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Denitrifying phosphorus removal in a step-feed CAST with alternating anoxic-oxic operational strategy

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Abstract

A bench-scale cyclic activated sludge technology (CAST) was operated to study the biological phosphorus removal performance and a series of batch tests was carried out to demonstrate the accumulation of denitrifying polyphosphate-accumulating organisms (DNPAOs) in CAST system. Under all operating conditions, step-feed CAST with enough carbon sources in influent had the highest nitrogen and phosphorus removal efficiency as well as good sludge settling performance. The average removal rate of COD, NH_4^+-N, PO_4^{3-}-P and total nitrogen (TN) was 88.2%, 98.7%, 97.5% and 92.1%, respectively. The average sludge volume index (SVI) was 133 mL/g. The optimum anaerobic/aerobic/anoxic (AOA) conditions for the cultivation of DNPAOs could be achieved by alternating anoxic/oxic operational strategy, thus a significant denitrifying phosphorus removal occurred in step-feed CAST. The denitrification of NOx^--N completed quickly due to step-feed operation and enough carbon sources, which could enhance phosphorus release and further phosphorus uptake capability of the system. Batch tests also proved that polyphosphate-accumulating organisms (PAOs) in the step-feed process had strong denitrifying phosphorus removal capacity. Both nitrate and nitrite could be used as electron acceptors in denitrifying phosphorus removal. Low COD supply with step-feed operation strategy would favor DNPAOs accumulation.

Key words: cyclic activated sludge technology; biological phosphorus removal; denitrifying polyphosphate-accumulating organisms

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Introduction

Over the past several decades, biological nutrient removal (BNR) processes in conjunction with chemical oxygen demand (COD) have been widely used to treat wastewater containing nitrogen and phosphorus due to their economic advantages. The developed BNR systems include sequencing batch reactor (SBR), the University of Cape Town (UCT) system, the Bardenpho process and the anaerobic/anoxic/oxic (A^2O) system, and so on (Tchobanoglous *et al*., 2003; Peng *et al*., 2006; Yang *et al*., 2007).

Cyclic activated sludge technology (CAST), a type of SBR, has attracted a great deal of interest in recent years, because it has a good performance in biological nutrient removal from wastewater and the ability of preventing sludge bulking with a selector. Being different from continuous flow activated sludge systems, various biological reactions are switchable in the same reactor. In CAST, clarifiers and flow equalization tanks are unnecessary, and thus, the costs of facilities and operation management are much lower than those with continuous flow activated sludge systems. Moreover, operation conditions can be changed easily in CAST (Irvine *et al*., 1997). Therefore, CAST is regarded as an effective technology, especially for small wastewater treatment plants.

Enhanced biological phosphorus removal (EBPR) is the most economical and sustainable process for removing phosphorus from wastewater. EBPR is achieved by recycling polyphosphate-accumulating organisms (PAOs) through alternating anaerobic and aerobic conditions. PAOs take up carbon sources while releasing orthophosphate in the anaerobic phase and store them in the form of polyhydroxyalkanoates (PHA), using the energy produced through the hydrolysis of intracellular polyphosphate. In the subsequent aerobic phase, PAOs grow and take up orthophosphate by using the stored PHA as the carbon and energy sources (Zeng *et al*., 2004).

Phosphorus uptake also occurs under anoxic condition. Previous studies have shown that at least some PAOs, called denitrifying PAOs (DNPAOs), are able to oxidize their intracellular PHA with nitrate and/or nitrite as the terminal electron acceptor, and thus provide energy for phosphorus uptake (Kuba *et al*., 1996, 1997; Mino *et al*., 1998; Ahn *et al*., 2001, 2002; Shoji *et al*., 2003). This means that the carbon source taken up by PAOs in the anaerobic phase is used for both denitrification and phosphorus removal, which is advantageous when

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the wastewater contains a relatively low level of organic carbon. Moreover, DNPAOs are 40% less efficient in generating energy and thus have a 20%–30% lower cell yield (Murnleitner *et al*., 1997). Consequently, the utilization of DNPAOs affords many advantages for BNR.

In CAST system, denitrifiers play the major role in nitrogen removal, and PAOs are responsible for EBPR. Therefore, these two kinds of organisms are necessary for simultaneous nitrogen and phosphorus removal. However, PAOs compete with denitrifiers for organic carbon in the influent because both denitrification and phosphate release require organic carbon. The phosphorus removal efficiency often decreases when the available organic carbon content is low (Morling, 2001). As a result, the step-feed CAST with multiple anoxic and oxic durations is put forward to enhance the utilization of influent chemical oxygen demand (COD) as much as possible because of the presence of anoxic phases. The proposed process is a single-sludge suspended growth system incorporating anaerobic, aerobic and anoxic stages in sequence, which favors the cultivation of DNPAOs in the system.

To date, several research works have confirmed that phosphorus uptake under anoxic conditions occurred in anaerobic/aerobic/anoxic (AOA) process in SBR (Tsuneda *et al*., 2006; Kishida *et al*., 2006). However, the role of DNPAOs in CAST system has not been reported. This study quantitatively evaluated nitrogen and phosphorus removal in the alternating anoxic/oxic (A/O) process using a step-feed CAST and the fraction of DNPAOs in the sludge was also determined.

1 Materials and methods

1.1 Bench-scale device

A schematic diagram of experimental equipment is shown in Fig. 1. The bench-scale CAST reactor was made of Plexiglas with a working volume of 72 L and composed of selector and complete mixed zone. The capacity ratio of selector to complete mix zone is 1:10. An air-compressor was used for aeration. Mechanical stirrers were used to provide liquid mixing in the two zones. The returned activated sludge (RAS) and influent pumped to selector and mixed by the ratio of 1:4. The pH, oxidation-reduction potential (ORP), and dissolved oxygen (DO) sensors were installed in reactor. Samples were collected at intervals according to pH and ORP variations. The aeration was controlled through manipulation of the aeration valves. RAS and 288 L/d fixed influent were controlled by variable speed peristaltic pumps. The mixed liquor suspended solids (MLSS) and solid retention time (SRT) were controlled at 3000–3500 mg/L and 10–15 d, respectively. The study was performed at (21 ± 1)°C.

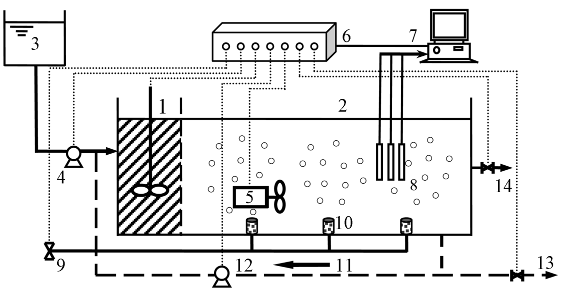

Fig. 1 Schematic diagram of experimental system and control equipment used in cyclic activated sludge technology. (1) selector; (2) complete mix zone; (3) inflow tank; (4) influent pump; (5) mixer; (6) timer; (7) computer; (8) DO, ORP, pH on-line sensors; (9) air pump; (10) air diffuser; (11) return activated sludge; (12) return activated sludge pump; (13) excess sludge; (14) effluent.

1.2 Phosphorus release and uptake batch tests

A series of batch experiments was performed using the sludge taken from the running CAST system during steady states under different operating conditions at the end of aerobic phase. In each test, 3 L of sludge was transferred to the 8 L laboratory SBR. After stewing overnight to deplete nitrate and nitrite via endogenous denitrification, a certain amount of acetate (300 mg COD/L) was added for anaerobic phosphorus release for 180 min. During the batch tests, COD, PO_4^{3-}-P, MLSS and mixed liquor volatile suspended solids (MLVSS) were monitored.

After three hours phosphorus release, the biomass was washed by deionized water to remove any acetate residue and sludge was used for the phosphorus uptake batch tests. Phosphate was then added and this initial phosphate concentration was 67.72 mg/L. Sludge (1 L each) was put into three 1.5 L laboratory SBRs, respectively. SBRs were performed under different conditions including aerobic (A), nitrate only (B, 40 mg/L) and nitrite only (C, 40 mg/L). The sludge was loaded with sufficient DO (2–4 mg/L). The phosphorus uptake capacities and uptake rates were measured and compared under identical experimental conditions. The batch tests were performed for 120 min at (21 ± 1)°C and pH 7.0–7.5.

1.3 Sludge and wastewater

The inoculated activated sludge for CAST system was taken in the oxidation ditch (OD) process in Jiuxianqiao Wastewater Treatment Plant, Beijing. After two-months cultivation of the activated sludge, a stable denitrifying phosphorus removal performance had been achieved. The activated sludge of the batch experiment was withdrawn from the complete mix zone of the CAST.

The municipal wastewater used in this study was taken from the septic tank of community in Beijing University of Technology. The practical municipal wastewater was obtained before and after adding acetate, with low COD/TN (total nitrogen) ratio (2.8) in Run 1 and Run 2, and high ratio (4.5) in Run 3 and Run 4. The main characteristics of the influent are shown in Table 1.

1.4 Sampling and analytical methods

Influent and effluent of the CAST were sampled every cycle for chemical analysis, and an activated sludge sample was sampled every 2 d for concentration measurement. When the step-feed CAST operation reached a steady state, a track analysis of an entire cycle was performed. The mixture of liquor samples (15 mL) was withdrawn from the reactor during the operating cycle at some special points,

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Table 1 Main characteristics of the influent

	Run 1	Run 2	Run 3	Run 4
Running time (d)	1–30	31–60	61–83	84–110
COD (mg/L)	203.7 ± 39.6	229.8 ± 41.2	368.7 ± 64.7	354.2 ± 57.6
NH_4^+-N (mg/L)	69.1 ± 3.6	70.7 ± 9.1	76.1 ± 6.6	74.9 ± 4.4
PO_4^{3-}-P (mg/L)	5.4 ± 1.2	5.1 ± 0.7	6.5 ± 1.5	6.2 ± 2.1
TN (mg/L)	72.3 ± 2.4	73.1 ± 5.6	78.7 ± 4.5	76.8 ± 3.8
Alkalinity ($mgCaCO_3$/L)	430 ± 20	460 ± 10	445 ± 18	452 ± 32

COD: chemical oxygen demand; TN: total nitrogen. Data are expressed as mean ± SD.

such as the end of feeding, the end of each non-aeration or aeration phase, and the beginning of effluent discharging. Once the sample was withdrawn from the reactor, it was immediately centrifuged at a 3000 r/min for 2 min and filtered through a 0.45-mm filter paper.

COD, TOC, TN, NH_4^+-N, NO_2^--N, NO_3^--N, PO_4^{3-}-P, MLSS, and MLVSS were analyzed according to standard methods (APHA, 1995). TOC and TN were analyzed with a Multi N/C 3400 TOC analyzer. DO, pH, and ORP were measured online using DO, pH, and ORP sensors (WTW 340i, WTW Company, Germany), respectively.

1.5 Operation procedure

In general, a typical CAST cycle is divided into four sequences, namely fill/react, settle, draw and idle. In this case, substantial amounts of nitrate and nitrite might remain in the effluent because denitrification in selector, simultaneous nitrification and denitrification (SND) in complete mix zone and endogenous denitrification would not remove nitrate and nitrite efficiently in the reaction and settle period due to the lack of an organic electron donor. In the present work, step-feeding strategy was mainly adopted to enhance denitrification, phosphorus release and removal efficiency, using the readily biodegradable organic substrates contained in wastewater. Figure 2 shows the operation procedure. The times of influent feeding included three feedings which fulfilled during the anoxic periods. There were three anoxic-oxic combinations and both of the durations were fixed at 40 min. Agitation and aeration of the reactor ceased during the settle phase and the activated sludge was allowed to settle under quiescent conditions. In the draw phase, supernatant was withdrawn through a valve fixed at the minimum liquid level. The

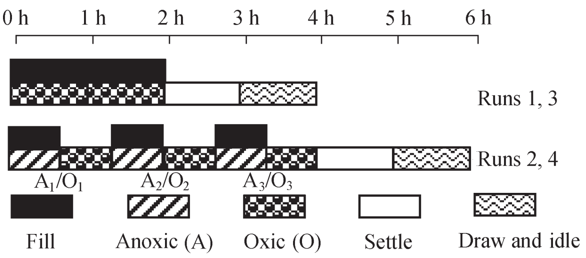

Fig. 2 Operation procedure in CAST.

sludge settling and effluent decanting were fixed at 60 and 10 min, respectively.

2 Results and discussion

2.1 Bench-scale test

Through the data analysis of phosphorus and nitrogen concentration in influent, effluent properties, and removal efficiency (Fig. 3 and Table 2), it can be concluded that not only the C/N ratio in influent but also the operation strategy had a great influence on phosphorus removal. Both step-feed operation and high influent C/N ratio can enhance phosphorus removal. The effluent phosphate in Run 4 was 0.04–0.48 mg/L and the average concentration was 0.17 mg/L. However, phosphorus could not be released completely in the selector of traditional CAST system. The denitrifiers competed carbon source in influent with PAOs and a strict anaerobic environment could not be established due to the existence of nitrate and dissolved oxygen carried by RAS, which affected the further aerobic phosphorus uptake in the complete mix zone.

It should be noticed that TN in effluent existed mainly in

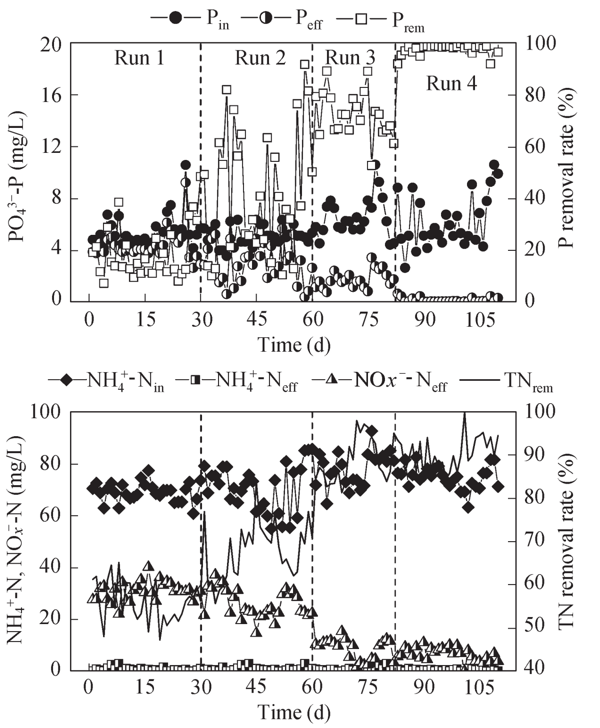

Fig. 3 Performance of phosphorus and nitrogen removal in CAST system under different conditions. in: influent; eff: effluent; rem: removal rate.

Table 2 Average removal efficiency and sludge settling performance in each run

Operation conditions	COD_{rem} (%)	NH_4^+-N_{rem} (%)	P_{rem} (%)	TN_{rem} (%)	SVI (mL/g)
Run 1	85.4	97.5	19.1	55.95	158
Run 2	87.5	99.7	42.6	69.92	122
Run 3	83.7	98.3	72.3	88.77	415
Run 4	88.2	98.7	97.5	92.10	133

The rem denotes the removal rate.

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nitrate form. Only when a high TN removal performance was achieved, high phosphorus removal rate could be realized in the system. The low TN removal rates in Run 1 and Run 2 caused by the COD scarcity in influent, affected the phosphorus removal efficiency in the EBPR system. Nevertheless, comparing with Run 1, Run 2 had a much higher average phosphorus removal rate (42.6%) because of efficient operation strategy. The effluent TN in Run 3 was quite low because of external carbon addition, the phosphorus removal performance was not stable. Moreover, traditional operation strategy exposed the system to the risk of filamentous bulking, which resulted in a sludge loss from effluent. However, both high and stable nutrients removal performance could be achieved in step-feed CAST system without that problem, which was proved by the lower average SVI of Run 2 and Run 4 (Table 2).

2.2 Step-feed CAST biological phosphorus removal characteristic

After the inoculated activated sludge acclimated for 30 d, the system was in a steady state. A typical plot of the chemical analysis in a cycle (day 103) along with pH and ORP in the complete mixed zone is shown in Fig. 4. The removal rate of COD, NH_4^+-N, PO_4^{3-}-P and TN was 90.1%, 99.1%, 96.1% and 89.9%, respectively (Fig. 3).

As shown in Fig. 4, the "ammonia valley" (point P_1 and P_2) in aerobic phases denotes the complete nitrification, and the "nitrate apex" or "nitrate knee" (point NP_1, NP_2 and NP_3) indicate the end of denitrification in anoxic phase. The anoxic phosphorus uptake was detected in each anoxic phase and phosphorus release only occurred when nitrate was denitrified completely.

In complete mix zone, the COD concentration in influent was higher than usual, and denitrifiers could not deplete the influent readily biodegradable COD (rbCOD). Thus, phosphorus release of the system would be enhanced by PAOs utilizing the remaining rbCOD in anoxic phase and resulting in a sustained phosphorus rise in duration.

In studied cycle, nitrate and nitrite remained from the former aerobic phase were used as electron acceptors. The utilization of NOx^- by DNPAOs uptaking phosphorus caused a non-increasing phosphate in the last anoxic phase when phosphorus was released completely in selector. As the system proceeded to the next aerobic phase, phosphorus was uptaken by PAOs under aerobic condition. Thus, a complete cycle of anaerobic phosphorus release and anoxic (aerobic) phosphorus uptake progressed. New PAOs produced in the cycle, and phosphorus in the wastewater was removed from the system through eliminating rich phosphorus wasted activated sludge.

2.3 Phosphorus releasing characteristic in the selector

In order to understand the details of the phosphorus release or uptake during anoxic phase in the complete mix zone of step-feed CAST process, variation of TOC, NOx^--N, PO_4^{3-}-P and pH, ORP profiles in the selector were investigated. As shown in Fig. 5, the trends of pH and ORP profiles in selector were similar to that in Fig. 4. However, the so-called "nitrate apex" in pH profile of selector presented earlier than that of complete mix zone in each feeding phase. It suggests that nitrate and nitrite (nitrite was barely detected) did not denitrified completely, whereas they were deoxidized to nitrogen gases very quickly because only 20% of the total flux of RAS circulated into the selector, which could be verified by the small amount of NOx^- in selector compare to that in complete mix zone.

It can be observed from Fig. 6 that phosphorus release

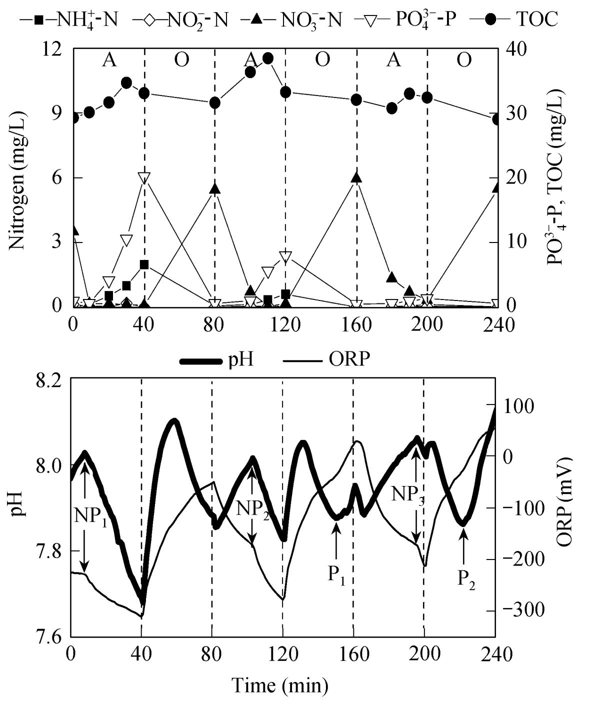

Fig. 4 Typical transformation of chemicals and pH, ORP profiles during one cycle in complete mix zone of the step-feed CAST system at day 103.

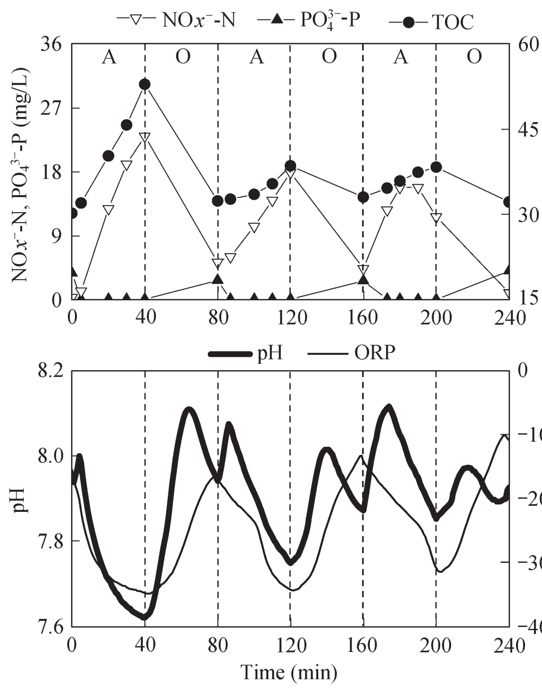

Fig. 5 Variation of TOC, NOx^--N, PO_4^{3-}-P and pH, ORP profiles in selector of the step-feed CAST system at day 103.

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of the system was enhanced due to enough rbCOD in influent that was not depleted by denitrifiers. As a result, the amount of phosphorus released at the end of anoxic duration was quite high, especially in the first A/O combination. Meanwhile, a significant phosphorus uptake took place before the end of denitrification in each anoxic phase. Phosphorus release amount in selector at the end of denitrification increased gradually and it was approximate to the amount of phosphorus released at the end of anoxic phase because of the time for denitrifying NOx^- in the system completely prolonging with the increase of feeding time.

2.4 Phosphorus uptake batch test under three different electron acceptors

The characteristics of denitrifying phosphorus removal bacteria enriched under the step-feed CAST process were investigated using three different electron acceptors (oxygen, nitrate and nitrite).

A series of batch tests was conducted to assess a large number of denitrifying phosphate removal bacteria. The tests were conducted in SBRs and the operation was mentioned above. The results are shown in Fig. 7. It is noted that the profiles of phosphorus declined progressively with time under the three different conditions. The internal poly-P was depleted and the PHA level in the biomass increased in anaerobic condition. Anoxic conditions were maintained by instant addition of nitrate or nitrite to 40 mg/L. Oxygen, nitrate and nitrite supplied to the reactors were enough at 150 min. The maximum specific phosphorus uptake rate under aerobic, anoxic with nitrate and anoxic with nitrite conditions were 30.39, 22.83 and 11.66 mg P/(gVSS·h), respectively (Fig. 7). The results indicated that nitrate and nitrite were able to act as electron acceptors successfully. The ratio of phosphorus uptake rate under nitrate to that under aerobic condition was 0.75, and the ratio of phosphorus uptake rate under nitrite to that under aerobic condition was 0.38, which indicated anoxic phosphorus uptake could take place under the favorable

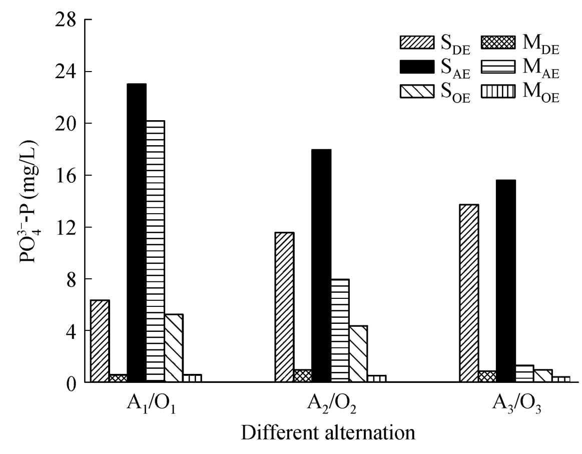

Fig. 6 Phosphorus release and uptake during different alternation of CAST system (at day 103). S: selector; M: complete mix zone; DE: nitrate apex or knee point; AE: end of the anoxic phase; OE: end of the oxic phase. A_1/O_2, A_2/O_2, and A_3/O_3 represent the three combinations of a typical cycle in Run 4 as shown in Fig. 2.

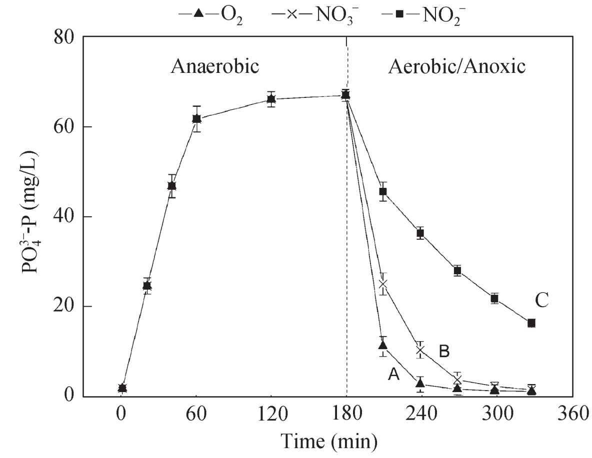

Fig. 7 Profile of phosphorus uptake under aerobic and anoxic conditions with three different types of electron acceptors after phosphorus release. A: aerobic; B: anoxic with nitrate; C: anoxic with nitrite.

conditions in the bench-scale step-feed CAST.

This observation suggested that nitrite was not an inhibitor to phosphorus removal process. Instead, it is an alternative electron acceptor to oxygen or nitrate (Hu *et al*., 2003; Zhou *et al*., 2007). The results verified the existence of a third group of phosphorus removal bacteria. That is, besides the two widely accepted groups of phosphorus removal bacteria, namely P_O which can only utilize oxygen to take up phosphorus and P_{ON} which can use both oxygen and nitrate (Kerrn-Jespersen *et al*., 1993), the third group that defined as P_{ONn} (Hu *et al*., 2003) could also take up phosphorus using nitrite as an electron acceptor (this group can use oxygen and nitrate, too). The relative population of these three types of bacteria (Table 3) could be calculated from results obtained from phosphorus uptake batch experiments with either oxygen or nitrate/nitrite as electron acceptor.

Table 3 shows that both phosphorus release rate and phosphorus removal population ratio were affected by the operation conditions. In terms of COD competition, P_{ONn} bacteria is the most capable group. This observation also suggested that a lower C/N ratio of influent with step-feed operation strategy would lead to a higher percentage of P_{ONn} bacteria. In other words, low level of COD supply and step-feed with alternating anoxic/oxic operation strategy would favor P_{ONn} cultivation. That is, under the second operation condition, bacteria were forced to utilize internal carbon source for denitrification and phosphorus removal which in turn would lead to more DNPAOs.

Table 3 Phosphorus release rate and fraction of PAOs in different procedures

	r_{max} (mg/gVSS·h)	P_O/P (%)	P_{ON}/P (%)	P_{ONn}/P (%)
Run 1	5.13	32	33	35
Run 2	7.89	9	21	70
Run 3	11.99	46	31	23
Run 4	20.01	12	32	56

r_{max} denotes the maximum specific phosphorus release rate under anaerobic condition.

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3 Conclusions

The step-feed CAST process with enough carbon sources in influent displayed an excellent nitrogen and phosphorus removal capacity. Alternating anoxic/oxic operation strategy could establish an AOA process in which P uptake under anoxic conditions occurred easily, thus significant denitrifying phosphorus removal efficiency was achieved and a carbon dual-use reached in step-feed CAST. The quick denitrification of NOx^- enhanced phosphorus release and further phosphorus uptake capability of the system. PAOs in the step-feed process were also proved to have strong denitrifying phosphorus removal capacity. Moreover, scarcity of carbon sources in influent with step-feed operation strategy would favor the cultivation of DNPAOs.

Acknowledgments

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