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Enhanced biological phosphorus removal by granular sludge in anaerobic/aerobic/anoxic SBR during start-up period

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ABSTRACT

Laboratory experiments were conducted to investigate the granulation process of the activated sludge in an anaerobic/aerobic/anoxic sequencing batch reactor (A/O/A SBR) during the start-up period. Under the condition of aeration intensity (6.5 L/min) and settling time (2 min), well-formed granular sludge was obtained in the A/O/A SBR, and the COD, phosphorus, and nitrogen removal efficiencies of the reactor for one typical cycle were as high as 90.1, 99.9, and 94.5%, respectively. Dynamics of microbial community in the reactor were monitored by polymerase chain reaction and denaturing gradient gel electrophoresis techniques. The results showed that microbial diversity of the granular sludge decreased compared with the initial sludge. However, strains which could assimilate excessive phosphorus became dominant in granular sludge, leading to the increase in the phosphorus removal efficiency of the reactor. Batch tests were conducted with three different electron acceptors, that is, oxygen, nitrate, and nitrite. It was observed that phosphorus removal efficiency of the reactor supplied with nitrite was only 43.4%, indicating that nitrite could be used as electron acceptor for phosphorus removal, but was less efficient than nitrate and oxygen.

Keywords: Enhanced biological phosphorus removal; Granular sludge; Anaerobic/aerobic/anoxic SBR; Start-up period; PCR–DGGE

1. Introduction

As one of the indispensable nutrients for organisms, phosphorus is capable of simulating the growth of aquatic plants and accelerating eutrophication. Therefore, to fulfill more restrictive permit requirement, it is essential to remove the phosphorus from wastewater effectively. As one type of the most economically and environmentally sustainable technologies, enhanced biological phosphorus removal (EBPR) processes, such as anaerobic/anoxic/oxic (A^2O) and University of Cape Town (UCT) processes, have been applied in the wastewater treatment [1–6].

The EBPR process is based on the enrichment of polyphosphate-accumulating organisms (PAOs), which

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can take up significantly more phosphorus than the normal requirement for cell growth through circulating anaerobic and aerobic phase. In general, PAOs assimilate superfluous orthophosphate to form polyphosphate bonds in cell storage with the concomitant oxidation [7,8]. Recently, a new type of organisms denitrifying phosphorus-accumulating organisms, (DNPAOs) has been found to be capable of utilizing nitrate or nitrite as electron acceptor besides oxygen [9–13], which has similar metabolism to PAOs. These researches of DNPAOs provided a new way to solve the problem of carbon source scarcity in domestic wastewater for simultaneous nitrogen and phosphorus removal. DNPAOs exhibited significant advantages on traditional biological wastewater treatment processes, such as less organic substrates and energy requirements, and less sludge production [14].

DNPAOs have been applied in several processes such as BCFs and dephanox in recent years [15,16], but the addition of more reactors and mixed liquid recycling streams lead to complicated operation for these processes. Biological phosphorus removal in sequencing batch reactor (SBR) has been widely investigated for its flexible operation. In comparison with continuous activated sludge process, the main trait of SBR is shifting operation easily, such as alternating anaerobic, aerobic, and anoxic conditions according to substrate feast and famine. Furthermore, operational conditions such as high aeration intensity, short settling time, and large volume exchange ratios could be implemented easily in SBR, and these favorable conditions would provide a selection pressure for shaping granular sludge in SBR system [17,18].

Granules are aggregated microbial flocs formed by self-immobilization under certain conditions in the wastewater treatment process [18,19]. With regular and dense-spherical structure, the aerobic granular sludge has many advantages , including excellent settleability, high biomass level, strong tolerance to shock loadings [20–25]. If DNPAOs are enriched in granular sludge, it would have the advantages of both DNPAOs and granular sludge, which would help diverse functional organisms' growth and maintain the efficient and stable nutrient removal in the reactor. Recently, it has been reported that the granular sludge enriched with PAOs can be formed in anaerobic/aerobic (A/O) SBR easily [18,26]. But the anaerobic/anoxic (A/A) conditions are more suitable for DNPAOs [27–29], so the anoxic phase was added subsequently to the aerobic phase for the enrichment of DNPAOs in this study. It was hypothesized that the granular sludge enriched with DNPAOs could be formed in the A/O/A SBR, which could enhance the efficiency and stability of the process. The performance of the A/O/A SBR, the transformations of the activated sludge from flocs to granules, and the succession of microbial community in the sludge were investigated to better understand the granulation during the start-up period.

2. Materials and methods

2.1. SBR operating conditions

A cylindrical vessel was used for the SBR operation, which had a working volume of 18 L with an internal diameter of 24 cm and a height of 50 cm. The SBR was operated in three cycles a day, and each cycle had a total operational time of 8 h, comprising feeding and anaerobic phase (mixing), aerobic phase (mixing and aeration), anoxic phase (mixing), settling and decant phase. During the decant phase, 12 L of the supernatant was discharged from the reactor. The hydraulic retention time was kept as 12 h. There was no excess sludge discharged from the A/O/A SBR process throughout the start-up period, except that a third of activated sludge was taken from the system on day 30. The pH of the influent was adjusted to 7.0 ± 0.2 by addition of 1 M HCl or NaOH, and the ambient temperature was controlled at 21 ± 2℃ in the experiments. During the start-up period, the operational parameters were not fixed, as shown in detail in Table 1.

2.2. Synthetic wastewater and activated sludge

The synthetic wastewater used in the experiments consisted of 0.60–0.95 g/L sodium acetate (COD = 240–380 mg/L), 0.20–0.28 g/L NH_4Cl (NH_4^+-N = 50–70 mg/L), 0.020–0.065 g/L KH_2PO_4 (PO_4^{3-}-P = 4.5–14.5 mg/L), 0.08 g/L $MgSO_4{\cdot}7H_2O$, 0.04 g/L $CaCl_2$, and 1 mL/L trace elements solution. The composition of the trace element solution was the same as that described by Smolders et al. [30]. The seed sludge was obtained from a local municipal wastewater treatment plant in Harbin, and the initial mixed liquid suspended sludge (MLSS) in the reactor was about 4,290 mg/L.

2.3. Analytical methods

Orthophosphate phosphorus (PO_4^{3-}-P), nitrate nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N), ammonia nitrogen (NH_4^+-N), COD, MLSS, and sludge volume index (SVI) were analyzed according to the standard methods [31], and the total nitrogen (TN) was calculated as the summation of NH_4^+-N, NO_3^--N and NO_2^--N. The pretreatment of granular sludge for scanning electron microscopy (SEM) observation was carried out using a modified method [18]. Optical microscopy images were taken using an Olympus BX51/52.

Table 1
Experimental scheme in A/O/A SBR process during start-up period

Operation	Anaerobic (min)	Aerobic (min)	Anoxic (min)	Settling (min)	Decant (min)	Air flow rate (L/min)
I (0–30 d)	180	180	80	30	10	2.5
II (30–60 d)	180	140	120	30	10	6.5
III[a] (60–70 d)	180	140	140	10	10	6.5
III[b] (70–80 d)	180	140	148	2	10	6.5

[a]The settling time was 10 minutes.
[b]The settling time was 2 minutes.

2.4. PCR–DGGE

2.4.1. Sample preparation and DNA extraction

The sludge samples were collected from the reactor on day 0, 30, 60, and 80 and concentrated by centrifugation at 9,000 rpm (2-16PK, SIGMA, German) for 5 min at 4℃ immediately. Then, the sludge samples were washed twice with phosphate buffered saline solution (137 mmol/L NaCl, 2.7 mmol/L KCl, 10 mmol/L Na_2HPO_4, 2 mmol/L KH_2PO_4, pH 7.4). The extraction of total genomic DNA was carried out by a bacterial genomic DNA extraction kit (Takara, Dalian, China) according to the instruction.

2.4.2. PCR amplification

The V3 region of 16S rDNA genes was PCR-amplified using forward primer BA101f (5′-TGGCGGACGGGTGAGTAA-3′) with a GC clamp (5′-CGCCCGCCGCGCGCGGCGGGC- GGGGCGGGG-GCACGGGGGGATTACCGCGGCTGCTGG-3′) and the reverse primer BA534r (5′-ATTACCGCGGCTG-CTGG-3′). The final PCR mixture (50 μL) contained 100 ng DNA extract, 2 μL of each primer, 4 μL deoxynucleoside triphosphates, 5 μL 10 × PCR buffer (Mg^{2+} plus), 0.5 μL Taq polymerase, and 0.5 μL bovine serum albumin. The touchdown PCR amplification was conducted following the protocol: initial denaturation of DNA at 94℃ for 8 min; 30 cycles of 40 s at 94℃ (denaturation), 40 s at 55℃ (annealing), and 30 s at 72℃ (extension); decreasing 0.5℃ per cycle to 55℃, and 10 min at 72℃ (final extension). After PCR amplification, PCR products were stored at 4℃ and electrophoresed on a 2% (wt/vol) agarose gel stained with ethidium bromide. The biochemical reagents were totally from Takara, Dalian, China.

2.4.3. DGGE analysis

DGGE was performed using the Dcode™ Universal Mutation Detection System (BioRad, USA). The PCR products and loading buffer were loaded onto 8% (w/v) poly acrylamide gels using a linear gradient ranging form 30 to 60% denaturant (100% denaturant contains 7 M urea and 40% (v/v) formamide). Electrophoresis was run at 60℃ with an initial voltage of 20 V for 0.5 h and a constant voltage of 150 V for 9 h. Finally, gels were stained with SYBR Green 1 and visualized by a trans illumination scanner (Image scanner III, GE, USA). The DNA sequences were determined by a commercial service (Takara, Dalian, China). The sequences obtained were compared with the similar 16S rDNA fragments by BLAST program in Genbank.

2.5. Batch tests

To confirm the enrichment of the DNPAOs in the granular sludge, and to investigate the capacity of the DNPAOs using different electron acceptors, batch tests were carried out after the start-up period. Three-stirred glass vessels of 800 mL were used as reactors. On the 92nd day, the A/O/A SBR was operated stably and efficiently. At the end of the anaerobic phase, the granular sludge was transferred from the SBR to the three reactors averagely and supplied with oxygen, nitrate, and nitrite for phosphorus removal in reactors, respectively. The first reactor was supplied with compressed air, and the oxygen concentration was kept around 6 mg/L. The concentration of NO_3^--N or NO_2^--N added in the other reactors was 30 mg/L, respectively, and nitrogen gas was bubbled from the bottoms of the two reactors to strip off oxygen and secure anoxic condition strictly. The pH of the influent was controlled at 7.0 ± 0.2 by addition of 1 M HCl or NaOH to avoid chemical phosphate precipitation, and the temperature in the three reactors was kept as 22℃.

3. Results and discussion

3.1. EBPR performance

In the first 26 d, the average influent COD of 372.5 mg/L was fed in the reactor as shown in Fig. 1(a). At the beginning of the experiments, sufficient amounts of organics in the anaerobic phase were

essential for the PAOs proliferation, on account of denitrifying bacteria and PAOs competing for the organic substrates. During this time, the COD concentration in the effluent was 46.1 mg/L, which illustrated that the original activated sludge could remove organics efficiently. After the 26 d, the COD concentration in the influent was reduced to 245.7 mg/L, based on the consideration that less organics flowing into aerobic phase favoring the nitrification. Along with the reduction of the influent COD, the average COD concentration in the effluent was decreased from 46.1 to 34.9 mg/L. During the start-up phase of the reactor, although the reactor operation shifted from phase I to III (the time of different phases changed a lot and the airflow rate increased from 2.5 to 6.5 L/min, as shown in Table 1), the average COD removal efficiencies of the reactor in the different phases were 86.8, 86.1, and 86.4%, respectively, and negligible changes were observed in the COD removal efficiency of the reactor. It could be concluded that varying the operation of the reactor had little effect on the COD removal.

Performance of the A/O/A SBR for PO_4^{3-}-P removal was shown in Fig. 1(b). During the initial 16 d, the phosphorus removal efficiency was improved to 87.9% under low phosphorus loading with two exceptional cases on days 8 and 9. The abrupt deterioration in phosphorus removal of the reactor was caused by the broken aeration device. After 16 d, the phosphorus concentration in the influent increased from 4.3 to 14.2 mg/L on average. During the 17–30th days, the phosphorus concentration in the effluent ascended along with the increase of that in the influent, and the removal efficiency decreased from 85.5 to 70.9% accordingly. After 30 d, the airflow rate was adjusted from 2.5 to 6.5 L/min, which was favorable for nitrification. With the increasing nitrate nitrogen in the anoxic phase, more phosphorus was taken up by DNPAOs and the phosphorus removal was enhanced gradually. At the end of the experiments, the

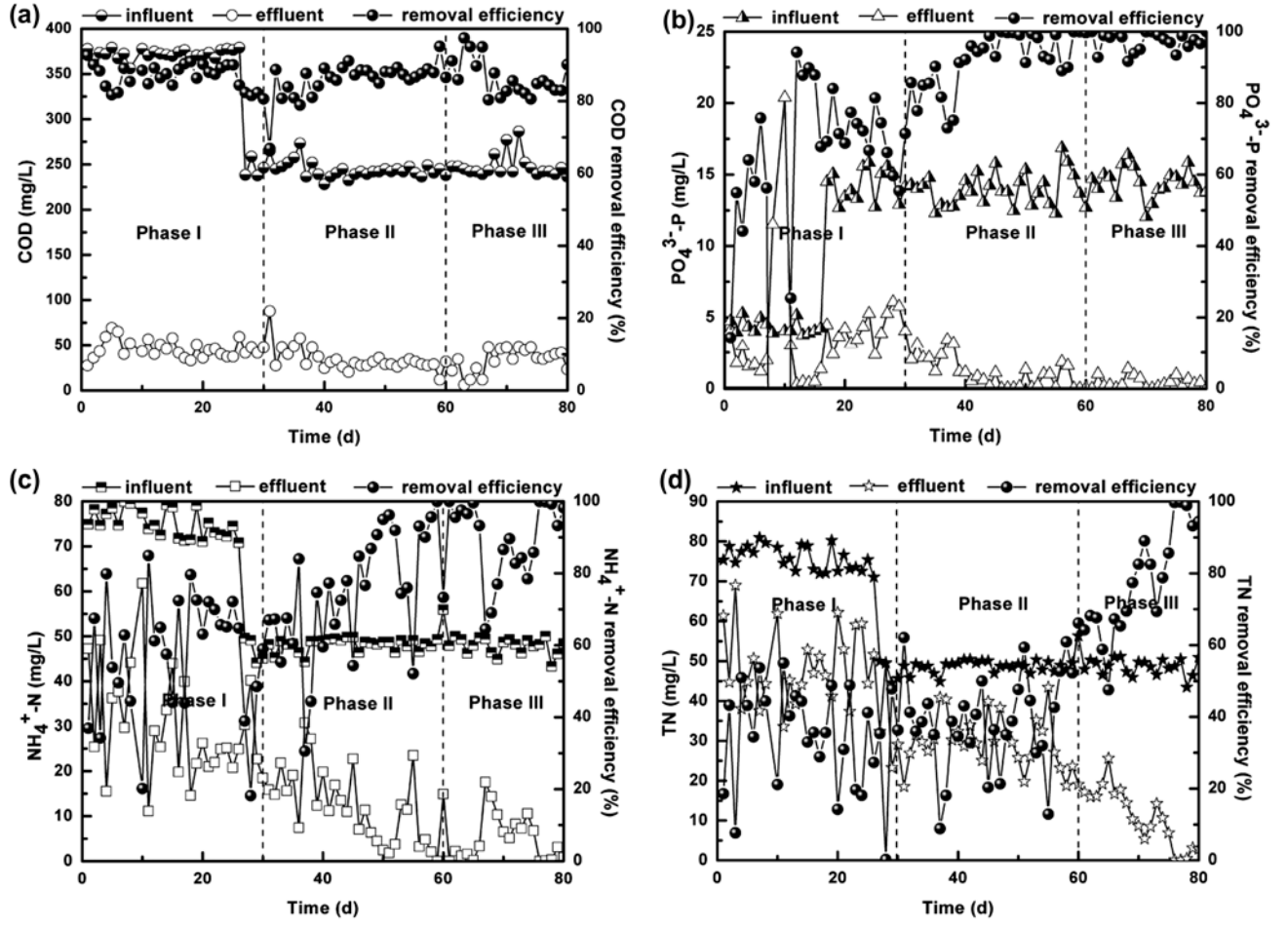

Fig. 1. Performance of A/O/A SBR process during the experiments: (a) COD; (b) PO_4^{3-}-P; (c) NH_4^+-N; (d) TN.

phosphorus concentration in the effluent was maintained below 1.0 mg/L, and the removal efficiency was always higher than 90%.

Fig. 1(c) presents the variations of influent and effluent NH_4^+-N through the experiments. In the first 26 d, the mean ammonia nitrogen concentration in the effluent was 30.0 mg/L with the influent ammonia nitrogen of 75.1 mg/L. Although the influent ammonia nitrogen was reduced to 48.1 mg/L during the 27–30th days, the ammonia nitrogen concentration in the effluent was still higher than 18.5 mg/L, and there was no significant improvement on the ammonia nitrogen removal in the reactor. From the 30th day, the ammonia nitrogen removal efficiency was up to 80.5% as airflow rate increased from 2.5 to 6.5 L/min. During the last 10 d of the experiments, a high average ammonia nitrogen removal efficiency of 90.7% was maintained. It could be concluded that high airflow rate was favorable for ammonia nitrogen removal in A/O/A SBR process.

Fig. 1(d) displays the performance of the TN removal in the reactor during the experimental period. The ammonia nitrogen removal efficiency increased after 30 d with the enhancement of aeration, but the TN removal efficiency was kept less than 50% until 57 d. The result suggested that the ammonia nitrogen was just converted to nitrate nitrogen or nitrite nitrogen in the solution rather than completely removed. After 60 d, the TN concentration in the effluent decreased from 38.0 to 10.7 mg/L, which was accompanied by the formation of granular sludge. At the end of the start-up period, the TN concentration in the effluent was 5.6 mg/L, corresponding to an excellent TN removal efficiency of 88.5%.

3.2. Characteristics of activated sludge in different phase

To better understand the start-up characteristics of the A/O/A SBR process, the variation of the activated sludge in different phase was investigated from two aspects. Firstly, morphology of the activated sludge was observed using the optical microscopy or scanning electron microscopy. Secondly, the corresponding performance of the activated sludge was investigated in a typical cycle.

3.2.1. Characteristics of activated sludge in phase I

As shown in Fig. 2, the activated sludge taken on day 27 was in the form of irregular brown flocs, and it had a SVI30 of 78.2 mL/g. At this time, as shown in Fig. 1, the effluent quality was dissatisfied, and protozoa or metazoa as the indicator of purified water cannot be observed in the system.

Fig. 3 shows the cycle performance of the activated sludge on day 27. During the anaerobic phase, COD was consumed quickly from the initial 237.7 to the final 32.0 mg/L, indicating the majority of organic substrates were taken up by the activated sludge, and the phosphorus concentration increased from 12.9 to 114.9 mg/L along with the decrease of the COD concentration in the system. The excess phosphorus release in the anaerobic phase could be mainly attributed to the extremely high biomass concentration, as no excess sludge discharge in the first 30 d, which resulted in the MLSS of the system up to 6,310 mg/L. The TN concentration decreased little in this phase. In the sequent aerobic phase, the remaining organics were further removed, and the COD concentration decreased to the minimum. During this stage, the ammonia nitrogen should be oxidized to nitrite, and then, the nitrite was oxidized to nitrate. As shown in Fig. 3, the ammonia nitrogen concentration was decreased from 42.6 to 22.4 mg/L, but the nitrite and nitrate concentrations were just 5.4 and 0.4 mg/L, respectively. It was speculated that simultaneous nitrification and denitrification might take place in the reactor under the condition of lower aeration intensity 2.5 L/min [32]. The TN concentration decreased to 28.1 mg/L with the oxidation of ammonia nitrogen, and the phosphorus concentration decreased to 2.3 mg/L at the end of the aerobic phase. In the anoxic phase, the COD concentration in the system increased gradually. Considering the depletion of organics in the aerobic phase, it was assumed that the organics in the system originated from the decay of the microorganisms. The nitrite nitrogen was assimilated, leading to the further decrease of TN, which could be possibly utilized by conventional denitrifying bacteria with carbon sources, or by glycogen-accumulating organisms (GAOs) with intracellular carbon sources, or by

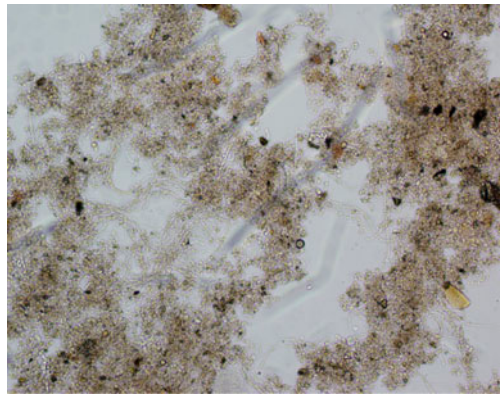

Fig. 2. Photograph of the sludge in the SBR in phase I (×100).

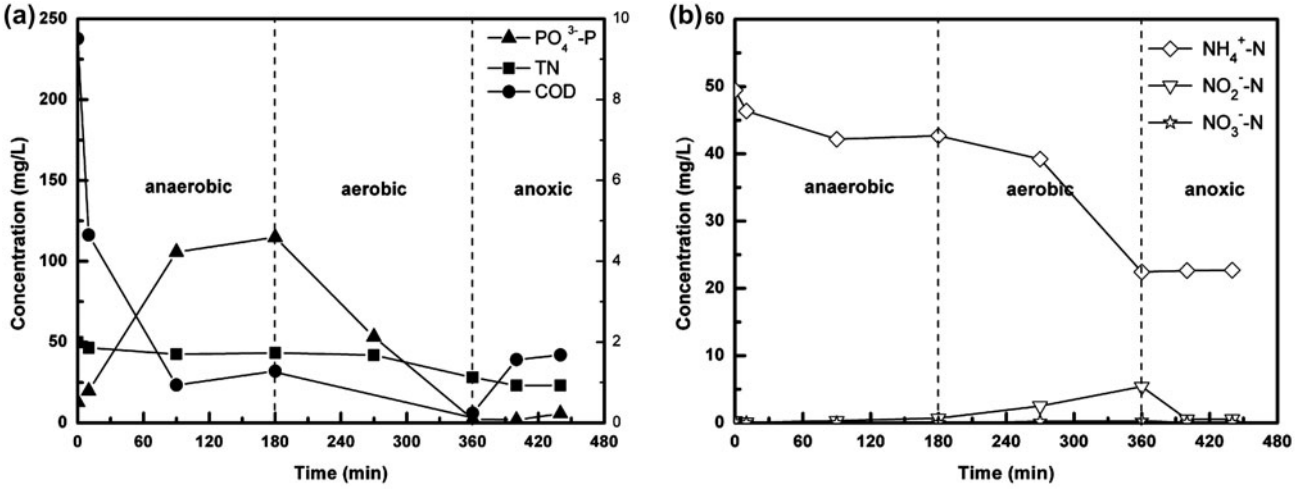

Fig. 3. COD, phosphorus, and nitrogen transformations during a typical cycle of the SBR in phase I: (a) COD, PO_4^{3-}-P, TN; (b) NH_4^+-N, NO_3^--N, NO_3^--N.

DNPAOs for phosphorus uptake. The phosphorus concentration decreased slowly and reached the minimum of 1.8 mg/L at 400 min. There were two possible reasons for the further phosphorus uptake in the anoxic phase: one is that PAOs utilized the residual oxygen in the solution for phosphorus removal, and the other is that a few DNPAOs existed in the activated sludge assimilating nitrite as electron acceptor for denitrifying phosphorus removal. After the depletion of nitrite nitrogen, the system converted from an anoxic phase to a strict anaerobic phase leading to a second endogenous phosphorous release, and the phosphorus concentration increased to 5.7 mg/L at the end of the anoxic phase.

3.2.2. Characteristics of activated sludge in phase II

As illustrated in Fig. 4, the activated sludge taken on day 60 was in the form of yellow particle instead of brown flocs, which had a diameter of 0.5–0.8 mm, and the SVI30 was reduced to 45.2 mL/g. The effluent quality was much better than that in the phase I, and protozoa such as *Paramecium* and *Vorticella* was also observed in the activated sludge.

The profiles of COD, phosphorus, and nitrogen (total, ammonia, nitrate, and nitrite) removal in a cycle on day 60 are plotted in Fig. 5. In the first 30 min of the anaerobic phase, it was found that the COD concentration was degraded more rapidly than that in phase I with a lower MLSS of 4,940 mg/L, but the phosphorus released in the reactor was lower than anticipated, suggesting that the COD might not be consumed only by PAOs alone, but also by conventional denitrifying bacteria, GAOs. Furthermore, phosphorus accumulated in the extracellular polymeric substances of the sludge particles might be another reason leading to less phosphorus in the liquid [33]. In the sequent anaerobic phase, the variations of the nutrients were similar to those in the phase I. In the aerobic phase, nitrate nitrogen was accumulated as high as 29.6 mg/L along with the oxidization of ammonia nitrogen, but little nitrite nitrogen was observed. It was obvious that higher airflow rate facilitated ammonia oxidation and nitrate accumulation. In the initial 30 min of the anoxic phase, the ammonia nitrogen in the reactor was reduced further, while there was little change in nitrate nitrogen concentration, suggesting that residual oxygen that entered into the anoxic phase and the rate of nitrification was almost equal to that of denitrification. Subsequently, as the remaining oxygen was exhausted, the nitrate nitrogen decreased gradually and the TN decreased accordingly. An obvious anoxic denitrification happened in the reactor (shown in Fig. 5), while the remaining phosphorus and carbon source existed in the solution were rather low, indicating that only a small part of nitrate was utilized by conventional denitrifying bacteria, and most of nitrate was utilized by novel denitrifying bacteria GAOs with endogenous carbon source. It was found that nitrate in the reactor could inhibit the endogenous phosphorous release effectively during the anoxic phase.

3.2.3 Characteristics of activated sludge in phase III

After phase II, the settling time was reduced from 30 to 2 min providing a selection pressure for granular sludge (shown in Table 1). The sludge particles were

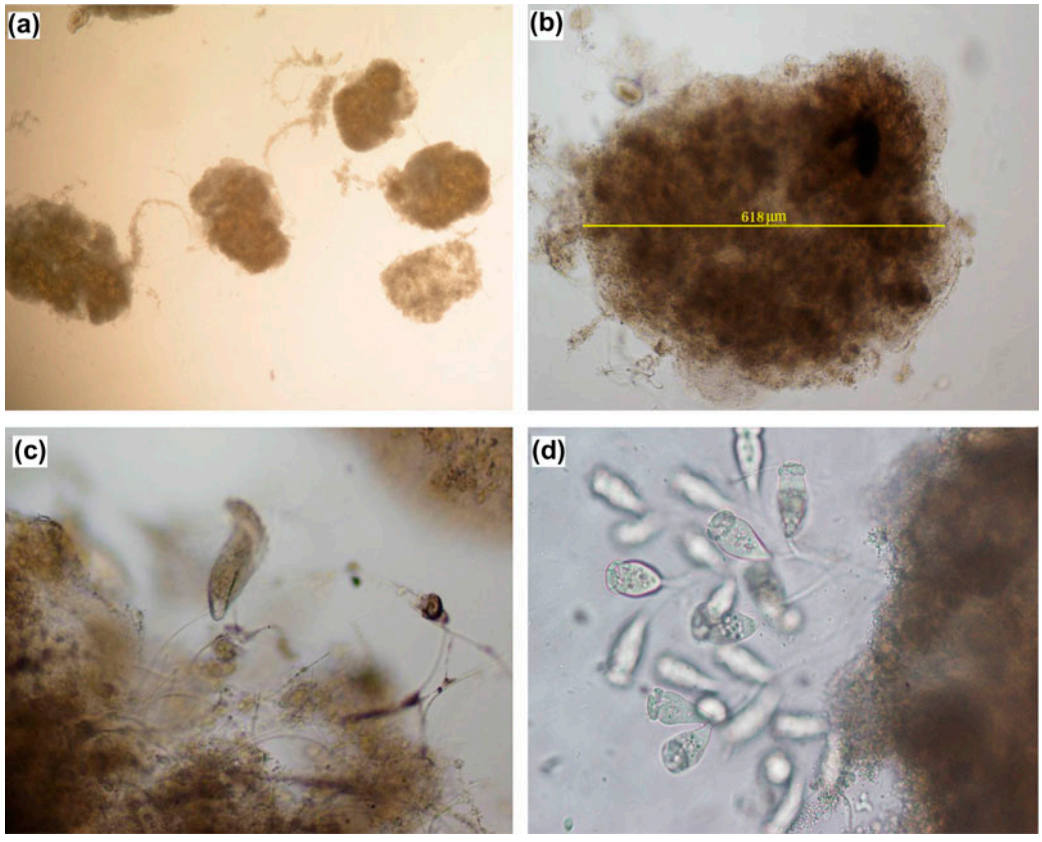

Fig. 4. Photographs of the sludge in the SBR in phase II: (a) ×40; (b) ×100; (c) *Paramecium*; (d) *Vorticella*.

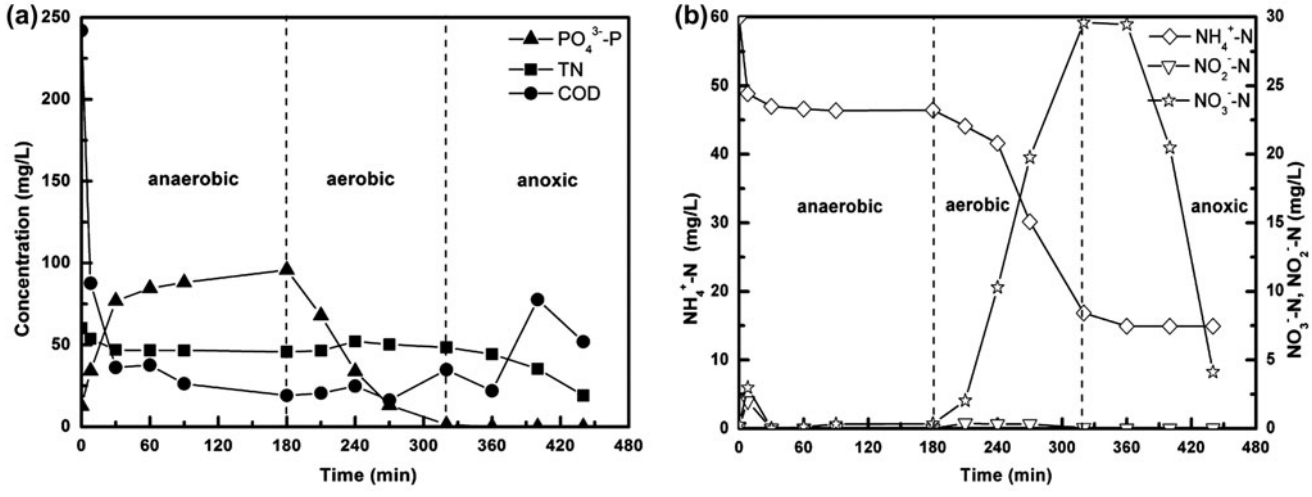

Fig. 5. COD, phosphorus, and nitrogen transformations during a typical cycle of the SBR in phase II: (a) COD, PO_4^{3-}-P, TN; (b) NH_4^+-N, NO_3^--N, NO_3^--N.

transformed into granular sludge at the end of the phase III on day 80. As shown in Fig. 6, the granular sludge was yellow and spherical shaped with compact structure, which was mainly composed of rod-shaped bacteria, a few filamentous bacteria as the framework, and some protozoa on the surface. The diameter of

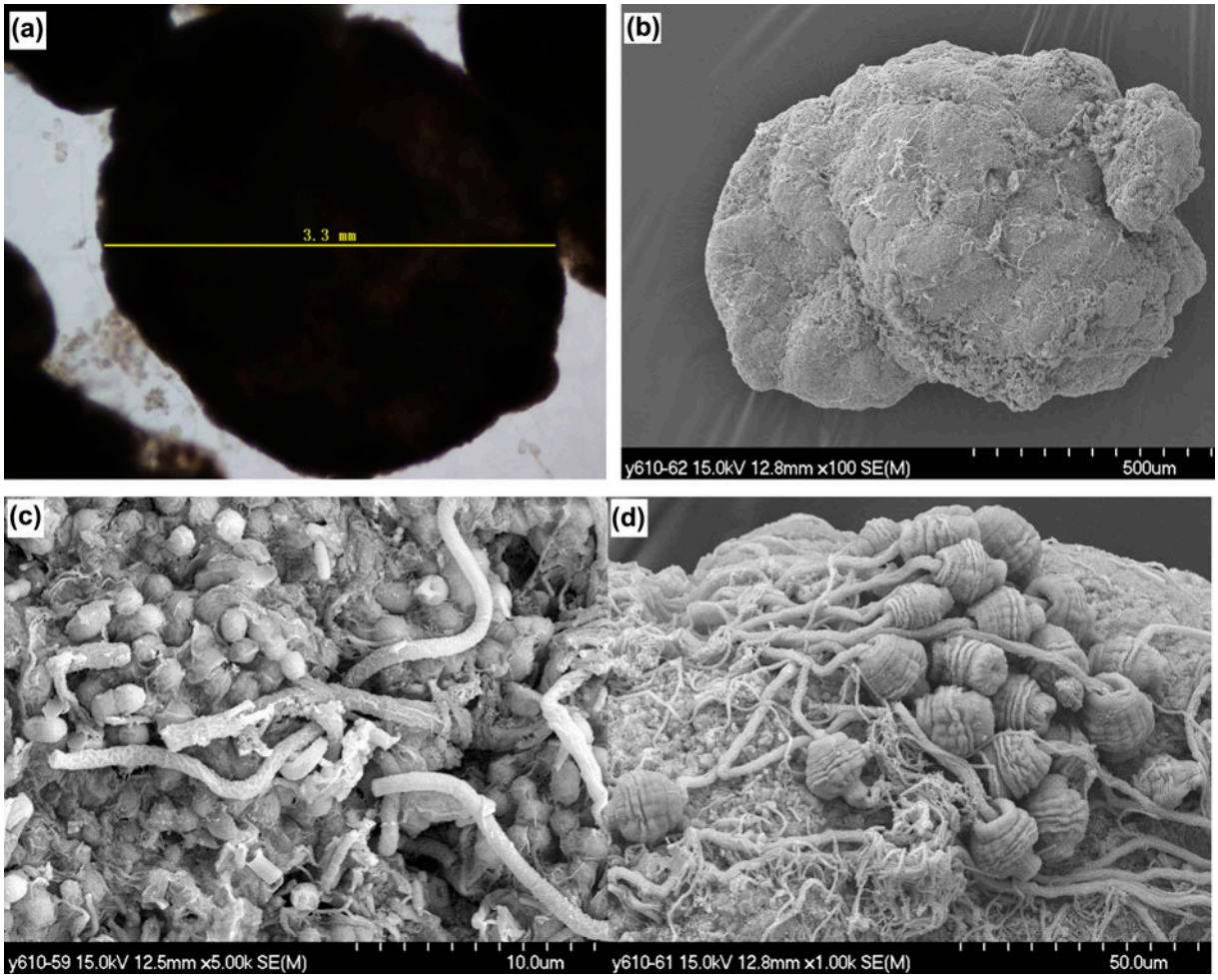

Fig. 6. Photographs of the sludge in the SBR in phase III: (a) digital image of granular sludge; (b) SEM image of granular sludge; (c) internal structure of granular sludge; (d) vorticella outside of granular sludge.

the granular sludge ranged from 1.5 to 3.5 mm, with a rather low SVI30 of 23.9 mL/g.

Fig. 7 shows changes of COD, phosphorus, and nitrogen during a typical cycle on day 80. In the anaerobic phase, variations of the nutrients were similar to that in phase II, except that more ammonia nitrogen was adsorbed with a lower MLSS of 3,890 mg/L in the anaerobic phase, suggesting that the mature granular sludge with the microporous structure had a higher adsorption capacity than sludge particles. In the initial 30 min of the aerobic phase, nitrite nitrogen was accumulated in the reactor instead of nitrate nitrogen. At the end of the aerobic phase, the nitrate nitrogen concentration increased to 29.1 mg/L as ammonia nitrogen decreased to 1.9 mg/L. At the end of the cycle, concentrations of COD and TN in the effluent were 23.4 and 2.8 mg/L, respectively; phosphorus concentration was shown to be lower than detection limit.

3.3. Microbial community analysis by PCR–DGGE

The PCR–DGGE method was employed to investigate the change in microbial community during the start-up period of the A/O/A SBR.

Fig. 8 shows DGGE profiles of the sludge samples obtained from the reactor on different days. The DGGE banding pattern at the end of the experiment was fairly different from that of the initial sample, suggesting that there existed dynamic succession in the structure of the microbial community throughout the start-up process.

Compared with the other lanes, there were more bands in lane A, showing that microbial communities were diverse in the initial sludge. With the augment of aeration, new bands W15 (*Rhodocyclus-related bacteria*) and W17 (*Chloroflexi bacterium*) appeared in lanes C and D, and these strains were reported to play an important role in phosphorus removal [34]. Accordingly, the

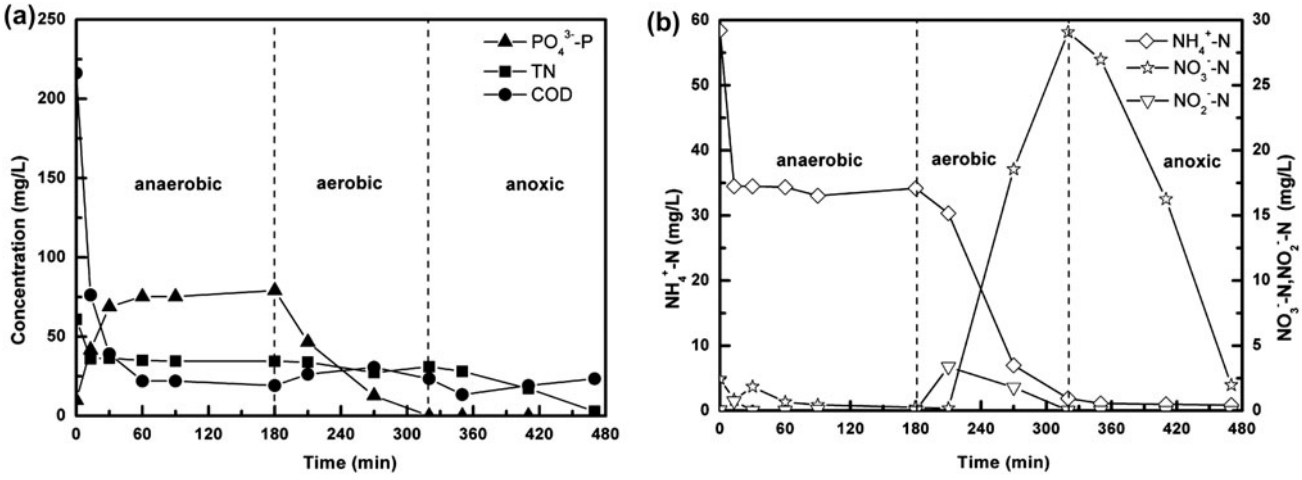

Fig. 7. COD, phosphorus, and nitrogen transformations during a typical cycle of the SBR in phase III: (a) COD, PO_4^{3-}-P, TN; (b) NH_4^+-N, NO_3^--N, NO_3^--N.

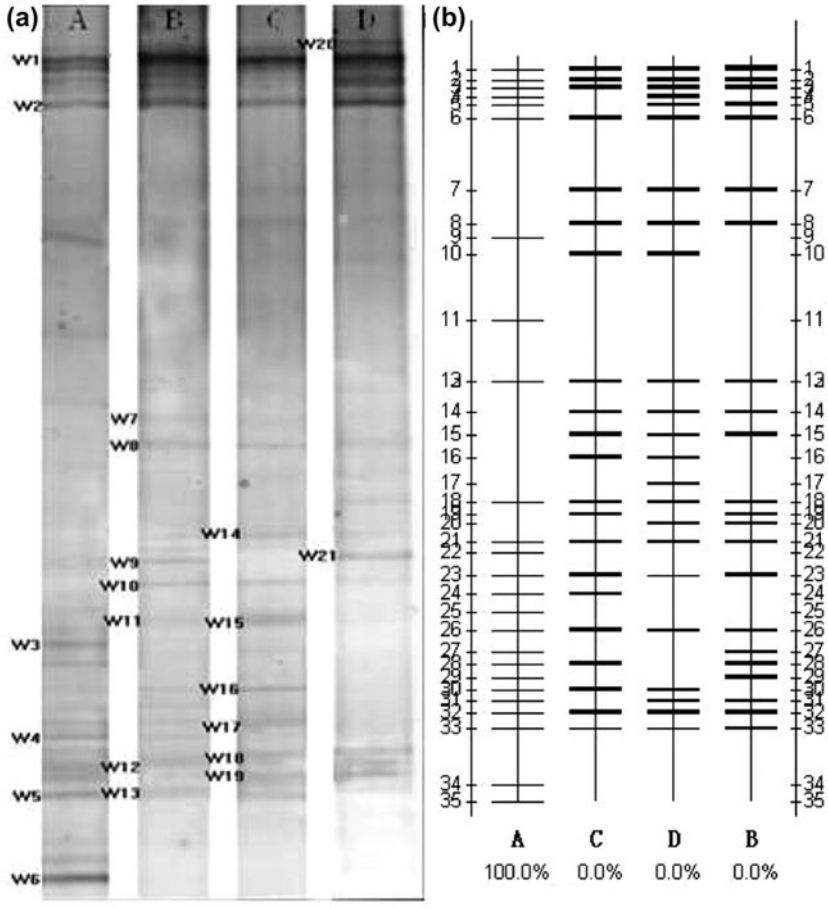

Fig. 8. DGGE profiles of the microbial communities in the SBR in start-up stage: (Lane A) day 1, (Lane B) day 27, (Lane C) day 60, (Lane D) day 80 ((a) image; (b) schematic).

Table 2
Sequences analysis and species identification of selected DGGE bands

Band No.	Closest relatives (accession No.)	Subclass	Max ident (%)
W1	Candidatus accumulibacter phosphatis clade (CP001715)	*β*-proteobacteria	95
W2	Uncultured gamma proteobacterium clone (GQ203645)	*γ*-proteobacteria	99
W3	Uncultured beta proteobacterium (EF203860)	*β*-proteobacteria	98
W4	Uncultured Rhodospirillales bacterium (EU300149)	*α*-proteobacteria	98
W5	*Rhizobium* sp. (DQ395341)	*α*-proteobacteria	94
W6	Uncultured gamma proteobacterium (FJ517040)	*γ*-proteobacteria	95
W7	Uncultured gamma proteobacterium (FJ516904)	*γ*-proteobacteria	92
W8	*Rubrivivax* sp. (EF028103)	*β*-proteobacteria	96
W9	Uncultured bacterium clone (GU911599)	–	95
W10	*Thiothrix* sp. (AF148516)	*γ*-proteobacteria	99
W11	Uncultured alpha proteobacteria (CU919551)	*α*-proteobacteria	91
W12	Uncultured alpha proteobacteria bacterium (CU918710)	*α*-proteobacteria	94
W13	Uncultured alpha proteobacterium (AF255640)	*α*-proteobacteria	97
W14	Uncultured bacterium clone (FJ660577)	–	100
W15	Uncultured rhodocyclaceae bacterium (HQ184363)	*β*-proteobacteria	98
W16	Uncultured bacterium clone (DQ640692)	–	91
W17	Uncultured chloroflexi bacterium (CU923254)	*β*-proteobacteria	95
W18	Candidatus accumulibacter phosphates (CP001715)	*β*-proteobacteria	97
W19	*Dechloromonas* sp. (AF170356)	*β*-proteobacteria	95
W20	Uncultured delta proteobacterium (FN668062)	*δ*-proteobacteria	87
W21	Uncultured proteobacterium (AB010602)	proteobacteria	99

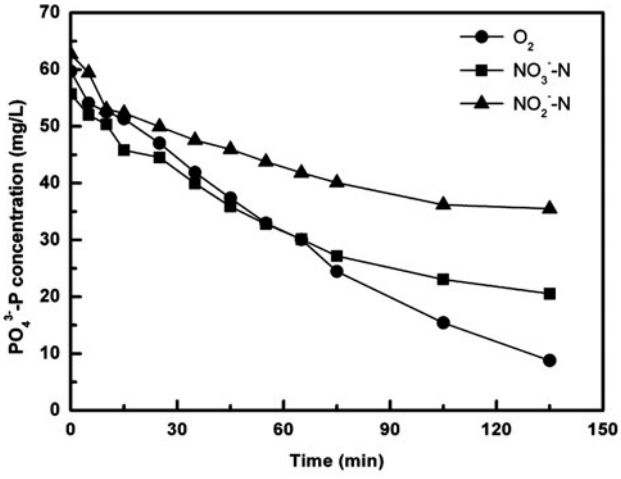

Fig. 9. Phosphorus uptake under different electron acceptor conditions.

phosphorus removal efficiency of the reactor became better. The bands in lane D were less but intensified, suggesting that the microbial community became less diverse but some groups of bacteria responsible for pollution removal were enriched in the reactor. As shown in Fig. 8, some bands such as W1 and W2 existed in all lanes and grew darker through the experiments, indicating that the operational condition of the reactor was favorable for the growth of the strains W1 and W2. The strain W1 (*Candidatus accumulibacter phosphate*) could assimilate excessive phosphorus [35], which was in consistent with the performance of phosphorus removal of the reactor at the beginning of the experiments.

Partial 16S rDNA sequences were successfully obtained, and the closest relative strains available in the GenBank database were summarized in Table 2. In the sequences, 18 strains belong to the *Proteobacteria*, comprising 5 to *α-proteobacteria*, 7 to *β-proteobacteria*, 4 to *γ-proteobacteria* and 1 to *δ-proteobacteria*. The other three strains belong to uncultured bacteria.

3.4. Effect of different electron acceptors on granular denitrifying phosphorus removal

As shown in Fig. 9, phosphorus removal efficiency in the reactors with oxygen, nitrate, and nitrite was 85.3, 63.1, and 43.4%, respectively. At the beginning of the tests, phosphorus uptake in the reactors with oxygen and nitrate exhibited little differences, but that with nitrite was much lower than the former two. These results indicated that a relative high portion of PAOs existed in the granular sludge could utilize nitrate as electron acceptor, that is DNPAOs. After 65 min, phosphorus uptake rate in the reactor with

nitrate decreased gradually, although the concentration of the nitrate was as high as 8.3 mg/L. After 105 min, phosphorous assimilation in the reactor with nitrite almost ceased, and the nitrite concentration was 12.1 mg/L. It demonstrated that nitrite could be utilized by DNPAOs for phosphorus uptake, but it was not an efficient electron acceptor.

4. Conclusions

Performance of the reactor and the characteristics of activated sludge in different phases during the start-up period were investigated. Dynamic succession of the microbial community throughout the experiment was observed by microbial analysis. The following conclusions were obtained:

(1) The aeration intensity had little influence on the COD removal efficiency in the A/O/A SBR, but the phosphorus and nitrogen (especially ammonia nitrogen) removal efficiencies could be significantly improved under higher airflow rate (6.5 L/min).
(2) Higher aeration intensity and shorter settling time could enhance the granulation of sludge. After the formation of granular sludge enriched with DNPAOs, nitrification, denitrification, and phosphorus uptake were achieved in the A/O/A SBR simultaneously, and excellent performance was obtained.
(3) The results of DGGE analysis indicated that the microbial community became less diverse at the end of the experiment compared with the initial period. Most of the strains belonged to the *Proteobacteria*. Some strains which could take up excessive phosphorus became predominant bacteria gradually, and phosphorus removal efficiency of the reactor increased accordingly.
(4) In this reactor, a majority of PAOs could utilize nitrate as electron acceptor for phosphorus uptake, which seemed to be DNPAOs, but only a small part of them could assimilate nitrite. In other words, oxygen, nitrate, and nitrite all could be used as electron acceptor for phosphorus removal, but nitrite was less efficient than oxygen and nitrate.

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