RESEARCH ARTICLE

Optimization of denitrifying phosphorus removal in a pre-denitrification anaerobic/anoxic/post-aeration + nitrification sequence batch reactor (pre-A$_2$NSBR) system: Nitrate recycling, carbon/nitrogen ratio and carbon source type

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HIGHLIGHTS

- A novel two sludge pre-A$_2$NSBR system was developed.
- Advanced N and P removal was optimized to treat real domestic wastewater.
- Nitrifiers and PAOs were enriched with 19.41% and 26.48%, respectively.
- Acetate was demonstrated as the high-quality carbon source type.

GRAPHIC ABSTRACT

ABSTRACT

Because the efficiency of biological nutrient removal is always limited by the deficient carbon source for the low carbon/nitrogen (C/N) ratio in real domestic sewage, the denitrifying phosphorus removal (DNPR) was developed as a simple and efficient method to remove nitrogen and phosphorous. In addition, this method has the advantage of saving aeration energy while reducing the sludge production. In this context, a pre-denitrification anaerobic/anoxic/post-aeration + nitrification sequence batch reactor (pre-A$_2$NSBR) system, which could also reduce high ammonia effluent concentration in the traditional two-sludge DNPR process, is proposed in this work. The pre-A$_2$NSBR process was mainly composed of a DNPR SBR and a nitrifying SBR, operating as alternating anaerobic/anoxic/post-aeration + nitrification sequence. Herein, the long-term performance of different nitrate recycling ratios (0–300%) and C/N ratios (2.5–8.8), carbon source type, and functional microbial community were studied. The results showed that the removal efficiency of total inorganic nitrogen (TIN, including NH_4^+-N, NO_2^--N, and NO_3^--N) gradually increased with the nitrate recycling ratios, and the system reached the highest DNPR efficiency of 94.45% at the nitrate recycling ratio of 300%. The optimum C/N ratio was around 3.9–7.3 with a nitrogen and phosphorus removal efficiency of 80.15% and 93.57%, respectively. The acetate was proved to be a high-quality carbon source for DNPR process. The results of fluorescence in situ hybridization (FISH) analysis indicated that nitrifiers and phosphorus accumulating organisms (PAOs) were accumulated with a proportion of 19.41% and 26.48%, respectively.

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1   Introduction

Biological nutrient removal in wastewater treatment plants (WWTPs) has been an important strategy to prevent eutrophication of waterbodies. Nevertheless, the low amount of organics in real domestic wastewaters is detrimental for an efficient nutrient removal inefficient for carbon sources competition between denitrifiers and phosphorus accumulating organisms (PAOs) (Ahn et al., 2002; Ma et al., 2009; 2013). Denitrifying phosphorus removal organisms (DPAOs) can achieve simultaneous denitrification and phosphorus removal under anoxic condition by using a single carbon source, conditions in which the aeration energy and carbon source saved, while the sludge production is reduced (Kuba et al., 1996). Our previous study (Zhao et al., 2016) showed the superiority of the pre-A$_2$NSBR system, SRT between nitrifiers and PAOs was separated in this two-sludge system, and we first solved the bottle-neck problem of high NH$_4^+$-N effluent concentration in the traditional two-sludge denitrifying phosphorus removal (DNPR) process, for example, A$_2$NSBR process (Kuba et al., 1996), A$_2$N process and Dephanox process (Wanner et al., 1992; Bortone et al., 1996).

Sewage quality such as carbon/nitrogen (C/N) ratio (Wang et al., 2013), type of carbon source (Pijuan et al., 2009), and nitrate recycling ratio (Chen et al., 2011) were important parameters for WWTPs design and optimization of operation conditions, because, to a certain extent, they directly influence the biological process and effluent quality. WWTPs influent quality fluctuated daily and seasonally, and they are frequently deprived of carbon source, which decreases the efficient nitrogen and phosphorus removal (Sun et al., 2010). The type of carbon source determines the denitrification rate and PHA formation, and influences the nutrient removal efficiency (Lee and Welander, 1996; Yagci et al., 2007). However, the research performed so far was mostly focused on traditional exogenous denitrification, whereas the endogenous denitrification alongside the phosphorus removal was scarcely studied.

The specific techniques of molecular biology, such as fluorescence in situ hybridization (FISH) analysis, were developed rapidly in the last few years. These techniques were mainly used to identify and quantify the microbial population structure (Crocetti et al., 2000; Paungfoo et al., 2007; Wang et al., 2015), which highlight the relationship between the nutrient removal performance and functional microorganisms.

However, the operational factors and sewage quality of the pre-A$_2$NSBR are far to be deeply understood. Little is known about the sewage quality with nutrient removal performance in real domestic wastewater treatment. In this study, nitrate recycling ratio and C/N ratio were adjusted to evaluate the process performance. The carbon source type,

such as acetate, propionate and glucose, was investigated by batch test to understand the inherent mechanism. The functional microbial communities were identified and quantified by FISH analysis.

2   Materials and methods

2.1   Process setup and operation

The pre-A$_2$NSBR system was composed of two independent SBRs. One was filled with suspended sludge and operated for DNPR as anaerobic/anoxic mode (A$_2$SBR), with a SRT relatively short. The other was filled with suspended carrier for aerobic nitrification (N-SBR). The volume of each SBR was 12 L. Both SBRs were made from plexiglass and equipped with IKA agitator. The aeration was conducted by air pump with glass rotameter to adjust the gas flow rate. Influent, recycling was conducted by peristaltic pumps. The start-up of the system lasted around 2 months, aiming to ensure a high and stable performance of the nutrient removal. Then the study was conducted.

At run 1, the A$_2$SBR was operated at alternating anaerobic/oxic (2 h/1 h) conditions for P- removal. After settlement (0.5 h), the supernatant, rich in ammonia, was fed into N-SBR (4 h) for nitrification and finally drained out without nitrate reflux. From run 2 to run 9, the duration of one cycle of pre-A$_2$NSBR system was 6 h, including 1 h anaerobic/ 2 h anoxic/ 0.5 h post-aeration/ 0.5 h settlement and decanting/ 3 h aerobic nitrification phase. A detailed description of each of these steps, includes the following steps. 1) Anaerobic phase, when 2 L raw wastewater was pumped into the A$_2$SBR from an influent tank, then the agitator was started to mix the suspended sludge. 2) Anoxic phase, when the nitrate liquid was recycled from the N-SBR effluent, while the mixing was constant mixing to ensure the denitrifying phosphorus removal. From run 2 to run 4, the C/N ratio was set around 4.0, the reflux ratio was adjusted from 100% to 300%. From run 5 to run 9, the reflux ratio was set to 200%, the C/N ratio was adjust at 2.5, 3.9, 5.3, 7.3, and 8.8. 3) Post-aeration, when 0.5 h post-aeration was allowed, followed by the 2 h anoxic phase. 4) Settlement phase, sludge and ammonia rich-in supernatant was separated. 5) Aerobic nitrification phase, when the supernatant rich in ammonia was pumped from A$_2$SBR to N-SBR, NH$_4^+$-N was almost completely removed. Finally, a part of nitrate liquid was recycled to the anoxic phase of the A$_2$SBR in the next cycle, while the remaining part was drained out of the system. Detailed operation scheme and wastewater characteristics are displayed in Fig. 1 and Table 1.

2.2   Inoculated sludge and sewage characteristics

The seeding activated sludge was inoculated from
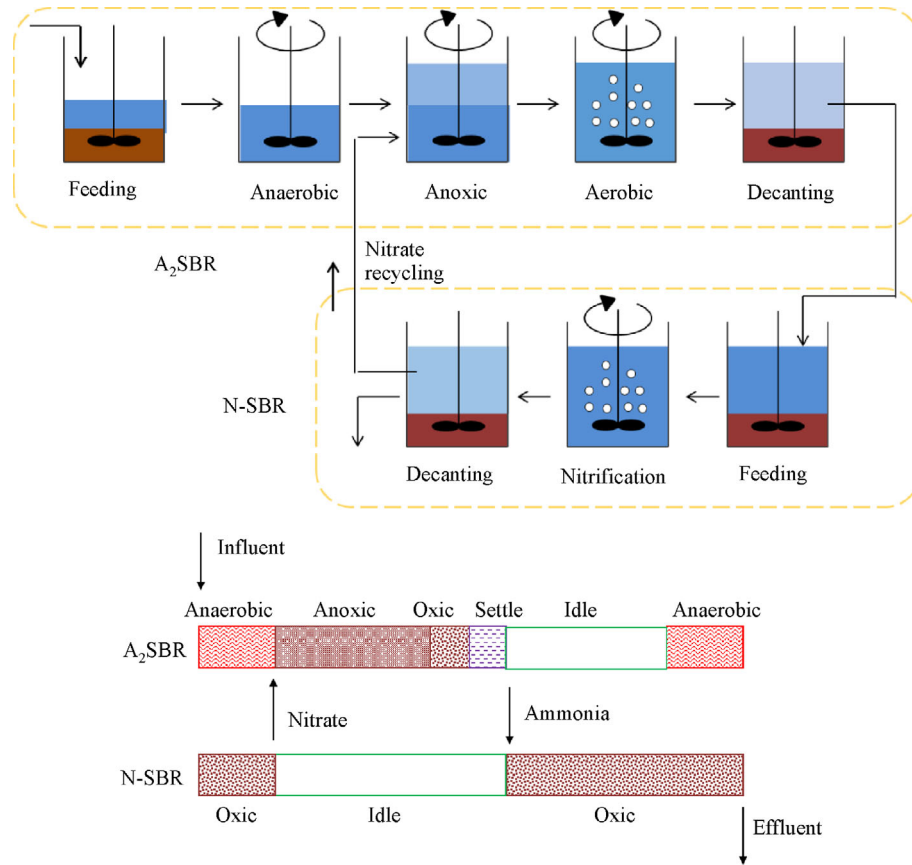
Fig. 1 Operational sequence and mechanism of the pre-A₂NSBR process.

Table 1 Operation scheme and wastewater characteristics (From run 1 to run 4, the C/N ratio was set around 4.0. The reflux ratio was adjusted as 0, 100%, 200%, and 300%. From run 5 to run 9, the reflux ratio was set to 200%, the C/N ratio was adjust as 2.5, 3.9, 5.3, 7.3 and 8.8)

No.	Parameter (d)	COD			NH₄⁺-N			TIN			PO₄³⁻-P		
		Influent (mg/L)	Effluent (mg/L)	Removal (%)	Influent (mg/L)	Effluent (mg/L)	Removal (%)	Influent (mg/L)	Effluent (mg/L)	Removal (%)	Influent (mg/L)	Effluent (mg/L)	Removal (%)
Run 1	1–32	192.44	47.35	75.39	45.50	0.53	98.84	45.94	37.26	18.89	3.64	0.13	96.43
Run 2	33–60	183.90	44.70	75.69	48.89	0.76	98.45	49.12	20.34	58.59	4.67	0.57	87.79
Run 3	61–94	216.40	46.35	78.58	53.28	0.48	99.10	53.84	13.36	75.19	3.98	0.22	94.47
Run 4	95–121	204.93	48.20	76.48	56.37	0.46	99.18	56.89	13.49	76.29	4.42	0.28	93.67
Run 5	122–169	128.4	39.46	69.27	51.36	0.37	99.28	51.45	22.45	56.37	4.22	0.31	92.65
Run 6	170–204	193.13	41.24	78.65	49.52	0.66	98.67	49.87	15.86	68.20	4.03	0.37	90.82
Run 7	205–253	291.02	44.93	84.56	54.91	0.43	99.22	55.16	10.95	80.15	4.51	0.29	93.57
Run 8	254–277	366.90	48.33	86.83	50.26	1.76	96.50	50.35	11.39	77.38	4.33	0.97	77.60
Run 9	278–297	475.89	57.47.	87.92	53.47	14.20	73.53	53.84	28.21	47.74	4.83	3.00	38.29

GaoBeiDian WWTPs in Beijing, China, which achieved a good performance of the nutrient removal with a daily flow rate of one million m³/d. The sewage influent was pumped from a septic pool in the living area of Beijing University of Technology. The suspended biofilm carrier filling ratio of the N-SBR was 40% to ensure complete nitrification, while the MLSS of the A₂SBR was maintained around 2200–2800 mg/L. Tap water and sodium acetate were added into the influent tank to change the C/N ratios. Wastewater characteristics are summarized in Table 1.

2.3 Analytical methods

In this study, daily wastewater samples were filtrated by

0.45 μm filter paper. COD, NH_4^+-N, NO_2^--N, NO_3^--N, and PO_4^{3-}-P were measured according to instruments and methods mentioned by Zhao (Zhao et al., 2016; 2018). The sludge samples were analyzed for MLSS and VSS by standard methods.

To detect the microbial community structure, the sludge and biofilm samples were taken from the A_2SBR and the N-SBR, respectively, on day 117. The functional PAOs and nitrifiers were identified and quantified according to previously described FISH analysis methods (Crocetti et al., 2000).

PHA was analyzed by a gas chromatograph (Agilent 6890N, USA). The critical water quality parameters, such as DO, ORP, pH and temperature, were measured by the WTW multi-parameter instrument (pH/Oxi 340i, Germany).

2.4 The effect of different carbon sources on denitrifying phosphorus removal performance

In the batch test, acetate, propionate and glucose were chosen as external carbon sources for denitrifying phosphorus removal. PHA conversion, P- release and DNPR rate were measured to evaluate the influence of each type of carbon source.

The activated sludge was taken from the A_2SBR and was divided into three parts after being washed by tap water. The acetate, propionate and glucose were added into three 1.5 L SBRs to supply 100 mg/L of initial COD concentration. One hour later, samples were taken out for quantification of P-release amount and PHA composition. Then 30 mg/L NO_3^--N (sodium nitrate) was added to perform the DNPR reaction. Samples were periodically taken, and COD, PHA, PO_4^{3-}-P, NO_3^--N and VSS were measured.

3   Results and discussion

3.1 The effect of the nitrate recycling ratios on variations of organics and nutrient removal

Figure 2 displays the variation of organics and nutrient removal under different nitrate recycling ratios.

As Fig. 2 (a) shows, from run 1 to run 4, the organic substrate was used with an average COD removal efficiency of 76.54%. In this pre-A_2NSBR process, easily biodegradable organic compounds, such as VFA, were anaerobically transformed into PHA with P- release (Zhang et al., 2016b). DNPR consisted of simultaneous nitrate and phosphorus removal, which were performed under anoxic conditions. During the post-aeration phase, the residual phosphorus was further uptaken (Zhang et al., 2013) and nitrogen gas was blown off. As seen in Fig. 2 (b), the system achieved advanced P removal with an average efficiency of 93.09%, while no nitrification was observed

during the post-aeration phase of 0.5 h, maybe due to the short HRT and SRT (Chen et al., 2015). Yang achieved an advanced phosphorus removal at short SRT of 2–3 days without nitrification occurring in an EBPR-PN/A process (Yang et al., 2017). Then, after settlement, the supernatant rich in ammonia was pumped into N-SBR, when, the occasionally seldom available COD and long SRT favored the nitrification (Walters et al., 2009; Ge et al., 2012), and thus, the nitrifiers were enriched. As Fig. 2 (c) illustrates, from run 1 to run 4, the NH_4^+-N removal efficiency was high and maintained at 98.89%.

3.2 The relationship between the nitrate recycling ratio and denitrifying phosphorus removal (runs 1 to 4)

Nitrogen removal was mainly conducted by nitrification and denitrification processes (Gong et al., 2014). The anoxic nitrate loading played a major role in the DNPR efficiency (Zhang et al., 2016a). In this study, TIN removal efficiency showed an upward tendency with the increase of nitrate recycling ratio (Carucci et al., 1996; Chen et al., 2011). As Table 1 shows, when the recycling ratios changed from $R = 0$ to 300%, the TIN removal efficiency was gradually increased from 18.89%, 58.59%, 75.19%, to 76.29%. When $R = 0$, the TIN removal was conducted mainly by simultaneous nitrification and denitrification, whereas when $R = 100\%$ and $R = 200\%$, the TIN removal efficiency was improved substantially. Further increased of the recycling ratios from $R = 200\%$ to 300%, did not change the TIN removal efficiency. This could be explained by the fact that the stored PHA is not enough for anoxic denitrification. Meanwhile, higher DO concentration, carried by the large recycling ratio, destroyed the anoxic environment. When $R = 200\%$, the effluent concentration of COD, NH_4^+-N, TIN, and PO_4^{3-}-P was 46.35 mg/L, 0.48 mg/L, 13.36 mg/L, and 0.22 mg/L, respectively, with removal efficiency of 78.58%, 99.1%, 75.19%, and 94.47%, respectively. The removal of excellent organics and nutrient were achieved, moreover, higher recycling ratios result in higher energy consumption, situation which is also detrimental to the anoxic condition ensured by high DO (Zhao et al., 2018). Hence, on the base of the results obtained in this study, a ratio of 200% is suggested, from economic point of view. Chen achieved a TN removal efficiency of 64.9%, 77.0%, 82.0%, and 87.0% under respective nitrate recycling ratios (increasing from 100% to 400%) (Chen et al., 2011), which are similar to those used this study.

As Fig. 2 (d) shows, the denitrifying phosphorus removal efficiency (ratio of anoxic P- uptake to total P-uptake) improved when the recycling ratios increased, because much more electronic acceptors were supplied. The average denitrifying phosphorus removal efficiency under various recycling ratios of 100%, 200%, and 300% was 72.63%, 88.65%, and 94.45%, respectively. Obviously, anoxic denitrifying phosphorus removal was
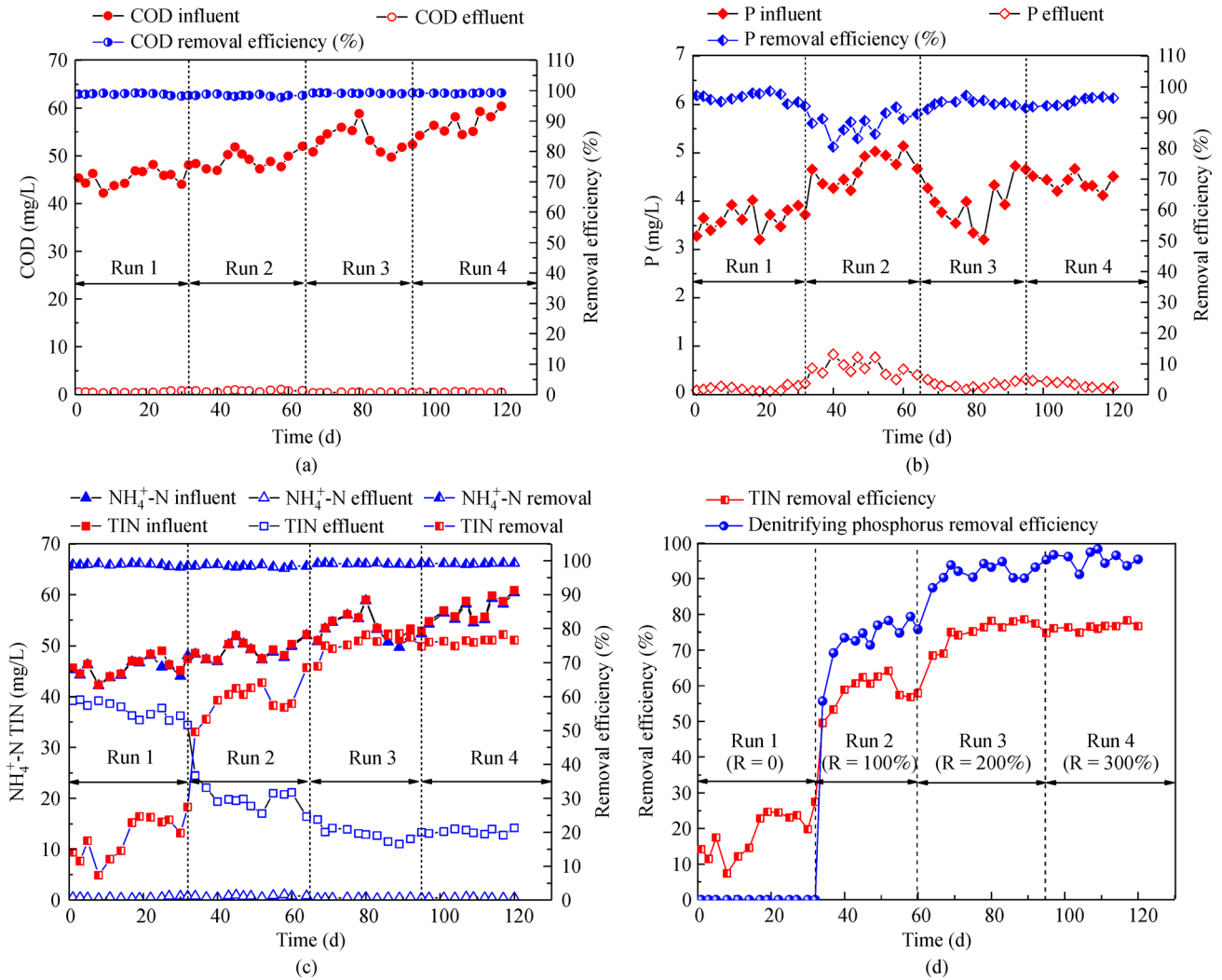
Fig. 2 Variations of organics and nutrient removal under different nitrate recycling ratios: (a) COD removal performance; (b) P removal performance; (c) NH$_4^+$-N and TIN removal performance; (d) relationship between TIN removal and denitrifying phosphorus removal efficiency.

the dominant way for P removal, which implies an efficient utilization of limited carbon source and energy-saving in comparison to the aerobic P-uptake consumption (Zhang et al., 2016c).

3.3 Identification of the functional microbial structure by FISH

P removal was performed by PAOs in the A$_2$SBR, whereas NH$_4^+$-N removal was performed by nitrification in the N-SBR. The biofilm easily accumulate nitrifiers with long SRT, the amount of functional microorganisms being important for the nutrient removal. In this study, FISH analysis was used to determine the microorganisms based on gene probes as follows. EUBmix was used for all bacteria (EUB338, EUB338-II, and EUB338-III), whereas PAO462, PAO651, and PAO846 probes were used for PAOs. For nitrifiers, NSO1225 was used for AOB, and NIT3 and Ntspa662 probes were used for NOB. Details on probes and detection methods are available in previously published works (Zhao et al., 2016; 2018). On day 117, the sludge and biofilm samples were taken from the pre-A$_2$NSBR system subjected to the FISH analysis. The results (Fig. 3) show that PAOs and nitrifiers were enriched with 26.48% and 19.41%, respectively, who contributed greatly to the enhanced removal performance of phosphorus and nitrogen. In addition, these results demonstrated that a two sludge process is more advantageous in enriching functional microorganisms with SRT separation (van Loosdrecht et al., 1998). The proportions of PAOs are higher than similar denitrifying phosphorus removal processes, such as A^{2}O + BCO process (20.67%) (Zhang et al., 2016b), SBR with nitrite pathway (7.6%) and SBR with nitrate pathway (6.5%) (Zeng et al., 2014). Previous
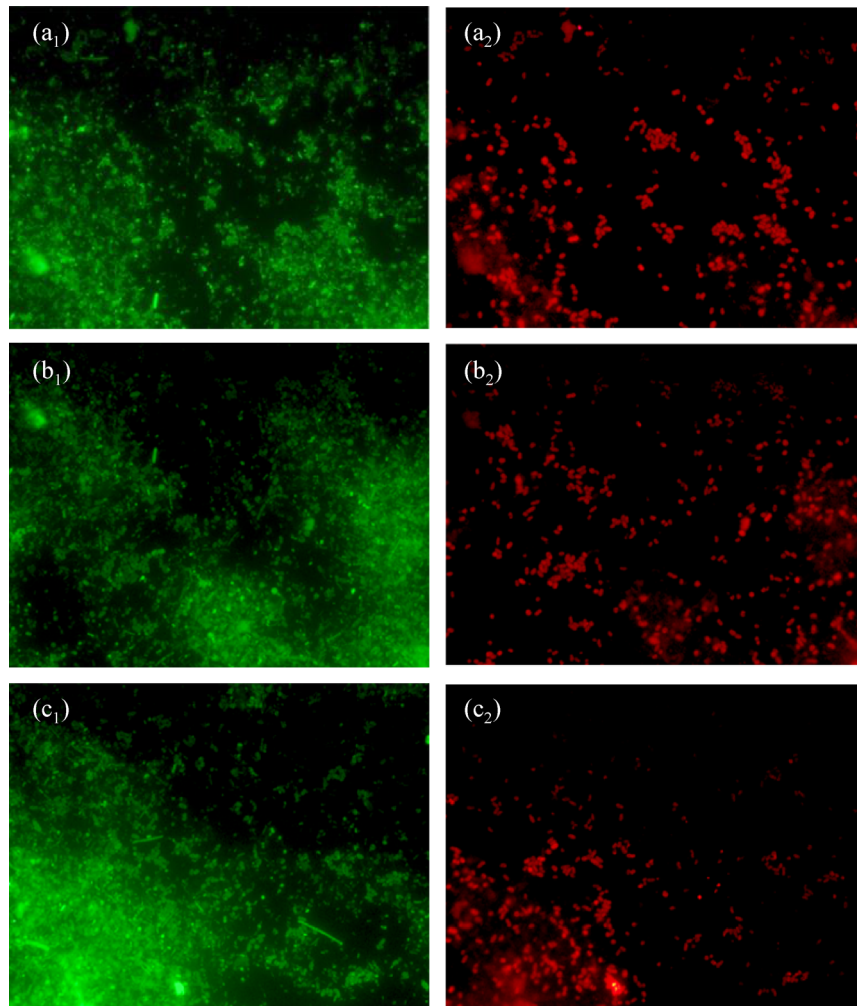
Fig. 3 FISH experiment analysis of PAOs (a_1 and a_2: total bacteria and PAOs) and AOB (b_1 and b_2: total bacteria and AOB) & NOB (c_1 and c_2: total bacteria and NOB).

FISH results also indicated that GAOs accounted for 4.7% in the pre-A_2NSBR system (Zhao et al., 2016), which may conduct endogenous denitrification.

3.4 The effect of the C/N ratios on variations of organics and nutrient removal

Figure 4 illustrates the effect of the different C/N ratios on the organics and nutrient removal efficiency.

The COD removal efficiency during various influent organics loadings is presented in Fig. 4(a). For C/N ratios varying from 2.5 to 8.8, the COD removal efficiency was high and maintained constant at a value of 80.65%, although the COD effluent deteriorated slightly, which exceeds the first A discharge standard (50 mg/L) in China for a ratio of 8.8. Therefore, the system exhibited an excellent capability to resist shocks caused by the organic loading. When the C/N ratio varied from 2.5 to 5.3, the COD was mainly removed during the anaerobic phase, where most biodegradable organics were transformed into

PHA as a last step in the endogenous denitrifying phosphorus removal, which facilitates the DNPR and enhances the nitrification performance (Zhang et al., 2014). The average P and NH_4^+-N removal efficiency was 92.56% and 99.26%, respectively. For a C/N ratio of 7.3, the residual COD was transferred from the anaerobic area to anoxic area. However, exogenous denitrification is superior to compete for the electronic acceptors of nitrate compared with denitrifying phosphorus removal (Zeng et al., 2003). As Figs. 4(b) and 4(d) show, the effluent P concentration increased slightly when the P removal decreased to 77.43%, but the TIN removal was still high, with a removal efficiency of 77.38%. For a C/N ratio of 8.8, the huge loading of exorbitant organics inhibited the P removal, whose efficiency was of 38.29%. The residual COD was transferred into N-SBR, which restricted the nitrification performance, because the nitrification occurred only when COD was degraded to a low level (Boiran et al., 1996; Zeng et al., 2004). As Fig. 4(c) shows, the NH_4^+-N removal efficiency deteriorated to 73.53%.
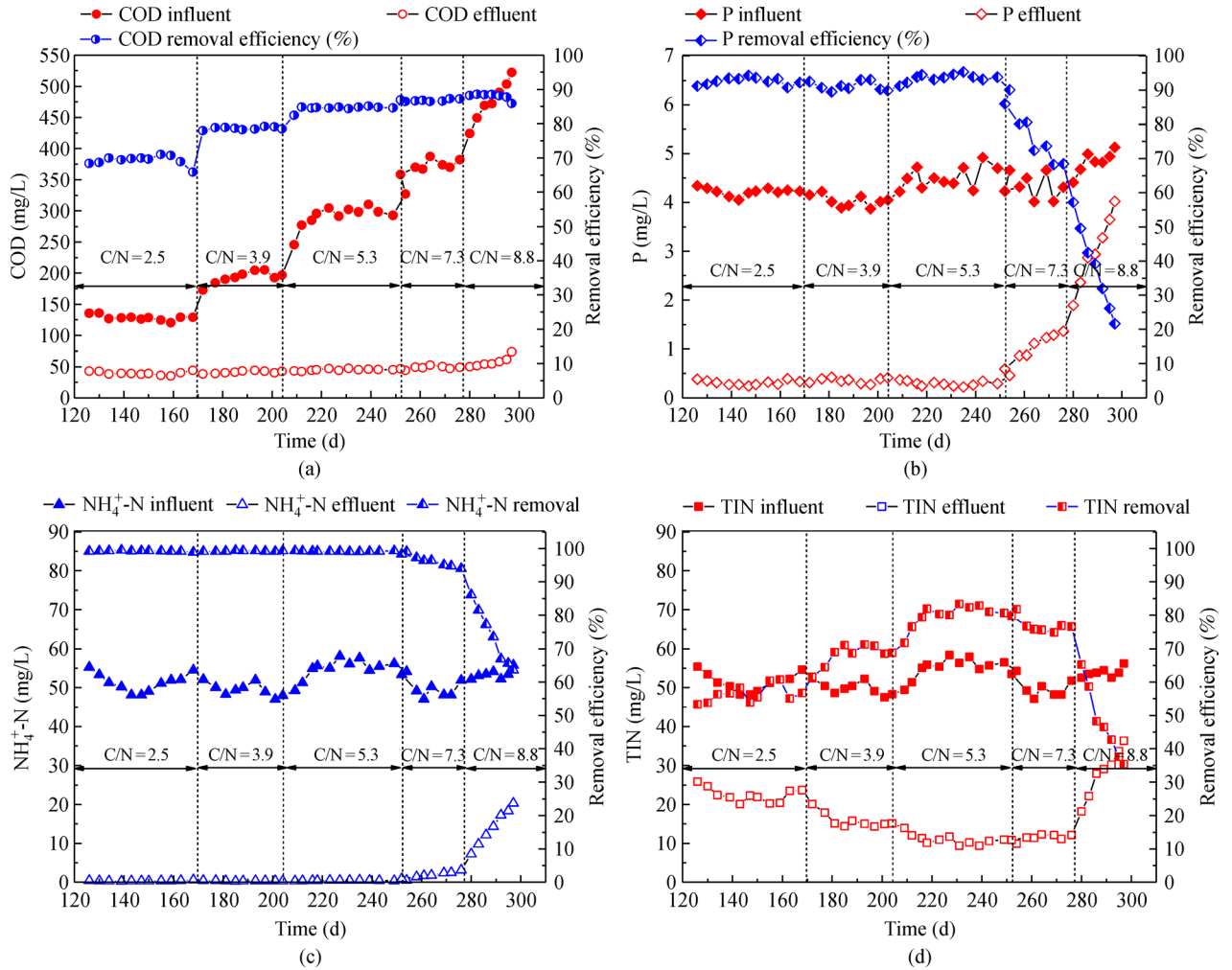
Fig. 4 Variations of organics and nutrient removal under different C/N ratios: (a) COD removal performance; (b) P removal performance; (c) NH₄⁺-N removal performance; (d) TIN removal efficiency.

TIN removal was completed through nitrification in N-SBR and DNPR in A₂SBR. The system achieved almost 100% of ammonia removal when C/N varied from 2.5 to 7.9. Hence, TIN removal was influenced by denitrification and increased with the variation of C/N ratios. When the C/N ratio was 5.3, TIN removal efficiency reached to 80.15%, while for a C/N ratio of 8.8, TIN removal was low, because the nitrification performance was limited by the huge loadings of organics (Lee et al., 2002). It is thus feasible to enlarge MLSS and aeration rate, as well as to extend or adjust HRT, so that to improve the organic removal.

3.5 The effect of carbon source on the P- release, DNPR rate and PHA composition

Figure 5 displays the effect of the carbon source on the P release and uptake performance.

The type of carbon source type influences the denitrification rate and PHA conversion, and plays an important role in the nutrient removal (Yu and Si, 2004). Actually, acetate and propionate were the main composition of VFA in real sewage, and glucose was also the common organics (Thomas et al., 2003; Chen et al., 2004). As Fig. 5(a) illustrates, the average rates of P- release of acetate, propionate and glucose were 17.40 mg P/(g MLSS·h), 11.84 mg P/(g MLSS·h), and 10.0 mg P/(g MLSS·h), respectively, and the corresponding PHA production was 64.73 mg COD/g VSS, 42.88 mg COD/g VSS, and 37.52 mg COD/g VSS, respectively. The PHA production is important, because the amount of PHA controls the DNPR capacity. On the basis of the results obtained in this study, it can be affirmed that the acetate is the optimal carbon source in terms of PHA synthesis and P removal. Therefore, the acetate could be added into WWTPs when the C/N ratio is low, and this is in accordance with studies of Lee and Welander (1996) and Elefsiniotis and Li (2006), Zhang et al. also found a similar P- release rate of 11.28 mg P/(g MLSS·h) when acetate was used as carbon source (Zhang et al., 2016a). Du et al. also demonstrated that the
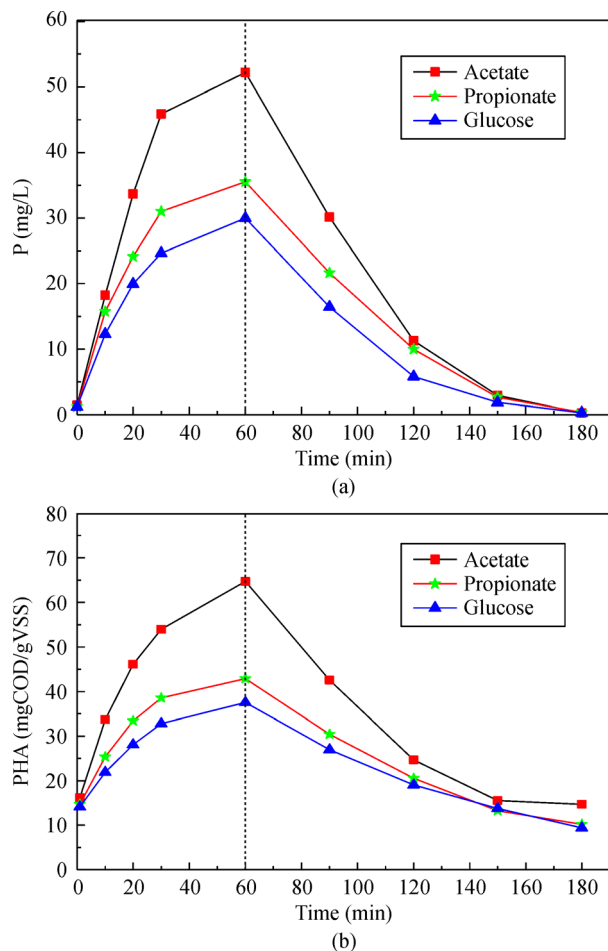
Fig. 5 (a) P-release and uptake performance under different carbon source of acetate, propionate and glucose; (b) PHA synthesis and consumption performance under different carbon source of acetate, propionate and glucose

acetate was beneficial to the growth of PAOs (Du et al., 2017). An accurate acetate dosage could be performed by using a novel, simple strategy that allows an online control of the acetate amount, as proposed by Liu et al. (2017).

4   Conclusions

In summary, this study showed that the nitrogen removal and DNPR efficiency increased with the increasing of the nitrate recycling ratios. The highest denitrifying phosphorus removal efficiency of 94.45% was reached for the nitrate recycling ratio of 300%. The optimum C/N ratio was 3.9–7.3 with a highest nitrogen and phosphorus removal efficiency of 80.15% and 93.57%, respectively. The system exhibited excellent P and NH_4^+-N removal performance at low C/N ratio, but it was declined at huge organic loadings. It was found that the acetate is a high-quality carbon source for DNPR process, and it could be added into WWTPs when the C/N ratio is low. FISH

analysis indicated that nitrifiers and PAOs were accumulated with a proportion of 19.41% and 26.48%, respectively, which accounted for the efficient and stable nutrient removal.

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