

Effect of influent nutrient ratios and hydraulic retention time (HRT) on simultaneous phosphorus and nitrogen removal in two-sludge sequencing batch reactor

Yayi Wang<sup>a,b</sup>, Yongzhen Peng<sup>b,\*</sup>, Tom Stephenson<sup>c</sup>

<sup>a</sup>State Key Laboratory of Pollution Control and Resources Reuse, Tongji University, Siping Road, Shanghai 200092, P R China (e-mail: yayi.wang@tongji.edu.cn)

<sup>b</sup>Key Laboratory of Beijing for Water Quality Science and Water Environment Recovery Engineering, Beijing University of Technology, Beijing 100022, P R China (e-mail: pyz@bjut.edu.cn)

<sup>c</sup>Cranfield University, Cranfield, MK43 0AL, UK (e-mail: t.stephenson@cranfield.ac.uk)

\* Correspondence author

**Abstract:** A laboratory-scale anaerobic-anoxic/nitrification sequencing batch reactor (A<sub>2</sub>N-SBR) fed with domestic wastewater was operated to examine the effect of varying ratios of influent COD/P, COD/TN and TN/P on the nutrient removal. With the increased COD/P, the phosphorus removals exhibited an upward trend. The influent TN/P ratios had a positive linear correlation with the phosphorus removal efficiencies, mainly because nitrates act as electron acceptors for the phosphorus uptake in the A<sub>2</sub>N-SBR. Moreover, it was found that lower COD/TN ratio, e.g. 3.5, did not significantly weaken the phosphorus removal, though the nitrogen removal first decreased greatly. The optimal phosphorus and nitrogen removals of 94% and 91% respectively were achieved with influent COD/P and COD/TN ratios of 19.9 and 9.9 respectively. Additionally, a real-time control strategy for A<sub>2</sub>N-SBR can be undertaken based on some characteristic points of pH, redox potential (ORP) and dissolved oxygen (DO) profiles in order to obtain the optimum hydraulic retention time (HRT) and improve the operating reliability.

**Key words:** sequencing batch reactor (SBR); anaerobic-anoxic/nitrification (A<sub>2</sub>N); denitrifying phosphorus removal organisms (DNPAOs); nutrient ratios; real-time control

## 1. Introduction

For conventional biological nutrient removal (BNR) processes, Chemical oxygen demand (COD) acts as a limiting factor for phosphorus release and denitrification, especially for wastewaters with lower COD/N ratios (Kuba et al., 1993, 1996; Meinhold et al., 1999). The discovery of denitrifying phosphate-accumulating organisms (DNPAOs) in enhanced biological phosphorus removal (EBPR) processes

offers an appropriate solution to the problem associated with the limitation of COD (Kuba et al., 1993; 1994; 1996). DNPAOs are capable of anoxic phosphorus uptake by using nitrate/nitrite ( $\text{NO}_3^-$  or  $\text{NO}_2^-$ ) instead of oxygen as electron acceptors, thereby removing nitrogen and phosphorus simultaneously without any extracellular carbon source (Kuba et al., 1994; 1996). They are activated under the alternate anaerobic/anoxic conditions and have metabolic characteristics similar to those of phosphate-accumulating organisms (PAOs) (Murnleitner et al., 1997; Mino et al., 1998; Ahn et al., 2001). Applying DNPAOs in BNR processes makes it possible to save COD (50%) and energy (aeration) (30%) with less sludge production (50%) (Kuba et al., 1997; Copp et al., 1998).

Some new denitrifying phosphorus removal processes, such as the anaerobic-anoxic/nitrifying sequencing batch reactor ( $\text{A}_2\text{N-SBR}$ ), DEPHANOX (continuous-flow), modified UCT (University of Cape Town) and biological-chemical phosphorus and nitrogen removal (BCFs), based on the activities of DNPAOs have been proposed in the past (Vlekke et al., 1988; Wanner et al., 1992; Kuba et al., 1996; 1997; Bortone et al., 1999; Hao et al., 2006). The processes promote DNPAOs and are suitable for low-COD wastewater treatment. The  $\text{A}_2\text{N-SBR}$  process, which couples an anaerobic-anoxic SBR with a nitrification SBR as a two-sludge system, is one of the prominent denitrifying phosphorus nitrogen removal processes. A laboratory-scale study by Kuba et al. (1997) indicated that  $\text{A}_2\text{N-SBR}$  showed steady phosphorus and nitrogen removals which enabled the removal of 15mg P/L and 105mg N/L at the expense of only 400 mg COD/L acetate (HAc), i.e. the optimal influent ratio was as low as 3.4 g COD/g N. Additionally,  $\text{A}_2\text{N-SBR}$  integrates two SBRs which means it is easy to change operations, such as cycle times and flow rates (Irvine et al., 1997; Del Solar et al., 2004; Bungay et al., 2007). Therefore, the  $\text{A}_2\text{N-SBR}$  has higher flexibility and has been regarded as effective, especially for small wastewater treatment plants.

Many studies have been dedicated to the behavior of DNPAOs, most of which were carried out with enriched PAO/DNPAO cultures using acetate as a sole source of volatile fatty acids (VFAs) in the influent synthetic wastewater (Kuba et al., 1994; Bond et al., 1999; Sudiana et al., 1999; Merzouki et al., 2001; Harper et al., 2003; Mahendraker et al., 2005). However, few investigations have been conducted on the real wastewater. The quality of real sewage is known to fluctuate widely throughout day and night, easily resulting in unstable treatment performance. On the other hand, the wastewater characteristics, e.g. influent ratios of C, N and P, are of primary importance for designing BNR processes. These guide the process design and configuration required to achieve the desired treatment. Moreover, the fraction of nitrogen and phosphorus, which is removed by each biological mechanism, depends strongly on the COD/N and COD/P ratios of the wastewater (Mahendraker et al., 2001). In this instance, the effect of the influent nutrient ratio is one of the most significant issues in assessment of the performance of the  $\text{A}_2\text{N-SBR}$  process, especially in regard to guaranteeing the steady denitrifying phosphorus removal.

In addition, it has been found that the monitoring and regulation of online parameters such as pH, DO, ORP and nutrient pollutants (phosphorus and  $\text{NH}_4^+\text{-N/NO}_2^-\text{-N/NO}_3^-\text{-N}$ ) can be helpful in achieving and maintaining high nutrient removal for treating real municipal wastewater (Lee et al., 2001; Wu et al., 2007). Therefore, a real-time control for process optimization is expected to be considered in the  $\text{A}_2\text{N-SBR}$  process.

In this study, an  $\text{A}_2\text{N-SBR}$  system was operated in order to gain a better understanding of the correlation between the influent nutrient ratios and the final nutrient removal efficiency; seek a reliable hydraulic retention time (HRT) for complete phosphorus removal with the variable influent nutrient ratios and examine the feasibility of establishing a real-time control strategy for  $\text{A}_2\text{N-SBR}$  for steadier operating.

## 2. Materials and Methods

### 2.1. Process setup and operation

A laboratory-scale  $\text{A}_2\text{N-SBR}$  characterized by the two-sludge system consisted of two reactors, i.e.  $\text{A}_2\text{-SBR}$  and  $\text{N-SBR}$ . The SBR was made from transparent Plexiglas with a tapered bottom. The working volumes of the two SBRs were both 12 L with the inner diameter of 190 mm and a height of 700 mm. A peristaltic pump was used to fill the  $\text{A}_2\text{-SBR}$  with the domestic wastewater from the influent tank during the fill phase. A mechanical mixer with a rectangular paddle was installed over the  $\text{A}_2\text{-SBR}$  tank and an air pump supplied air through a porous stone diffuser, located at the bottoms of these two SBRs.

The  $\text{A}_2\text{-SBR}$ , operating for denitrifying phosphorus removal, was inoculated with sludge from Hangzhou Sibao Sewage Treatment Plant (China). The sludge retention time (SRT) of the  $\text{A}_2\text{-SBR}$  was 18-20 days and the concentration of mixed liquor suspended solids (MLSS) ranged between 3800 mg/L and 4400 mg/L. The  $\text{N-SBR}$  packed with “Pall rings” as a biofilm carrier was operated under aerobic conditions for nitrification. The whole operation cycle for  $\text{A}_2\text{N-SBR}$  was approximately 13 h. A typical cycle consisted of six phases as described in Table 1. The reaction time for each phase was controlled by a timer automatically and the whole experiment

(including set-up) was conducted at a temperature of 22 to 25°C. Reactor control and data acquisition (pH, DO and ORP) were taken with the software package (WTW inolab). pH was not controlled throughout the experiment.

### 2.2. Domestic wastewater

The domestic wastewater for this experiment was taken from a septic tank on the

campus of Zhejiang University of Technology (China). The average concentrations of COD, Biochemical oxygen demand (BOD<sub>5</sub>), total nitrogen (TN), NH<sub>4</sub><sup>+</sup>-N and phosphorus in the influent wastewater over the experimental period were 237±58 mg/L, 145±98 mg/L, 37±21 mg/L, 35±20 mg/L and 12±5 mg/L, respectively. The pH of the influent varied between 6.81 and 7.88 with the mean value of 7.20. As the mean influent NH<sub>4</sub><sup>+</sup>-N concentration during the spring semester was found to be approximately 25 mg/L lower than that of the autumn semester, a spike of nitrate (KNO<sub>3</sub>) of 10mg/L NO<sub>3</sub><sup>-</sup>-N was added to the initial anoxic phase to ensure there was no limitation of electron acceptors for a complete phosphorus uptake. Meanwhile, KH<sub>2</sub>PO<sub>4</sub> was added to obtain the different influent phosphorus loading.

### **Table 1 the operating procedure for the A<sub>2</sub>N-SBR process**

#### 2.3. Experimental approach

The operational conditions for A<sub>2</sub>N-SBR during two experimental periods, defined as Run 1–7, are summarized in Table 2. During period I undertaken within the autumn semester, KH<sub>2</sub>PO<sub>4</sub> was added to vary influent phosphorus loads. For period II conducted during spring semester, the anoxic and aerobic reaction times were adjusted manually according to the varied effluent quality to determine the optimal HRT. After each cycle, the PAO/DNPAO sludge was washed twice by tap water to keep the parallel experimental conditions.

### **Table 2 Experimental approach for A<sub>2</sub>N-SBR process (superscript a adding nitrate in the initial anoxic phase; superscript b T<sub>ano</sub>-anoxic reaction time; superscript c T<sub>n</sub>-nitrification reaction time)**

#### 2.4. Analytical methods

BOD<sub>5</sub>, PO<sub>4</sub><sup>3-</sup>-P, TN, NH<sub>4</sub><sup>+</sup>-N, NO<sub>2</sub><sup>-</sup>-N, NO<sub>3</sub><sup>-</sup>-N and MLSS were analyzed in accordance with standard methods (APHA, 1998). The COD was measured with a 5B-1 type COD quick-analysis instrument (Lanzhou Environmental Technology Co., Ltd). The DO and temperature were monitored online using a Wissenschaftlich-Technische-Werkstätte (WTW) oxygen probe. Continuous monitoring of pH and ORP were carried out using two WTW inolab<sup>®</sup> pH level 2 meters with an ORP electrode and a pH probe (WTW).

### 3. Results and Discussion

During the start-up period, the A<sub>2</sub>N-SBR process operated for 63 days until PO<sub>4</sub><sup>3-</sup>-P, NH<sub>4</sub><sup>+</sup>-N and COD removal efficiencies reached 85%, 99% and 89%, respectively. Mixed liquor suspended solids (MLSS) in the A<sub>2</sub>-SBR remained between 3800–4400 mg/L throughout the experimental periods.

#### 3.1. A<sub>2</sub>N-SBR performance under the varying influent nutrient ratios

Despite the great fluctuation in the feed quality, effluent COD and  $\text{NH}_4^+\text{-N}$  concentrations for the  $\text{A}_2\text{N-SBR}$  were as lower as 39 mg/L and 6.8 mg/L (Table 3), which had met the discharge consents. The biofilm N-SBR performed well throughout the experimental period with  $\text{NH}_3\text{-N}$  ions almost completely converting to  $\text{NO}_3^-$  or  $\text{NO}_2^-$ . Also, no significant phosphorus removal was observed in N-SBR during nitrification phase.  $\text{NO}_3^-\text{-N}$  was seldom detected in the anoxic effluent, whereas, the TN concentration in the final effluent was as high as 8.6 mg/L (Table 3), mainly due to the anaerobic residue  $\text{NH}_4^+\text{-N}$  existing in the settled sludge, which was transferred to anoxic phase directly without being nitrified by the N-SBR. Later, during the post-aeration phase in  $\text{A}_2\text{-SBR}$ , a fraction of this residual  $\text{NH}_4^+\text{-N}$  converted to  $\text{NO}_2^-\text{-N}/\text{NO}_3^-\text{-N}$  without being denitrified.

**Table 3 Performance of  $\text{A}_2\text{N-SBR}$  process for removal of COD,  $\text{NH}_4^+\text{-N}$ , TN and P (average data are in parenthesis)**

For the phosphorus removal, as the influent phosphorus concentration was adjusted in a broad range to gain the different COD/P, this subsequently caused a significant fluctuation of phosphorus removal efficiency even though the average phosphorus removal efficiency had reached 89% (Table 3). Phosphorus removal efficiency for each run will be discussed at the following sections. During the experimental phase the effluent pH ranged from 7.17 to 7.45 with an average value of 7.28.

### 3.2. Relationships between measured online parameters (ORP, DO and pH) and nutrient-containing ions during one typical cycle of the $\text{A}_2\text{N-SBR}$

Figs.1 and 2 show the relationship between the measured online parameters (ORP, DO and pH) and the nutrient-containing ions ( $\text{NH}_4^+\text{-N}/\text{NO}_2^-\text{-N}/\text{NO}_3^-\text{-N}$ ,  $\text{PO}_4^{3-}\text{-P}$ ) during a typical cycle at day 241.

In  $\text{A}_2\text{-SBR}$ , an immediate and sharp increase in pH ranging from 7.06 to a peak of 7.21 (shown with point A in Fig. 2) was observed at the initial 15 min of anaerobic phase which was attributable to the VFAs uptake by DNPAO/PAO. Then, pH decreased continuously mainly due to the release of orthophosphate ( $\text{PO}_4^{3-}\text{-P}$ ) ion. It was also found that the decrease rate of pH had a close correlation with the P-release rate (Figs. 1 and 2). After anaerobic reaction for about 70 min, the P-release rate became relatively low and pH reached a constant value (point B in Fig. 2) that probably indicated the termination of the P-release. Meanwhile, the ORP curve dropped continuously throughout the anaerobic process and reached -212mV at the end of anaerobic phase. Clearly, the ORP profiles can not exhibit a characteristic point identifying the end of the phosphorus release but online monitoring of the ORP favors to guarantee the strict anaerobic conditions for the better phosphorus release. During the subsequent anoxic process, a sharp increase of pH was observed along with the prolonged reaction as a result of anoxic phosphorus uptake and

denitrification. As to the ORP curve, it was observed to decline initially and then approach a plateau (point E in Fig. 2) where the  $\text{NO}_3^-/\text{NO}_2^-$  concentrations both reached the relatively consistent values (Fig. 1).

In the N-SBR, pH increased sharply during the initial 30 min of aeration reaction. It was also found that only the undegradable organic compounds remained in the SBR after the aeration reaction for 30 min, which could be confirmed by the COD measurement in Fig. 1. Further, after nitrification became the dominant reaction in N-SBR, pH dropped initially and then increased to the point C (Fig. 2) corresponding to the end of the nitrification (Fig. 1).

**Fig. 1. Profiles of COD,  $\text{PO}_4^{3-}\text{-P}$ ,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  and  $\text{NO}_2^-\text{-N}$  variations during one typical cycle of  $\text{A}_2\text{N}$ -SBR process (on day 241) (MLSS=3875 mg/L; T=23-25.5°C)**

**Fig. 2. Variations of DO, ORP and pH during one typical cycle of  $\text{A}_2\text{N}$ -SBR process (on day 241) (MLSS=3875 mg/L; T=23-25.5°C)**

For the DO variations, in N-SBR a DO plateau (point D in Fig.2) occurred during the latter of nitrification stage, which coincided with the complete ammonia conversion, confirmed by the  $\text{NH}_3^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  measurements in Fig. 1. In  $\text{A}_2\text{N}$ -SBR, during the post-aeration phase the end of nitrification was also identified based on the bending-points of DO (point F in Fig. 2) and pH (point G in Fig. 2) profiles when the oxidation of  $\text{NH}_4^+\text{-N}$  finished (Fig. 1).

### 3.3. Effect of influent COD/P on the $\text{A}_2\text{N}$ -SBR process performance

Although with the great fluctuation of the influent COD/P ratios, steady anaerobic P-release was observed during four operational periods (Runs 1–4, period I) with the average concentrations of approximately 40.8 mg P/L at the end of anaerobic phase (Fig. 3). At the beginning of anoxic phase, phosphorus concentration was normally 42 mg/L on average, and then decreased to 20.8 mg/L at the end of anoxic phase as a result of the denitrifying phosphorus uptake. The percentage of the amount of anoxic P uptake to overall P uptake was close to 51%. After 2h post-aeration, the final effluent P concentration was stable at 2.0 mg/L (mean value). This finding revealed that the post-aeration phase was indispensable to  $\text{A}_2\text{N}$ -SBR process for polishing the effluent, as the post aerobic phosphorus uptake accounted for approximately 45% of the phosphorus removal when electron acceptors as  $\text{NO}_3^-\text{-N}$  were not available.

**Fig.3. Variations of phosphorus concentration at different reaction phases with different influent COD/P in the  $\text{A}_2\text{N}$ -SBR process (The horizontal line A**

**represents the mean anaerobic phosphorus concentration and the line B represents the mean anoxic phosphorus concentration)**

The phosphate release and uptake capacity as well as the phosphorus removal in the A<sub>2</sub>N-SBR exhibited different patterns with the different influent COD/P, as seen from Fig. 3. In Run 1, the influent COD/P was 19.4 on average. The mean phosphorus concentrations at the beginning of the anaerobic phase and the final effluent were 11.9 and 0.5 mg/L respectively, with the phosphorus removal efficiency of 96% (Table 4). For Run 2, the influent COD/P decreased to 15.4 by the addition of KH<sub>2</sub>PO<sub>4</sub>. The mean influent and effluent phosphorus concentrations thereby increased to 15.7 and 1.6 mg/L respectively, with the removal efficiency of 90%. Since the phosphorus removal efficiency of Run 2 decreased compared with Run 1 (Table 4), the influent COD/P was returned to 30.8 in the subsequent Run 3 to recover the phosphorus removal capacity. The phosphorus concentrations of influent and effluent in Run 3 both declined to 6.9 and 0.3 mg/L, respectively, with the increased phosphorus removal efficiency of 97%. For Run 4, the influent phosphorus concentration was as high as 21.6 mg/L and the influent COD/P thus dropped down to 9.5. Consequently, with this shock load the effluent P concentration was above 8.5 mg/L, resulting in the lower phosphorus removal efficiency of 62%.

**Table 4 Comparison of COD/TN, COD/P and TN/P with nutrients removal efficiencies for A<sub>2</sub>N-SBR process (<sup>a</sup>adding 10 mg/L nitrate at the beginning of the anoxic phase)**

Phosphorus removal efficiencies exhibited an increase trend with the increasing of influent COD/P and a linear positive relationship was found between COD/P and phosphorus removal with COD/P in the range of 7.2 and 19.4 (Fig. 4). When COD/P researched approximately 13.1, higher phosphorus removal of above 85% was achieved in A<sub>2</sub>N-SBR. Further, when the influent COD/P continued to be raised up to 19.4, the phosphorus removal kept at a constant value. This means that further increase of the COD/P can not promote the phosphorus removal efficiency, mainly because PAOs or DNPAOs have the maximum capacity for carbon source consumption to synthesis inner Poly-β-hydroxybutyrate (PHB) that determines the phosphorus uptake capacity, and can not consume extra carbon once their PHB assimilation capacity researches the threshold.

**Fig.4. Relationships between the influent COD/P and phosphorus removal efficiencies in A<sub>2</sub>N-SBR process**

In contrast, there were several days when the influent COD/P varying between 8.8 and 10.9 in Run 1 (signified by arrow A in Fig. 4), where the phosphorus removal efficiencies of over 90% were achieved, which seems debatable. We checked the data and found the influent COD, NH<sub>4</sub><sup>+</sup>-N as well as phosphorus concentrations during

these days were lower than the normal days due to the weekend holidays. Particularly, the influent  $\text{NH}_4^+$ -N concentrations were approximately 10 mg/L lower than that of normal days and most of the released phosphorus was removed mainly during the post-aeration by using oxygen as the final electron acceptors. This means that during these days most of the influent carbon source available had been used only for the phosphorus removal, thereby favored to enhance the phosphorus removal performance. In this instance, in the  $\text{A}_2$ -SBR process the impact of the influent COD/N ratio on the final P removal efficiency should not be neglected.

#### 3.4. Effect of influent COD/N and TN/P on the $\text{A}_2$ N-SBR process performance

As with COD/P, COD/TN and TN/P also play key roles in  $\text{A}_2$ N-SBR for the efficient nitrogen and phosphorus removals (Table 5). The TN removal efficiencies have positive linear correlation with the influent COD/TN ratios ( $n=7$ ) (Fig. 5): the greater the influent COD/TN, the better the TN removal was obtained. Further, TN/P ratios were found to be closely correlated to the phosphorus removals with the correlation coefficient ( $r$ ) of 0.8345 ( $n=7$ ), which confirmed that in  $\text{A}_2$ N-SBR where denitrifying phosphorus uptake mechanism is applied, the TN/P ratio has greater influence on the final phosphorus removal efficiencies.

**Fig.5. Influence of COD/TN and TN/P on the nitrogen and phosphorus removal (a: the relationships between the influent COD/TN and TN removal efficiencies; b: the relationships between the influent TN/P and phosphorus removal efficiencies; data from Table 5)**

In our study, no distinct relationship between COD/TN and phosphorus removal efficiencies was found except that it was found the low influent COD/TN did not significantly weaken the final phosphorus removals. For example, in Run 2 with the lowest COD/TN of 3.5, the phosphorus removal efficiency maintained around 88%, although TN removal efficiency had declined to 66%. This finding is opposite to the conventional BNR processes, where the nitrogen removal process always takes priority over the phosphorus removal process to utilize the limited carbon source in the influent.

#### 3.5. Effect of anoxic HRT on the $\text{A}_2$ N-SBR performance

In Run 6 the anoxic HRT was prolonged to 4h with the aim of obtaining the desired HRT for the optimal nutrient removal, since in Run 5 the phosphorus concentration at the end of the anoxic phase was as high as 16.7 mg/L and the corresponding  $\text{NO}_x$ -N (electron acceptors) concentration was around 3 mg/L, which seems that the anoxic reaction time of 3h may not be sufficient for the complete anoxic P-uptake and denitrification. Additionally, increase of MLSS by prolonging SRT was also undertaken in the  $\text{A}_2$ -SBR to promote the denitrification rate and phosphorus uptake rate.



**Table 5 Effect of the anoxic HRT on the A<sub>2</sub>N-SBR process performance (mean values)**

With the prolonged anoxic HRT in Run 6, the anaerobic P-release and aerobic P-uptake both increased greatly (Table 5). A larger amount of phosphorus released under anaerobic condition was suspected to be related to the applied operating condition of long SRT which favored the more accumulation of the PAO/DNPAO. Nitrate in the effluent of anoxic phase was under detection limit. Compared with Run 5, the TN and phosphorus removal efficiencies of Run 6 were improved by approximately 12.6% and 25.3%, respectively.

In the subsequent Run 7, the anoxic reaction time was returned to 3h to examine the operating reliability of the A<sub>2</sub>N-SBR when the reaction time was shortened. The net anaerobic P-release for Run 7 was similar to that of Run 6, i.e. both around 50 mg/L. This indicated that PHB had been sufficiently synthesized by PAO/DNPAO during the anaerobic phase. For the net anoxic P-uptake of Runs 6 and 7, it was 36.0 and 33.7 mg/L, respectively. The slight difference of the anoxic phosphorus uptake for these two tests was suspected to due to the “endogenous” phosphorus release during anoxic react period. Actually, the “endogenous” phosphorus release did occur in our study when no NO<sub>3</sub><sup>-</sup>-N was available for DNPAOs in anoxic phase. On day 261 (during Run 6), it was found that phosphorus concentration was 20.5 mg/L at 3h of the anoxic reaction, and the corresponding NO<sub>3</sub><sup>-</sup>-N concentration was close to 0 mg/L. Further, by extending the anoxic reaction time to 4h, the effluent phosphorus concentration increased to 23.9 mg/L, indicating that the “second” phosphorus had taken place.

Since the real domestic wastewater was used in this study, the nutrient concentrations as well as their ratios might vary significantly; thus, the fix-time strategy can not ensure the optimum reaction time for each biological procedure based on the varying influent nutrient ratios.

## 4. Discussion

### 4.1. Effect of the nutrient ratios on the A<sub>2</sub>N-SBR performance

The experiments performed with the different COD/P revealed that the higher the influent phosphorus concentrations, the more the phosphorus was released due to the more phosphorus available for PAO/DNPAO to accumulate as internal poly-P (Fig. 3), despite the similar COD loadings adopted in all tests (Table 4). Same phenomenon was also presented by Suidiana et al. (1999) and Sevour et al. (2003), who explained that more PAOs could be accumulated and activated in the EBPR system with the higher phosphorus loading.

Compared with the conventional simultaneous phosphorus and nitrogen removal

processes, the A<sub>2</sub>N-SBR process has saved greater amount of COD consumption for the optimum phosphorus removal by using DNPAOs as a portion of functional PAOs. For example, in Run 1 with the mean phosphorus and COD concentrations of 13.5 mg/L and 260 mg/L respectively, it is impossible to obtain the desired phosphorus removal efficiency as high as 96% in case of the conventional biological phosphorus removal (BPR) processes. Jr Grady et al. (1999) reported that at least 15–20 mg BOD (or 26–34 mg COD) is required for removal of 1 mg P in conventional BPR processes (without nitrification), such as Anaerobic-Oxide (A/O) and Virginia Initiative Process (VIP). In the case of five-stage Bardenpho process with the long SRT, more BOD or COD, i.e. over 25 mg BOD/ 43 mg COD, is required to remove 1 mg P. Therefore, in Run 1 the minimum theoretical required COD is estimated to be approximately 338–442 mg/L for removal of 13.0 mg P /L, supposing the effluent phosphorus concentration was 0.5 mg/L, which is much higher than the actual consumed COD of 220 mg/L.

In general, high COD/P favors the improvement of phosphorus removal (Fig. 4); however, in the A<sub>2</sub>N-SBR process that based on the denitrifying phosphorus removal, the final P-uptake capacity also greatly depends on the amount of electron acceptors (NO<sub>x</sub>-N), i.e. the influent N/P ratios. This can be shown from Fig.5b that phosphorus removal efficiencies had close positive relationship with the TN/P ratios with the correlation coefficient (r) of 0.8345.

With respect to the case of the low influent COD/TN, it was phosphorus removal rather than nitrogen removal that still maintained at a relatively higher level, i.e. the low influent COD/TN did not deteriorate the phosphorus removal significantly. This finding is contrary to the conventional BNR processes, where better nitrogen removal can be primarily guaranteed if the influent nutrient ratios are lower (Metcalf and Eddy, 2003). Apparently, the specific A<sub>2</sub>N-SBR operation mode has maximized the phosphorus removal capacity especially with the limitation of carbon source.

To promote TN removal in the case of low influent COD/TN, adding extra carbon source into the anaerobic phase or extending the nitrification reaction time (if the influent NH<sub>4</sub><sup>+</sup>-N can not convert to NO<sub>2</sub><sup>-</sup>-N/NO<sub>3</sub><sup>-</sup>-N completely in N-SBR) are appropriate alternatives. By addition of carbon source in anaerobic phase, more readily biodegradable compounds are available to PAO/DNPAO for synthesis internal PHB; thereby the N and P removal efficiencies can be promoted simultaneously. However, it is worthy noting that the excessively high COD/TN is prone to result in a portion of unconsumed COD residual to the subsequent anoxic phase. This will provide denitrifying ordinary heterotrophic organisms (DNOs) a competitive advantage over DNPAO to utilize a fraction of COD for denitrification (Jr Grady et al., 1999). As a result, the electron donors (COD) available to DNPAO are reduced, finally leading to the deterioration of phosphorus removal capacity.

4.2. Establishing a real-time control strategy for more efficient nutrient removal in

## A<sub>2</sub>N-SBR

The observation of the concentration profiles through a typical cycle for A<sub>2</sub>N-SBR favors to understand the biochemical reaction and to determine the optimal condition for the individual reaction time (Mahendraker et al., 2005). Yoo et al (2004) reported that in wastewater treatment process the control strategy can be based on the identification of the endpoint after biological reaction. Switching to the next phase shortly after the detection of the reaction endpoint provides an optimum solution for both the process performance and the economies of the plant. For the SBR process, Bungay et al (2007) demonstrated that the flow-proportional cycle times control strategy use the treatment volume more efficiently than fixed cycle times. In our study, the on-line monitoring ORP and pH profiles indicated accurately the biochemical reaction procedures for anaerobic P-release, nitrifying denitrification and P-uptake. Whilst, the banding-points of DO and pH profiles would make it possible to determine the end of phosphate release and nitrification. Therefore, it is promising to utilize DO, pH and ORP as real-time parameters in A<sub>2</sub>N-SBR process to obtain the optimum react time for each procedure with the varying influent characteristics.

Nevertheless, it is worthy noting that on day 241 the end of the P-uptake was not pointed out by DO or pH variations (Figs. 1 and 2), probably due to the fact that the 2h post-aeration reaction was not sufficient for complete phosphorus uptake. This can be shown from Fig. 1 that the final effluent P concentration was still around 3 mg/L at the end of cycle. The similar study has been conducted by Lee et al. (2001) using an anaerobic–aerobic–anoxic–aerobic sequencing batch reactor ((AO)<sub>2</sub> SBR, where a bending-point occurred on the pH curve indicating the end of P-uptake with a prolonged post-aeration reaction. Our experimental findings of a fixed-time control proved that a real-time control strategy is essential for A<sub>2</sub>N-SBR process to obtain more efficient nutrient removal.

Further, the tests of HRT effect indicated that the duration for the anaerobic and anoxic reactions significantly affected the final nitrogen and phosphorus removals especially for the P removal that is prone to occur “second phosphorus” release in the absence of electron donors (e.g. COD) and acceptors (e.g. O<sub>2</sub> or NO<sub>x</sub>-N), thereby lowering the final phosphorus removal efficiency. Meanwhile, domestic wastewater is subject to wide variations in flow and load (Bungay et al., 2007), certainly resulting in the wide variations of the influent nutrient ratios, which has been proved significantly impact the final nutrient performance of the A<sub>2</sub>N-SBR. Therefore, in the A<sub>2</sub>N-SBR process retrofitted from the SBR technology, the unsteady performance due to the variation of nutrient ratios should overcome by altering the cycle times of an SBR based on the real-time control strategy.

## 4. Conclusions

1. The combined effect of COD/P, COD/TN and TN/P determines the final

- nutrient removal performance in the A<sub>2</sub>N-SBR process. With the increased C/P, the phosphorus removal efficiency exhibited an upward trend wholly. The experiment with influent COD/P and COD/TN of 19.9 and 9.9 achieved simultaneous optimal phosphorus and nitrogen removal of 94 % and 91 %, respectively.
2. pH, ORP, and DO variations were closely related to the dynamic variations of nutrient pollutants in the separate A<sub>2</sub>-SBR and N-SBR. From the tests of varying anoxic HRT, it demonstrated that the application of pH, DO and ORP as real time control parameters to adjust the duration of each case in A<sub>2</sub>N-SBR appears promising, which favors to promote the reliability and stability for the system operating. The real-time control strategy is complex compared with the fixed-time control, but enables the treatment to adjust automatically to match the influent wastewater quality.
  3. Relatively steady nitrogen and phosphorus removals of 86% and 89 % were achieved by introduction of DNPAOs in the A<sub>2</sub>N-SBR despite the significant fluctuation in the feed qualities. This indicates that A<sub>2</sub>N-SBR has great potential to treat nutrient pollutant wastewater.

#### Acknowledgements

This work was financed by the National Natural Science Foundation of China (NSFC) (no.50608064; 50628808), Excellent Doctoral Dissertation Nationwide Foundation (Grant no. 200756) and the national key Laboratory Foundation. We also would like to acknowledge the fellowship with Center for Water Science, Cranfield University.

#### References

- Ahn, J., Daidou, T., Tsuneda, S., Hirata, A., 2001. Metabolic behavior of denitrifying phosphate-accumulating organisms under nitrate and nitrite electron acceptor conditions. *J. Biosci. Bioeng.* 92 (5), 442–446.
- Bond, P.L., Keller, J., Blackall, L.L., 1999. Anaerobic phosphate release from activated sludge with enhanced biological phosphorus removal: a possible mechanism of intracellular pH control. *Biotechnol Bioeng.* 63 (5), 507–515.
- Bortone, G., Libelli, SM., Tilche, A., Wanner, J., 1999. Anoxic phosphate uptake in the Dephanox process. *Water Sci. Technol.* 40 (4-5), 177–183.
- Bungay, S, Humphries, M, and Stephenson, T., 2007. Operating strategies for variable flow sequencing batch reactors. *Water Environ. J.* 21, 1–8.
- Copp, J.B., Dold, P.L., 1998. Confirming the nitrate-to-oxygen conversion factor for denitrification. *Water Res.* 32 (4), 1296–1304.
- Del Solar, J., Hudson, S., and Stephenson, T., 2005 On-site treatment of a motorway service area wastewater using a package sequencing batch reactor (SBR) *Water Sci. Technol.* 51 (10), 311–316.
- Hao, X. D. Sustainable treatment technologies of wastewater-wastes, 1<sup>st</sup> ed., China Architecture & Building Press, Beijing, China, 2006 (in Chinese).
- Harper, W.F., 2003. The effect of an initial anaerobic zone on the kinetics and stoichiometry of acetate removal during nutrient-limiting conditions. *Water SA.* 29

(4), 443–450.

Irvine, R.L. P., Wilderer, A., Flemming, H. C., 1997. Controlled unsteady state processes and technologies—an overview. *Water Sci. Technol.* 35 (1), 1–10.

Grady, C. P. L., Daigger, G. T., Lim, H. C., 1999. *Biological wastewater treatment: 2nd ed., revised and expanded.*, Marcel Dekker, Inc. New York, NY, USA.

Kuba, T., Smolders, G., van Loosdrecht, M.C.M., 1993. Biological phosphorus removal from wastewater by anaerobic-anoxic sequencing batch reactor. *Water Sci. Technol.* 27 (5-6), 241–252.

Kuba, T., Wachtmeister, A., van Loosdrecht, M.C.M., Heijnen, J.J., 1994. Effect of nitrate on phosphorus release in biological phosphorus removal systems. *Water Sci. Technol.* 30 (6), 263–269.

Kuba, T., van Loosdrecht, M.C.M., Heijnen, J.J., 1996. Phosphorus and nitrogen removal with minimal COD requirement by integration of nitrification in a two-sludge system. *Water Res.* 30 (7), 1702–1710.

Kuba, T., van Loosdrecht, M.C.M., Brandse, F.A., Heijnen, J.J., 1997. Occurrence of denitrifying phosphorus removing bacteria in modified UCT-type wastewater treatment plants. *Water Res.* 31 (4), 777–786.

Kuba, T., van Loosdrecht, M.C.M. and Heijnen, J.J., 1997. Biological dephosphatation by activated sludge under denitrifying conditions: pH influence and occurrence of denitrifying dephosphatation in a full-scale waste water treatment plant. *Water Sci. Technol.* 36 (12), 75–82.

Lee, D. S., Jeon, C.O., Park, J. M., 2001. Biological nitrogen removal with enhanced phosphate uptake in a sequencing batch reactor using single sludge system. *Water Res.* 35 (16), 3968–3976.

Mahendraker, V., Mavinic, S Donald., Rabinowitz, B., Kenneth Hall, J., 2005. The impact of influent nutrient ratios and biochemical reactions on oxygen transfer in an EBPR process—a theoretical explanation. *Biotechnol. Bioeng.* 91 (1), 22–42.

Meinhold, J., Filipe, C.D.M., Daigger, G.T., Isaacs, S., 1999. Characterization of the denitrifying fraction of phosphate accumulating organisms in biological phosphate removal. *Water Sci. Technol.* 39 (1), 31–42.

Merzouki, M., Bernet, N., Delgene's, J.P., Moletta, R., Benlemlih, M., 2001. Effect of operating parameters on anoxic biological phosphorus removal in anaerobic–anoxic sequencing batch reactor. *Environ. Technol.* 22 (4), 397–408.

Mino, T., van Loosdrecht, M.C.M., Heijnen, J.J., 1998. Microbiology and biochemistry of the enhanced biological phosphate removal process. *Water Res.* 32 (11), 3193–3207.

Murnleitner, E., Kuba, T., van Loosdrecht, M.C.M., Heijnen, J.J., 1997. An integrated metabolic model for the aerobic and denitrifying biological phosphorus removal. *Biotechnol. Bioeng.* 54 (5), 434–450.

Seviour, R. J., Mino, T., Onuki, M., 2003. The microbiology of biological phosphorus removal in activated sludge systems. *FEMS Microbiology Reviews.* 27 (1), 99–127.

Sorm, R., Bortone, G., Wanner, J., Tilche, A., 1998. Behaviour of activated sludge from a system with anoxic phosphate uptake. *Water Sci. Technol.* 37 (4-5), 563–566.

Standard Methods for the Examination of Water and Wastewater (1998). 20th edn, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.

Sudiana, I.M., Mino, T., Satoh, H., Nakamura, K., Matsuo, T., 1999. Metabolism of enhanced biological phosphorus removal and non-enhanced biological phosphorus removal sludge with acetate and glucose as carbon source. *Water Sci. Technol.* 39 (6), 29–35.

Vlekke, G.J.F.M., Comeau, Y., Oldham, W.K., 1988. Biological phosphate removal from wastewater with oxygen or nitrate in sequencing batch reactors. *Environmental Technology Letters*. 9 (8), 791–796.

Wanner, J., Cech, J.S., Kos, M., 1992. New process design for biological nutrient removal. *Water Sci. Technol.* 25 (4-5), 445–448.

Wu, C.Y., Chen, Z.Y., Liu, X.H., Peng, Y.Z., 2007. Nitrification-denitrification via nitrite in SBR using real-time control strategy when treating domestic wastewater. *Biochem. Eng. J.* 36 (2), 87–92.

### Figure list

Fig. 1. Profiles of COD,  $\text{PO}_4^{3-}\text{-P}$ ,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  and  $\text{NO}_2^-\text{-N}$  variations during one typical cycle of  $\text{A}_2\text{N-SBR}$  process (on day 241) (MLSS=3875mg/L; T=23-25.5°C)

Fig. 2. Variations of DO, ORP and pH during one typical cycle of  $\text{A}_2\text{N-SBR}$  process (on day 241) (MLSS=3875mg/L; T=23-25.5°C)

Fig.3. Variations of phosphorus concentration at different reaction phases with different influent COD/P in  $\text{A}_2\text{N-SBR}$  process (The horizontal line A represents the mean anaerobic phosphorus concentration and the line B represents the mean anoxic phosphorus concentration)

Fig.4. Relationships between the influent COD/P and phosphorus removal efficiencies in the  $\text{A}_2\text{N-SBR}$  process

Fig.5 Influence of COD/TN and TN/P on the nitrogen and phosphorus removals (a: the relationships between the influent COD/TN and TN removal efficiencies; b: the relationships between the influent TN/P and phosphorus removal efficiencies; data from Table 5)

### Table list

Table 1 the operating procedure for the  $\text{A}_2\text{N-SBR}$  process

Table 2 Experimental approach for  $\text{A}_2\text{N-SBR}$  process (<sup>a</sup> adding nitrate in the initial anoxic phase; <sup>b</sup>  $T_{\text{ano}}$ -anoxic reaction time; <sup>c</sup>  $T_{\text{n}}$ -nitrification reaction time)

Table 3 Performance of  $\text{A}_2\text{N-SBR}$  process for removal of COD,  $\text{NH}_4^+\text{-N}$ , TN and P (<sup>a</sup>average data are in parenthesis)

Table 4 Comparison of COD/TN, COD/P and TN/P with nutrients removal efficiency for  $\text{A}_2\text{N-SBR}$  process (<sup>a</sup>adding 10 mg/L nitrate at the beginning of the anoxic phase)

Table 5 Effect of the anoxic HRT on the  $\text{A}_2\text{N-SBR}$  process performance (mean value)

Fig. 1

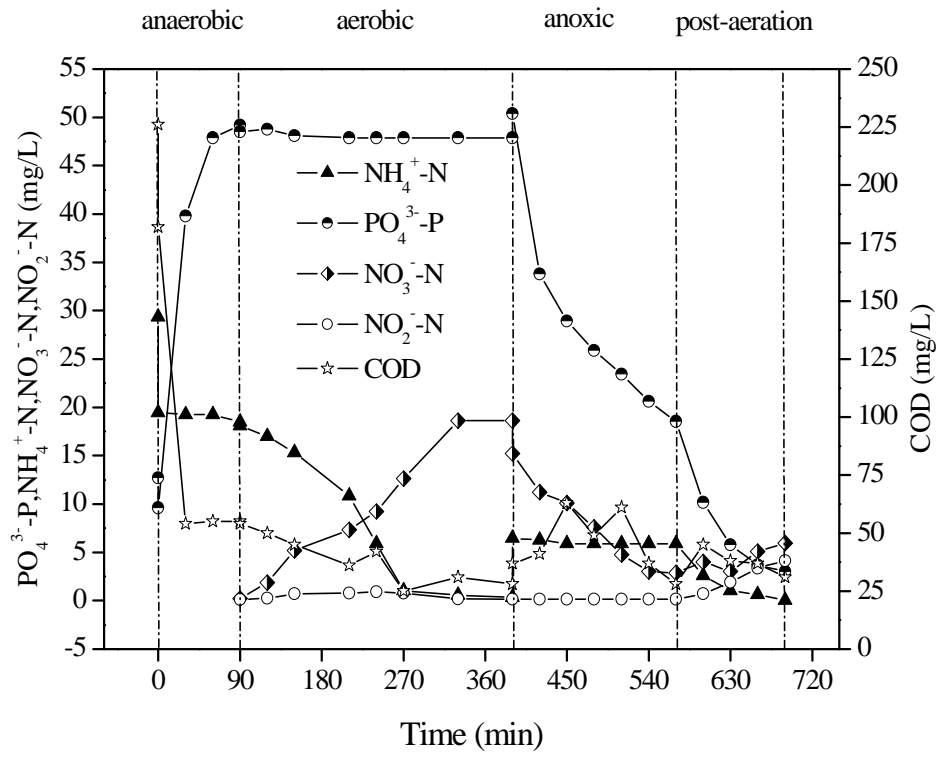




Fig. 2

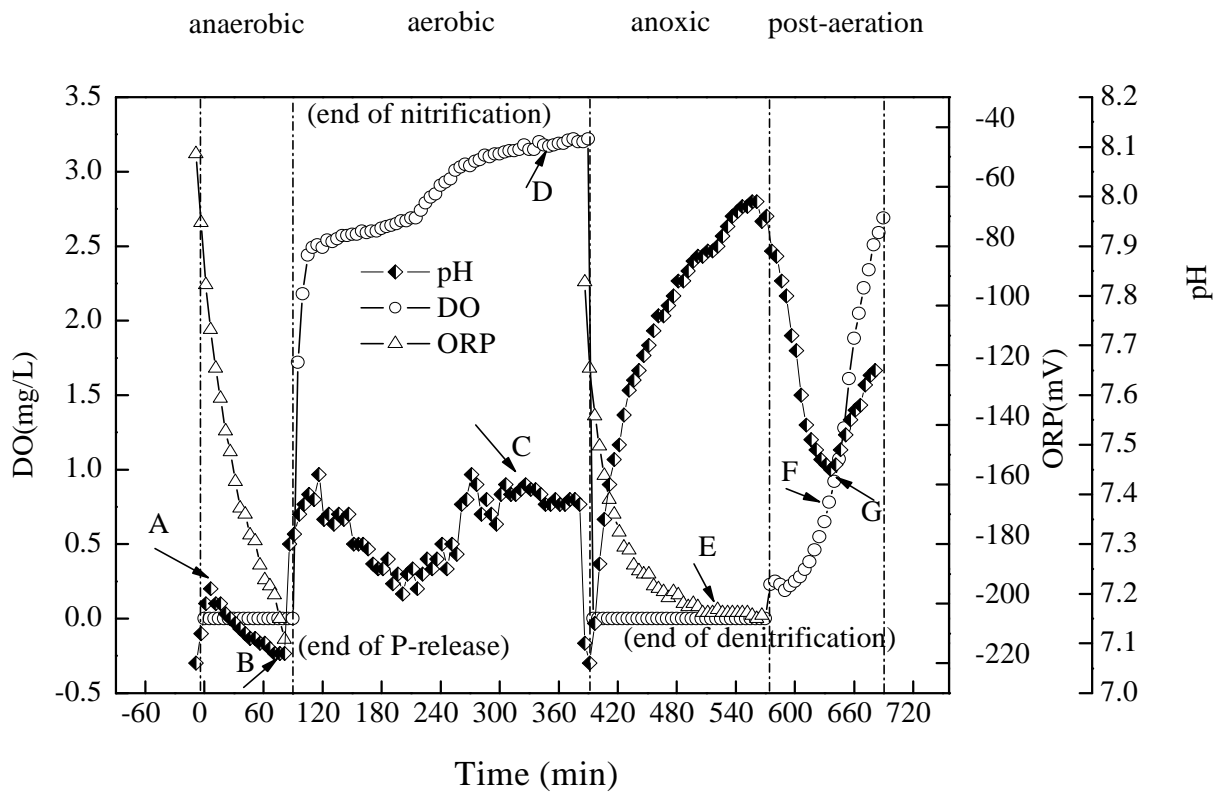
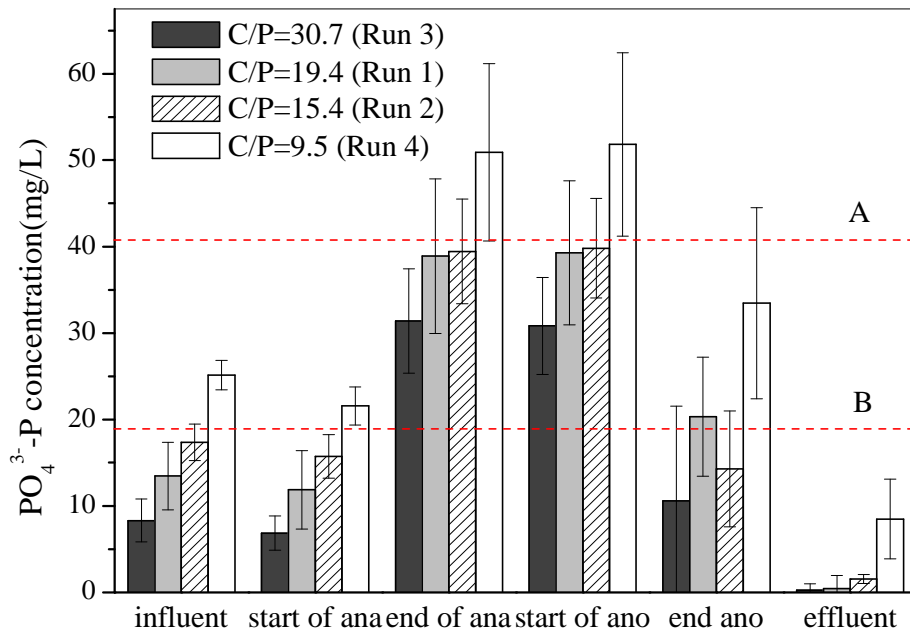
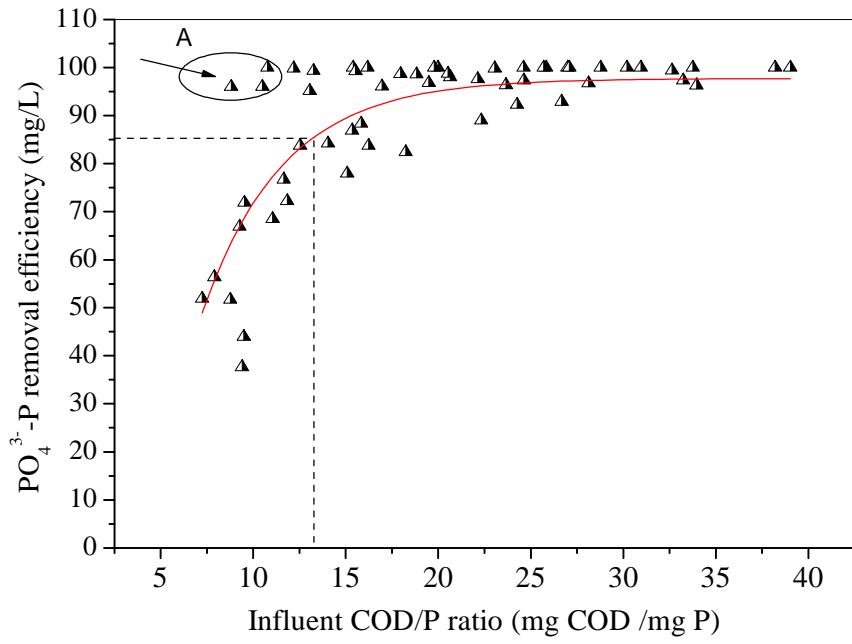


Fig. 3



**Fig. 4**



**Fig.5**

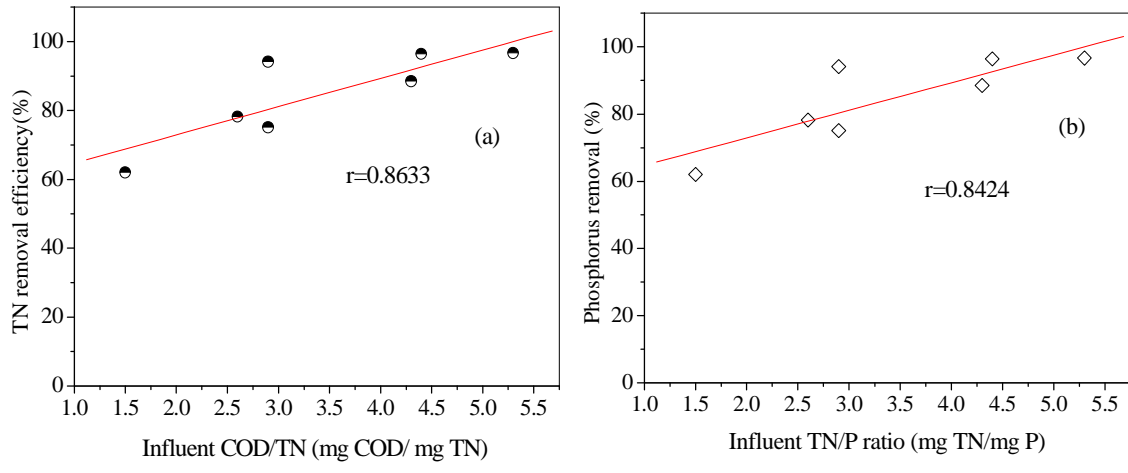


Table 1

Reactor	Procedure	Operation description
A <sub>2</sub> -SBR	i) anaerobic phase for 90min	9 L clarified sewage was added into A <sub>2</sub> -SBR, mixing with 3 L of settled activated sludge; biological phosphorus release and COD consumption take place in this phase
	ii) Settling for 30–45min	After settling, 9L anaerobic supernatant abundant of PO <sub>4</sub> <sup>3-</sup> and NH <sub>4</sub> <sup>+</sup> was transferred into the biofilm N-SBR, thus giving the volume exchange ratio as around 0.75
N-SBR	iii) nitrification	nitrification for 315 minutes including a final 5min settling period; DO was maintained at 3–4 mg/L
A <sub>2</sub> -SBR	iv) anoxic reaction for 180min	9 L supernatant with high nitrate concentrations was transferred from the N-SBR to the A <sub>2</sub> -SBR, providing DNPAOs/PAO with electron acceptors for the anoxic P uptake
	v) A <sub>2</sub> -SBR aeration phase for 120min	DO was kept at 2–3 mg/L. At the end of the aeration phase, 100ml P-rich sludge was discharged to keep the solid retention time (SRT) at 18–22d
	vi) settling/decanting	settling for 30min and decanting for 10min

Table 2

Period	Run	Time of operation (d)	Operating parameters
I	1	64 - 94	P=10 mg/L, C/P=19.4
C/P influence	2	95 - 123	P=15 mg/L, C/P=15.4
	3	124 - 161	P=5 mg/L, C/P=30.8
	4	162 - 173	P=20 mg/L, C/P=9.5
II <sup>a</sup> HRT influence	5	219 - 246	T <sub>ano</sub> <sup>b</sup> =3h, T <sub>n</sub> <sup>c</sup> =5h; HRT=11.5h
	6	247 - 261	T <sub>ano</sub> <sup>b</sup> =4h, T <sub>n</sub> =4h; HRT=11.5h
	7	262 - 272	T <sub>ano</sub> <sup>b</sup> =3h, T <sub>n</sub> <sup>c</sup> =4h; HRT=10.5h

Note: <sup>a</sup>adding nitrate in the initial anoxic phase; <sup>b</sup>T<sub>ano</sub>-anoxic reaction time; <sup>c</sup>T<sub>n</sub>-nitrification reaction time

Table 3

Parameter	Concentration (mg/l)		Removal (%)	
	Range	Mean	Range	Mean
BOD <sub>5</sub>	15 - 65	32	62 - 89	78
COD	15 - 91	39	65 - 90	85
NH <sub>4</sub> <sup>+</sup> -N	0.4 - 16.4	6.8	55 - 100	86
TN	6.7 - 18.9	8.6	61 - 90	82
P	0 - 13.9	2.0	38 - 100	89

Table 4

	Run	COD loading (mg COD/g MLSS)	C/N	C/P	N/P	P removal (%)	TN removal (%)
Period	1	64.3	4.5	19.4	4.4	96	81
(I)	2	65.3	3.5	15.4	4.3	89	67
	3	63.1	5.8	30.8	5.3	97	79.4
	4	58.9	6.2	9.5	1.5	62	82
	Average	62.9	5.0	18.8	3.87	86	77
Period	5	59.2	7.9	16.2	2.1/2.9 <sup>a</sup>	75	83 <sup>a</sup>
(II)	6	66.6	9.9	19.9	2.0/2.9 <sup>a</sup>	94	91
	7	58.6	10.8	18.4	1.7/2.6 <sup>a</sup>	78	88 <sup>a</sup>
	Average	61.5	9.5	18.2	1.9/2.8 <sup>a</sup>	83	87 <sup>a</sup>

Note: <sup>a</sup>adding 10mg/L nitrate at the beginning of the anoxic phase



Table 5

Items		Run		
		5 <sup>a</sup>	6 <sup>b</sup>	7 <sup>c</sup>
Reaction	T <sub>an</sub> (h)	3.0	4.0	3.0
time	T <sub>n</sub> (h)	5.0	4.0	4.0
	HRT (h)	11.5	11.5	10.5
COD	influent (mg·L <sup>-1</sup> )	211	238	209
	effluent (mg·L <sup>-1</sup> )	35	34	25
	removal (%)	83	86	88
p	influent (mg·L <sup>-1</sup> )	13.0	12.0	11.4
	end of ana <sup>d</sup> (mg·L <sup>-1</sup> )	46.8	62.8	61.4
	end of ano <sup>e</sup> (mg·L <sup>-1</sup> )	16.7	26.8	27.7
	effluent (mg·L <sup>-1</sup> )	3.3	0.7	2.5
	removal (%)	75	94	78
NH <sub>4</sub> <sup>+</sup> -N	influent (mg·L <sup>-1</sup> )	24.9	20.4	17.1
	effluent (mg·L <sup>-1</sup> )	0.0	0.0	0.0
	removal (%)	100	100	100
NO <sub>3</sub> <sup>-</sup> -N	initial of ano (mg·L <sup>-1</sup> )	20.3 <sup>*</sup>	21.3 <sup>*</sup>	17.2 <sup>*</sup>
	end of ano (mg·L <sup>-1</sup> )	3.0	0.0	0.0
	effluent (mg·L <sup>-1</sup> )	3.1	0.9	0.6
NO <sub>2</sub> <sup>-</sup> -N	initial of ano (mg·L <sup>-1</sup> )	0.2	0.2	0.1
	end of ano (mg·L <sup>-1</sup> )	0.2	0.1	0.1
	effluent (mg·L <sup>-1</sup> )	3.4	1.32	1.1
TN	influent (mg·L <sup>-1</sup> )	26.9/37.7 <sup>*</sup>	24.1/34.8 <sup>*</sup>	19.3/30.1 <sup>*</sup>
	effluent (mg·L <sup>-1</sup> )	6.3	3.3	3.6
	removal (%)	77/8 <sup>*</sup>	86/9 <sup>*</sup>	82/88 <sup>*</sup>

Note: <sup>a</sup> operations with anoxic reaction time and nitrification reaction time of 3h and 5h; <sup>b</sup> operations with anoxic reaction time and nitrification reaction time of 4h and 4h; <sup>c</sup> operations with anoxic reaction time and nitrification reaction time of 3h and 4h; <sup>d</sup> ana: anaerobic reaction; <sup>e</sup> ano: anoxic reaction; <sup>\*</sup> adding nitrate (around 10 mg/L) in the initial anoxic phase.