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Biological phosphorus removal from real wastewater in a sequencing batch reactor operated as aerobic/extended-idle regime

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ABSTRACT

Recently, it has been reported that biological phosphorus removal (BPR) could be achieved in a sequencing batch reactor (SBR) with aerobic/extended-idle (A/EI) regime using synthetic medium. This paper first examined the feasibility and stability of the A/EI regime treating real domestic wastewater. The results showed that the A/EI-SBR removed 1.32 ± 0.03–3.55 ± 0.04 mg of phosphorus per g of volatile suspended solids during the steady-state operation, suggesting that BPR from domestic wastewater could be well realised in the A/EI regime. Then, another SBR operated as the conventional anaerobic/oxic (A/O) regime was conducted to compare the soluble orthophosphate (SOP) removal with the A/EI regime. The results clearly showed that the A/EI regime achieved higher SOP removal than the A/O regime. Finally, the mechanism for the A/EI-SBR driving superior SOP removal was investigated. It was found that the sludge cultured by the A/EI regime had more polyphosphate accumulating organisms and less glycogen accumulating organisms than that by the A/O regime. Further investigations showed that the A/EI-SBR had a lower glycogen transformation and a higher PHB/PHV ratio, which correlated well with the superior phosphorus removal.

1. Introduction

Enhanced biological phosphorus removal (EBPR) is currently considered to be one of the most economical and sustainable methods for removing phosphorus (P) from wastewater [1,2]. The conventional EBPR processes are based on the enrichment of activated sludge with polyphosphate accumulating organisms (PAOs) and, in the EBPR processes, biomass is subjected to alternating anaerobic and aerobic conditions so that the PAOs are favoured over other organisms. In the anaerobic phase, PAOs take up volatile fatty acids (VFAs) and store them as polyhydroxyalkanoates (PHAs). During the subsequent aerobic zone, PAOs take up excessive amounts of phosphate using the stored PHAs as both energy and carbon sources [3]. By wasting the P-rich sludge, excess P removal from wastewater can be achieved.

It is widely accepted that an anaerobic/aerobic sequence is a necessity to initiate and sustain EBPR [4]. In this regard, Comeau et al. [5], Mino et al. [6] and Arun et al. [7] developed two models for EBPR (i.e. the "Comeau" model, and the "Mino" model). Both of the two models together with several subsequent update models all described EBPR as system with a characteristic array of an anaerobic basin followed by an aerobic phase.

The EBPR system with cyclic changes of anaerobic and aerobic conditions has an economical advantage of lower sludge production and less use of chemicals, therefore it plays an increasingly important role in controlling eutrophication of aquatic water system and is widely used all over the world [8]. Especially, it is popularly applied for treatment of domestic wastewaters, which contain typical phosphorus concentrations of 4–12 mg PO_4^{3-}-P/L [1]. But at the same time, it is a complex process when compared to biological removal of organic matter [9]. Moreover problems related to the process stability and impairment of P removal efficiencies are often reported in many full-scale applications [8,10].

In some cases, external disturbances such as nutrient limitation or excessive nitrate loading to the anaerobic reactor explained the process upsets. In other cases, microbial competition between PAOs and another group of organisms known as the glycogen (non-polyphosphate) accumulating organisms (GAOs) was considered to be the cause of the degradation in P removal [11]. Like PAOs, GAOs are able to proliferate under alternating anaerobic and aerobic conditions without performing anaerobic P release or aerobic P uptake [12]. Since GAOs consume VFAs without contributing to P removal,

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they are highly undesirable organisms in EBPR system, and minimising the growth of GAOs has the potential to be an effective strategy to promote the performance of EBPR systems [8].

Recently, it has been reported that excess P removal could be achieved without specific anaerobic period in activated sludge system if the idle period is extended properly, and this operation was defined as the aerobic/extended-idle (A/EI) regime [13,14]. Compared with conventional anaerobic/oxic (A/O) techniques, the anaerobic period was cancelled and an extended-idle phase (210–450 min) was operated in the A/EI regime. Up to now, the feasibility of using propionate as the carbon source for BPR in A/EI process has been examined [15], and the inducing metabolism of poly-P accumulation in the A/EI regime has also been addressed [16]. The previous studies suggested that the A/EI regime could achieve a good BPR performance and might be a promising alternative approach for P removal. Nevertheless, all of the experimental studies focused on the A/EI regime were conducted in simulated municipal wastewaters. In view of the complex composition and high P and nitrogen contents of real wastewater, these studies would not be able to accurately and comprehensively reflect the real situations and further examinations are needed.

This work, therefore, used domestic sewage as research object. First, a sequencing batch reactor (SBR) operated as the A/EI regime was conducted to examine the feasibility and stability for the treatment of a real domestic wastewater. Then, another SBR operated as the conventional A/O regime was conducted, and the soluble orthophosphate (SOP) removal efficiencies between the A/EI-SBR and A/O-SBR were compared. Finally, Variations of SOP and sludge glycogen as well as PHAs in a typical cycle in the two SBRs were discussed, and the mechanism for the A/EI-SBR achieving superior SOP removal performance was investigated.

2. Materials and methods

2.1. Wastewater

The wastewater used in this study was collected from a residential district of Changsha, PR China. It characterized by 100–400 mg/L chemical oxygen demand (COD), 20–40 mg/L ammonia-nitrogen (PO_4^{3-}-N), 2–8 mg/L PO_4^{3-}-P. In addition, the wastewater had typical VFAs contents of 68–186 mg/L acetic acid, 21–90 mg/L propionic acid and a few other acids. Its pH level was about 7.0.

2.2. Reactor setup and operation

Two identical SBRs, each with a 2.0 L working volume, were seeded with activated sludge from the second wastewater treatment plant of Changsha, PR China, which routinely achieves EBPR. The initial mixed liquor suspended solids (MLSS) of activated sludge was 4000 mg/L. The two SBRs were operated with three cycles per day. The A/EI-SBR cycle consisted of a 240 min aerobic period, followed by 30 min settling, 1 min decanting and 209 min idle periods. As a control, the A/O-SBR cycle consisted of an anaerobic period (2 h), and an aerobic period (4 h), with the remainder of the cycle time for settling (30 min), decanting (1 min), and idle (89 min). After settling period 1 L supernatant was discharged from both reactors and was replaced with 1 L of domestic wastewater during the first 1 min of the aerobic period (A/EI-SBR) and the anaerobic period (A/O-SBR), respectively. The A/O-SBR was mixed using a magnetic stirrer in the anaerobic stage. During the aerobic time, air was supplied into both SBRs at a flow rate of 2 L/min. Mean cell residence time (MCRT) was controlled at approximately 14 days in both reactors by withdrawing the sludge from the reactors at the end of the aerobic period, but before settling.

2.3. Cycle studies

The performance of the two SBRs was monitored through cycle studies in which both liquid and solid phase samples were collected at an interval of 5–10 min during the first 30 min of the anaerobic and/or aerobic period and each 30 min afterwards for the analysis of SOP, glycogen, PHAs, as well as dissolved organic carbon (DOC). Total suspended solids (TSS) and volatile suspended solids (VSS) were determined at the end of the aerobic periods.

2.4. Analytical methods

Sludge samples from the two reactors were immediately filtered through a Whatmann GF/C glass microfiber filter (1.2 μm). The filtrate was analyzed for SOP, DOC, and the filter was assayed for TSS, VSS, sludge total phosphate (TP), PHAs and glycogen.

SOP, NH_4^+-N, nitrite, nitrate, VSS and TSS were measured according to Standard Methods [17], and DOC was determined after membrane filtration (0.45 mm cellulose nitrate filter) using a total organic carbon (TOC) analyzer (ShimadzuTOC-500, Japan) according to the literature [18]. Element analysis of sludge sample was conducted by an analyzer of scanning electron microscope with X-ray energy dispersive microanalysis (JSM-5910, Japan) after lyophilization pretreatment. The measurements of sludge glycogen, poly-3-hydroxybutyrate (PHB), poly-3-hydroxyvalerate (PHV), and poly-3-hydroxy-2-methylvalerate (PH2MV) were the same as described in the literatures [14,19].

DAPI (4′,6′-diamidino-2-phenyl indol dihydrochloride) staining was carried out to analyze the presence of intracellular poly-P granules as described previously [20]. Sludge samples taken at the end of the aerobic period were used for staining.

The fluorescence in situ hybridization (FISH) technique with 16S rRNA-targeted oligonucleotide probes was employed to quantify PAOs and GAOs in both systems. FISH analyses were the same as described by Carvalho et al. [21] and Wong et al. [22]. Sludge samples were taken from the reactors at the end of aerobic zone during steady operation period. The oligonucleotide probes used for FISH are listed in Supporting Information (SI) Table S1.

3. Results

3.1. SOP removal performance in the A/EI-SBR during long-term operation

Experiments for SOP removal were conducted and lasted for about 180 days. The efficiencies of SOP removal during this period were shown in Fig. 1.

As shown in Fig. 1, it can be clearly observed that although influent SOP concentration ranged from 2 to 8 mg/L, SOP concentration in effluent were kept among 0–0.5 mg/L mostly and the removal efficiencies were about 94%, with a highest value of 98.6% during the stable operation. This proved that SOP removal from this type of domestic wastewater could be achieved without anaerobic phase, which was conventionally considered as an absolutely necessary phase for EBPR.

Fig. 2 shows that the variations of the SOP uptake per g of volatile suspended solids (SOP/VSS) in the A/EI-SBR were consistent with the variations of influent SOP concentration during long-term operation. As influent SOP concentration changed from 2 mg/L at day 14 to 6 mg/L at day 56, the SOP/VSS progressively increased from 0.98 ± 0.02 to 2.62 ± 0.03 mg of P per g of VSS. The SOP/VSS ratios were maintained stably at approximately 1.02 ± 0.03–3.69 ± 0.05 mg of P per g of VSS when influent SOP concentrations ranged from 2 to 8 mg/L. With the increase of SOP/VSS, TP content of the activated sludge in A/EI-SBR increased from

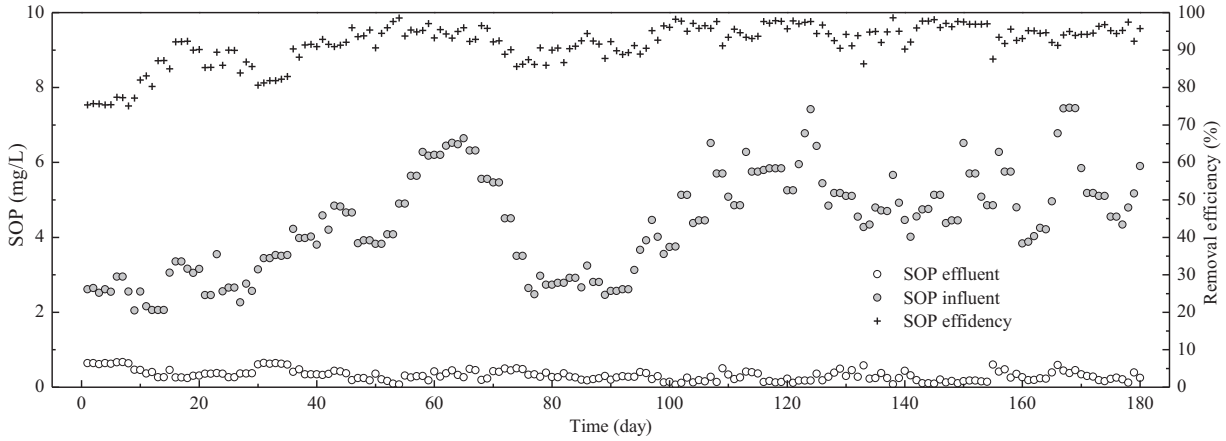

Fig. 1. Phosphorus removal during 180 days long-term operation in the A/EI-SBR.

39 ± 2 mg P per g of TSS on day 14 to 61 ± 3 mg P per g of TSS on day 63 (Fig. S1, Supporting Information). Element analysis of sludge sample (Fig. S2, Supporting Information) showed that the sludge contained large quantities of P, K, Na and Ca, which were proved to be the main components of poly-P granules [23]. All these facts demonstrated the good stability of SOP removal in the A/EI-SBR.

3.2. Comparison of SOP removal performance between the A/EI-SBR and the A/O-SBR

Comparisons of SOP removal efficiencies between the two SBRs were illustrated in Fig. 3a. On the 10th day, the SOP removal efficiencies in the AEI-SBR and the A/O-SBR were 75.4% and 81.7%, respectively, with the A/O-SBR 6.3% higher than the AEI-SBR (a difference of about 0.61 mg-P/L). However, on the 30th day, the SOP removal efficiency in the AEI-SBR became just 1.3% (0.05 mg-P/L) lower than the A/O-SBR. With the increase of cultivated time, it can be seen that the SOP removal efficiency in the AEI-SBR on day 110 became, on the contrary, 8.7% (0.73 mg-P/L) greater than the A/O-SBR. After day 130, SOP removal efficiency in the A/EI-SBR maintained an average 9.0% higher than the A/O-SBR. It seemed that it required at least 2–3 MCRTs for the AEI-SBR microbial populations to reach a fairly stable level of SOP removal efficiency.

Fig. 3b shows the comparisons of the SOP uptake rates between the two SBRs. On day 10, the SOP uptake rates are 0.53 mg-P/g-VSS/h in the A/EI-SBR and 0.72 mg-P/g-VSS/h in the A/O-SBR, with A/O-SBR 34.3% higher than the A/EI-SBR. However, on day 30, this advantage was reduced to just 2.1% (0.02 mg-P/g-VSS/h). And on day 50, the SOP uptake rate in the A/EI-SBR became, on the contrary, 29.2% (0.31 mg-P/g-VSS/h) greater than that in the A/O-SBR. The difference increased to 33.9% and 0.62 mg-P/g-VSS/h on day 70, and drop on day 90 as a result of low influent SOP concentration. After day 110, the rate of SOP uptake in the A/EI-SBR maintained an average 29.5% (0.51 mg-P/g-VSS/h) higher than that in the A/O-SBR. These findings were consistent with the comparisons of SOP removal efficiencies in Fig. 3a.

The results in Fig. 3 indicated that deleting the anaerobic phase in EBPR process benefited SOP removal after the biomass was cultured for a long-term period (after day 110), but for a brief transient period after anaerobic phase was cancelled the opposite result was observed (see day 10 in Fig. 3). The clear difference between the two SBRs, as shown in the above text, was that the A/O-SBR was equipped with an anaerobic phase before aeration whereas the A/EI-SBR was not.

Table 1 further summarized reactor performances between the A/EI-SBR and the A/O-SBR in a 28-day period during steady-state operation. The effluent SOP concentration in the A/EI-SBR was 0.44 ± 0.19 mg/L, which was lower than the 1.19 ± 0.37 mg/L in the A/O-SBR. This indicated that the A/EI-SBR had a lower SOP concentration in effluent, thus had a higher SOP removal than the A/O-SBR (0.44 ± 0.19 vs 1.19 ± 0.37 mg/L and 94.3 ± 1.6 vs 82.2 ± 2.3%, respectively), which was in correspondence

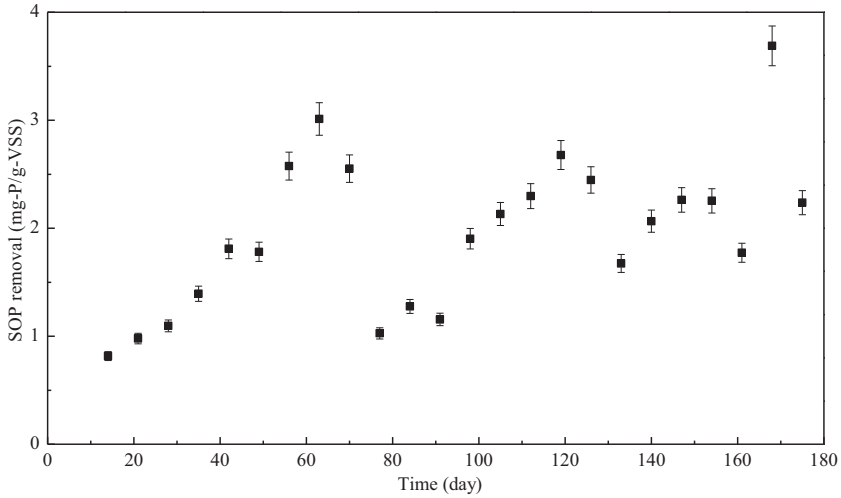

Fig. 2. Variations of SOP/MLVSS ratio in the A/EI-SBR during long-term operation.

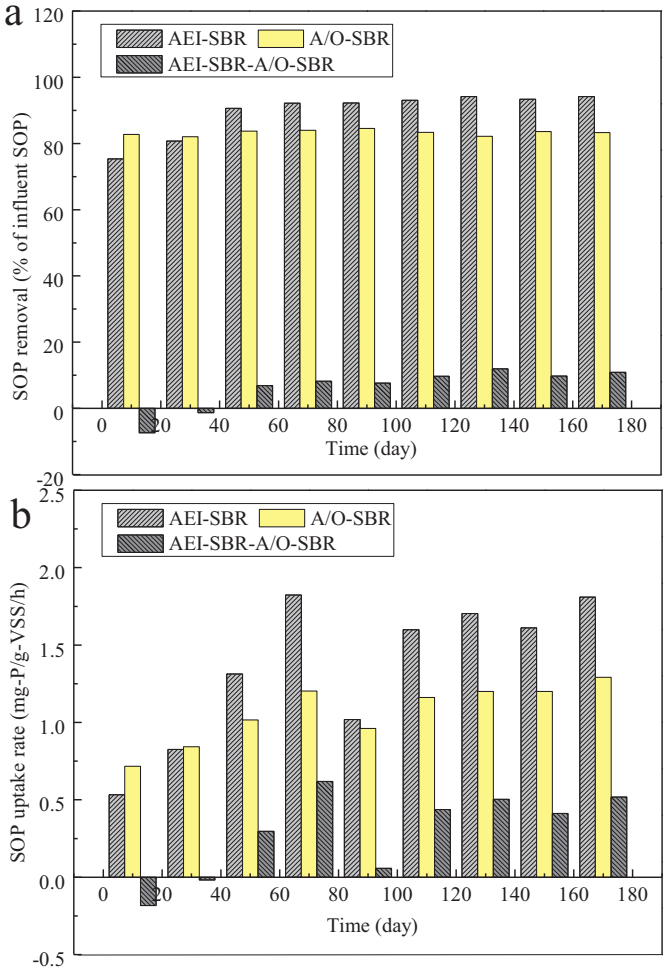

Fig. 3. Variations of SOP removal efficiencies (a) and SOP uptake rates (b) with operating time in the two SBRs.

with a greater sludge TP content in the A/EI-SBR (61 ± 3 vs 29 ± 2 mg-P per g-TSS). Moreover, the SOP removal efficiency remained around 94% in the A/EI-SBR and 82% in the A/O-SBR, and the average SOP removal rate maintained 316 ± 9 and 264 ± 5 mg-P/m^3/h in the A/EI-SBR and the A/O-SBR, respectively, during the steady-state operation. These results clearly showed that though a good SOP removal performance could be achieved in both reactors, the A/EI regime could drive better. The mechanism for the A/EI-SBR driving higher SOP removal than the A/O-SBR would be discussed in the following text.

Table 1
Summary of reactor performances between the AEI-SBR and A/O-SBR during steady-state operation.[a]

Item	AEI-SBR	A/O-SBR
Effluent SOP (mg/L)	0.44 ± 0.19	1.19 ± 0.37
SOP removal efficiency (%)	94.3 ± 1.6	83.2 ± 2.3
TP content of TSS (mg-P/g-TSS)	61 ± 3	29 ± 2
SOP removal rate (mg-P/m^3/h)	316 ± 9	264 ± 5
Effluent NH_4^+-N (mg/L)	1.01 ± 0.56	1.24 ± 0.63
Effluent NO_2^--N (mg/L)	0.11 ± 0.03	0.15 ± 0.02
Effluent NO_3^--N (mg/L)	8.66 ± 0.55	9.73 ± 0.61
NH_4^+-N removal efficiency (%)	97.1 ± 1.7	96.5 ± 1.6
TN removal efficiency (%)	72.8 ± 2.1	69.1 ± 1.9

[a] Results are averages and their standard deviations, and these data are obtained from day 120 to day 147.

4. Discussion

As shown in Table 1, the mean effluent ammonia nitrogen concentrations in the A/EI-SBR and the A/O-SBR remained at 1.01 ± 0.56 and 1.24 ± 0.63 mg/L, respectively. For the effluent nitrite and nitrate concentrations, they averaged at 0.11 ± 0.03 and 8.66 ± 0.55 in the A/EI-SBR and 0.15 ± 0.02 and 9.73 ± 0.61 mg/L in the A/O-SBR during steady-state operation. The ammonia and total nitrogen removal efficiencies were 97.1 ± 1.7% and 72.8 ± 2.1% in the A/EI-SBR, and were 96.5 ± 1.6% and 69.1 ± 1.9% in the A/O-SBR. Because nitrogen removal efficiencies in the two SBRs were similar, their effects on SOP removal were negligible.

Fig. 4 shows the results of DAPI staining of the sludge samples taken from both SBRs on day 128. From Fig. 4, it could be observed that though the activated sludge biomass contained many cells with visible poly-P inclusions in both systems, abundance in the A/EI-SBR was much higher than that in the A/O-SBR. The percentage of white cell area in total cell area in two SBRs were about 46.6% and 22.1%, respectively, indicating that the A/EI-SBR had a higher rate of poly-P containing cells than the A/O-SBR. Thus, one reason for the A/EI-SBR achieving superior SOP removal performance was the more poly-P containing cells cultured in the activated sludge.

FISH analysis revealed that *Accumulibacter* (PAOs) and *Competibacter*- or *Defluviicoccus*-related bacteria (GAOs) accounted for, respectively, 33.6% and 18.8% of all bacteria in the sludge of the A/EI-SBR, whereas those in the A/O-SBR were 16.2% and 29.7% of total biomass, respectively (Fig. 5). The data supported the results of DAPI staining and further showed that less GAOs contained in biomass might be another reason for the A/EI-SBR achieving superior SOP removal. At present the composition of the remainder of cells in the sludge is not well understood, ordinary heterotrophs and a small amount of other PAOs and GAOs may be presented.

To further reveal the difference of SOP removal performance between the A/EI-SBR and the A/O-SBR, SOP, glycogen and PHAs as well as DOC were analyzed periodically. The variations of the concentrations of these compounds during one quasi-steady cycle in the A/O-SBR (a) and the A/EI-SBR (b) were illustrated in Fig. 6.

The results in Fig. 6a show a rapid DOC decrease along with much PHAs accumulation and high glycogen hydrolysis as well as much SOP release was observed during the anaerobic phase in the A/O-SBR. The DOC and glycogen were utilized rapidly at the beginning of anaerobic time and consumed to low levels after 120 min. During this time a very fast SOP release was observed and the released amount was about 50 mg-P/L, corresponding to ten times more than the influent (the influent SOP concentration was about 5 mg/L). It is known that PAOs consumed glycogen and poly-P during the anaerobic phase for reducing power and energy production necessary to take up VFAs and their final reduction to PHAs. In the subsequent aerobic stage SOP was taken up very quickly within the initial 60 min, and 80% of the SOP uptake occurred during this time. There was a considerable PHAs biosynthesis (3.8 mM-C/g-VSS) during the anaerobic period, and those synthesized PHAs were utilized in the subsequent aerobic phase. The glycogen decreased from the initial 2.9 to 1.3 mM-C/g-VSS during the anaerobic phase, then was replenished to 3.1 mM-C/g-VSS in the subsequent aerobic phase, and in idle period, a bit of glycogen consumption with a small DOC decrease and PHAs accumulation was detected. These observations were consistent in all cycle experiments and were similar to the existing studies [15,24].

Fig. 6b shows that along with rapid decreases of DOC and SOP during the initial 60 min of aeration, glycogen in activated sludge was replenished after a transient hydrolysis in the A/EI-SBR. After DOC and SOP completely got their minimum values, glycogen was slightly synthesized and then became to be consumed gradually

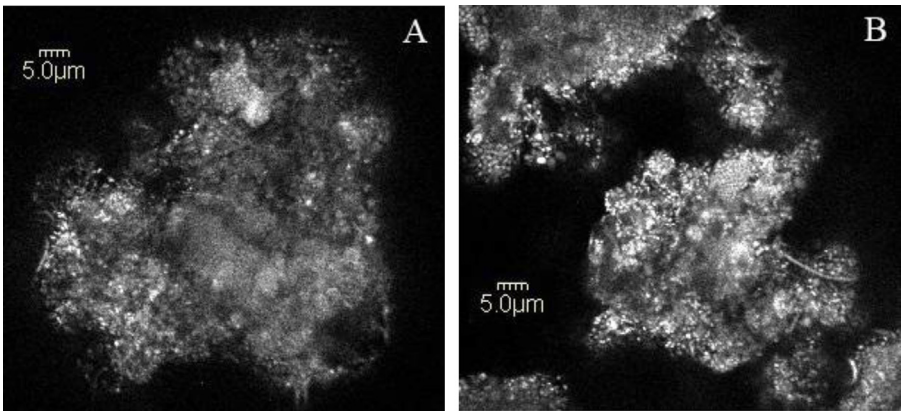

Fig. 4. Micrographs of DAPI stained dispersed sludge at the end of the aerobic period in the A/O-SBR (A) and the A/EI-SBR (B) on day 128. White cells are those containing polyphosphate.

after the aeration was terminated. Accompanied with the glycogen consumption, about 4 mg/L SOP released during the idle phase. As DOC was consumed to a very low level, energy generated from the SOP release was not used for substrate uptake/PHAs accumulation but for bacterial maintenance, which thereby resulted in only a slight PHAs synthesis. In the literature, it was reported that an idle SOP release accompanied by a low idle PHA production could induce PAOs to effectively take up SOP in excess of metabolic requirement in the A/EI process [16]. Also, it was worth noting that cyclic transformations of PHAs and glycogen in the A/EI-SBR were observed to be lower than those in the A/O-SBR.

It was reported that the transformations of PHAs and glycogen were associated with the activities of PAOs and GAOs, and higher transformation of glycogen suggested that the metabolism of GAOs might be activated [25,26]. So the lower glycogen transformation observed in the A/EI-SBR indicated that the biomass cultured in the A/EI-SBR contained less GAOs activities than that in the A/O-SBR. Since GAOs consume VFAs without contributing to SOP removal, they are highly undesirable organisms in EBPR systems. The transformation of glycogen was in agreement with the results of the FISH analysis.

In the aerobic period of anaerobic/aerobic EBPR processes, as there is almost no external carbon addition, the internally stored PHAs which are biosynthesized in the anaerobic phase are oxidized and used for SOP uptake, glycogen synthesis, and cell growth. PHAs oxidation has been studied and observed to be directly correlated with SOP uptake from the bulk liquid, and decreased initial aerobic PHAs content can lead to a lower SOP uptake for a given population [24,27]. However, PHAs synthesis was lower in the A/EI-SBR than that in the A/O-SBR (2.1 vs 3.8 mM-C/g-VSS), but SOP uptake was higher and phosphorus removal performance was more stable in long-term running. One possible reason is that the A/O-SBR synthesized more PHV than the A/EI-SBR. As stated above the major VFAs in the wastewater were acetic and propionic acids. When fed with acetate, PAOs produce mainly PHB but little PHV while GAOs produce approximately 75% PHB and 25% PHV as well as a very

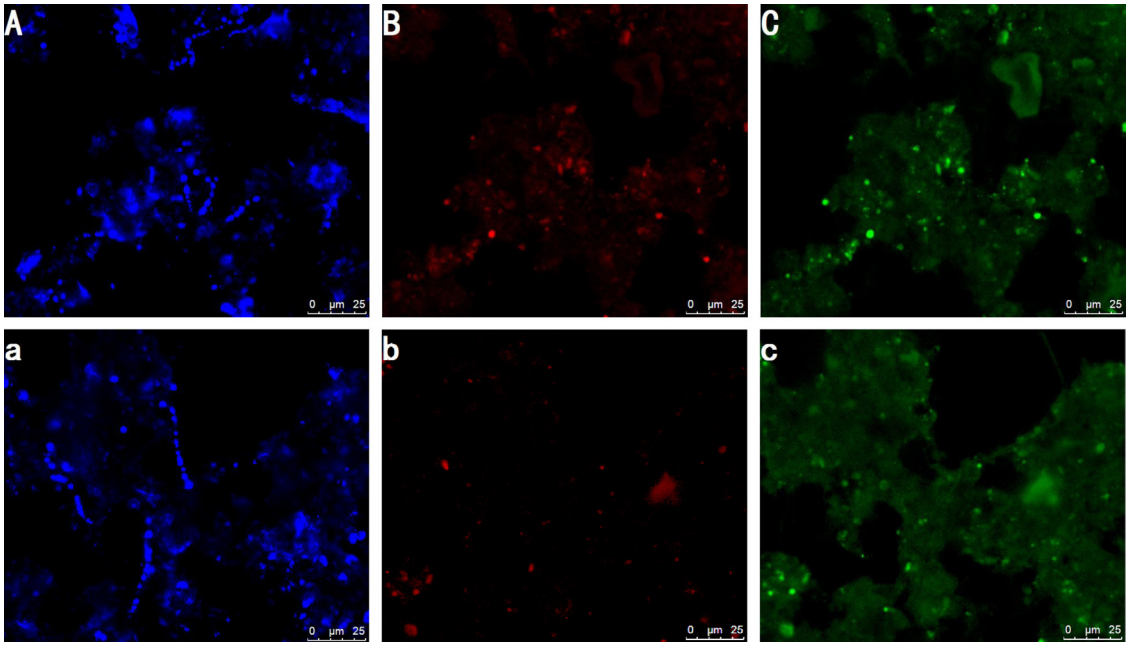

Fig. 5. Confocal laser scanning micrographs (CLSM) of activated sludge samples obtained from the A/O-SBR (A–C) and the A/EI-SBR (a–c) on day 128. The samples were hybridized with PAOmix (blue, A and a), GAOmix (red, B and b) and EUBmix (green, C and c) probes specific for *Accumulibacter* (PAOs), *Competibacter*- and *Defluviicoccus*-related organisms (GAOs), and the dominant bacteria, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

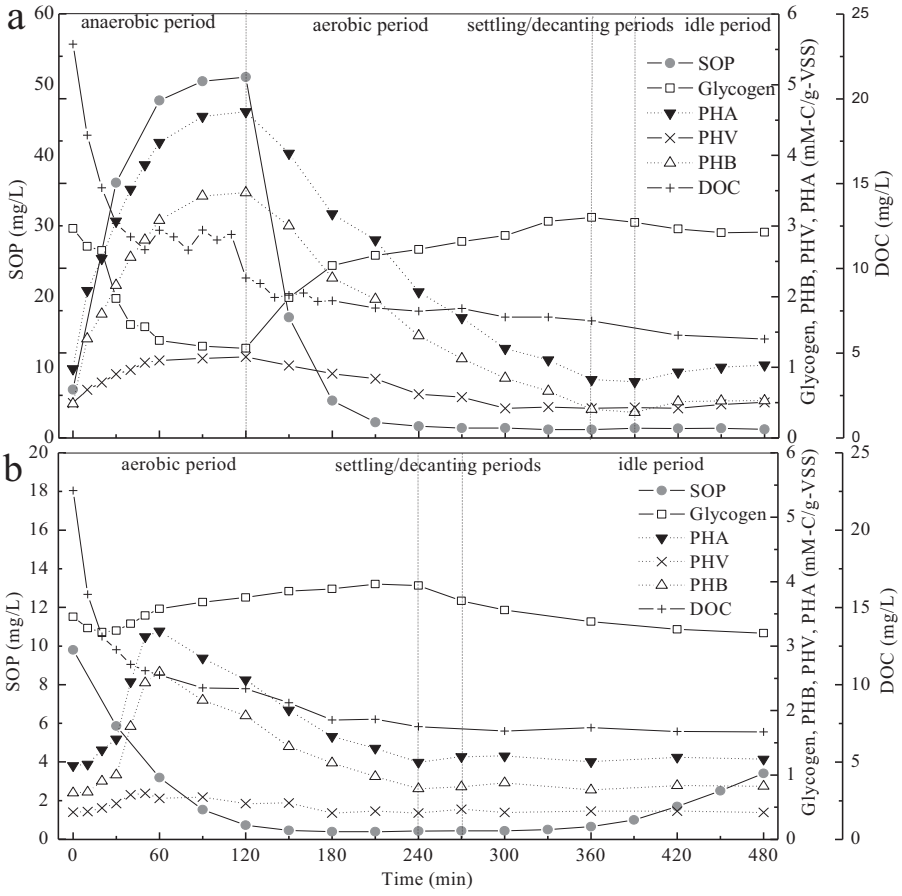

Fig. 6. Variations of SOP, sludge glycogen and PHAs as well as DOC during a cycle in the A/O-SBR (a) and the A/EI-SBR (b).

small fraction of PH2MV (C-mol basis, the PHB/PHV is about 3). However, both PAOs and GAOs produce mainly PHV and PH2MV with propionate uptake, with a very low amount of PHB [25,28]. According to Zeng et al. [28], the presence of PHV in the cycle could be an indicator for the presence and functioning of GAOs. The PHAs composition in the A/EI-SBR comprised of 2.9 mM-C/g-VSS PHB and 0.6 mM-C/g-VSS PHV, with the PHB/PHV of about 5, greater than either the 3 above-mentioned or the 2 in the A/O-SBR (the PHB and PHV in the A/O-SBR were 3.0 and 1.5 mM-C/g-VSS, respectively). These facts were consistent with the results of FISH analysis that less GAOs were contained and PAOs were dominant organisms in the A/EI-SBR, which benefited the superior phosphorus removal.

The above studies clearly showed that the A/EI regime could achieve a good BPR when treating the real domestic wastewater. In contrast to the conventional A/O process, the anaerobic period was cancelled and an extended-idle starvation phase was operated in the A/EI regime, so the effect of the extended-idle period upon the SOP removal performance also deserved to be discussed. As is well known, glycogen degradation, PHAs biosynthesis and SOP release are three important features for the anaerobic period of the A/O system. During the short idle stage of the A/O-SBR, obvious glycogen degradation and PHAs synthesis but no SOP release was observed. This proved that the idle stage played the role of the anaerobic period to some extent although the two were not absolutely the same as each other. However, in the extended-idle stage of the A/EI regime, in addition to glycogen degradation and slight PHAs synthesis, appreciable SOP release was also observed. This indicated that the extended-idle enhanced SOP release from poly-P containing cells, thus might promote SOP uptake in the subsequent aerobic period of next cycle. As shown in Fig. 6b, VFAs were depleted during the aeration, and PHAs were also decreased to very low levels at the end of the aerobic period, the energy necessary for bacterial maintenance in the idle phase was supplied partly by glycogen utilisation but mostly by the hydrolysis of the intracellular stored poly-P. Thus, poly-P degradation could nicely meet the energy requirement for bacterial maintenance and seemed as the major suitable energy source during the idle phase. It was reported that the capability of BPR was largely dependent on the role of poly-P playing in their biochemical metabolism [16,29]. With the increase of the idle time, energy requirement for bacterial maintenance would increase, and the role of poly-P would be enhanced consequently. That is, the A/EI regime can well meet the need of PAOs' metabolism and thus provide a strong advantage for PAOs when competing with GAOs or other bacteria.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bej.2013.06.005.

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