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Improved biological phosphorus removal performance driven by the aerobic/extended-idle regime with propionate as the sole carbon source

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ABSTRACT

Our previous studies proved that biological phosphorus removal (BPR) could be achieved in an aerobic/extended-idle (AEI) process employing two typical substrates of glucose and acetate as the carbon sources. This paper further evaluated the feasibility of another important substrate, propionate, serving as the carbon source for BPR in the AEI process, and compared the BPR performance between the AEI and anaerobic/oxic (A/O) processes. Two sequencing batch reactors (SBRs) were operated, respectively, as the AEI and A/O regimes for BPR using propionate as the sole substrate. The results showed that the AEI-reactor removed 2.98 ± 0.04–4.06 ± 0.06 mg of phosphorus per g of total suspended solids during the course of the steady operational trial, and the phosphorus content of the dried sludge was reached 8.0 ± 0.4% after 56-day operation, demonstrating the good performance of phosphorus removal. Then, the efficiencies of BPR and the transformations of the intracellular storages were compared between two SBRs. It was observed that the phosphorus removal efficiency was maintained around 95% in the AEI-reactor, and about 83% in the A/O-reactor, although the latter showed much greater transformations of both polyhydroxyalkanoates and glycogen. The facts clearly showed that BPR could be enhanced by the AEI regime using propionate as the carbon source. Finally, the mechanisms for the propionate fed AEI-reactor improving BPR were investigated. It was found that the sludge cultured by the AEI regime had more polyphosphate containing cells than that by the A/O regime. Further investigation revealed that the residual nitrate generated in the last aerobic period was readily deteriorated BPR in the A/O-SBR, but a slight deterioration was observed in the AEI-SBR. Moreover, the lower glycogen transformation measured in the AEI-SBR indicated that the biomass cultured by the AEI regime contained less glycogen accumulating organisms activities than that by the A/O regime.

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1. Introduction

Enhanced biological phosphorus removal (EBPR), which is usually based on the principle that the microbes need to be recycled repeatedly through alternating anaerobic and aerobic stages, is often considered as the effectively preferred approach for removing phosphorus to control eutrophication in receiving waters. The alternating anaerobic and aerobic operation can exploit the ability of some microbes (e.g. *Candidatus Accumulibacter phosphatis*) to uptake phosphorus in excess of normal metabolic requirement and to store this as the intracellular biopolymer polyphosphate (poly-P). Under anaerobic condition, poly-P accumulating organisms (PAOs) utilize the energy generated from poly-P cleavage and reducing power from glycogen glycolysis to uptake available carbon sources such as volatile fatty acids (VFAs) and store them as polyhydroxyalkanoates (PHAs); and under the subsequent aerobic condition, PAOs use their stored PHAs as energy and carbon sources for biomass growth, glycogen replenishment, phosphate uptake and poly-P accumulation (Majed et al., 2009). Net phosphate removal can be achieved via the discharge of the phosphorus-rich sludge.

Currently, EBPR is widely applied in full-scale wastewater treatment plants owing to its economical operation and low environmental impact, but is prone to unpredictable failures due to reduced or loss biomass of PAOs (Blackall et al., 2002; Oehmen et al., 2007). Especially in some volatile fatty acids (VFAs) deficiency regions, EBPR capability is easier to deteriorate (Crocetti et al., 2002; Wong et al., 2004; Mullan et al., 2006), weakening its application scope. Accordingly, any improvement in existing phosphorus removal technologies should be of significance to control eutrophication in freshwater ecosystems.

Our recent study has demonstrated that some cells can be induced to store phosphate as intracellular poly-P in a common activated sludge system using both glucose and acetate as the sole carbon source if the idle period is extended suitably (Here we defined this operation as the aerobic/extended-idle (AEI) regime). As compared with the current anaerobic/oxic (A/O) technique, a strict anaerobic period is not performed while an extended-idle period (210–450 min) is operated between the decanting period and the next aerobic period (Wang et al., 2008, 2009). The extended-idle phase looks like a post anaerobic zone of the A/O process as it is also not aerated. However, mixture stirring does not need to be conducted during the extended-idle phase, showing that the AEI process is a simpler strategy for phosphorus removal. Furthermore, the good performance of phosphorus removal achieved in glucose-fed system (Wang et al., 2008, 2009) indicates that the AEI process might be less dependence upon VFAs contents than the A/O process. The previous results obtained by us suggest that the AEI regime might serve as an alternatively effective supplement to the existing method of phosphorus removal.

As is known, many types of carbon sources are present in real wastewaters, and different carbon sources have been shown to have significant impacts on the performances of phosphorus removal (Oehmen et al., 2007). Acetate, the most prevalent VFAs in EBPR plants, and glucose, the detrimental model substrate for EBPR which is often used in laboratory-scale studies, have been tested successfully in the AEI process. Besides, propionate is another important VFAs abundant in many prefermenters and is often present in substantial quantities in real septic domestic wastewaters. According to Von Muench's summarization, propionate ranged from 24% to 33% of the total VFAs in influents of four full-scale systems (Chen et al., 2004). Naik (1999) and Shah (2001) found the carbon molar ratio of propionate to acetate varied between 0.28 and 0.75 in a full-scale plant in Orange County, Florida. Additionally, we monitored the composition of the domestic wastewater from a municipal wastewater treatment plant in Shanghai, China, and found propionate ranged from 19% to 27% of the total VFAs during a two-month period. Though many recent studies have proved that a propionate fed A/O system can provide a more selective advantage to PAOs over glycogen accumulating organisms (GAOs) than an acetate fed A/O system during long-term enrichment, thus decreasing phosphorus level in effluent (Pijuan et al., 2004; Oehmen et al., 2006), whether a propionate fed AEI system can achieve a good phosphorus removal efficiency remains unknown as yet. Therefore, this study first examined the feasibility of propionate using as the carbon source for phosphorus removal in an AEI process, and then compared the performances of phosphorus removal between the propionate fed A/O and AEI-reactors. Because the AEI-reactor showed a better performance of phosphorus removal than the A/O-reactor and the results presented in this study were different from those of previous studies of phosphorus removal, the mechanisms of increased phosphorus removal in the AEI-reactor were also discussed.

2. Materials and methods

2.1. *Parent sequencing batch reactors (SBRs) operation*

Seed sludge was inoculated into two reproductive SBRs with working volumes of 40 L simultaneously. Both SBRs were fed with propionate as the sole carbon source. One was operated as the A/O regime; the other was operated as the AEI regime. The AEI-SBR cycle consisted of a 210 min aerobic period, followed by 55 min settling, 5 min decanting and 210 min idle periods. As a control, the A/O-SBR cycle consisted of approximately a 120 min anaerobic period, a 180 min aerobic period, a 55 min settling period, 5 min decanting and 120 min idle phases according to the literatures (Oehmen et al., 2005a; Tong and Chen, 2007). 20 L supernatant was discharged from both SBRs after settling period, and was replaced with 20 L of the identical synthetic medium (composition detailed below) during the first 5 min of the aerobic period (AEI-SBR) and the anaerobic period (A/O-SBR), respectively. The AEI-SBR was mixed with a mechanical stirrer during the idle period when cycle studies were performed, whereas the A/O-SBR was mechanically stirred during the anaerobic period all the time. During the aerobic time, air was supplied into both SBRs at a flowrate of 40 L min^{-1}. The hydraulic retention time in the two SBRs was 16 h, while the sludge retention time was maintained at approximately 13 days by withdrawing 3 L of

the sludge mixtures (once per day) from the reactors at the end of the aerobic period but before settling.

The synthetic medium was prepared daily and contained 315 mg CH_3CH_2COONa $L^{-1}$and 15 mg PO_4–P L^{-1}, yielding a theoretically influent chemical oxygen demand (COD): PO_4–P ratio of 20 mg COD mg PO_4–P^{-1}, which was considered as being favorable for PAOs. Oehmen et al. (2007) suggested that a low COD/PO_4–P ratio (e.g. 10–20 mgCOD/mgP) should be more favorable to the growth of PAOs. The concentrations of the other nutrients in the synthetic medium were presented as below (per liter): 0.12 g NH_4Cl, 0.01 g $MgSO_4 \cdot 7H_2O$, 0.005 g $CaCl_2$, and 0.5 mL of a trace metals solution. The trace metals solution containing metal-chelator of ethylenediaminetetraacetic acid has been described in our previous publication (Wang et al., 2008).

2.2. Batch experiment

Since nitrification was not inhibited in this study as operated in full-scale wastewater treatment plants, NO_x^-–N (mainly as nitrate) would be accumulated in substantial quantities at the end of aerobiosis in both SBRs, and half of the accumulated nitrate would be entered into the next cycle via the remaining. To compare the tolerance of nitrate levels between the AEI and A/O regimes, batch experiments were conducted. Two totals of 9 L of activated sludge mixtures were, respectively, taken from the parent SBRs at the end of aerobiosis but before settling, centrifuged (5000 rpm for 5 min) and washed three times with tap water to remove the residual nitrate, PO_4–P and COD, and then resuspended in tap water with a final volume of 4.5 L before being divided equally into six reactors (working volumes of 1.5 L each). Two totals of 4.5 L of the synthetic medium described above were added to each reactor equally. KNO_3 was supplied to control the average concentration of nitrate (0, 2.5, 5, 7.5, 10, 20 mg L^{-1}) in the two groups (six each) of reactors. The two groups of reactors were then operated the same as the parent SBRs, respectively. When effluent Pi concentration reached stable, cycle studies were performed once per week.

2.3. Analytical methods

Sludge samples from the reactors were immediately filtered through a Whatman GF/C glass microfiber filter (1.2 μm). The filtrate was analyzed for propionate, soluble orthophosphate (SOP), ammonia, nitrite and nitrate, and the filter was assayed for total suspended solids (TSS), volatile suspended solids (VSS), glycogen, PHAs, sludge total phosphate (TP) content and element.

SOP, ammonia, nitrite, nitrate, TSS and VSS were measured according to Standard Methods (APHA, 1995). 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 our previous publication (Wang et al., 2009). The total PHA was calculated as the sum of measured PHB, PHV, and PH2MV. 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 analysis of propionate was conducted according to the method of Yuan et al. (2006). Briefly, the pH of the filtrate was adjusted to approximately 4.0 using 3% H_3PO_4, and then the filtrate was collected in a 1.5 mL gas chromatography (GC) vial. Nitrogen was the carrier gas and the flux was 50 mL min^{-1}. The injection port and the detector were maintained at 200 and 220 °C, respectively. The oven of the GC was programmed to begin at 110 °C and to remain there 2 min, then to increase at a rate of 10 °C/min to 200 °C, and to hold at 200 °C for 2 min.

For microscopic analysis of the presence of intracellular poly-P granules, grab samples of activated sludge from the AEI- and A/O-SBRs were examined by a confocal scanning laser microscope (FV 500). Intracellular poly-P granules can be visualized by either Neisser staining or using the fluorescent dye 4′,6-diamidino-2-phenylindole (DAPI) (Mullan et al., 2006). DAPI, which was used in this study, stains poly-P emitting a distinct bright white fluorescence (Liu et al., 2001). For estimation of all the abundance of PAOs in sludge samples, visible cell counts of total and poly-P positive cells were taken, as previously used by Mullan et al. (2006). Cell counts were arithmetically averaged from five random fields at each test.

3. Results and discussion

3.1. Variations of pH and dissolved oxygen (DO) in the AEI-reactor

pH and DO were not held constant in this study so that pH and DO varied during a cycle as they would in a full-scale wastewater treatment plant. Therefore, it was necessary to measure cyclic variations of pH and DO to obtain a more deep understanding of the operational condition in the AEI-reactor. Fig. 1 showed the cyclic profiles of pH and DO variations on day 56 and day 109. In the experiments of day 56, the pH increased gradually from 7.67 to 8.43 during the initial 60 min of the aerobic period and decreased slightly to 8.31 at the end of aeration, then a slow decrease to a final pH of 7.94 was followed during the subsequent idle period. DO decreased sharply when the synthetic medium was pumped into the reactor during the initial 5 min of aeration (from 6.05 mg L^{-1} to 0.45 mg L^{-1}) and kept approximately constant until to 30 min, then increased gradually to a final DO of 6.12 mg L^{-1} at the end

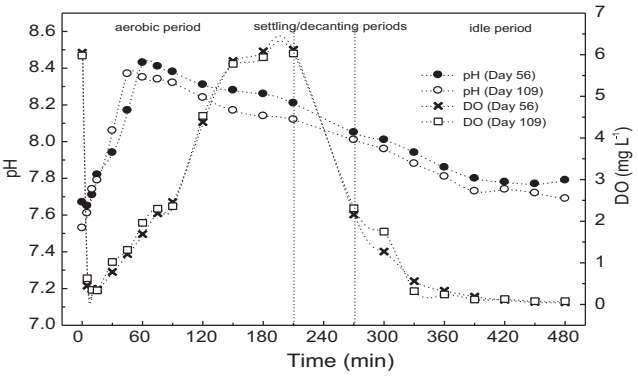

Fig. 1 – Profiles of pH and DO variations in AEI-SBR during one cycle of Day 56 and Day 109 experiments.

of the aerobic period. During the initial 60 min of the idle period, DO decreased gradually to a low level (0.56 mg L^{-1}) and further decreased below 0.1 mg L^{-1} during the remainder of the period. Similar results were observed in the experiments of day 109 as well as in all other experiments, showing that little difference in the cyclic variations of pH and DO during the whole operation.

3.2. *Phosphorus removal performance in the AEI-reactor*

Fig. 2 showed the data of the effluent SOP concentration and phosphorus removal efficiency during the period of 151 days experiments. It was clearly observed that a poor level of SOP removal was achieved during the initial 2 days. 9.76 (day 1) and 6.28 mg L^{-1} (day 2) of SOP concentrations were monitored in the effluent, respectively, which suggested that only 33.9% (day 1) and 58.1% (day 2) of influent SOP were removed, respectively. However, effluent SOP decreased substantially in the following 6 days, and a high level of SOP removal was achieved throughout the test period. After only a one-week period of domestication, effluent SOP was kept among 0.22–1.46 mg L^{-1} and the phosphorus removal efficiency was above 90.3% during the remainder of the tests except for the experiments of day 64 (3.46 mg L^{-1}, 76.9%) and 146 (2.37 mg L^{-1}, 84.2%).

To express the phosphorus removal performance of the AEI-reactor more accurately, phosphorus removal was also transformed relative to the mean TSS during the experiment period (Fig. 3). It could be observed that 2.98 ± 0.04–4.06 ± 0.06 mg phosphorus was removed per g of TSS during the course of the steady operational trial, reflecting the good phosphorus removal performance of the reactor. Also as shown in Fig. 3, a low VSS/TSS ratio implied a high level of phosphorus was stored in the sludge (Oehmen et al., 2005a), and supported the data of the phosphorus removal efficiency presented in Fig. 2.

Verification of the phosphorus removal patterns shown in Figs. 2 and 3 was provided by analysis of the TP content of the activated sludge during the whole operation (Fig. 4). This value increased from 47 ± 2 mg TP per g of TSS on day 2–80 ± 4 mg TP per g of TSS on day 56, and held approximately constant during the course of the remaining experiments. The high TP content of activated sludge was in agreement with the data of phosphorus removal displayed in Figs. 2 and 3. Additionally, energy-dispersive X-ray spectroscopy analysis of the sludge sample (on day 47, Fig. S1, Supporting Information) clearly showed that the activated sludge accumulated substantial quantities of P, K, Ca and Mg, which were proved to be the main elements of poly-P granule (Liu et al., 2001; Martín et al., 2006). All above facts strongly showed that the AEI regime could drive an excellent phosphorus removal performance selecting with propionate as the sole carbon source.

3.3. *Comparison of phosphorus removal performance between the A/O- and AEI- reactors*

Table 1 summarized reactor performances between the AEI- and A/O-reactors during a 24-day period after reaching steady-state operation. Nitrification appeared largely unaffected by the operated regimes, because both regimes contained a relatively long aerobic period. Negligible effluent nitrite was detected in both reactors whereas nitrate was measured in substantial quantities (9.75 ± 0.61 mg L^{-1} in the AEI-reactor vs 11.93 ± 0.57 mg L^{-1} in the A/O-reactor), which indicated that 63.6% and 57.2% of total nitrogen were removed in the AEI- and A/O-reactors, respectively. These results suggested aerobic denitrification occurred to a certain extent in both reactors. Also from Table 1, it could be observed that the AEI-reactor had a lower phosphorus concentration in effluent, thus had a higher SOP removal efficiency than the A/O-reactor (0.76 ± 0.17 vs 2.53 ± 0.37 mg L^{-1} and 94.9 ± 1.2 vs 83.1 ± 2.5%, respectively), which were in correspondence with a greater phosphorus content in the sludge of the AEI-SBR than that of the A/O-SBR (79 ± 3 vs 65 ± 4 mg phosphorus per g of TSS). Moreover, the SOP removal efficiency remained around 95% in the AEI-SBR and 83% in the A/O-SBR during a period more than 4 months after steady-state operation. The results clearly showed that though a good SOP removal performance could be achieved in the A/O-SBR, the AEI regime could drive better. The reasons for the AEI-SBR driving higher phosphorus removal efficiency than the A/O-SBR would be discussed in the following text.

The profiles of changes of propionate, SOP, sludge PHAs and glycogen in the A/O- and AEI-SBRs were shown in Figs. 5 and 6, respectively. There was a quick depletion of propionate and an obvious SOP release, coupled with PHAs accumulation and glycogen degradation during the anaerobic period of the A/O-SBR. During the following aerobic phase, SOP uptake, PHAs utilization, and glycogen replenishment

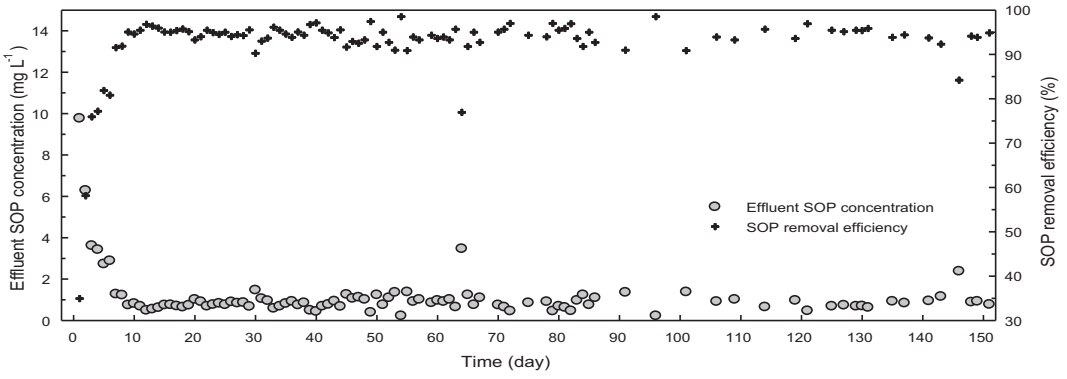

Fig. 2 – Variations in the effluent SOP concentration and the % SOP removed in the AEI-SBR during the 151 days tests.

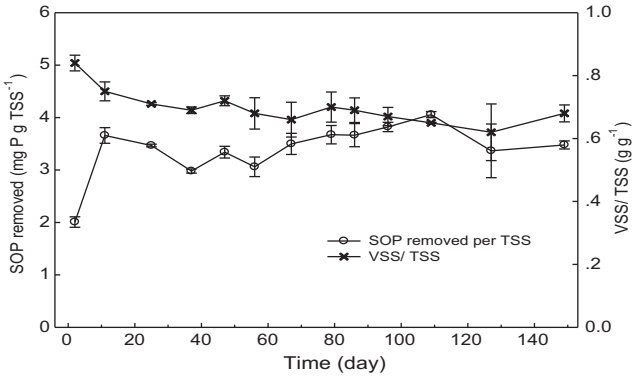

Fig. 3 – Changes of SOP removed per gram of TSS and the VSS/TSS ratio in the AEI-SBR during the 151 days tests.

Table 1 – Comparison of reactor performances between AEI- and A/O-reactors during steady-state operation[a].

Item	AEI-mediated reactor	A/O-mediated reactor
Effluent SOP (mg L^{-1})	0.76 ± 0.17	2.53 ± 0.37
SOP removal efficiency (%)	94.9 ± 1.2	83.1 ± 2.5
TP content of TSS (mg P g TSS^{-1})	79 ± 3	65 ± 4
Effluent NH_4^+–N (mg L^{-1})	1.38 ± 0.63	1.22 ± 0.59
Effluent NO_2^-–N (mg L^{-1})	0.15 ± 0.06	0.12 ± 0.04
Effluent NO_3^-–N (mg L^{-1})	9.75 ± 0.61	11.93 ± 0.57
Effluent pH	8.03 ± 0.08	8.15 ± 0.11

a Results are the averages and their standard deviations, and data are obtained during day 106 and day 129.

took place concurrently, and the SOP aerobic uptake was greater than the anaerobic release. This behavior was the classical phenotype of PAOs-enriched cultures in the A/O system and was similar to that observed by other researchers (Smolders et al., 1994; Pijuan et al., 2004; Tong and Chen, 2007). In addition, the cyclic variations of pH and DO (Fig. S2, Supporting Information) were also similar with the data monitored by other researchers (Chen et al., 2004; Zhang, 2007). Compared with the A/O-SBR, propionate was fully taken up in 45 min of the aeration accompanied by the substantial accumulation of PHAs in the AEI-SBR, obvious SOP release and glycogen degradation were not occurred as they were in the A/O-SBR. After propionate was consumed, PHAs degradation and SOP uptake were observed simultaneously. Glycogen was slightly synthesized during the aerobic period. During the idle period, about 6 mg L^{-1} phosphorus released, the aerobic accumulated glycogen was also consumed coupled with a slight synthesis of PHAs.

To further understand the differences between the AEI- and A/O-SBRs, the experimental results obtained in the AEI-SBR were compared with the stoichiometric coefficients monitored in the A/O-SBR (this study) and other previous studies, as outlined in Table 2. It was clear that the stoichiometry of the A/O-SBR from this study correlated well with the metabolic stoichiometric coefficients displayed in other A/O studies for both acetate and propionate. However, the PHAs-up/VFA ratio detected in the AEI-SBR was much lower than that in the A/O-SBR (0.46 ± 0.07 vs 1.16 ± 0.09 mmol-C/mmol-C). According to the metabolic pathway from VFA to PHAs, the uptake of VFA and the conversion to acetyl-CoA (or propionyl-CoA) required adenosine triphosphate (ATP), and the subsequent conversion to PHAs required $NADH_2$ to maintain the redox balance (Smolders et al., 1994; Oehmen et al., 2005b). In the metabolic models of biological phosphorus removal (BPR), two sources for $NADH_2$ production (the tricarboxylic acid cycle (TCA) and glycogen degradation, Fig. S3, Supporting Information) were documented in the literatures. Glycogen degradation and poly-P hydrolysis were considered as the primary sources for $NADH_2$ and ATP generations required for PHAs accumulation in the A/O system, respectively, but the TCA cycle seemed to provide both $NADH_2$ and ATP for PHAs synthesis in the AEI-reactor for negligible glycogen degradation and SOP release were measured during the period of PHAs accumulation. That is, a part of propionate would be oxidized though the TCA cycle to supply $NADH_2$ and ATP for PHAs storage, which was one reason for the much lower PHAs-up/VFA ratio monitored in the AEI-SBR. Furthermore, since PHAs accumulation/VFAs consumption in the AEI-SBR were occurred during the aerobic period, cell growth would take place along with those transformations and consumed some propionate and/or PHAs, which also reduced the PHAs-up/VFA ratio. Consequently, the aerobic glycogen synthesis in the AEI-SBR was also lower than that in the A/O-SBR (0.52 ± 0.06 vs 1.83 ± 0.12 mmol-C/g VSS). Since propionate was consumed completely during the aerobic period, the dominating ATP consumption during the idle period of the AEI-SBR was for the purpose of cell maintenance, and this energy seemed to be supplied via poly-P cleavage and glycogen degradation. From the idle transformations of glycogen and PHAs measured in the AEI-SBR, "3HV fermentation" might occur in the idle period. This metabolism is a combination of glycolysis, succinate-propionate pathway, and PHV production, and a net production of ATP is produced from the conversion of glycogen to 3HV (Fig. S4, Supporting Information). Lopez et al. (2006) indicated that the anaerobic conversion of glycogen to PHV could generate energy for maintenance purpose during anaerobic starvation. Furthermore, SOP release rate in the idle period of the AEI-reactor was much lower than that in the anaerobic period of the

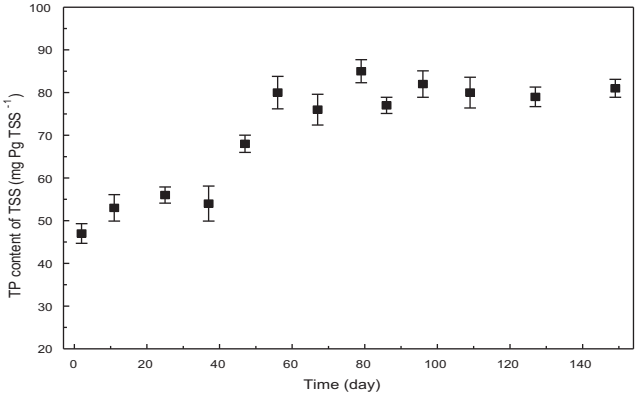

Fig. 4 – Variations in TP content of TSS during the 151 days tests.

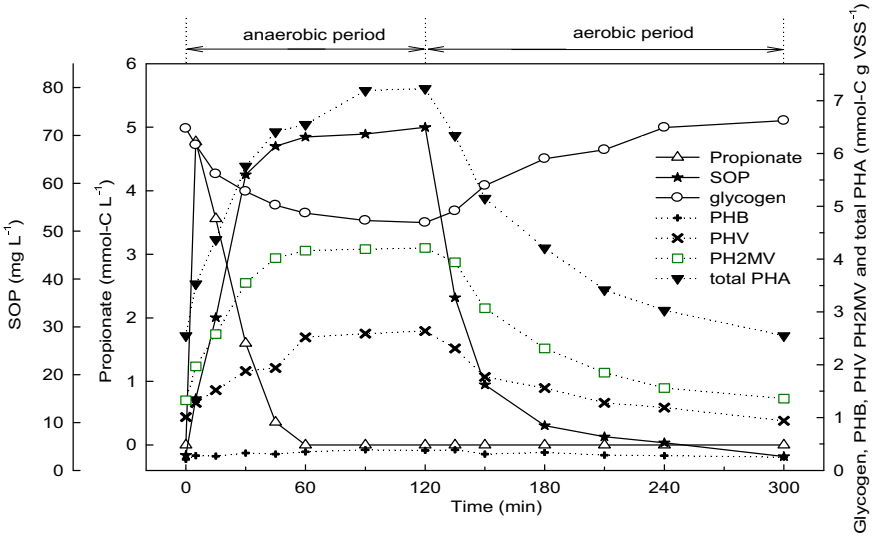

Fig. 5 – Variations of SOP, propionate and sludge glycogen as well as sludge PHAs during one cycle (day 109) in the A/O-reactor.

A/O-reactor owing to the lower energy required for maintenance purpose, thus resulting in a much lower SOP uptake ratio in the subsequent aerobic zone.

3.4. *Mechanism of the AEI-SBR achieving higher phosphorus removal performance*

In this study higher phosphorus removal efficiency was obtained in the AEI-SBR than that in the A/O-SBR. It was therefore necessary to investigate the reasons for improved phosphorus removal efficiency achieved in the AEI-SBR.

Fig. 7 showed the results of DAPI staining of the sludge samples taken from both reactors on day 109. Samples of the activated sludge biomass maintained in the AEI-SBR contained a more abundance of cells (45.3%) with visible poly-P inclusions than was found in sludge sampled from the A/O-SBR (36.7%). Also from Fig. 7, it could be observed that samples from the A/O-SBR seemed to contain a greater abundance of bacteria with tetrad-type morphology, which looked like a group of GAOs, *Competibacter*, belonging to the *Alphaproteobacteria* phylum (Oehmen et al., 2005a; Schroeder et al., 2008). The *Alphaproteobacteria* GAOs, *Competibacter*, are well-known organisms capable of competing with PAOs for anaerobic VFA uptake but do not performing anaerobic SOP release and aerobic SOP uptake (Tsai and Liu, 2002). Thus, one reason for the AEI-SBR showing higher phosphorus removal performance was due to the more poly-P containing cells cultured in the activated sludge.

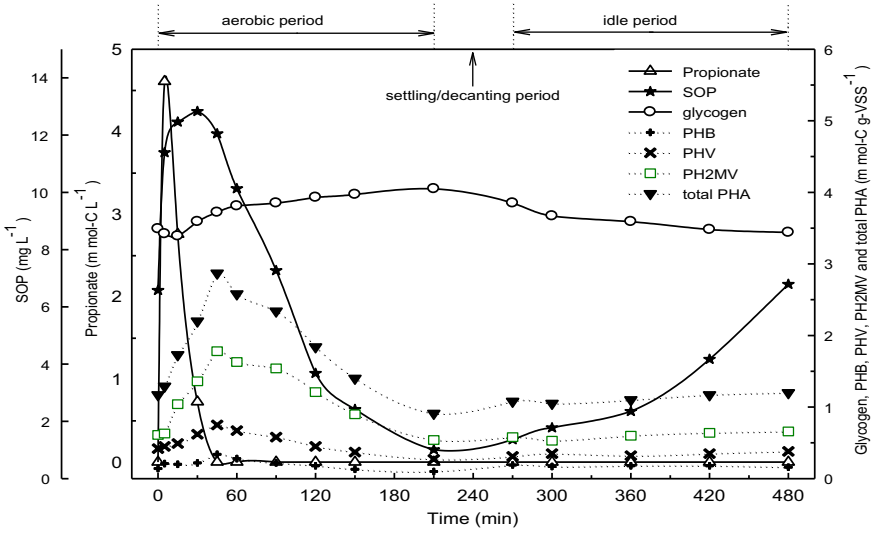

Fig. 6 – Variations of SOP, propionate and sludge glycogen as well as sludge PHAs during one cycle (day 109) in the AEI-reactor. 20 L supernatant was discharged during min 265 to 270, thus aqueous volume calculated for the release of SOP concentrations during idle period was 20 L. Additionally, 20 L synthetic wastewater was fed to reactor during min 0 to 5, and data measured at min 0 represented the values which were obtained at the end of the idle period of last cycle.

Table 2 – Comparison of the stoichiometric coefficients and operational conditions between AEI- (this study) and A/O-mediated reactors (this study and previous studies).

Study	A/O operated reactor					AEI operated reactor						Substrate type	COD/P rate in feed (mg COD/mg P)	pH
	Anaerobic transformations			Aerobic transformations		Aerobic transformations			Idle transformations					
	PHA-up/VFA (C-mol/C-mol)	Gly-de/VFA (C-mol/C-mol)	P-rel/VFA (P-mol/C-mol)	Gly-syn (mmol-C/g VSS)	P uptake rate (mmol-P/g VSS h)	PHA-up/VFA (C-mol/C-mol)	Gly-syn/VFA (mmol-C/g VSS)	P uptake rate (mmol-P/g VSS h)	Gly-de (mmol-C/g VSS)	PHA-up (mmol-C/g VSS)	P release rate (mmol-P/g VSS h)			
This study[a]	1.16 ± 0.09	0.47 ± 0.05	0.41 ± 0.06	1.83 ± 0.12	0.39 ± 0.04	0.46 ± 0.07	0.52 ± 0.06	0.036 ± 0.002	0.57 ± 0.08	0.38 ± 0.06	0.022 ± 0.001	Propionate	20	Not control
Vargas et al. (2011)[b]	0.94	0.15	0.38 ± 0.08	1.68	0.41	–	–	–	–	–	–	Propionate	15	7.50 ± 0.05
Vargas et al. (2011)[b]	1.23	0.08	0.55 ± 0.07	0.9	0.48	–	–	–	–	–	–	Acetate	15	7.50 ± 0.05
Vargas et al. (2009)	1.18	0.58	0.56	–	–	–	–	–	–	–	–	Propionate	15	7.50
Pijuan et al. (2009)	1.39	0.91	0.107	–	–	–	–	–	–	–	–	Acetate	4.18	7.0 ± 0.1
Pijuan et al. (2009)	0.64	0.45	0.268	–	–	–	–	–	–	–	–	Propionate	4.17	7.0 ± 0.1
Tong and Chen (2007)	1.19	0.55	0.29	2.44	0.33	–	–	–	–	–	–	Acetate	24.93	Not control
Lu et al. (2006)	1.22	0.29	0.44	–	–	–	–	–	–	–	–	Propionate	20	7.0–8.0
Lu et al. (2006)	1.26	0.46	0.62	–	–	–	–	–	–	–	–	Acetate	20	7.0–8.0
Oehmen et al. (2005a)	1.22	0.33	0.42	–	–	–	–	–	–	–	–	Acetate	15	7.0
Oehmen et al. (2005b)	1.23	0.32	0.42	–	–	–	–	–	–	–	–	Propionate	15	7.0 ± 0.1
Pijuan et al. (2004)	0.91	0.21	0.42	–	0.72	–	–	–	–	–	–	Propionate	14	Not control
Filipe et al. (2001)	1.30	0.53	0.73	–	–	–	–	–	–	–	–	Acetate	23.6	8.0 ± 0.1

Smolders et al. (1994)	1.30	–	0.60	–	–	–	–	–	–	–	Acetate	26.67	7.4	
Smolders et al. (1994)	1.22	–	0.50	–	–	–	–	–	–	–	Acetate	26.67		7.0 ± 0.05

a Data reported in this study are the averages and their standard deviations of 3 cyclic studies.
b Operated as a nitrite-based anaerobic anoxic regime.

As shown in Table 1, substantial quantities of effluent nitrate were measured in both reactors and half of the accumulated nitrate would be entered into the next cycle via the remaining. According to the views summarized in the literature (Guerrero et al., 2011), the availability of nitrate in the anaerobic reactor would deteriorate the performance of BPR in the A/O system owing to (a) the presence of some denitrification intermediates (e.g. nitrite) which would have an inhibitory effect on PAOs and (b) consuming substrate for denitrification which would result in less carbon source available for PAOs growth. However, the effect of nitrate on the phosphorus removal performance in the AEI-SBR remained unknown. Thus it was necessary to investigate if nitrate would bring different effects on phosphorus removal between the AEI- and A/O-SBRs, and the results were displayed in Table 3. It can be seen that with the increase of influent nitrate maximal anaerobic nitrite was gradually increased in the A/O-reactors, and the PHAs-up/VFA ratio and anaerobic SOP release were substantially decreased, resulting in an obviously increase of aerobic end phosphorus. Especially at the influent nitrate concentration of 20 mg L^{-1}, phosphorus removal failure was almost detected. The data monitored in the AEI-reactors, however, showed a much weaker influence of nitrate on the phosphorus removal. The PHAs-up/VFA ratio and nitrite accumulation were slightly affected by the influent nitrate increase, and a few increase of aerobic end phosphorus was detected. Even at the influent nitrate concentration of 20 mg L^{-1}, 2.76 ± 0.17 mg L^{-1} of phosphorus was only measured in effluent, which indicated about 81% of influent SOP could be removed. It can be easily understood therefore that the high tolerance of nitrate of the AEI-SBR could alleviate the deterioration of phosphorus removal due to nitrate recirculation as compared with the A/O-SBR. This character further suggested that the AEI regime might be a promising approach to phosphorus removal with a higher stability in operation.

It is well-known that BPR is related to the transformations of intracellular PHAs and glycogen (Smolders et al., 1994; Mino et al., 1998). As shown in Figs. 5 and 6, A/O-SBR showed much greater cycle variations of PHAs and glycogen. It is reported that the transformations of PHA and glycogen in BPR are associated with the activities of PAOs and GAOs, and higher transformation of glycogen indicates that the metabolism of GAO might be activated (Mino et al., 1998; Zheng et al., 2011). Thus, the lower glycogen transformation measured in the AEI-SBR indicated that the biomass cultured in the AEI-SBR contained less GAOs activities than that in the A/O-SBR, which might be another reason for the higher phosphorus removal performance in the AEI-SBR. This hypothesis was consistent with the results of DAPI staining shown in Fig. 7.

Additionally, the current metabolic models of phosphorus removal considered that the energy for SOP uptake came from PHAs degradation, and PHAs degradation in the A/O-SBR was higher than that in the AEI-SBR (4.68 vs 1.96 mmol-C/g VSS, Figs. 5 and 6). Therefore, one might want to know why higher PHAs degradation in the A/O-SBR resulted in a lower SOP removal. One possible reason is that the degradation of PHAs is used not only for SOP uptake but for glycogen synthesis as well as cell growth. If a higher glycogen synthesis and/or cell

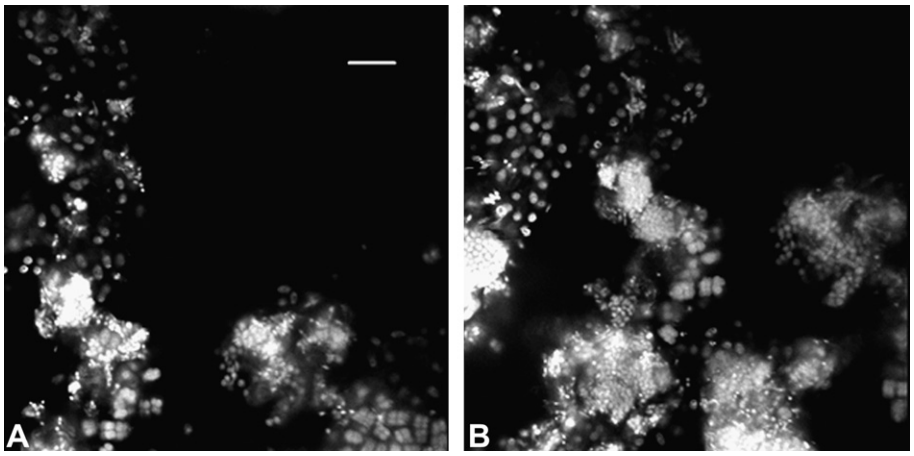

Fig. 7 – Micrographs of DAPI staining of the sludge samples taken from the A/O- (A) and the AEI- (B) reactors (Sludge samples were obtained on day 109). Poly-P inclusions were found to be bright white with DAPI staining. Bar = 5 μm.

Table 3 – Effect of influent NO_3–N on the phosphorus removal performances of AEI- and A/O- reactors[a].

Influent NO_3–N (mg L^{-1})	A/O operated reactor				AEI operated reactor		
	PHA-up/VFA (C-mol/C-mol)	Maximal anaerobic nitrite (mg L^{-1})	Anaerobic end phosphorus (mg L^{-1})	Aerobic end phosphorus (mg L^{-1})	PHA-up/VFA (C-mol/C-mol)	Maximal aerobic nitrite (mg L^{-1})	Aerobic end phosphorus (mg L^{-1})
0	1.31 ± 0.08	0.15 ± 0.04	80.11 ± 2.14	0.37 ± 0.07	0.48 ± 0.08	0.23 ± 0.07	0.41 ± 0.06
2.5	1.29 ± 0.06	0.39 ± 0.05	77.39 ± 1.45	1.96 ± 0.16	0.52 ± 0.05	0.22 ± 0.04	0.53 ± 0.05
5	1.20 ± 0.09	0.46 ± 0.07	72.46 ± 1.89	2.84 ± 0.32	0.49 ± 0.06	0.25 ± 0.08	0.72 ± 0.12
7.5	1.12 ± 0.06	0.57 ± 0.08	69.32 ± 1.77	3.26 ± 0.36	0.42 ± 0.08	0.38 ± 0.06	0.78 ± 0.19
10	0.97 ± 0.05	1.33 ± 0.10	57.79 ± 1.16	5.91 ± 0.43	0.40 ± 0.07	0.67 ± 0.09	1.13 ± 0.21
20	0.76 ± 0.07	3.52 ± 0.11	39.78 ± 1.22	11.31 ± 0.65	0.33 ± 0.04	2.39 ± 0.13	2.76 ± 0.17

a Data reported are the averages and their standard deviations of 3 cyclic studies.

growth occurred, then less PHAs could be left for taking up SOP even though the PHA degradation was higher. Though cell growth was approximate in both reactor (because similar VSS concentration was measured in both reactor, data not shown), the aerobic synthesis of glycogen in the A/O-SBR was much higher than that in the AEI-SBR (1.93 vs 0.56 mmol-C/g VSS). Accordingly, more PHAs were used for glycogen synthesis, and less PHAs were used for SOP uptake in the A/O-SBR. Besides, it was found that there was much greater SOP release in the anaerobic period (the A/O-SBR, Fig. 5), as compared with that in the idle and initial aerobic periods (the AEI-SBR, Fig. 6). Consequently, at the beginning of PHAs degradation, SOP concentration in the A/O-SBR was much higher than that in the AEI-SBR (71.70 vs 12.05 mg L^{-1}). That is, more PHAs was consumed for taking up the released SOP in the A/O-SBR, which might affected the energy supply for SOP uptake from influent wastewater (i.e., synthetic medium in this study).

According to the above studies, it was clearly showed that the AEI regime could drive a good BPR. Compared with the current A/O regime, a strict anaerobic period is not performed while an extended-idle period (210–450 min) is operated in the AEI regime. Thus, another question as to why the AEI regime induced BPR required to be discussed. It is known that the essential of BPR is to enrich some specific microorganisms to accumulate SOP in excess of metabolic requirement and to store this as poly-P (i.e., poly-P containing bacteria) (Mullan et al., 2006). The quantity of these bacteria is largely dependent on whether the operational condition can provide a selective advantage to poly-P containing cells over other populations. As is well-known, PHA, glycogen and poly-P are the three most common intracellular energy storages. Among them, poly-P is hardly utilized under aerobic (even aerobic starvation) conditions, but easily degraded under anaerobic conditions (Lopez et al., 2006), which can ensure poly-P to keep high levels even at the end of an aerobic starvation period. In the AEI-SBR, propionate is depleted during the initial aeration, and PHA and glycogen are also decreased to very low levels at the end of the aerobic period, which indicated that energy generated from those is deficient in the extended-idle period. However, poly-P degradation can nicely meet the energy requirement for bacterial maintenance in the idle period, and seems as the only suitable energy source during this period. With the idle time increase, energy requirement for bacterial maintenance in the idle period increased. That is, AEI regime correlates well with poly-P containing cells' metabolism, and can provide a selective advantage to poly-P containing cells over other populations.

4. Conclusions

The results obtained in this study showed that the AEI regime could achieve successfully BPR. During the steady-state operation, the AEI-SBR had higher phosphorus removal efficiency than the A/O-SBR (around 95% in the AEI-SBR and 83% in the A/O-SBR, respectively). Further investigations showed that the AEI-SBR had more poly-P containing cells in sludge, forbore higher level of nitrate, and had lower glycogen transformation, which were the reasons for the higher phosphorus removal performance.

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Appendix A. Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.watres.2012.04.036.

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