-DNPAOs anaerobic-anoxic SBR system

(0.14 mole P/mole electron). In case of nitrate, the ratio was two times lower than in the previous work (0.12 mole P/mole electron [17]). These values confirmed that denitrifiers which utilized nitrite were DNPAOs, while nitrate was competitively used by ordinary denitrifiers.

Table 3 Specific phosphorus uptake and nitrogen oxide utilization rates of SBBRNO_2L sludge in batch experiments

Activities	O_2	NO_3^-	NO_2^-
Specific P uptake rate (mg P/g VSS.h)	3.7	12.3	16.3
Specific NO_x^- utilization rate (mmole e/g VSS.h)	-	7.0	4.8
P uptake NO_x^- utilization (mole P/mole e)	-	0.06	0.11

The *Nitrosomonas* and *Nitrobacter* were the other group of microorganisms which were identified by FISH technique. The activities of *Nitrosomonas* and *Nitrobacter* resulted to remove ammonia in aerobic period (Fig. 2). The proportion of *Nitrosomonas* of 20.8% was three times higher than *Nitrobacter.* The low proportion of *Nitrobacter* indicated that nitrite which was substrate of *Nitrobacter* may be competitively used by denitrifiers. Moreover, anammox microbes could be detected but low proportion of only 3 %. The reduction of ammonia in anoxic period (Fig. 2) could be uptake by the anammox microbes as followed equation [18].

$$NH_4^+ + 1.26NO_2^- + 0.085CO_2 + 0.02H^+ \rightarrow 0.017C_5H_7NO_2 + 0.24NO_3^- + N_2 + 1.95H_2O$$

Table 4 Proportion of microorganisms in SBBRNO_2L

Microorganisms	Range of proportion (%)	Number of sample
PAO	16.5 + 3.8	13
Denitrifier	19.3 + 5.2	13
Nitrosomonas	20.8 + 4.0	13
Nitrobacter	6.6 + 2.2	13
Anammox	2.4 + 1.2	13

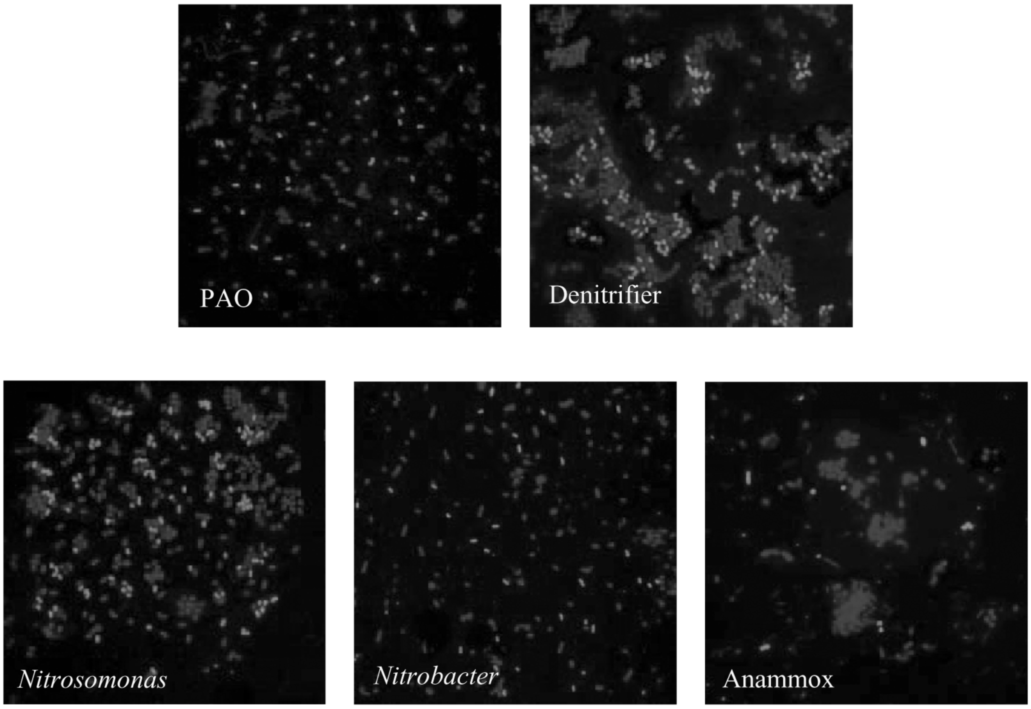

Fig. 5 FISH images of SBBRNO$_2$L sludge; gray is hybridized with DAPI and white is hybridized with target probes

4. Conclusions

This work was conducted to study the effect of nitrite on nitrogen and phosphorus removal in SBBRs operated under anaerobic-anoxic-aerobic system. The results showed that nitrite could serve as an electron acceptor for phosphorus uptake process as effectively as nitrate. However, high accumulation of nitrite was observed in high nitrogen oxide loading and low phosphorus uptake occurred under this loading due to the inhibiting effect of nitrite. The removal efficiency in SBBRNO$_2$L applied only nitrite (60 mg N/l of reactor volume) was 95% for COD, 53% for phosphorus and 84% for ammonia removal. The activity test results indicated that amount of denitrifiers that could utilize nitrite (DNPAOs) were predominant. *Nitrosomonas,* denitrifier and PAO were high proportion in system, while *Nitrobacter* were about three times lower than *Nitrosomonas.* Low *Nitrobacter* and no accumulation of nitrite in low nitrite loading system implied that nitrite may be competitively used by denitrifiers.

5. Acknowledgments

The authors would like to thank the Joint Graduate School of Energy and Environment of KMUTT for financially supporting this research.

6. References

1. Kuba, T., Smolders, G., van Loosdrecht, M.C.M., and Heijnen, J.J., 1993, “Biological Phosphorus Removal from Wastewater by Anaerobic-Anoxic Sequencing Batch Reactor”, *Water Science and Technology*, Vol. 27, No. 5-6, pp. 241-252.

2. Kuba, T., Wachtmeister, A., van Loosdrecht, M.C.M., and Heijnen, J.J., 1994, “Effect of Nitrate on Phosphorus Release in Biological Phosphorus Removal Systems”, *Water Science and Technology,* Vol. 30, No. 6, pp. 263-269.

3. Meinhold, J., Arnold, E., and Isaacs, S., 1999, “Effect of Nitrite on Anoxic Phosphorus Uptake in Biological Phosphorus Removal Activated Sludge”, *Water Research,* Vol. 33, No. 8, pp. 1871-1883.

4. Lee, D.S., Jeon, C.O., and Park, J.M., 2001, “Biological Nitrogen Removal with Enhanced Phosphate Uptake in a Sequencing Batch Reactor using Single Sludge System”, *Water Research,* Vol. 35, No. 16, pp. 3968-3976.

5. Ahn, J., Daidou, T., Tsuneda, S., and Hirata, A., 2001, “Metabolic Behavior of Denitrifying Phosphate-accumulating Organisms under Nitrate and Nitrite Electron Acceptor Conditions”, *Journal of Bioscience and Bioengineering,* Vol. 92, No. 5, pp. 442-446.

6. Saito, T., Brdjanovic, D., and van Loosdrecht, M.C.M., 2004, “Effect of Nitrite on Phosphate Uptake by Phosphate Accumulating Organisms”, *Water Research,* Vol. 38, pp. 3760-3768.

7. Hu, J.Y., Ong, S.L., Ng, W.J., Lu, F., and Fan, X.J., 2003, “A New Method for Characterizing Denitrifying Phosphorus Removal Bacteria by Using Three Different Types of Electron Acceptors”, *Water Research,* Vol. 37, pp. 3463-3471.

8. Ahn, J., Daidou, T., Tsuneda, S., and Hirata, A., 2002, “Characterization of Denitrifying Phosphate-accumulating Organisms Cultivated under Different Electron Acceptor Conditions using Polymerase Chain Reaction-denaturing Gradient Gel Electrophoresis Assay”, *Water Research,* Vol. 36, pp. 403-412.

9. Juretschko, S., Timmermann, G., Schmid, M., Schleifer, K.H., Pommerening-Röser, A., Koops, H.P., and Wagner, M., 1998, “Combined Molecular and Conventional Analyses of Nitrifying Bacterium Diversity in Activated Sludge: *Nitrosococcus mobilis* and *Nitrospira*-like bacteria as dominant populations”, *Applied and Environmental Microbiology,* Vol. 64, No. 8, pp. 3042-3051.

10. Wagner, M., Rath, G., Koops, H.P., Flood, J., and Amann, R, 1996, “In situ Analysis of Nitrifying Bacteria in Sewage Treatment Plants”, *Water Science and Technology,* Vol. 34, pp. 237-244.

11. Crocetti, G.R., Hugenholtz, P., Bond, P.L., Schuler, A., Keller, J., Jenkins, D., and Blackall, L.L., 2000, "Identification of Polyphosphate-accumulating Organisms and Design of 16S rRNA-directed Probes for Their Detection and Quantitation", *Applied and Environmental Microbiology,* Vol. 66, No. 3, pp. 1175-1182.

12. Neef, A., Zaglauer, A., Meier, H., Amann, R., Lemmer, H., and Schleifer, K.H., 1996, "Population Analysis in a Denitrifying Sand Filter: Conventional and in situ Identification of *Paracoccus* spp. in Methanol-fed Biofilms", *Applied and Environmental Microbiology,* Vol. 62, No. 12, pp. 4329-4339.

13. Egli, K., Fanger, U., Alvarez, P.J.J., Siegrist, H., van der Meer, J.R., and Zehnder, A.J.B., 2001, "Enrichment and Characterization of an Anammox Bacterium from a Rotating Biological Contactor Treating Ammonium-rich Leachate", *Archivcs of Microbiology,* Vol. 175, No. 3, pp. 198-207.

14. APHA, AWWA, and WEF., 1998, *Standard Methods for the Examination of Water and Wastewater,* 20th ed., American Public Health Association, Washington, D.C., USA.

15. Randall, C.W., Barnard, J.L., and Stensel, H.D., 1992, *Design and Retrofit of Wastewater Treatment Plants for Biological Nutrient Removal,* Technomic Publishing Company Inc., Pennsylvania, USA.

16. Wutthirat, W., 2003, *Use of Nitrite as an Electron Acceptor for Biological Phosphorus Removal,* M.Eng. Thesis, King Mongkut's University of Technology Thonburi, Bangkok, Thailand.

17. Shoji, T., Satoh, H., and Mino, T., 2003, "Biological Phosphorus Removal using Nitrite as Electron Acceptor", *IWA Asia-Pacific regional conference,* Bangkok, Thailand.

18. Rittmann, B.E., and McCarty, P.L., 2001, *Environmental Biotechnology : Principles and Applications,* McGraw-Hill, Boston.