Desalination and Water Treatment
www.deswater.com
47 (2012) 42–49
September
1944-3994/1944-3986
doi: 10.1080/19443994.2012.696418
Taylor & Francis
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High phosphorus shock loading induced glycogen accumulating organisms in anaerobic/oxic sequencing batch reactor

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Received 30 September 2011; Accepted 1 March 2012

ABSTRACT

This study investigated the long-term adaptability of an acetate-fed anaerobic–oxic sequencing batch reactor, operated at 15 days of solid retention time (SRT), to the influent P/COD loading raised from 15/400 to 40/400 mg/mg. Experiment results showed that after four SRTs operation, the anaerobic P release, aerobic P uptake and sludge P content decreased apparently, implying the metabolism of phosphate-accumulating organisms (PAOs) was inhibited. In contrast, anaerobic glycogen consumption and polyhydroxyalkanoates (PHAs) accumulation per acetate uptake increased observably, and 3HV/PHAs ratio elevated gradually to 21.8%. These results indicated that the proliferation of glycogen-accumulating organisms (GAOs) was encouraged. When influent phosphorus was reduced back to 15 mg/L, sludge P content diminished and a low P release/acetate uptake ratio was achieved, suggesting that PAOs' metabolism was not restored. The above findings concluded that long-term operation of the high phosphorus influent inhibited the proliferation of PAOs, and more importantly provided GAOs a competitive advantage over PAOs.

Keywords: Phosphorus removal; Phosphate-accumulating organisms; Glycogen-accumulating organisms; Biological treatment; High phosphorus loading

1. Introduction

Enhanced biological phosphorus removal (EBPR) process has been widely recognized as one of the most economical and sustainable methods to remove phosphate from wastewater. The phosphate removal function of this biological process occurs as a result of the predominance of a group of bacteria, called phosphate-accumulating organisms (PAOs). These microorganisms are capable of storing extracellular phosphate as intracellular polyphosphate, leading to phosphorus removal from the bulk liquid phase. Phosphorus removal is, therefore, achieved by wasting excess sludge with high phosphorus content. However, the failure of phosphorus removal was occasionally observed in laboratory-scale and full-scale EBPR systems. Previous studies [1,2] have attributed the deterioration of the EBPR processes to the appearance of another specific organisms, i.e. glycogen-accumulating organisms (GAOs). GAOs, like PAOs but without contribution of phosphorus removal, are also capable of

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sequestrating volatile fatty acids (VFAs) anaerobically and converting them to polyhydroxyalkanoates (PHAs). The anaerobic metabolism of GAOs is similar to that of PAOs, except for energy required for anaerobic VFAs uptake. GAOs use glycogen as their sole energy source, whereas PAOs generate energy by both poly-p degradation and glycogen hydrolysis. Due to the competition between PAOs and GAOs occurs in the anaerobic phase of the EBPR process, the presence of GAOs in EBPR processes can potentially affect the capability of the process to remove phosphorus.

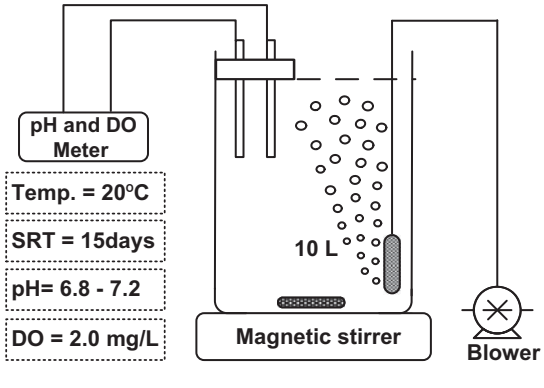
It is important to identify operational conditions that positively support PAOs to take up VFAs, while negatively affect GAOs. The influent phosphorus/chemical oxygen demand (P/COD) ratio (wt/wt) was found to be one of the most important factors influencing the competition between PAOs and GAOs [3]. When the P/COD feeding ratio of 30/400 mg/mg was provided, PAOs' metabolism could be enhanced to successfully out-compete GAOs. In contrast, reducing the P/COD feeding ratio to 3/400 mg/mg eventually led to the enrichment of GAOs [4]. Sudiana et al. [5] also observed that the growth of PAOs was suppressed under limited P/COD influent loading, whereas PAOs were encouraged under phosphorus-rich loading condition. Besides, a high P/COD ratio (e.g. 5/100–10/100 mg/mg) was indicated by Oehmen et al. [6] to favor the growth of PAOs. So far, high P/COD influent was confirmed by most of the previous studies [7–11] to enhance the proliferation of PAOs. However, experiments for 5.5 years operation showed that doubling the influent phosphate to 20 mg P/L brought about an immediate increase of phosphate removal in a sequencing batch reactor (SBR), and then the reactor exhibited a long-term downward trend in the efficiency of phosphate removal [12]. Besides, after a period of 2 months operation under an influent P/COD ratio of 30/400 mg/mg, an anaerobic-oxic (A/O) reactor unexpectedly suffered a decrease of sludge P content and an increase of the aerobic phosphate concentration [4].

The deterioration of the above reactors seems to occur at a long-term operation under high P/COD influent. Besides, the failure of these reactors under high P/COD influent has not been well characterized, particularly in terms of the competition between PAOs and GAOs in these processes. This study, therefore, investigated the long-term adaptability of an acetate-fed A/O SBR to the influent P/COD loading raised from 15/400 to 40/400 mg/mg. The cyclic studies of the reactor were also implemented at different cultivating periods so as to evaluate the PAOs–GAOs competition in the A/O SBR under high phosphorus loading. Besides, the recovery of reactor performance was examined after the influent P/COD was reduced back to 15/400 mg/mg.

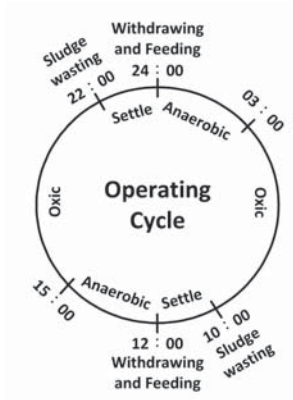
2. Materials and methods

2.1. Reactor operation

A laboratory-scale A/O SBR with a working volume of 10 L was constructed to investigate the effect of high phosphorus loading on EBPR process. This A/O reactor was inoculated with an activated sludge from the sludge source SBR which was operated at the influent P/COD loading of 15/400 mg/mg (the same influent as the A/O reactor) and exhibited a stable performance of phosphorus removal in our laboratory. Fig. 1 displays the schematic diagram and daily operating mode of the A/O reactor. The A/O reactor was situated in a room with a constant temperature of 20°C. This A/O reactor, operated 2 cycles per day,



(a) Schematic diagram of the A/O reactor.



(b) Daily operating mode of the A/O reactor.

Fig. 1. The schematic diagram and daily operating mode of the A/O reactor.

consisted of 3 h anaerobic phase, 7 h aerobic phase, and 2 h settle/decant period. At the end of the oxic phase, 330 ml of mixed liquor was removed so as to maintain a 15 days of SRT. After settling, 4,670 ml of supernatant was withdrawn and 5 L of fresh medium was supplied. When the A/O reactor reached a steady-state performance of phosphorus removal, the influent P/COD loading was raised from 15/400 to 40/400 mg/mg, and then two stages of the experiments were implemented. The stage 1 was operated under an influent P/COD loading of 40/400 mg/mg for a 132 days operation and the stage 2 was performed by reducing the influent P/COD back to 15/400 mg/mg for a 38 days operation.

2.2. Culture medium

The culture medium of A/O reactor consisted of a carbon source and nutrients. Sodium acetate (400 mg COD/L of $CH_3COONa{\cdot}6H_2O$) was used as the carbon source in influent. The nutrient solution was composed of 48 mg $MgSO_4{\cdot}7H_2O$, 21.6 mg $CaCl_2$, 72 mg NH_4Cl, 24 mg peptone, 110.67 mg KH_2PO_4, 84 mg K_2HPO_4, and 0.28 ml of the trace elements solution (per liter of distilled water). Each liter of the trace elements solution consisted of 6 g $FeCl_3$, 0.6 g H_3BO_3, 0.12 g $CuSO_4$, 0.72 g KI, 0.48 g $MnCl_2$, 0.24 g Na_2MoO_4, 0.48 g $ZnSO_4$, 0.6 g $CoCl_2$, and 40 g EDTA. These medium solutions were mixed together and adjusted to pH 7.0 ± 0.2 by addition of 1 M HCl and 1 M NaOH. The synthetic influent into the SBR contained 400 mg/L of chemical oxygen demand (COD), 20 mg/L of ammonia-nitrogen (NH_4^+-N) and 40 mg/L of phosphate (PO_4^{3-}-P), respectively. Besides, the influent phosphorus loading of 15 mg/L was prepared by adjusting the amount of KH_2PO_4 and K_2HPO_4 in the above nutrient solution to 41.5 mg and 31.5 mg.

2.3. Analytical techniques

The mixed liquor sample taken from reactor was immediately filtered through a Whatman GF/C fiberglass membrane (0.45 μm) so as to examine the concentration of soluble chemical oxygen demand (SCOD), acetate, orthophosphate (PO_4^{3-}-P). SCOD, PO_4^{3-}-P, and mixed liquor suspended solid (MLSS) were analyzed according to standard methods [13]. Acetate was analyzed with ion chromatography (Dionex Corporation, DX-120) equipped with an organic acids analysis column (AS1). Polyhydroxyalkanoates analysis was performed using the method proposed by Brandl et al. [14], Comeau et al. [15], and Liu et al. [16]. Intracellular carbohydrate was determined by the method of Herbert et al. [17].

3. Result and discussion

3.1. Effect of high P/COD influent on EBPR process

3.1.1. Long-term operation of the A/O reactor

The operating results of the A/O reactor during the whole experimental period were demonstrated in Fig. 2. As was shown in this figure, a period of steady-state performance of the A/O reactor was obtained before the P/COD ratio in influent was increased from 15/400 to 40/400 mg/mg. This operating period exhibited a stable SCOD removal at the end of anaerobic and aerobic phases. Besides, anaerobic phosphate release and aerobic phosphate uptake were steadily sustained in the A/O reactor, and the sludge P content was maintained stably at 8.1%. This stable performance of the A/O reactor, therefore, demonstrated a typical pattern of phosphorus metabolism in an EBPR process. When the influent phosphorus loading was raised to 40 mg/L, the anaerobic phosphate concentration and sludge P content, as is shown in Fig. 2, increased rapidly at the initial period of stage 1. These results apparently indicated that PAOs were enhanced in a short-term period of adaptation to the suddenly increase of the influent P/COD loading from 15/400 to 40/400 mg/mg.

However, continuously feeding high P/COD influent led gradually to the decrease of anaerobic phosphate release and aerobic phosphate uptake, as is indicated in the later period of stage 1 (Fig. 2). The anaerobic phosphate concentration declined significantly from 120 to 39 mg/L and the aerobic phosphate concentration increased progressively from zero to 9.6 mg/L. Consequently, the sludge P content dropped dramatically from 16.0 to 10.1%; that is, PAOs' metabolism was observably inhibited in the A/O reactor by the long-term cultivating with high P/COD influent. Nevertheless, the sequestration of anaerobic SCOD remained unchanged and the metabolism of glycogen elevated steadily, implying that PAOs' competitor, i.e. GAOs, was enhanced. The above findings suggested that continuously feeding this high P/COD influent eventually resulted in the deterioration of phosphorus removal, and more importantly caused the PAOs to gradually lose the dominance in the A/O reactor.

When the influent P/COD loading was reduced back to 15/400 mg/mg, merely 36.8 mg/L of the anaerobic phosphate concentration was observed in the A/O reactor (Fig. 2). This anaerobic phosphate concentration was relatively lower as compared with the concentration in steady-state period. Besides, the sludge P content decreased again from 10.1 to 7.0% in this reactor. These results suggested that PAOs'

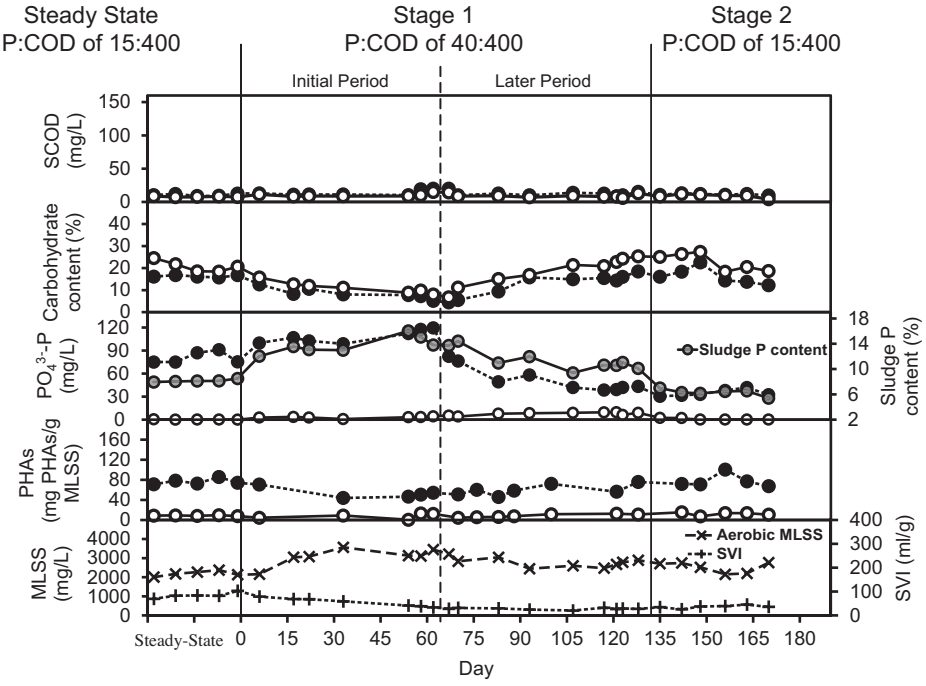


Fig. 2. Operating performance of the A/O reactor during the whole experimental period (•) end of anaerobic phase, (○) end of aerobic phase.

metabolism was not recovered. Nevertheless, the gradual increase of glycogen metabolism accompanying with higher PHAs accumulation seems to imply that GAOs were promoted in this A/O reactor. Since the phosphate metabolism of PAOs was still observed in this A/O reactor, a low residual phosphate concentration was obtained at the end of aerobic phase.

3.1.2. Cyclic studies of the A/O SBR

The cyclic operating performances of the A/O reactor were further utilized to investigate the metabolism of PAOs and GAOs. Fig. 3(a) illustrates the cyclic study of the steady-state performance of the A/O reactor operated at the P/COD ratio of 15/400 mg/mg in influent. As is shown in Fig. 3(a), the profiles of phosphate release and uptake demonstrated a typical pattern of PAOs in an EBPR process. Acetate was totally depleted in the anaerobic phase, concurrently with the phosphate concentration of 81.4 mg/L and the PHAs accumulation of 72.0 mg PHAs/g MLSS. Then, during the aerobic phase, complete phosphate uptake was achieved and the stored PHAs were used for cell growth and cell maintenance.

Fig. 3(b) presents the cyclic operating performances of the A/O reactor at initial period of stage 1 (54th day). The anaerobic phosphate concentration, as observed from a comparison of Fig. 3(b) with Fig. 3(a), increased observably from 81.4 mg/L (P/COD = 15/400 mg/mg, steady-state period) to 112.1 mg/L (P/COD = 40/400 mg/mg, initial period of stage 1). Besides, anaerobic PHAs accumulation decreased from 72.0 to 46.5 mg PHAs/g MLSS. These results indicated that PAO's behavior was supported by the increase of influent phosphorus loading. Nevertheless, the duration required for complete acetate uptake was delayed to 40 min, implying that this influent phosphorus loading had been probably over the capability of PAOs in the A/O reactor.

The cyclic operating performances on 123th day (later period of stage 1) are shown in Fig. 3(c). The anaerobic phosphate concentration is shown in this figure to be only 41.2 mg/L. This phosphate concentration was significantly lower than that (112.1 mg/L) in Fig. 3(b). Moreover, 6.2 mg/L of phosphate is indicated in Fig. 3(c) to be leftover at the end of aerobic phase. That is, the phosphorus metabolism of the sludge was significantly suppressed in the A/O reactor during this period. Furthermore, the PHAs accumulation and glycogen degradation increased visibly during the anaerobic phase, as indicated from a comparison of Fig. 3(c) with Fig. 3(b). This suggested that GAOs' metabolism was raised in the A/O reactor. The above findings concluded that long-term operation with high P/COD influent can seriously inhibit the proliferation of PAOs in an A/O process, and provide GAOs a selective advantage over PAOs.

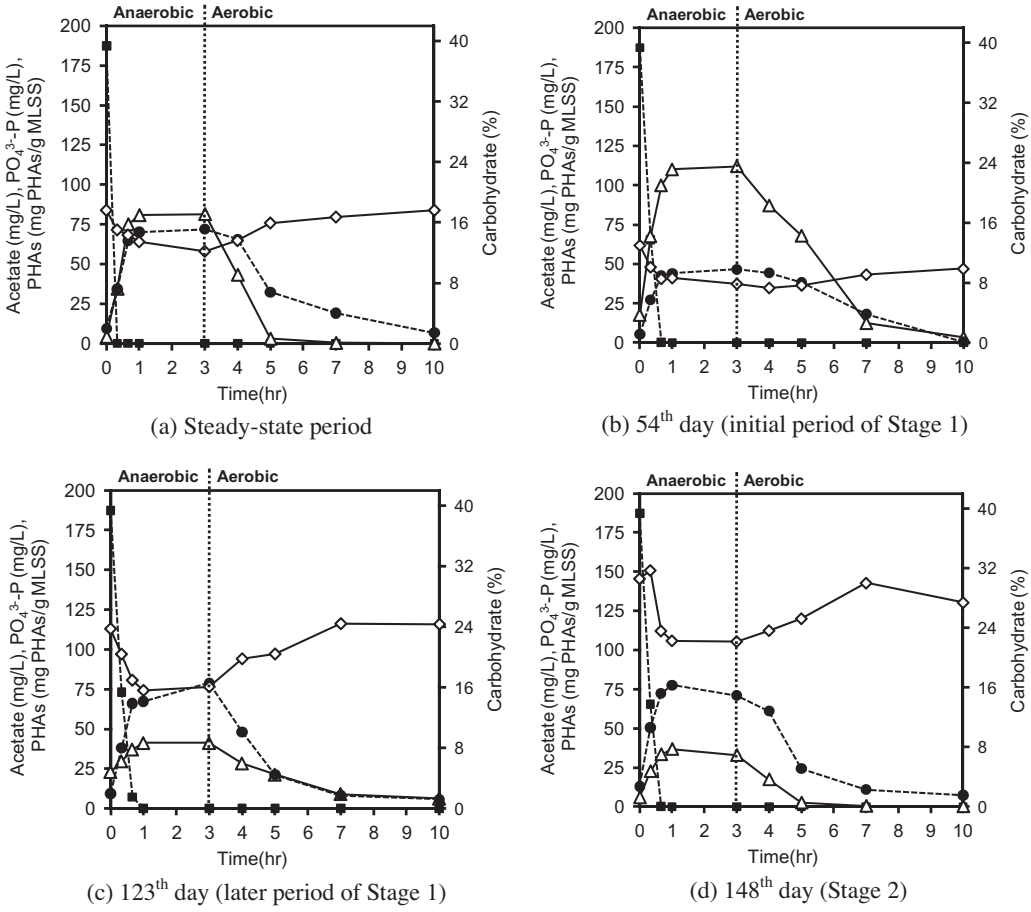


Fig. 3. Cyclic performances of the A/O reactor at different operational periods (■) acetate, (△) PO_4^{3-}-P, (●) PHAs, (◇) carbohydrate.

When the influent P/COD loading was reduced back to 15/400 mg/mg, the cyclic operating performance of the A/O reactor on 148th day (stage 2) was examined and is displayed in Fig. 3(d). Acetate was indicated in this figure to be totally sequestrated; however, only 36.8 mg/L of the phosphate concentration was achieved during the anaerobic phase. This anaerobic phosphate concentration was significantly lower than that of steady-state period (81.4 mg/L); that is, PAOs' metabolism was not restored. On the contrary, PHAs accumulation and glycogen degradation in the anaerobic phase still maintained at high levels (similar to later period of stage 1). These results indicated that GAOs, instead of PAOs, dominated this A/O reactor. The above results suggested that PAOs lost the competitive advantage and GAOs became the predominant microorganisms in the A/O reactor.

3.2. Effect of high P/COD influent on the competition between PAOs and GAOs

The stoichiometries of anaerobic acetate metabolism at different operation periods were summarized in Table 1, so as to investigate the cause for the dramatic transition of reactor performance under high P/COD influent operation. As Table 1 depicts, the anaerobic phosphate released/acetate uptake (P/HAc) ratio was steadily increased from 0.36 (steady-state period) to 0.40 and 0.49 P-mol/C-mol (the initial period of stage 1) when influent phosphorus loading was elevated to 40 mg/L. The P/HAc ratio was suggested by Saunders et al. [18] and Schuler and Jenkins [19] to assess the relative activity of PAOs and GAOs in an EBPR process. Besides, the P/HAc ratio was estimated by Smolders et al. [20] to be approximately 0.5 P-mol/C-mol at pH 7.0 when PAOs were highly enriched in an A/O SBR. Thus, the steady increase of P/HAc ratio implied that raising P/COD ratio in influent promoted the proliferation of PAOs in the A/O reactor. Furthermore, the fraction of PAOs (f_{PAOs}) in the A/O reactor was determined by adopting the same calculation procedure proposed by Yagci et al. [21]. The f_{PAOs} value, as is shown in Table 1, increased from 0.32 to 0.47 during this period. This result further confirmed that high P/COD ratio in influent enhanced the PAOs in short-term period of adaptation.

Table 1
Stoichiometries of anaerobic acetate metabolism at different operational periods

Date	P/HAc (P-mol/C-mol)	Gly/HAc (C-mol/C-mol)	PHAs/HAc (C-mol/C-mol)	3HB/PHAs (wt.%)	3HV/PHAs (wt.%)	f_{PAOs}
Steady-state	0.36	0.75	0.74	81.1%	18.8%	0.32
23th d (initial period of stage 1)	0.40	0.45	0.65	83.3%	16.2%	0.47
54th d (initial period of stage 1)	0.49	0.73	0.78	82.6%	16.2%	0.37
123th d (later period of stage 1)	0.09	1.04	1.34	77.6%	21.8%	≈ 0
148th d (stage 2)	0.14	0.88	0.85	79.5%	19.7%	≈ 0

The P/HAc ratio, however, diminished to 0.09 P-mol/C-mol on 123th day (i.e. the later period of stage 1), as depicted in Table 1. The P/HAc ratio was dramatically lower than that in initial period of the same stage, suggesting that continuous operation of high P/COD influent seriously inhibited the metabolism of PAOs. Moreover, the glycogen consumption/acetate uptake (Gly/HAc) ratio and the PHAs accumulation/acetate uptake (PHAs/HAc) ratio increased to 1.04 and 1.34 C-mol/C-mol, respectively. An extremely lower P/HAc ratio associating with relatively higher Gly/HAc and PHAs/HAc ratios clearly illustrated the anaerobic phenotype of GAOs in the A/O reactor [4,19]. That is, long-term operation of high P/COD influent favored the growth of GAOs more than that of PAOs. Meanwhile, the value of 3HV/PHAs visibly increased to 21.8% on 123th day, as depicted in Table 1. According to the biochemical pathways proposed for PAOs and GAOs [20,22,23], PAOs produce 3HB when fed with acetate, while GAOs produce approximately 73% PHB and approximately 25% 3-hydroxyvalerate (3HV). Therefore, the increase of 3HV/PHAs value also verified the increase of GAOs in the A/O reactor. That is, PAOs' competitor, GAOs, had been induced to dominate this A/O reactor. These findings suggested that long-term operation of high P/COD influent seriously eliminated the proliferation of PAOs, and more importantly provided a competitive advantage of GAOs over PAOs.

The P/HAc ratio of 0.14 P-mol/C-mol was achieved on 148th day (stage 2) when the influent P/COD loading was transformed back to 15/400 mg/mg. This P/HAc value was significantly lower than the 0.36 P-mol/C-mol that was observed at the steady-state period of the A/O reactor (Table 1). This comparison indicated that PAOs' metabolism could not be restored although the influent P/COD loading has been reduced back to 15/400 mg/mg. In contrast, this stage achieved the relatively higher PHAs/HAc and Gly/HAc ratios than that in steady-state period of the A/O reactor. Besides, the value of 3HV/PHAs slightly increased to 22.0% at the end of experimental period (170th day), displaying again the high enrichment of GAOs in the A/O reactor. The above findings suggested that long-term operation on high P/COD influent provided GAOs with a selective advantage over PAOs, and accordingly GAOs dominated this EBPR process.

4. Discussion

This study investigated the long-term adaptability of an acetate-fed A/O SBR to the influent P/COD loading raised from 15/400 to 40/400 mg/mg and obtained two major results. Firstly, the increase of PAOs in short-term period of adaptation was indicated in experimental results by the decrease of anaerobic 3HV/PHAs ratio, and the increase of sludge P content, anaerobic P/HAc ratio, and f_{PAOs} value. The high P/COD ratio of 40/400 in influent indeed encouraged the proliferation of PAOs in the A/O reactor. This result corresponded with the other researches [4,5,8,9,11,14]; that is, high enrichment of PAOs was established in A/O process under the conditions of high P/COD influent.

Secondly, long-term operation of high P/COD influent to the A/O reactor seriously eliminated the proliferation of PAOs and visibly encouraged the growth of GAOs. Table 2 summaries the kinetics of the sludge in the A/O reactor at different operational periods. Continuous operation at high P/COD ratio in influent resulted in the decrease of sludge P content from 16.0 to 11.0% during the later period of stage 1 (Table 2). The sludge P content is considered to be an essential factor relating to energy storage of PAOs in a biological phosphorus removal system [24]. Besides, the increase of sludge P content was also verified by past studies [11,25–27] to be the enhancement of PAOs in an EBPR system. Thus, the visible decrease of sludge P content in the A/O reactor implied that PAOs were seriously inhibited and gradually washed out

Table 2
Kinetics of the sludge in the A/O reactor at different operational periods

Date	Sludge P content (%)	Specific acetate uptake rate (mg/g MLSS·h)	Specific phosphate release rate (mg/g MLSS·h)	Specific phosphate uptake rate (mg/g MLSS·h)
Steady-state	7.9	136.6	51.6	15.3
23th d (initial period of stage 1)	13.0	97.7	66.9	27.3
54th d (initial period of stage 1)	16.0	112.0	49.3	10.0
123th d (later period of stage 1)	11.0	106.0	8.3	5.1
148th d (stage 2)	6.2	144.6	21.3	6.2

through population selection under the long-term operation on high P/COD influent. The similar result was observed by Liu et al. [4] to indicate a shift of the dominating population from PAOs to GAOs during the decrease in sludge P content. Besides, the gradual decrease of PAOs also led to the decline of specific phosphate release rate during the later period of stage 1, as was shown in Table 2.

Furthermore, the specific acetate uptake rate of 106.0 mg/g MLSS·h was observed on 123th day (later period of stage 1), as shown in Table 2. This specific acetate uptake rate was observably lower than that of steady-state period (136.6 mg/g MLSS·h). Liu et al. [4] proposed that uptake of acetate by PAOs was apparently more and faster than GAOs. This result further verified that the dominance of the A/O reactor had changed from PAOs to GAOs during the stage 1. That is, long-term operation of an A/O process with high P/COD influent provided undesired GAOs a competitive advantage over PAOs.

5. Conclusions

(1) The metabolic behavior of PAOs could be observably enhanced in short-term period of adaptation to the influent P/COD loading elevated from 15/400 to 40/400 mg/mg.
(2) Long-term operation of high P/COD ratio in influent seriously inhibited the proliferation of PAOs, and more importantly induced the advantageous growth of GAOs.
(3) When the influent P/COD loading was transformed back to 15/400 mg/mg, PAOs' metabolism was not recovered and the A/O reactor was dominated by GAOs.

Acknowledgement

The authors would like to thank the National Science Council of Taiwan for financially supporting this research under No. NSC 98-2221-E-224-003-MY2.

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