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Comparison between acetate and propionate as carbon sources for phosphorus removal in the aerobic/extended-idle regime

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ABSTRACT

Lately, we have proved that biological phosphorus (P) removal can be achieved in the aerobic/extended-idle (AEI) regime using glucose as the sole carbon source, which might develop a potential simple strategy for simultaneous removal of P and organic substrates from wastewater. Since acetate and propionate are the two most common substrates present in real domestic wastewater, this paper further assesses the P removal performances in the AEI process using acetate and propionate as the sole carbon source. The results showed that 3.91 and 3.64 mg of P/g of total suspended solids were, respectively, removed in the acetate-reactor and propionate-reactor after 50 days, respectively. After 90 days P removal in the propionate-reactor increased to 4.91 mg P/g of total suspended solids whereas that in the acetate-reactor kept in the same level (3.98 mg/g). Though both acetate and propionate could be used as carbon sources for P removal in such a novel system, the latter was more effective after long-term operation. Further investigations showed that, after 90 days' acclimatization, sludge poly-P content in the propionate-system was more than that in the acetate-system, which was the primarily reason for the propionate-system showing higher P removal.

1. Introduction

In the traditional theory of wastewater treatment, biological phosphorus removal is accomplished through two modes: biological assimilation and enhanced biological phosphorus removal (EBPR) [1]. The biological assimilation is in the use of the microorganisms cultivated in wastewater to uptake phosphorus and convert it to microorganism component. In this process, the microorganisms could take about 5.5 mg P–PO_4^{3-}/L in the sewage [2]. To reduce P levels in freshwater ecosystems effectively, EBPR is prior to choose due to its economical and low environmental impact. This process is based on the selective enrichment of bacteria accumulating inorganic polyphosphate as an ingredient of their cells [2]. Polyphosphate accumulating organisms (PAOs) uptake volatile fatty acids (VFA) and convert it to poly-β-hydroxyalkanoates (PHA), decompose the intracellular stored polyphosphate (poly-P) and glycogen anaerobically [3,4]. In the aerobic phase, PAOs oxidize PHA to gain energy and carbon sources [5], reincorporate the orthophosphate into intracellular poly-P, thus EBPR is achieved. Although the current EBPR technology (i.e., anaerobic/aerobic process) is widely applied in full-scale wastewater treatment plants, its stability and reliability can still be a problem due to external disturbances such as high rainfall, excessive nitrate loading to the anaerobic reactor [6–8].

Recently, we have found excess phosphorus removal could be obtained in an activated sludge system with an extended idle period using glucose as the sole carbon source, and then Wang et al. [9–11] made further researches on this peculiar phenomenon, and named this process for the aerobic/extended-idle (AEI) process [12]. Compared with the conventional EBPR process, a strictly anaerobic period was not conducted whereas an extended-idle zone was operated between the decanting and the next aerobic phases. Though the extended-idle phase seems as a post anaerobic phase performed in the conventional anaerobic/oxic (A/O) regime for it is also not aerated, mixture stirring does not require to be performed during the idle period. Further, besides a significant idle phosphate release, external substrate consumption and PHA and glycogen transformations, however, are not obviously observed during this idle period [12]. This metabolic behavior indicates that the idle period operated in the AEI regime is different from the anaerobic period performed in the A/O regime. Unexpectedly, this extended-idle period was also proved to provide a selective advantage to PAOs

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over other populations [12]. The previous results obtained by us suggested that the AEI regime might serve as an alternatively effective supplement to the existing method of phosphorus removal.

As is known, carbon source plays an important role in biological phosphorus removal. The microbial-accumulated biopolymers (PHA, poly-P and glycogen) are vital for the metabolism of PAOs, and the formation of polymers composition is mainly dependent on the substrate [13]. Consequently, a mass of carbon sources in wastewater is a prerequisite for achieving EBPR process [2,4]. However, the components of real wastewater are complex, including several organic carbon sources such as macromolecular organic compounds, and volatile fatty acids (VFAs). Acetate and propionate are two kinds of familiar VFAs in wastewater, among which acetate accounts for 60–70% and propionate accounts for 20–30% [2,6]. Through summarizing data from four full-scale systems with prefermenters (in Canada and Australia), Von Muench [14] showed that acetate ranged from 49% to 71% of total influent VFAs (by weight), and propionate ranged from 24% to 33% of the total VFAs. Therefore, most of studies on traditional P removal focused on using acetate as the carbon source, which could obtain considerable performances of EBPR [14,15]. Moreover, Chen et al. [16] reported that a higher phosphorus removal level was achieved in a propionate fed system than that in an acetate fed system with a long-term cultivation, then the potential effect of propionate on EBPR behavior has been further studied [17–19], which suggested that propionate may be a more favorable substrate than acetate for successful EBPR performance. Although the effects of acetate and propionate on biological P removal induced by the conventional A/O regime are well investigated, information regarding the two most common VFAs as carbon sources for biological P removal driven by AEI regime is lacking.

Therefore, two kinds of typical VFAs (acetate and propionate) as the sole carbon source were selected to maintain the activated sludge system in this work, which aimed to assess P removal efficiencies and to evaluate the feasibility of the two VFAs as carbon sources for Pi removal in such a novel system. First, the efficiencies of BPR between the acetate- and the propionate-reactors were compared. Then, we analyzed the reasons for propionate-reactor showing higher P removal efficiency than acetate-reactor after long-term acclimatization. Finally, we compared the stoichiometric relation between acetate and propionate as the sole carbon source for P removal in the AEI and the A/O systems.

2. Materials and methods

2.1. Experimental device and operational method

Experiments were carried out in two identical sequencing batch reactors (SBRs) (R1 and R2) made of Lucite, with each reactor having a working volume of 2 L.

Inoculated sludge was taken from the First Municipal Wastewater Treatment Plant of Changsha, China, and the initial concentrations of mixed liquor suspended solids (MLSS) in the two reactors were all set at around 4000 mg/L. The operation of the two SBRs is described below: influent → aeration (4 h) → settling/decanting (0.5 h) → idle zone (7.5 h). During the aerobic time, air was supplied into both SBRs at a flow rate of 1.5 L min^{-1}.

For each SBR cycle, 1.5 L supernatant was discharged after the settling period, and the sludge retention time (SRT) in the two SBRs was maintained at approximately 20 days.

During the process, 2 mg/L EDTA was added to the synthetic media to eliminate the effects of metal phosphorus on biological P removal. The pH was around 7.5 during aerobic periods through the addition of 0.5 M HCl and $NaHCO_3$.

2.2. Synthetic media

Synthetic wastewater was used in this research. R1, R2 were fed, respectively, with acetate and propionate, but they had almost the same influent amount of carbon element (about 15 mmol/L). The concentrations of other nutrients in the synthetic media fed to the two SBRs are the same as below: PO_4–P (20 mg/L); NH_4Cl (100 mg/L); $MgSO_4$ (10 mg/L); $CaCl_2$ (5 mg/L) and 0.3 mg/L trace metals solution. The trace metals solution had been described in Smolders et al. [20].

2.3. Analytical methods

TP was determined by phosphoantimomolybdate ascorbic acid spectrophotometry, mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were measured by standard method [21], glycogen was measured by the phenol-sulfuric method with glucose as standard [22], total organic carbon (TOC) was determined using a TOC analyzer (ShimadzuTOC-500, Japan), and sludge TP content was measured by the method described in Wang et al. [9]. VFAs were measured using high performance liquid chromatography (HPLC) with a HPX-87H 300 mm × 7.8 mm, BioRad Aminex ion exclusion HPLC column operated at 65.1 °C. FIA and VFA samples were obtained through filtering mixed liquor from the SBR using 0.22 mm Millex GP syringe driven filters [22]. PHA was measured with gas chromatography (GC) [9]. Poly-P staining was carried out with 4–6-diamidino-2-phenylindole (DAPI) according to the method described by Liu et al. [23], and examined using a confocal scanning laser microscope (FV 500).

3. Results and discussion

3.1. P removal performances and related multi-parameter transformations in the acetate fed and the propionate fed AEI reactors during the steady-state operation

Table 1 showed the data of the effluent TP concentration and phosphorus removal efficiency during the period of 96 days experiments. It was clearly observed that a poor level of TP removal was achieved in both reactors during the initial 10 days. R1 operated steadily after 10 day operation, and 5.29–6.27 mg/L were measured in effluent between day 10 and day 96. Compared with R1, P removal efficiency in R2 was lower during the initial 50 days, but was higher during the next 50 days operation, and the average P removal increased to 18.41 mg/L during the operation of day 50–96. In this test, the researcher set the influent P concentration high in order to assess the feasibility of acetate and propionate serving as carbon source for phosphorus removal induced by AEI regime. Although the effluent P concentration was high, the net amount of P removal in both reactors was more than 14 mg/L after long-term operation. In the first phase (0–10 days), the average MLSS of the two reactors was 3348 mg/L and 3252 mg/L, respectively. During 10–50 day operation, the average MLSS of the two reactors, respectively, increased to 3582 mg/L and 3656 mg/L, while the ratio of MLVSS/MLSS in both reactors dropped below 0.7, corresponding with the increase of the P removal capability. MLVSS/MLSS ratio kept steadily in R1 after 50 days, whereas decline continued in R2, which was consistent with the improvement of TP removal. Oehmen et al. [24] suggested a low VSS/TSS ratio implied a high level of phosphorus was stored in the sludge. The results suggested there was an increase in the numbers of microorganisms performing P removal in R2 after 50 day operation.

Table 1
Comparison of multi-parameters in two AEI-reactors.

	R1			R2			Units
	0–10 days	10–50 days	50–96 days	0–10 days	10–50 days	50–96 days	
TP in effluent							
Avg	8.38	5.51	5.49	9.72	5.64	1.59	mg/L
Max	12.82	6.25	6.27	12.12	7.72	1.94	
Min	7.08	5.37	5.29	8.04	3.37	0.96	
TP release[a]							
Avg	13.95	11.68	11.64	8.49	5.51	11.36	
Max	19.63	12.09	12.27	15.32	7.93	12.57	
Min	11.45	11.04	10.68	5.25	4.37	9.34	
TP removal							
Avg	11.62	14.49	14.51	10.28	14.36	18.41	
Max	12.92	14.63	14.71	11.96	15.63	19.04	
Min	7.18	13.75	13.73	7.88	12.28	18.06	
Efficiency of TP removal							
Avg	58.1	72.4	72.6	51.4	71.8	92.1	%
Max	64.6	73.1	73.6	59.8	78.1	95.2	
Min	35.9	68.8	68.7	39.4	61.4	88.9	
MLVSS (Avg)	2578	2457	2432	2439	2523	2093	mg/L
MLSS (Avg)	3348	3582	3561	3252	3656	3610	
MLVSS/MLSS (Avg)	0.77	0.69	0.68	0.75	0.69	0.58	g/g

[a] TP release = end of idle zone TP – end of aerobic zone TP.

3.2. Transformations of TP, glycogen and PHA during one cycle after short-term acclimatization

As shown in Fig. 1, VFAs were fully exhausted in both reactors during the initial 30 min of the aerobic period. Meanwhile, glycogen and PHA were produced significantly. After the external substrate was exhausted, PHA was degraded accompanied by glycogen synthesis and P uptake. P concentration in effluent was 6.52 mg/L in the acetate-reactor (R1) and 7.25 mg/L in the propionate-reactor (R2), respectively.

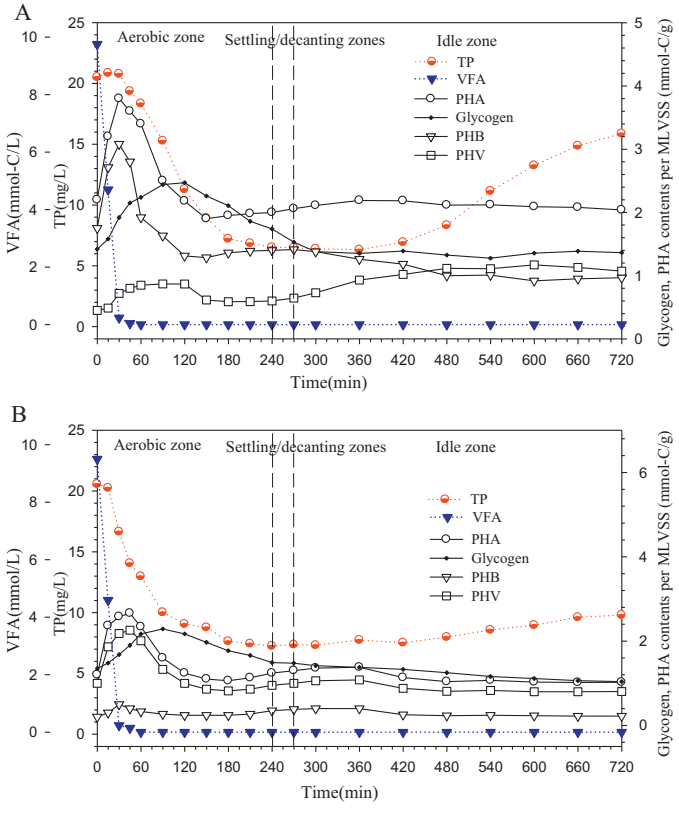

Fig. 1. Transformations of TP, VFA, PHA and sludge glycogen in the AEI-reactors during a typical cycle in 50th day. (A) Acetate (R1) and (B) propionate (R2).

In R1, the maximal accumulations of PHA and glycogen were, respectively, 3.8 mmol-C/g and 2.47 mmol-C/g, but the corresponding data were, respectively, 2.62 mmol-C/g and 2.29 mmol-C/g in R2. It can be clearly seen that the PHA and glycogen accumulated in R2 was lower than those accumulated in R1. Also, it can be found some different metabolic transformations between the AEI regime and the A/O regime. It is known that, external substrate is consumed in the anaerobic period [4,19,25]. However, external substrate is consumed aerobically, which might be the reason for the AEI regime showing some metabolic differences from the A/O regime. In R1, the main energy storage was PHA, while accumulations of PHA and glycogen were nearly at the same level in R2. In both reactors, the accumulation time of glycogen was longer than that of PHA. When external substrates were depleted, glycogen continued to be accumulated in a short time. R1 produced PHB (73.6%) as the main PHA while R2 produced PHV (83.8%) as the dominative PHA. The report of Albuquerque et al. [26] suggested PHV content related to the amount of propionate and valerate in the feed, which was consistent with the research results. During the idle phase, P release was observed obviously in R1, but the concentration of P release was lower in R2. PHV was accumulated in R1. However PHV did not change significantly in R2.

3.3. Transformations of P, glycogen and PHA during one cycle after long-term acclimatization

The carbon and P transformations during a cycle after long-term acclimatization in R1 were shown in Fig. 2(A). Compared with Fig. 1(A), transformations of related parameters were similar in R1. P in effluent was 5.78 mg/L. At the end of one cycle, 10.4 mg/L of P was released. The P removal microbe in R1 also produced PHB (75.7%) as the main PHA.

Transformations of P, VFA, PHA and sludge glycogen in R2 during a typical cycle on 90th day were shown in Fig. 2(B). P in effluent decreased to 2.77 mg/L. The maximal accumulation of PHA was 2.66 mmol-C/g, and PHV was also the main component, which accounted for 84.3% of the total PHA. In the research of Oehmen et al. [19], there was also only a little PHB accumulated in the propionate fed reactor. The accumulation of glycogen increased slightly, 3.67 mmol-C/g of glycogen was accumulated after 60 min of aeration. During the idle phase, phosphate release was observed to be higher in R2 than that in R1. At the end of the cycle, 9.36 mg $P–PO_4^{3-}$/L was released. It was reported that phosphorus removal

Table 2
Comparison between unadapted sludge and 40- and 90-day VFAs enriched sludge.

	0-Day	R1		R2		Unit
		40-day	90-day	40-day	90-day	
1st P release rate	0.42	0.34	0.07	–	–	mg P–PO_4^{3-}/g VSS h
P uptake rate	0.28	1.56	1.59	1.55	3.49	
2nd P release rate	0.87	0.47	0.45	0.11	0.50	

capability showed well correlation with idle phosphate release [12]. Thus the higher idle phosphorus release detected in R2 was consistent with the P removal capability.

A character of the EBPR process is the interaction between different microbial intracellular biopolymers [4,27]. Therefore, the understanding of the effects of these biopolymers on extended idle period performing enhanced biological phosphorus removal is helpful to further understand the mechanism of the process.

3.4. DAPI staining of the sludge samples taken from the two reactors during short- and long-term operations

According to the above studies, it seemed that after short-term acclimatization higher P removal efficiency was obtained in the acetate reactor than that in the propionate reactor whereas P removal efficiency of propionate reactor was greater than that in the acetate reactor after long-term acclimatization. Therefore, DAPI staining was further carried out to confirm the above data.

Fig. 3(A) and (C) showed the results of DAPI poly-P staining of the sludge samples taken from the acetate reactor in day 50 and day 90, respectively. Although both DNA and poly-P can be bound by fluorescent dye DAPI, the two binding states emit different fluorescence under epifluorescence microscopy. DNA-bound DAPI appears pale whereas poly-P-bound DAPI appears strongly bright white [23], which can distinguish the poly-P granules easily. From Fig. 3, it can be found that Fig. 3(A) and (C) have a lot of bright spots, so the activated sludge system with an extended idle period has a large number of accumulated poly-P granules (33% vs. 32%). Fig. 3(B) and (D) showed the poly-P staining graphs of activated sludge samples taken from the propionate reactor in day 50 and day 90, respectively. In Fig. 3(D), bright spots were more than that in Fig. 3(B) obviously (43% vs. 28%). It can be concluded that P in the wastewater was mainly converted to poly-P granules in this research. Further, the main reason for the long-term acclimated propionate reactor showing higher phosphorus removal performance was due to the more poly-P containing cells cultured in the activated sludge.

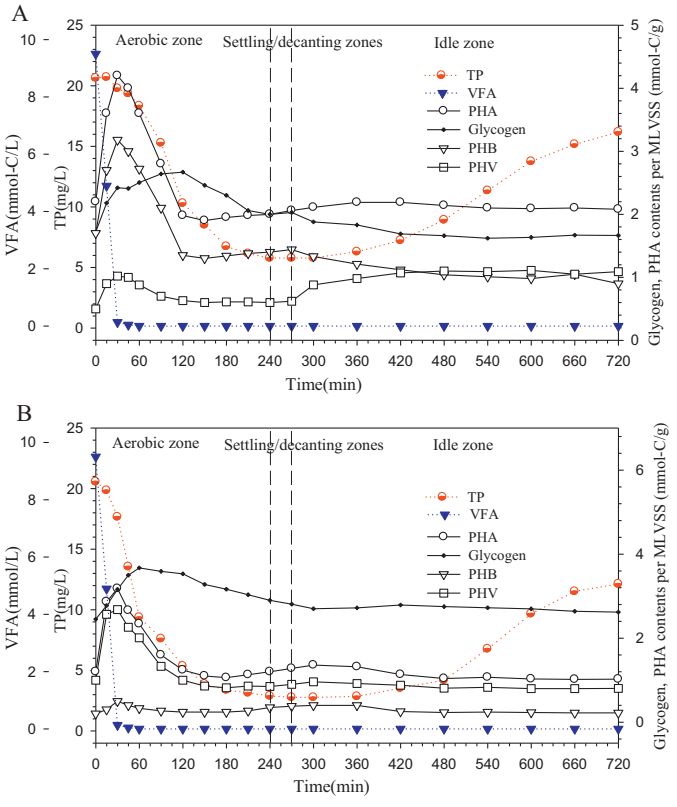

Fig. 2. (A and B) Transformations of TP, TOC, PHA and sludge glycogen in the AEI-reactors during a typical cycle in 90th day.

3.5. Effect of acclimated time on the specific phosphate release and uptake rates

Table 2 compares the P release and uptake rates of 40- and 90-day acetate and propionate adapted activated sludge in AEI system. Although an unacclimated biomass was not able to perform a suitable AEI, after a period of biomass adaptation (10–90 days), the population dynamics of the activated sludge evolved to an efficient phosphorus removal process.

When acetate was used as carbon source, the first P release rate was observed in the beginning of aerobic phase, the second P release rate was observed in idle phase. The first P release rate corresponded with the VFA depletion (0.34 and 0.07 mg P/g VSS h for acetate on 40- and 90-day), while the 2nd specific P release rates (0.47 and 0.45 mg P/g VSS h for acetate on 40- and 90-day) could result from the enriched P removal microbe using their excess intracellular poly-P to generate energy which was required for maintenance. Also from Table 2, it can be seen that when propionate was used as carbon source, the 2nd phosphate release rate was detected but the first P release rate was not observed. With the increase of the acclimated time, both the 2nd P release rate and P uptake rate increased.

3.6. Comparison of the stoichiometric values of P removal in the AEI and the A/O systems

The PHA produced during the aerobic phase for each carbon source tested (acetate, propionate) in the AEI system are presented in Table 4. PHA was synthesized in the anaerobic phase in the traditional A/O process, but was accumulated aerobically along with carbon source consumption in the AEI process.

In our study, the P removal microbes mainly accumulated PHB (75.7%) in the acetate reactor whereas in the propionate reactor the P removal microbes primarily accumulated PHV (84.3%). These results were in accord with the data reported in the literatures which were shown in Table 3. PHA production was higher in the acetate reactor (4.2 mmol-C/g VSS) than that in the propionate reactor (3.18 mmol-C/g VSS) (Fig. 1). The storage polymer produced by the P removal microbe in a 90-day propionate acclimated sludge was mainly PHV (84.3%; 2.68 mmol-C/g VSS). In the A/O regime, Pijuan et al. [22] found that PAOs also produced a low level of PHB (4%) in a 140-day propionate-acclimated biomass, and more glycogen is needed than acetate to produce PHV, which were in agreement with the data obtained in the AEI reactors (this study).

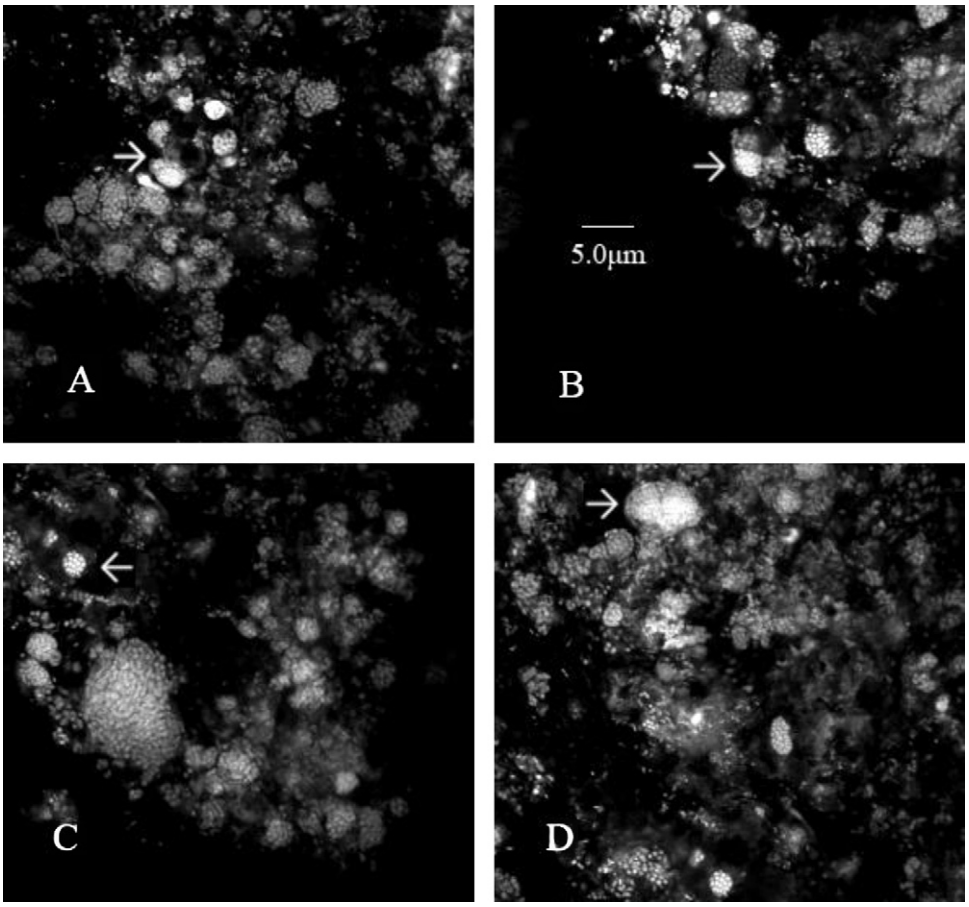

Fig. 3. The poly-P staining procedures on activated sludge samples taken from the AEI reactors. (A) Acetate, day 50; (B) propionate, day 50; (C) acetate, day 90; and (D) propionate, day 90.

Table 3
Date from the literature concerning the PHA accumulated and from produced in activated sludge system with an extended idle period using acetate and propionate as carbon sources.

	Carbon source	PHB (%)	PHV (%)	Other (%)	References
AEI	Acetate	75.7	24.3	NM	This study
	Propionate	15.7	84.3	NM	This study
A/O	Acetate	90.2	9.8	NM	Smolders et al. [20]
	Acetate	46.6	20.2	31.2	Puig et al. [28]
	Propionate	4.0	46.5	49.5	Pijuan et al. [22]
	Propionate	2.0	45.0	53.0	Oehmen et al. [24]
	Propionate	2.1	50.7	47.2	Puig et al. [28]

Table 4 showed that the amount of PHA stored in the sludge was higher in R1 than that in R2 at any investigated time. On day 50, the PHA-produced/VFA-uptake and the glycogen-produced/VFA-uptake ratios were 0.58 vs. 0.37, and 0.33 vs. 0.35 in the two reactors, respectively. On day 90, the accumulation of PHA/VFA did not increase obviously in R1, whereas this ratio increased to 0.52 in R2. This indicated that there was enough VFA available for P removal microbe, thus leaded to an increase in the intracellular storage of PHA as their dominated intracellular storage in R1. However P removal microbe took VFA to synthesize PHA and glycogen in R2 (dominated storage not obvious). In addition, the values obtained (Table 2) for the carbon recovery ratio increased from 0.8 and 0.76 C-mol/C-mol, after 50 days the values in the two reactors increased to 0.91 and 0.72, respectively. After long-term acclimatization, the carbon recovery ratio unceasingly increased to 0.98 and 1.09 in two reactors, respectively.

To further understand the differences between the AEI and A/O regimes, the experimental results obtained in the AEI-reactors were compared with the stoichiometric coefficients monitored in the A/O-reactors of previous studies, as outlined in Table 5. It was clear that the stoichiometry of the metabolic stoichiometric coefficients displayed in AEI reactors and other A/O studies for both acetate and propionate. PAOs of A/O system uptake VFAs to

Table 4
Ratios calculated from the experimental results obtained in R1 and R2.

Ratios	R1			R2			Units
	0-day	50-day	90-day	0-day	50-day	90-day	
PHA/VFA	0.54	0.58	0.62	0.52	0.37	0.52	C-mol/C-mol
PHB/VFA	0.32	0.48	0.56	0.29	0.05	0.07	
PHV/VFA	0.22	0.1	0.06	0.23	0.32	0.45	
Glycogen/VFA	0.26	0.33	0.36	0.24	0.35	0.53	
Carbon recovery ratio[a]	0.8	0.91	0.98	0.76	0.72	1.05	
P-release/VFA-uptake	0.053	0.032	0.028	0.042	0.0068	0.026	P-mol/C-mol

[a] Carbon recovery ratio = (PHA-synthesized + glycogen-synthesized)/VFA-uptake.

Table 5
Comparison of the stoichiometric coefficients between AEI- (this study) and A/O-mediated reactors (previous studies).

	References	Carbon source	Aerobic phase			Idle phase
			PHA-up/VFA (C-mol/C-mol)	Gly-syn/VFA (mmol-C/g VSS)	P uptake rate (mmol-P/g VSS h)	P release rate (mmol-P/g VSS h)
AEI	This study[a]	Acetate	0.62	0.36	0.049	0.018
		Propionate	0.52	0.53	0.068	0.020

	References	Carbon source	Anaerobic phase			Aerobic phase
			PHA-up/VFA (C-mol/C-mol)	Gly-de/VFA (C-mol/C-mol)	P-rel/VFA (P-mol/C-mol)	P uptake rate (mmol-P/g VSS h)
A/O	Vargas et al. [29]	Acetate	1.23	0.08	0.55	0.48
		Propionate	0.94	0.15	0.38	0.41
	Pijuan et al. [30]	Acetate	0.64	0.45	0.268	–
		Propionate	1.39	0.91	0.107	–
	Lu et al. [31]	Acetate	1.26	0.46	0.62	–
		Propionate	1.22	0.29	0.44	–
	Oehmen et al. [24]	Acetate	1.22	0.33	0.42	–
	Oehmen et al. [32]	Propionate	1.23	0.32	0.42	–
	Pijuan et al. [22]	Propionate	0.91	0.21	0.42	0.72

[a] The samples taken from AEI-SBR in 90-day.

synthesize PHA in anaerobic phase, however, the AEI system synthesize PHA in aerobic phase. The PHAs-up/VFA ratio detected in the AEI-reactors was much lower than that in the A/O-reactors (0.62 vs. 0.64–1.26 and 0.52 vs. 0.91–1.39 mol-C/mol-C, acetate and propionate respectively). Consequently, compared with the A/O-reactors the aerobic glycogen synthesis in the AEI-SBR was not higher or lower obviously, however the propionate-reactor was higher than acetate-reactor obviously. After long-term acclimatization, P uptake rate in propionate-reactor was 0.068 mmol-P/g VSS h and higher than 0.049 mmol-P/g VSS h in acetate. However in idle phase, P release rate in two reactors was closed (0.018 and 0.020 mmol-P/g VSS h). It is one reason that P removal efficiency in propionate-SBR higher than in acetate-SBR after long-term acclimatization. According to the above studies, it was clearly showed that the AEI regime using acetate or propionate as sole carbon source could drive a good P removal, but propionate was a more favorite substrate for P removal in the AEI regime after long-term acclimation.

4. Conclusion

A satisfied phosphorus removal performance was observed in the AEI process using acetate and propionate as the sole carbon source in this test. After short-term operation, the maximal P uptake rate was obtained in biomass adapted by acetate. For long-term addition, a higher P uptake rate could be obtained in the propionate reactor. If AEI-reactor needs to be incidentally supported by substrate addition, acetate and propionate was preferred for short-term addition and propionate can also for long-term addition.

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References

[1] J. Lv, Y. Chen, G. Gu, Enhanced biological removal of phosphate by aerobic granular sludge, Environ. Sci. Technol. 29 (2006) 106–108.
[2] E.L. de-Bashan, Y. Bashan, Recent advances in removing phosphorus from wastewater and its future use as fertilizer (1997–2003), Water Res. 38 (2004) 4222–4246.
[3] T.M. Louie, T.J. Mah, W. Oldham, W.D. Ramey, Use of metabolic inhibitors and gas chromatography/mass spectrometry to study poly-β-hydroxyalkanoates metabolism involving cryptic nutrients in enhanced biological phosphorus removal systems, Water Res. 34 (2000) 1507–1514.
[4] T. Mino, M.C.M. van Loosdrecht, J.J. Heijnen, Microbiology and biochemistry of the enhanced biological phosphate removal process, Water Res. 32 (1998) 3193–3207.
[5] G.R. Crocetti, P. Hugenholtz, P.L. Bond, A. Schuler, J. Keller, D. Jenkins, L.L. Blackall, Identification of polyphosphate-accumulating organisms and design of 16S rRNA-directed probes for their detection and quantitation, Appl. Environ. Microbiol. 66 (2000) 1175–1182.
[6] M. Thomas, P. Wright, L. Blackall, V. Urbain, J. Keller, Optimisation of Noosa BNR plant to improve performance and reduce operating costs, Water Sci. Technol. 47 (2003) 141–148.
[7] A. Oehmen, P.C. Lemos, G. Carvalho, Z. Yuan, J. Keller, L.L. Blackall, M.A.M. Reis, Advances in enhanced biological phosphorus removal: from micro to macro scale, Water Res. 41 (2007) 2271–2300.
[8] P. Kampas, A.P. Simon, P. Pete, L. Sandrine, V. Pete, C. Elise, S. Ana, An internal carbon source for improving biological nutrient removal, Bioresour. Technol. 100 (2009) 149–154.
[9] D. Wang, X. Li, Q. Yang, G. Zeng, D. Liao, J. Zhang, Biological phosphorus removal in sequencing batch reactor with single-stage oxic process, Bioresour. Technol. 99 (2008) 5466–5473.
[10] D. Wang, X. Li, Q. Yang, W. Zheng, J. Cao, G. Zeng, X. Yue, T. Shen, T. Zeng, Y. Ding, Effect and mechanism of carbon sources on phosphorus uptake by microorganisms in sequencing batch reactors with the single-stage oxic process, Sci. China Ser. B 52 (2009) 1–8.
[11] D. Wang, X. Li, Q. Yang, W. Zheng, J. Cao, X. Yue, T. Shen, G. Zeng, J. Deng, The probable metabolic relation between phosphate uptake and energy storages formations under single-stage oxic condition, Bioresour. Technol. 100 (2009) 4005–4011.
[12] D. Wang, G. Yang, X. Li, W. Zheng, Y. Wu, Q. Yang, G. Zeng, Inducing mechanism of biological phosphorus removal driven by the aerobic/extended-idle regime, Biotechnol. Bioeng. 109 (2012) 2798–2807.
[13] H. Satoh, T. Mino, T. Matsuo, Uptake of organic substrates and accumulation of polyhydroxyalkanoates linked with glycolysis of intracellular carbohydrates under anaerobic conditions in the biological excess phosphate removal process, Water Sci. Technol. 26 (1992) 933–942.
[14] E. Von Muench, DSP Prefermenter Technology Book, Science Traveller International CRC WMPC Ltd., Brisbane, QLD, Australia, 1998.
[15] C.D.M. Filipe, G.T. Daigger, C.P.L. Grady Jr., Stoichiometry and kinetics of acetate uptake under anaerobic conditions by an enriched culture of phosphorus-accumulating organisms at different pHs, Biotechnol. Bioeng. 76 (2001) 32–43.
[16] Y. Chen, A.A. Randall, T. McCue, The efficiency of enhanced biological phosphorus removal from real wastewater affected by different ratios of acetic to propionic acid, Water Res. 38 (2004) 27–36.
[17] A. Oehmen, Z. Yuan, L.L. Blackall, J. Keller, Short-term effects of carbon source on the competition of polyphosphate accumulating organisms and glycogen accumulating organisms, Water Sci. Technol. 50 (2004) 139–144.
[18] Y. Chen, Y. Liu, Q. Zhou, G. Gu, Enhanced phosphorus biological removal from wastewater––effect of microorganism acclimatization with different ratios of short-chain fatty acids mixture, Biochem. Eng. J. 27 (2005) 24–32.
[19] A. Oehmen, A.M. Saunders, M.T. Vives, Z. Yuan, J. Keller, Competition between polyphosphate and glycogen accumulating organisms in enhanced biological

phosphorus removal systems with acetate and propionate as carbon sources, J. Biotech. 123 (2006) 22–32.
[20] G.J.F. Smolders, J. Vandermeij, M.C.M. van Loosdrecht, Model of the anaerobic metabolism of the biological phosphorus removal process––stoichiometry and pH influence, Biotech. Bioeng. 43 (1994) 461–470.
[21] APHA, Standard Methods for the Examination of Water and Wastewater, 19th ed., American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, 1995.
[22] M. Pijuan, J.A. Baeza, C. Casas, J. Lafuente, Response of an EBPR population developed in an SBR with propionate to different carbon sources, Water Sci. Technol. 50 (2004) 131–138.
[23] W.T. Liu, A.T. Nielsen, J.H. Wu, C.S. Tsai, Y. Matsuo, S. Molin, In situ identification of polyphosphate- and polyhydroxyalkanoate-accumulating traits for microbial populations in a biological phosphorus removal process, Environ. Microbiol. 3 (2001) 110–122.
[24] A. Oehmen, M.T. Vives, H. Lu, Z. Yuan, J. Keller, The effect of pH on the competition between polyphosphate accumulating organisms and glycogen-accumulating organisms, Water Res. 39 (2005) 3727–3737.
[25] J. Pramanik, P.L. Trelstad, A.J. Schuler, D. Jenkins, J.D. Keasling, Development and validation of a flux-based stoichiometric model for enhanced biological phosphorus removal metabolism, Water Res. 33 (1999) 462–476.
[26] M.G.E. Albuquerque, C.A.V. Torres, M.A.M. Reis, Polyhydroxyalkanoate (PHA) production by a mixed microbial culture using sugar molasses: effect of the influent substrate concentration on culture selection, Water Res. 44 (2010) 3419–3433.
[27] M. Vargas, C. Casas, J.A. Baeza, Maintenance of phosphorus removal in an EBPR system under permanent aerobic conditions using propionate, Biochem. Eng. J. 43 (2009) 288–296.
[28] S. Puig, M. Coma, H. Monclus, M.C.M. van Loosdrecht, J. Colprim, M.D. Balaguer, Selection between alcohols and volatile fatty acids as external carbon sources for EBPR, Water Res. 42 (2008) 557–566.
[29] M. Vargas, A. Guisasola, A. Artigues, C. Casas, J.A. Baeza, Comparison of a nitrite-based anaerobic–anoxic EBPR system with propionate or acetate as electron donors, Process Biochem. 46 (2011) 714–720.
[30] M. Pijuan, C. Casas, J.A. Baeza, Polyhydroxyalkanoate synthesis using different carbon sources by two enhanced biological phosphorus removal microbial communities, Process Biochem. 44 (2009) 97–105.
[31] H. Lu, A. Oehmen, B. Virdis, J. Keller, Z. Yuan, Obtaining highly enriched cultures of Candidatus Accumulibacter phosphates through alternating carbon sources, Water Res. 40 (2006) 3838–3848.
[32] A. Oehmen, R.J. Zeng, Z. Yuan, J. Keller, Anaerobic metabolism of propionate by polyphosphate-accumulating organisms in enhanced biological phosphorus removal systems, Biotechnol. Bioeng. 91 (2005) 43–53.