Free Nitrous Acid-Based Nitrifying Sludge Treatment in a Two-Sludge System Enhances Nutrient Removal from Low-Carbon Wastewater

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Abstract: A new method to enhance nutrient removal from low carbon-wastewater was developed. The method consists of a two-sludge system (i.e., an anaerobic-anoxic-oxic reactor coupled to a nitrifying reactor (N-SBR)) and a nitrifying-sludge treatment unit using free nitrous acid (FNA). Initially, 65.1 ± 2.9% in total nitrogen removal and 69.6 ± 3.4% in phosphate removal were obtained without nitrite accumulation. When 1/16 of the nitrifying sludge was daily treated with FNA at 1.1 mg N/L for 24 h, ~28.5% of nitrite was accumulated in the N-SBR, and total nitrogen and phosphate removal increased to 72.4 ± 3.2% and 76.7 ± 2.9%, respectively. About 67.8% of nitrite was accumulated at 1.9 mg N/L FNA, resulting in 82.9 ± 3.8% in total nitrogen removal and 87.9 ± 3.5% in phosphate removal. Fluorescence in-situ hybridization analysis showed that FNA treatment reduced the abundance of nitrite oxidizing bacteria (NOB), especially that of *Nitrospira sp.*

Keywords: Nutrient removal, Low-carbon domestic wastewater, Free nitrous acid, Nitrite accumulation, Denitrifying phosphorus removal

1. Introduction

The increase of nitrogen and phosphorus concentrations in freshwater environments is the main reason for the occurrence of eutrophication; one of the most serious global water management issues (Li et al., 2014; Chen et al., 2013a, 2014a; Yi et al., 2017). Generally, three sources that lead to the increase of nitrogen and phosphorus concentrations in natural water bodies are: rainfall, agriculture discharge, and effluent of wastewater treatment plants (WWTPs). Among them, WWTPs is the only engineering source that could be readily manipulated by engineers. Therefore, removing nitrogen and phosphorus from municipal sewage to protect water bodies from eutrophication attracts much attention in the past few decades (Zeng et al., 2003; Xu et al., 2013; Chen et al., 2016; Wang et al., 2017a, 2017b).

Nitrogen and phosphorus removal from municipal sewage are mainly achieved via a series of biological processes such as nitrification, denitrification, anaerobic phosphate release, and oxic/anoxic phosphate uptake (Zeng et al., 2003; Wang et al., 2009; Lee et al., 2010; Majed et al., 2009). Except microbial nitrification, all other processes are closely relevant to organic carbon sources available in wastewaters (Wang et al., 2017c), thus organic carbon level present in wastewaters becomes a key factor affecting the performance of nutrient removal. However, wastewater biodegradable carbon sources are often insufficient in many areas worldwide, such as in South China, and it is a big challenge to maintain desirable nitrogen and phosphorus removal in these areas (Zhang and Chen, 2009).

Generally, two strategies that could enhance nutrient removal are: (i) adding external carbon source, and (ii) decreasing the waste of internal carbon source. The former strategy is widely investigated and often applied in real situations. Several external carbon sources such as acetate, propionate, alcohol and glycerol have been tested by researchers (Wang et al., 2012a; Chen et al., 2013b; Wang et al., 2013; Zhao et al., 2016a). Although this strategy is effective, it is uneconomical and unsustainable to implement it into long-term operation of full WWTPs.

In fact, readily biodegradable carbon matters in wastewaters are not completely used for nutrient removal. Besides being utilized for nutrient removal and biomass growth, a considerable amount of carbon source is squandered via being oxidizing into carbon dioxide without any contribution to nitrogen and phosphorus removal. For example, NH_4^+-N in wastewaters is required to be oxidized to NO_3^--N or NO_2^--N (i.e., nitrification) before it can be finally removed via microbial denitrification. The aerobic/aerobic condition of nitrification inevitably causes a large number of carbon source consumption. Moreover, compared with nitrite based denitrification, nitrogen removal via nitrate ($NH_4^+ \rightarrow NO_3^- \rightarrow N_2$) would consume 40% more carbon. Thus, if carbon consumption during nitrification could be minimized and nitrite based denitrifying phosphorus removal could be achieved, desirable nitrogen and phosphorus removal from low-carbon wastewater might be also realized without adding external carbon source.

The two-sludge process, which usually consists of an anaerobic-anoxic reactor and a nitrifying reactor, is the most promising technology for low-carbon wastewater treatment (Kuba et al., 1996). Wastewater is first fed to the anaerobic-anoxic reactor, where most of the organic matter in the wastewater is adsorbed and stored as polyhydroxyalkanoates (PHA) in the anaerobic phase by polyphosphate-accumulating organisms (PAOs). The supernatant containing NH_4^+-N and PO_4^{3-}-P is then entered into the nitrifying reactor, where NH_4^+-N is bio-converted to NO_3^--N by nitrifiers. Afterwards, the effluent of the nitrifying reactor containing NO_3^--N and PO_4^{3-}-P is returned to the anaerobic-anoxic reactor, where NO_3^--N and PO_4^{3-}-P are removed simultaneously via denitrifying phosphorus removal. As thus, the separation of nitrifiers from PAOs and denitrifiers effectively avoids the waste of accumulated internal carbon substrates in the nitrification process. Moreover, denitrifying phosphorus removal, which dominates in the anoxic phase of the anaerobic-anoxic reactor, also saves considerable carbon. Although the two-sludge process has been developed for more than 20 years, improvement potential is still existed. It was found that, in all previous investigations, nitrate-based denitrifying phosphorus removal dominated in the anaerobic-anoxic reactor. Therefore, substantial carbon would be further saved if the fraction of nitrite-denitrifying phosphorus removal could increase.

The fraction of nitrite-denitrifying phosphorus removal in the two-sludge process mainly depends on the ratio of nitrite accumulated in the nitrifying reactor. Although high levels of nitrite accumulation have been demonstrated to be achieved in anaerobic sludge digestion liquor treatment reactor and nitrification-denitrification bioreactor, it is a challenge to selectively retain ammonia-oxidizing bacteria (AOB) over nitrite-oxidizing bacteria (NOB) to achieve stable nitritation in such a nitrifying reactor. Recently, it was found that free nitrous acid (FNA), the protonated form of nitrite, had different inhibition threshold to AOB and NOB (Wang et al., 2014a, 2016a, 2016b). Based on this finding, it is assumed that nitrite-denitrifying phosphorus removal in the two-sludge system might be achieved if the nitrifying sludge is treated by FNA, thereby enhancing nutrient removal from low carbon-wastewater without addition of extra carbon source.

To evaluate this feasibility, two lab-scale wastewater treatment processes were conducted to treat synthetic municipal wastewater containing ~150 mg/L chemical oxygen demand, ~50 mg/L NH_4^+-N, and ~10 mg/L PO_4^{3-}-P, and their performances were compared. One was operated as the two-sludge system and the other was performed as the conventional anaerobic/oxic/anoxic/oxic/anoxic/oxic system according to our previous study (Chen et al., 2014b). The nitrogen and phosphorus removal performances, nitrite accumulation ratio, activities and abundances of AOB and NOB, and polyphosphate staining were carried out to explore how FNA treatment enhanced both nitrogen and phosphorus removal in the two-sludge system but deteriorated phosphorus removal in the conventional system. The strategy developed in this work provides an alternatively promising solution for enhanced nutrient removal from low-carbon domestic wastewater without external substrate addition.

2. Materials and Methods

2.1. The operations of two wastewater treatment systems

Two lab-scale wastewater treatment systems were implemented simultaneously in this experiment. One was operated as the modified two-sludge system while the other was performed as the conventional biological nitrogen and phosphorus removal system and set as the control. Two systems were operated together three cycles daily and seeded with the same inoculums, which were taken from the secondary sedimentation tank of a WWTP in Changsha, China.

The conventional system included only one reactor with a working volume of 5 L. Each 8-h cycle contained about 90 min anaerobic, 80 min oxic, 50 min anoxic, 30 min oxic, 40 min anoxic, and 30 min oxic periods, followed by 60 min settling, 10 min decanting, and 100 min idle periods according to the literature (Chen et al., 2014b). The sludge retention time (SRT) in this conventional system was maintained at about 20 d.

The modified two-sludge system composed of two reactors with a working volume of 5L each, one serving as the anaerobic-anoxic-oxic sequencing batch reactor (AAO-SBR) and the other as the nitrification sequencing batch reactor (N-SBR) (Fig. 1). The synthetic wastewater (see Section 2.3 for details) was first pumped into the AAO-SBR in the initial 5 min of anaerobic period. After 90 min anaerobic phosphate release, a 30 min settling period (Settling I) was followed. At the end of the Settling I, 4 L of supernatant (NH_4^+-N and PO_4^{3-}-P rich) was discharged into the nitrification SBR (N-SBR) for 180 min nitrification and 30 min settling (Settling II). Then, 4 L NO_3^--N/NO_2^--N and PO_4^{3-}-P rich supernatant was pumped back to the AAO-SBR for anoxic denitrification and phosphorus uptake in the initial 5 min of anoxic period (150 min). To enhance nutrient removal, a 30 min oxic period was followed. After 30 min of settling (Settling III), 4 L of supernatant was finally discharged in the decanting period (10 min). The SRT in the AAO-SBR was also maintained at 20 d approximately.

The N-SBR was operated with three cycles daily. Each cycle started with a 180 min feeding-oxic period, during which 4 L of supernatant was pumped into the reactor in the initial 5 min, followed by 30 min settling and 10 min decanting periods. Dissolved oxygen was controlled at

2.0-2.5 mg/L in the oxic period with a programmed logic controller, while the pH was maintained at ~7.5 by addition of 1 M $NaHCO_3$. No sludge wasting was carried out in the N-SBR. The SRT was estimated to be approximately 30 d during steady-state operation based on the measured total suspended solids (TSS) and effluent TSS concentrations in the N-reactor.

2.2. Overall experimental design

The two systems were operated for 225 days, during which the operation of the two systems was divided into three Phases. All the following three phases of tests were performed at room temperature of 22± 1 °C.

Phase I (day 0 - day 45): The two systems were operated the same as described above. After about 30 d operation, the two systems reached steady-state, which was indicated by the almost the same PO_4^{3-}-P, NH_4^+-N, NO_3^--N and NO_2^--N levels in the effluent. Then, the cycle studies were carried out. Cycle studies in the two systems were carried out to analyze PO_4^{3-}-P, NH_4^+-N (B), NO_3^--N (C), NO_2^--N, glycogen, acetate and PHA. TSS and volatile suspended solids (VSS) concentrations in all reactors were also analyzed periodically. In this phase, sludge treatment with FNA was not implemented in the N-SBR and the conventional reactor.

Phase II (day 46 - day 135): The two systems were operated the same as Phase I excepting sludge treatment with FNA implemented in the N-SBR and the conventional reactor, as described below. According to the previous findings (Wang et al., 2014a, 2016a, 2016b), a treatment time of 24 h and a sludge treatment ratio of 1/16 (i.e. 1/16 of the sludge from either the N-SBR or the conventional reactor was treated everyday) were chosen. The N-SBR and the conventional reactor were operated as described above, with the exception that 312.5 mL of sludge mixture (1/16 volume of N-SBR or the conventional reactor) was wasted daily from either the N-SBR or the conventional reactor at the end of an oxic period. The above two sludge mixtures were first thickened to 100 mL and then transferred into the two FNA treatment units, respectively. The pH was adjusted to 6 by dosing 2 M HCl in the units, and a $NaNO_2$ stock solution (40 g N/L) was added to achieve a NO_2^--N concentration of 450 mg N/L. This gives a calculated FNA concentration of 1.1 mg N/L (T= 22 °C) according to the reference (Anthonisen et al., 1976). The pH was controlled at 6.0 ± 0.05 with a programmable logic controller using 0.5 M HCl solution and 0.5 M NaOH solution during the entire treatment process. After 24h treatment, the two FNA-treated sludge mixtures were respectively returned to the N-SBR and the conventional reactor manually.

Phase III (day 136-day 225): The two systems and the two FNA treatment units were operated as described in Phase II, except that 1.9 mg N/L instead of 1.1 mg N/L FNA concentration was maintained in the FNA treatment units by controlling NO_2^--N concentration at 780 mg N/L, pH at 6, and temperature at 22 °C.

2.3. The composition of synthetic wastewater

Synthetic wastewater was used in this study and prepared daily. As acetate is the most common carbon source present in the real domestic wastewaters (Chen et al., 2004), it is selected as the sole carbon source in this work. The chemical oxygen demand (COD), PO_4^{3-}-P, and NH_4^+-N concentrations in the synthetic wastewater were 150, 10, and 50 mg/L, respectively. The other nutrients in the synthetic wastewater were also presented below (per liter): 10 mg $MgSO_4$, 5 mg $CaCl_2$, and 0.5 mL trace metal solution, which was detailed previously (Wang et al., 2008; Chen et al., 2015; Yang et al., 2016).

2.4. Batch tests to measure the activities of AOB, NOB, and PAO after FNA treatment

For the measurement of AOB and NOB activities, 500 mL mixed liquor withdrawn was first centrifuged (5000 revolutions per min for 5 min at 20 ℃) and washed with tap water to remove the residual NH_4^+-N , NO_3^--N and NO_2^--N and then re-suspended in tap water with a final volume of 500 mL before being transformed in a reactor. Afterwards, 3 mL NH_4^+-N stock solution (5 g N/L) and 3 mL NO_2^--N stock solution (5 g N/L) were respectively added to the reactor, resulting in an initial NH_4^+-N and NO_2^--N concentration of 30 mg NH_4^+-N/L and 30 mg NO_2^--N/L, respectively. The experiment was lasted for 60 min. During the entire experiment period, air was adequately supplied to guarantee that dissolved oxygen was not limiting (> 5 mg/L) while pH was controlled at 7.5 ± 0.1 by dosing 0.5 M HCl solution or 0.5 M NaOH solution. Samples were taken at 15 min intervals for NH_4^+-N and NO_2^--N measurements, and the VSS concentration was determined at the end of the test. The volumetric NH_4^+-N oxidation (or nitrate production) rate were measured through linear regression of the corresponding profile, and the AOB and NOB activities (indicated by biomass specific NH_4^+-N oxidation and NO_3^--N production rates) were then calculated by dividing the corresponding volumetric rates by the VSS concentration.

To measure the variation of PAO activity in conventional system before and after FNA treatment, the mixed liquor was also centrifuged (5000 revolutions per min for 5 min at 20 ℃) and washed with tap water to remove the residual organic substrates and PO_4^{3-}-P, and then re-suspended in tap water with a final volume of 500 mL before being transformed in a batch reactor. At the beginning of the anaerobic period, 500 mL synthetic wastewater, as described above, was added into the reactor. The batch reactor was operated as the anaerobic/oxic regime which contained a 2-h anaerobic period and a 3-h oxic period. Samples were taken at 30 min intervals for PO_4^{3-}-P measurement, and the VSS concentration was determined at the end of the test. The specific phosphate release and uptake rates were used to express the activity of PAO.

2.5. Batch tests to measure the specific phosphate uptake rates using oxygen, nitrate, and nitrite as the sole electron acceptor

When the AAO-SBR achieved stable operation in each phase, the following batch test was conducted. A total of 900 mL of activated sludge mixture was withdrawn from the AAO-SBR at the end of the anaerobic period, centrifuged (5000 revolutions per min for 5 min at 20 ℃) and washed with tap water to remove the residual PO_4^{3-}-P, NO_3^--N and NO_2^--N. Then the biomass was re-suspended in tap water with a final volume of 3 L before being divided equally into three reactors. Afterwards, 100 mL PO_4^{3-}-P stock solution (1 g/L) was respectively added into the three reactors, resulting in an initial PO_4^{3-}-P level of 91 mg/L each. Among the three reactors, one used oxygen (dissolved oxygen > 5 mg/L) as the sole electron acceptor to take up PO_4^{3-}-P while the other two reactors used NO_2^--N (40 mg/L) and NO_3^--N (40 mg/L) as the sole electron acceptor, respectively. Nitrogen gas was fed into the latter two anoxic reactors to maintain anoxic conditions. The reaction time was lasted for 60 min, and samples were taken at 15 min intervals for PO_4^{3-}-P measurement.

2.6. Analytical methods

All samples from the reactors were immediately filtered through a Whatman GF/C glass microfiber filter (0.22 <mu>m pore size) to analyze PO_4^{3-}-P, NH_4^+-N, NO_3^--N, NO_2^--N, TSS, and VSS according to standard methods (APHA, 2005). The determinations of poly-3-hydroxybutyrate (PHB), poly-3-hydroxyvalerate (PHV), poly-3-hydroxy-2-methylvalerate (PH2MV) and sludge glycogen were the same as described in our previous publications (Wang et al., 2009; Wang et al., 2014b), and the total PHA was calculated as the sum of measured PHB, PHV, and PH2MV. The analysis of acetate was conducted via a HP5890 GC with flame ionization detector, and the procedure was detailed in previous publications (Yuan et al., 2006; Zhao et al., 2015a). Fluorescence in situ hybridization (FISH) was employed to quantify PAO, AOB, and NOB abundances in activated sludge. 4′,6′-diamidino-2-phenylindole dihydrochloride (DAPI) staining was conducted to analyze intracellular polyphosphate (poly-P) granules. The analyses of FISH and DAPI were performed according to the methods documented in previous publications (Mullan et al., 2006; Wang et al., 2012b; Wang et al., 2012c; Zeng et al., 2013; Zhao et al., 2015a).

3. Results and discussion

3.1. Phosphorus and nitrogen removal performance in the two-sludge and conventional systems

Fig. 2 shows the data of the effluent PO_4^{3-}-P, NH_4^+-N, NO_3^--N, and NO_2^--N concentrations of the two systems during the period of 225 days experiments, and the steady-state data in all phases were further summarized in Table 1.

In phase I, it can be seen from Fig.2A that high concentrations of PO_4^{3-}-P in effluent were detected in both systems during the initial 5 days, and effluent PO_4^{3-}-P in both systems decreased gradually in the following 15 days. In steady-state operation, 3.06± 0.16 and 3.60 ± 0.23 mg/L PO_4^{3-}-P were respectively achieved in the two-sludge system and the conventional system, suggesting that 69.6 ± 3.4% and 65.1 ± 3.1% of influent PO_4^{3-}-P were removed in the two-sludge and conventional systems, respectively. Effluent NH_4^+-N and NO_2^--N in the two systems were very similar and maintained at low levels (~1.0 mg/L in effluent NH_4^+-N and ~0.6 mg/L in effluent NO_2^--N). However, effluent NO_3^--N in the conventional system was higher than that in the two-sludge system (18.10± 0.54 vs 14.70± 0.59 mg/L), which thereby resulted in a lower total nitrogen (TN) removal efficiency (60.9 ± 3.4 vs 65.1 ± 2.9%).

In phase II, both 1/16 of the nitrifying sludge of two-sludge system and 1/16 of the sludge of the conventional system were daily treated with FNA at 1.1 mg N/L for 24 h. In the two-sludge system, effluent NH_4^+-N and NO_2^--N did not affected by the FNA treatment while effluent PO_4^{3-}-P and NO_3^--N decreased gradually, with 2.86 ± 0.63 mg/L PO_4^{3-}-P and 11.86± 0.63 NO_3^--N measured in the effluent after stable operation. As a result, nitrogen and phosphorus removal efficiencies of the two-sludge system increased to 72.4 ± 3.2 and 76.7 ± 2.9%, respectively. In the conventional system, however, the NH_4^+-N and NO_2^--N concentrations in the effluent increased while the NO_3^--N concentrations in the effluent decreased slightly after FNA treatment was implemented, with 5.04 ± 0.38 mg/L NH_4^+-N, 6.75± 0.81 mg/L NO_2^--N, and 13.91± 0.59mg/L NO_3^--N measured in the effluent in the steady-state operation, resulting in a decrease of nitrogen removal efficiency (44.7 ± 4.3%). Effluent PO_4^{3-}-P concentration increased to 7.84 ± 0.15 mg/L in the stable operation, which was close to the influent PO_4^{3-}-P level. DAPI staining showed that the biomass taken from the conventional system contained no poly-P granule. All the results suggested that phosphorus removal was completely collapsed in the conventional system.

In phase III, FNA concentration increased from 1.1 mg N/L to 1.9 mg N/L. This increase of FNA level further decreased the TN removal efficiency of the conventional system to 36.8 ± 4.8%. However, in comparison to Phase II, although the effluent NH_4^+-N and NO_2^--N did not vary, the effluent PO_4^{3-}-P and NO_3^--N in the two-sludge system decreased to 1.21 ± 0.14 and 6.98± 0.69 mg/L, respectively. Further calculations showed that 87.9 ± 3.5% phosphorus removal efficiency and 82.9 ± 3.8% nitrogen removal were achieved in this system.

The results presented above showed that FNA-based nitrifying sludge treatment in the two-sludge system could enhance both nitrogen and phosphorus removal from low-carbon wastewater. In the following text, details of how FNA-based nitrifying sludge treatment enhances nutrient removal would be explored.

3.2. Details of how FNA-based nitrifying sludge treatment enhances nutrient removal in the two-sludge systems

As shown in Fig. 1, the two-sludge system consisted of an AAO-SBR and an N-SBR, and NO_x^--N in the effluent of N-SBR was the major electron acceptor for phosphate uptake in AAO-SBR. It is known that compared with nitrate-based denitrifying phosphorus removal, nitrite-based denitrifying phosphorus removal would save carbon source, which benefits nutrient removal from low-carbon wastewaters. If more nitrite could be accumulated in N-SBR, better nutrient removal would be obtained in AAO-SBR. Thus, variations in effluent NH_4^+-N, NO_3^--N, and NO_2^--N of N-SBR in the three phases were first compared, and the results are shown in Fig. 3.

In phase I, N-SBR reached stable operation after 15-day operation, with the effluent NH_4^+-N and NO_2^--N concentration maintaining among 0.05-0.77 mg/L and 0.10-0.62 mg/L, respectively. It can be seen that NH_4^+-N was almost fully converted to NO_3^--N at the end of the oxic period, suggesting that full nitrification was achieved in this phase. In phase II, 1/16 of the sludge was daily treated with FNA at 1.1 mg N/L for 24 h. Upon implementation of this FNA treatment, effluent NO_3^--N level decreased gradually accompanied by an increase of effluent NO_2^--N concentration. After stable operation, ~9.1 mg/L of NO_2^--N (~28.5% of the effluent total nitrogen) and ~20.9 mg/L of NO_3^--N (70.2 ± 0.7% of effluent total nitrogen) were measured in the effluent. In phase III, FNA treatment at 1.9 mg N/L was implemented. This level of FNA treatment did not also lead to deterioration of NH_4^+-N conversion but further inhibit the conversion from NO_2^--N to NO_3^--N. As a result, the NH_4^+-N of nitrite increased from ~9.1 mg/L (~28.5% of the effluent total nitrogen) in Phase II to ~21.7 ± 0.6 mg/L (~67.8% of the effluent total nitrogen) in Phase III.

The data presented in Fig. 3 clearly showed that FNA-based nitrifying sludge treatment could cause NO_2^--N accumulation effectively, which could provide more NO_2^--N as the electron acceptor for subsequent denitrifying phosphorus uptake. Due to the similar growth kinetics of AOB and NOB, we further investigated the mechanisms of how this FNA treatment resulted in a substantial nitrite accumulation. NO_2^--N accumulation is closely relevant to the abundance and activity of NOB. It was measured that the abundance of NOB in phase I accounted for 21.2 ± 3.3% of the total bacteria, which decreased to 9.4 ± 2.5% at the steady-state in phase II (Table 2). Correspondingly, the activity of NOB decreased from 47.7 ± 3.8 (phase I) to 28.2 ± 2.4 mg N/g VSS•h (Phase II). When FNA concentration further increased to 1.9 mg N/L (i.e., phase III), the population and activity of NOB further decreased to 5.1 ± 1.9% and 21.5 ± 1.6 mg N/g VSS•h. On the contrary, the population of AOB increased from 51.6 ± 3.8% (phase I) to 57.1 ± 4.5% (phase II) and 63.8 ± 6.4% (phase III) of the total bacteria. It should be noted that this shift caused a slight decrease of the AOB activity from 63.6 ± 4.9 (phase I) to 60.5 ± 5.2 (phase II) and 58.4 ± 3.4 mg N/g VSS•h (phase III), suggesting that the increase of AOB population measured might be owing to the decrease of the relative NOB population rather than an absolute increase in AOB numbers. These results indicated that FNA treatment could effectively inhibit the growth of NOB but did not suppress the growth or activity of AOB, which was the reason for substantial nitrite accumulation achieved in N-SBR.

Two types of NOB, i.e., *Nitrospira sp.* and *Nitrobacter sp.,* were previously reported to be the main NOB in nitrifying systems (Wang et al., 2016a; Wang et al., 2016b; Chen et al., 2014). In this study, both *Nitrospira sp.* and *Nitrobacter sp.* were detected in substantial levels in phase I, accounting for 14.1 ± 3.6% and 7.1 ± 2.4% of the total bacteria, respectively (Table 2). In phase II, the abundance of *Nitrospira sp.* and *Nitrobacter sp.* decreased to 3.5 ± 1.1% and 5.9 ± 1.7%, respectively. In phase III, *Nitrospira sp.* was almost at a non-detectable level, while *Nitrobacter sp.* further dropped to 5.1 ± 1.9% of the total bacteria. The results suggest that FNA treatment is an effective approach to wash out *Nitrospira sp.*, a major type of NOB present in nitrifying reactors. Although FNA could also suppress the growth of *Nitrobacter sp.*, this type of NOB is more tolerant to FNA, as compared with *Nitrospira sp.*.

Fig. 4 presents the cyclic variations of sludge PHA, glycogen, PO_4^{3-}-P, NH_4^+-N, NO_3^--N and NO_2^--N of AAO-SBR in the two-sludge system. It can be seen that there was an obvious PO_4^{3-}-P release, accompanied with substantial PHA accumulation and glycogen degradation during the anaerobic period in all phases. In the subsequent anaerobic period, PO_4^{3-}-P uptake, nitrate (or nitrite) reduction, PHA degradation, and glycogen replenishment took place simultaneously. In the following oxic period, the remaining NH_4^+-N was further oxidized to nitrate. The synthesized PHA in the anaerobic period was mainly oxidized for phosphorus uptake, denitrification, and glycogen replenishment in the anoxic/oxic periods. It was found that the changes of PHA and glycogen among the three phases were almost the same. However, compared with phase I, the ratio of nitrite-based denitrification increased but nitrate-based denitrification decreased in phase III. It is well-known that nitrite-based denitrification could save 40% of carbon than nitrate-based denitrification. Thus, in phase III, more PHA could be left for both denitrification and phosphorus uptake, as compared with phase I. It can be therefore understood why FNA-based nitrifying sludge treatment executed in N-SBR enhances nutrient removal in the two-sludge system.

Table 3 further shows the specific phosphate uptake rates using oxygen, NO_3^--N and NO_2^--N as the sole electron acceptor in AAO-SBR during steady-state operation of different phases. When oxygen was used as the sole electron acceptor, the specific phosphate uptake rate among the three phases was very similar and maintained at 14-15 mg P/g VSS•h. However, with the shift from phase I to phase III, the specific phosphate uptake rate using NO_3^--N as the sole electron acceptor decreased from 10.71 ± 0.29 to 6.43 ± 0.21 mg P/g VSS•h. On the contrary, the specific phosphate uptake rate using NO_2^--N as the sole electron acceptor increased from 5.71 ± 0.24 to 13.72 ± 0.38 mg P/g VSS•h. The results suggested that although the total amount of PAOs was unaffected significantly (Table 2), the fraction of NO_2^--N -based PAO increased, which was consistent with the data presented above.

3.3. What happen in the FNA treatment units ?

To reveal the facts of what happen in the FNA treatment unit treating nitrifying-sludge, the changes of total COD, soluble COD, NH_4^+-N, NO_3^--N, NO_2^--N and activities of AOB and NOB were detected in the unit before and after FNA treatment at steady-state in Phase III (Table 4). It can be seen that there was no significant change in the total COD, NH_4^+-N, NO_3^--N and NO_2^--N concentrations before and after the FNA treatment. However, the activities of AOB and NOB and soluble COD concentration were affected. FNA treatment caused a 18% decrease in AOB activity and a 55% decrease in NOB activity. Moreover, the soluble COD level increased from 12 ± 3 mg/L to 335 ± 28 mg/L after 24 h treatment at 1.9 mg N/L FNA concentration. The increase of soluble COD may be owing to the solublization of extracellular materials and cell lysis, as demonstrated by previous investigations (Zhao et al., 2015b; Zhang et al., 2015).

In the FNA treatment unit treating sludge withdrawn from the conventional system, the activity of PAO, which was indicated by the specific rates of anaerobic phosphorus release and oxic/anoxic phosphorus uptake, was completely inhibited by 24 h of treatment at 1.1 mg N/L FNA concentration, which may be the reason for the collapse of biological phosphorus removal in the conventional system. Although similar decreases were observed in AOB and NOB activities, it should be noticed that the oxic time in the conventional system was shorter than that in the N-SBR of the two-sludge system (140 vs 180 min), which might be the reason for the increased ammonium level in effluent of the conventional system.

3.4. Benefits

With the increasing energy crisis worldwide, there is an ongoing paradigm shift in the operation of WWTPs from waste removal to both nutrient removal and energy recovery (Li et al., 2016; Xu et al., 2017; Zhao et al., 2016b, 2017). Thus, wastewater treatment in a sustainable way is urgently required. FNA-based nitrifying sludge treatment in a two-sludge system is a promising method to enhance nutrient removal from low-carbon wastewater, because this method not only improves removal efficiency of nitrogen and phosphorus but also saves the cost of sewage treatment through saving external carbon source addition.

The strategy obtained in this study could have tremendous benefits for treatment of low-carbon domestic wastewater. Nitrite-denitrifying phosphorus removal can save approximately 25% oxidation costs and 40% carbon consumption compared with conventional nitrification–denitrification technology. Based on the results achieved in this work, it is estimated that 15 tons carbon source will be added per year in a mainstream A_2O WWTP (Q: 10^5 m^3/d; COD: ~150 mg/L; NH_4^+-N: ~50 mg/L; PO_4^{3-}-P: ~10 mg/L) if 85% nitrogen and phosphorus removal efficiency is achieved by conventional methods. However, this value will be saved if the 85% removal efficiency of nitrogen and phosphorus is achieved by the above FNA treatment based approach, as presented in the current study. It should be emphasized that a cost-reduction of 15 tons carbon source would result in a decrease of 4000 Euro of wastewater treatment. FNA is a waste-generated, renewable compound that can be produced in situ in WWTPs as a byproduct of wastewater treatment by nitritation of the anaerobic digestion liquor, which makes it easily obtained in WWTPs (Law et al., 2015). Considering the huge municipal wastewater daily treated worldwide, this FNA-based nitrifying sludge treatment in a two-sludge system method may have significant benefits. However, this strategy has so far been tested only in the laboratory scale. Its technical performance and economic evaluations need to be further evaluated under real-wastewater conditions in the future.

4. Conclusion

This study develops a new strategy about nitrite-denitrifying phosphorus removal in the two-sludge system for the enhancement of nutrient removal from low-carbon wastewaters.

Experimental results showed that when 1/16 of the nitrifying sludge was daily treated with 1.1-1.9 mg N/L FNA for 24 h, ~29.5%-69.2% of nitrite accumulation was achieved in the nitrifying reactor, which resulted in 70.4%-84.5% in total nitrogen removal and 75.3%-88.7% in phosphate removal of the two-sludge system, respectively. FNA treatment could effectively reduce the abundance and activity of NOB. Although the total amount of PAOs was not affected significantly, the fraction of nitrite-based PAOs increased.

Acknowledgments

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Supporting Information

Useful supplementary data associated with this article can be found in the online version.

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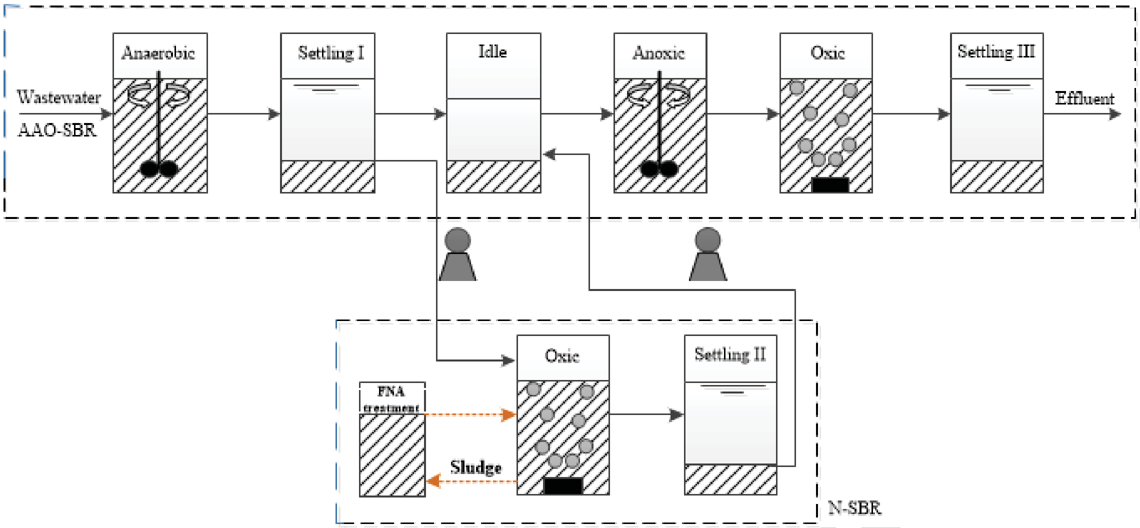
Figure Captions

Fig. 1. Schematic diagram of the operation of the modified two-sludge system

Fig. 2. The long-term variations in effluent PO_4^{3-}-P (A), NH_4^+-N (B), NO_3^--N (C) and NO_2^--N (D) in the two systems under the different operational conditions.

Fig. 3. The long-term variations in effluent NH_4^+-N, NO_3^--N, and NO_2^--N in the N-SBR of two-sludge system under the different operational conditions.

Fig. 4. Variations of sludge PHA, glycogen, PO_4^{3-}-P, NH_4^+-N, NO_3^--N and NO_2^--N during one typical cycle in AAO-SBR of the two-sludge system in Phase I (A), Phase II (B) and Phase III (C).



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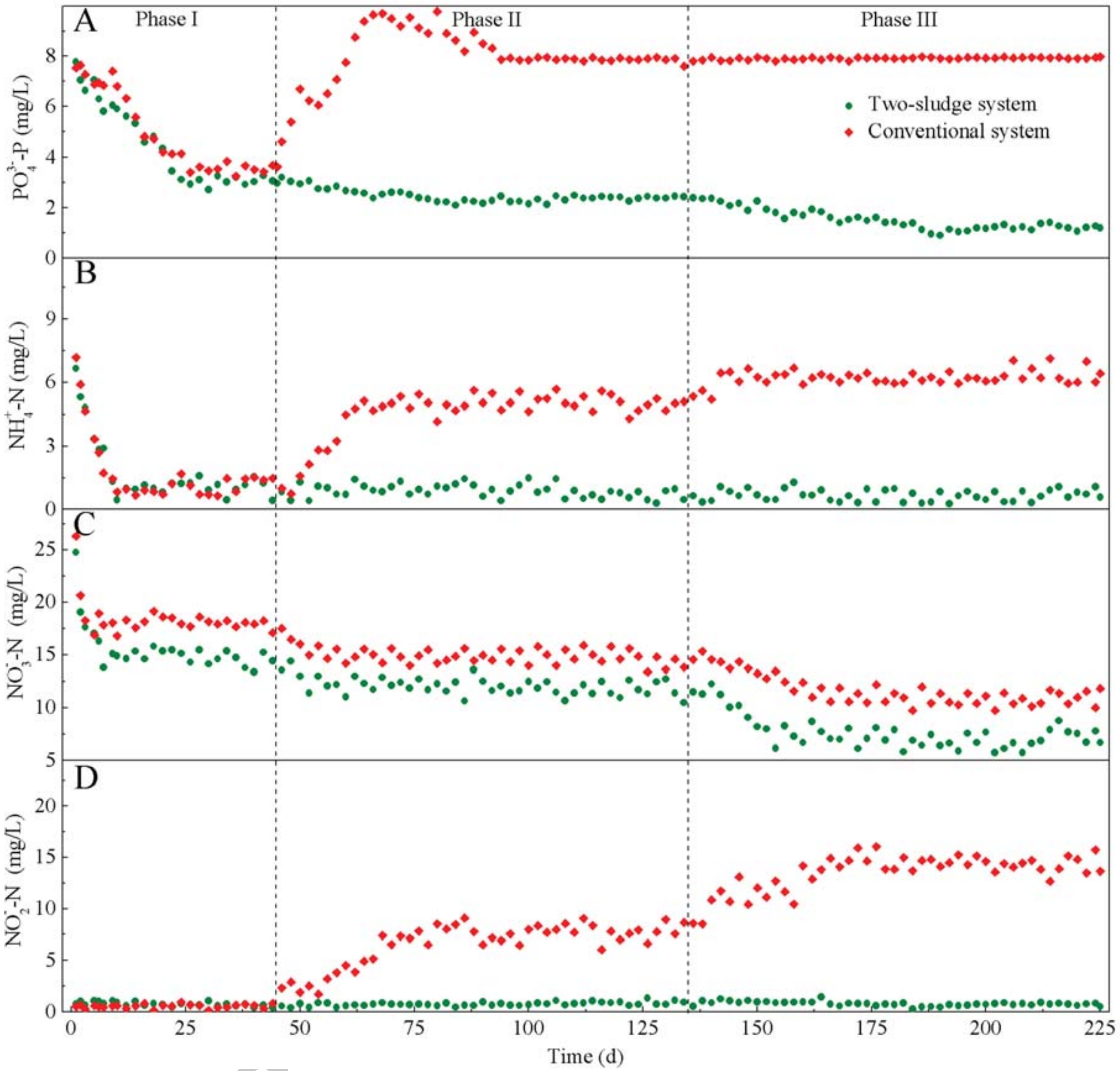

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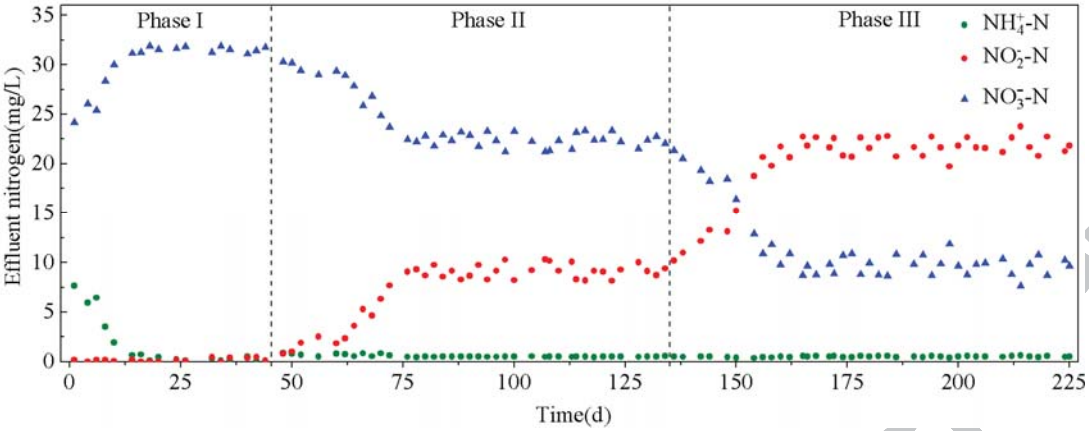

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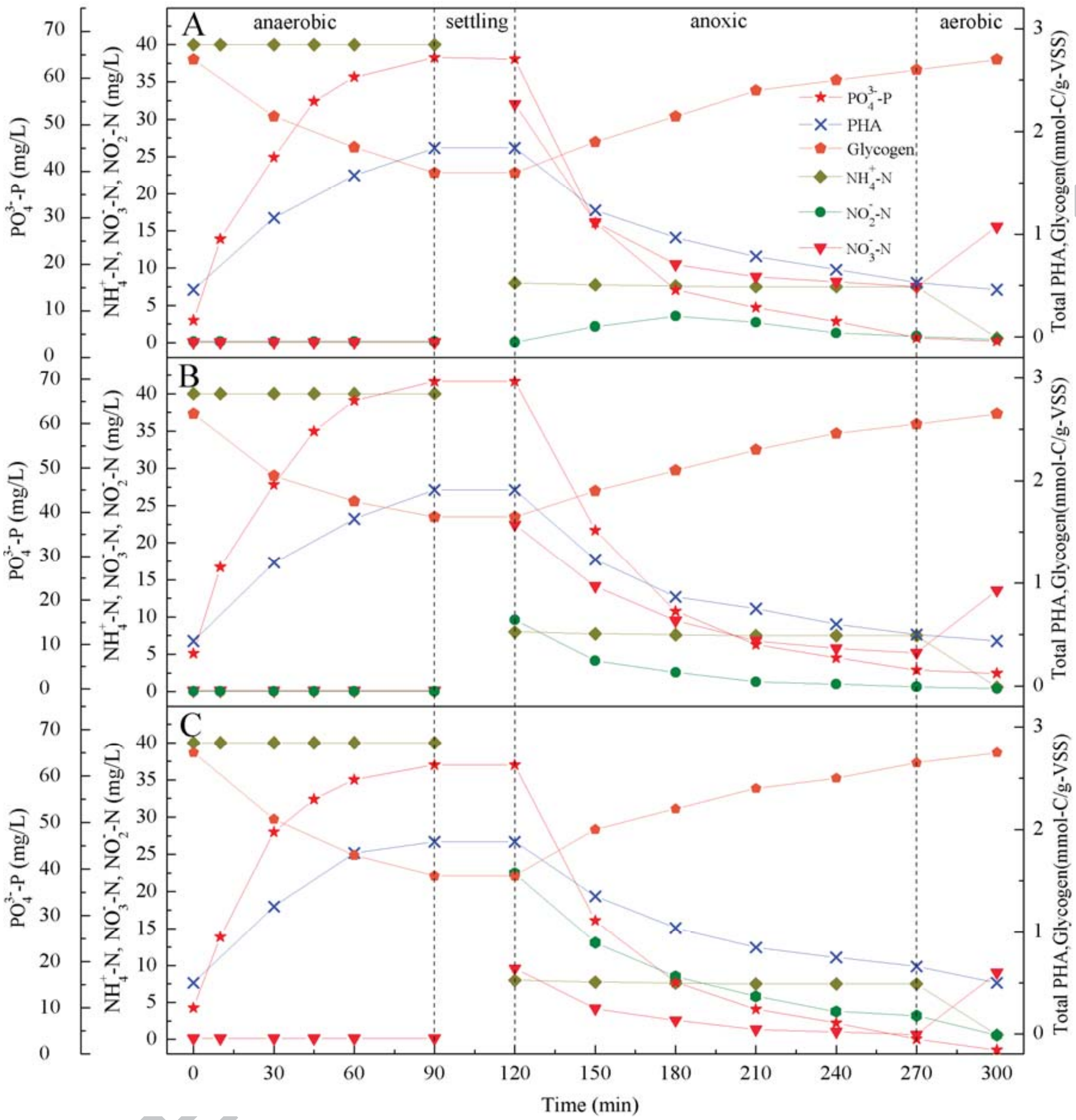

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Table Captions

Table 1 Comparison of the performances between the two-sludge system and the conventional system in steady-state operation of different phases [a].

Table 2 The abundances and activities of PAO, AOB, and NOB in the two-sludge system at steady-state in different phases [a].

Table 3 Comparison of the specific phosphate uptake rates using oxygen, nitrate, and nitrite as the sole electron acceptor in AAO-SBR during steady-state operation of different phases [a].

Table 4 Variations of total COD, soluble COD, NH_4^+-N, NO_3^--N, NO_2^--N, and activities of AOB and NOB in FNA treatment unit [a].

Table 1

Comparison of the performances between the two-sludge system and the conventional system in steady-state operation of different phases [a].

Item	Two-sludge system			Conventional system		
	Phase I	Phase II	Phase III	Phase I	Phase II	Phase III
Effluent PO_4^{3-} (mg/L)	3.06± 0.16	2.35 ± 0.15	1.21 ± 0.14	3.60 ± 0.23	7.84 ± 0.15	7.94 ± 0.03
Effluent NH_4^+ (mg/L)	1.03 ± 0.29	0.84 ± 0.32	0.65± 0.26	1.07± 0.35	5.04 ± 0.38	6.27 ± 0.34
Effluent NO_3^- (mg/L)	14.70± 0.59	11.86± 0.63	6.98± 0.69	18.10± 0.54	13.91± 0.59	9.39± 0.67
Effluent NO_2^- (mg/L)	0.67± 0.19	0.80± 0.19	0.70± 0.15	0.58± 0.22	6.75± 0.81	12.96± 0.77
PO_4^{3-} removal (%)	69.6 ± 3.4	76.7 ± 2.9	87.9 ± 3.5	65.1 ± 3.1	-	-
TN removal (%)	65.1 ± 2.9	72.4 ± 3.2	82.9 ± 3.8	60.9 ± 3.4	44.7 ± 4.3	36.8 ± 4.8
VSS (mg/L)	3238 ± 115	3032 ± 127	3102 ± 140	3252 ± 138	2125 ± 173	1476 ± 164
TSS (mg/L)	4560 ± 138	4458 ± 146	4492 ± 169	4645 ± 152	2258 ± 192	1528 ± 159

[a] Results are the averages and their standard deviations, and data are obtained during steady-state operation.

Table 2

The abundances and activities of PAO, AOB, and NOB in the two-sludge system at steady-state in different phases [a].

Item	Two-sludge system		
	Phase I	Phase II	Phase III
PAO population (%)	44.8 ± 0.6	42.6 ± 0.8	45.3 ± 0.7
P release rate (mg-P/g VSS•h)	24.6 ± 0.4	23.5 ± 0.5	21.8 ± 0.3
Anoxic P uptake rate (mg-P/g VSS•h)	12.5 ± 0.5	11.9 ± 0.3	12.7 ± 0.4
Aerobic P uptake rate (mg-P/g VSS•h)	15.6 ± 0.7	15.1 ± 0. 5	15.9 ± 0.3
AOB population (%)	51.6 ± 3.8	57.1 ± 4.5	63.8 ± 6.4
AOB activity (mg N/g VSS•h)	63.6 ± 4.9	60.5 ± 5.2	58.4 ± 3.4
NOB population (%)	21.2 ± 3.3	9.4 ± 2.5	9.4 ± 2.5
Nitrospira abundance (%)	14.1 ± 3.6	3.5 ± 1.1	Not detected
Nitrobacter abundance (%)	7.1 ± 2.4	5.9 ± 1.7	5.1 ± 1.9
NOB activity (mg N/g VSS•h)	47.7 ± 3.8	28.2 ± 2.4	21.5 ± 1.6

[a] Results are the averages and their standard deviations. PAO-based data were measured from the AAO-SBR while AOB- and NOB-based data were measured from the N-SBR.

Table 3

Comparison of the specific phosphate uptake rates using oxygen, NO_2^--N and NO_3^--N as the sole electron acceptor in AAO-SBR during steady-state operation of different phases [a].

Electron acceptor	Phosphate uptake rates (mg P/g VSS•h)		
	Phase I	Phase II	Phase III
NO_2^--N	5.71 ± 0.24	7.71 ± 0.26	13.72 ± 0.38
Oxygen	14.03 ± 0.31	14.67 ± 0.35	14.75 ± 0.36
NO_3^--N	10.71 ± 0.29	9.25 ± 0.33	6.43 ± 0.21

[a] Results are the averages and their standard deviations. PAO-based data were measured from the AAO-SBR while AOB- and NOB-based data were measured from the N-SBR.

Table 4

Variations of total COD, soluble COD, NH_4^+-N, NO_3^--N, NO_2^--N, and activities of AOB and NOB in FNA treatment unit [a].

Treatment time	Total COD (mg/L)	Soluble COD (mg/L)	NH_4^+-N (mg/L)	NO_3^--N (mg/L)	NO_2^--N (mg/L)	AOB activity (mg N/g VSS·h)	NOB activity (mg N/g VSS·h)
0	2468±108	12±3	0.13±0.03	798±28	1.25±0.05	58.7±3.6	23.6±1.8
24h	2450±125	335±28	0.18±0.04	790±24	1.02±0.04	48.2±2.8	10.8±0.9

[a] Results are the averages and standard deviations from triplicate measurements during steady-state in Phase III. The pH in the FNA treatment unit was 6.0 during the entire treatment period.

Highlights:

- A new strategy was developed to enhance nutrient removal from low carbon-wastewater
- A FNA-based nitrifying-sludge treatment was used in a two-sludge system
- 1.9 mg N/L FNA caused ~67.8% of nitrite accumulation in the nitrifying reactor
- 1.9 mg N/L FNA caused ~83% in nitrogen removal and ~88% in phosphate removal
- FNA treatment reduced the abundance of nitrite oxidizing bacteria