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The probable metabolic relation between phosphate uptake and energy storages formations under single-stage oxic condition

Dong-bo Wang^a, Xiao-ming Li^{a,*}, Qi Yang^a, Wei Zheng^a, Zong-yao Liu^b, Yi-lin Liu^a, Jian-bin Cao^a, Xiu Yue^a, Ting-ting Shen^a, Guang-ming Zeng^a, Jiu-hua Deng^a

^a College of Environmental Science and Engineering, Hunan University, Changsha 410082, PR China ^b Scivic Engineering Corporation, Luoyang 471079, PR China

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ABSTRACT

To investigate the possible biochemical metabolisms for excess phosphate uptake in a sequencing batch reactor (SBR) with single-stage oxic process, which was reported using glucose as the sole carbon source previously, glucose and acetate were fed to two SBRs as the sole carbon source, respectively. The changes of polyhydroxyalkanoates (PHAs), glycogen and the removal of phosphorus were compared between two SBRs. It was observed that the phosphorus removal efficiency was 91.8–94.4% with glucose, and 23.3–28.5% with acetate, although the former showed much lower accumulations/transformations of PHAs. Instead, the former showed a much higher transformation of glycogen. The facts suggested that glycogen could replace PHAs to supply energy for phosphate uptake under the single-stage oxic condition. Furthermore, the possible biochemical metabolisms were proposed to describe the relation between phosphate uptake and energy storages formations under such a single-stage oxic process. Such a process may serve as a prototype for the development of alternative biological and chemical options for phosphate removal from wastewaters.

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1. Introduction

Excessive phosphate (Pi) supply to freshwater negatively affects water quality and ecosystem balance through a process known as eutrophication. This can lead to the increase of wastewater treatment costs, a reduction in the biological diversity and recreational value of natural water bodies through algal blooms, loss of livestock and human health issues (Mullan et al., 2006). Increasingly stringent Pi limits for effluent wastewater are expected in the future, and therefore efficient and reliable Pi removal methods are required. Any improvements in existing methods should have tangible economic and ecological consequences for the massive quantity of wastewater treated daily.

Enhanced biological phosphorus removal (EBPR), conventionally conducted by alternating anaerobic and aerobic conditions and widely applied in real wastewater treatment processes, exploited the ability of certain microorganisms to accumulate Pi in excess of metabolic requirement and to store this intracellularly as the biopolymer polyphosphate (poly-P) (Mullan et al., 2006; Chen et al., 2004). Recently, many researchers have investigated the microbiology and biochemistry of EBPR to make it a more reliable industrial process (Pantelis et al., 2009; Lu et al., 2007; Pijuan et al., 2008), others have focused on the process improvement to obtained higher efficiency or more economy of Pi removal (Fu et al., 2009; Zhang et al., 2009). Compared with chemical Pi removal, EBPR is more economical in the long term and has a lower environmental impact (Martín et al., 2006), but is prone to failure due to low concentrations of volatile fatty acids (VFAs) in the influent wastewater. In South China and other temperate regions, the VFAs concentrations of wastewater are low, and periodic organic matter supplementation and/or chemical "polishing" may be required to attain compliance. Moreover, anaerobic pretreatment zones required for EBPR may make the retrofitting of existing activated sludge processes problematic (Mullan et al., 2006).

Our recent study on possible alternatives to the conventional EBPR process has demonstrated that it is possible to increase the level of Pi removal using glucose as the sole carbon source under single-stage oxic conditions (Wang et al., 2008). This phenomenon may form a potentially novel strategy for the "one-step" removal of Pi from wastewater. Nevertheless, the previous study has shown that PHAs which are supplied as the energy storages for Pi uptake in traditional EBPR process are almost kept constant at a low level during the process, and has also indicated that other storages are synthesized to provide energy for Pi uptake. Before this potential "one-step" process of Pi removal could successfully apply in real wastewater treatment, the energy storages supplied for Pi removal needed to be clearly investigated. The purpose of this study was



^{*} Corresponding author. Tel.: +86 731 8823967; fax: +86 731 8822829. *E-mail address*: wwyy44339745@yahoo.com.cn (X.-m. Li).

therefore to investigate the energy storages supplied for Pi uptake and polyphosphate accumulation, and to propose the probable metabolic relation between Pi uptake and storage product formation under single-stage oxic condition.

2. Methods

2.1. Experimental device and synthetic media

Experiments were carried out in two reproductive sequencing batch reactors (SBRs) with a working volume of 12 l, which were made of Lucite.

Synthetic wastewater was used in this research. The G-SBR and A-SBR were fed, respectively, with glucose and acetate which were mostly considered as detrimental and favorable substrates for EBPR (Cech and Hartman, 1993; Tong and Chen, 2007), but they had almost the same influent amount of carbon element (0.45 g $C_6H_{12}O_6/I$ and 0.62 g CH₃COONa/I, respectively, implied about 14.96 and 14.83 mmol of C/I or 479 and 481 mg of chemical oxygen demand/I) and Pi concentration (35 mg/I). The concentrations of the other nutrients in the synthetic media fed to two SBRs are the same as below (per liter): 0.12 g NH₄Cl, 0.01 g MgSO₄ · 7H₂O, 0.005 g CaCl₂, and 0.5 ml of a trace metals solution. The trace metals solution has also been described in our previous publication (Wang et al., 2008). An aliquot of 1 mg/l of allylthiourea was added to the synthetic wastewater to inhibit nitrification in the SBRs.

2.2. Operational methods

Activated sludge, taken from the first municipal wastewater treatment plant of Changsha, PR China, which was operated with a anaerobic (1 h)-aerobic (4 h) process, was seeded and acclimated according to the way described below, and the initial concentrations of mix liquor suspended solids (MLSSs) in two reactors were set both around 4000 mg/l. The operation of two SBRs was the same as described in our previous publication (Wang et al., 2008) with minor revisions. The 720 min cycles of both SBRs consisted of approximately 240 min aerobic period, followed by 28 min settling, 2 min decanting and 450 min idle periods. Each of the reactors was constantly mixed with a stirrer except during the settling and decanting periods. Synthetic wastewater was fed to reactor during the first 2 min of the aerobic periods. For each SBR cycle, 7.81 supernatant was discharged after the settling period, resulting in a hydraulic retention time of 18 h approximately. In contrast to the conventional EBPR of anaerobic-aerobic process, anaerobic phase did not exist but long-term idle zone (450 min) was operated during two aerobic zones. In aerobic phases, dissolved oxygen concentration was controlled at 3 ± 0.2 mg/l using an on/off control valve that was connected with a compressed air supply. The pH was controlled at 7-8 during aerobic periods through addition of 0.5 M HCl and 0.5 M NaOH. The sludge retention time (SRT) in the two SBRs was maintained at approximately 12 days.

2.3. Analytical methods

Sludge samples from the reactors were immediately filtered through a Whatmann GF/C glass microfiber filter (1.2 μ m). The filtrate was analyzed for total phosphorus (TP), total organic carbon (TOC), and the filter was assayed for MLSS, mix liquor volatile suspended solids (MLVSSs), polyhydroxyalkanoates (PHAs) and sludge TP content. For analyses of cell glycogen and PHAs, two drops of 1 N hydrochloric acid were immediately added to the samples to stop the bacterial activity. Glycogen was measured by the phenol-sulfuric method with glucose as standard (Herbert et al.,

1971). Saturated HgCl₂ of 0.2 ml and 1 ml test mixture were added to several heat-resistant tubes, and then heated with 70–80 °C water for 15 min. After cooling, the samples were centrifuged at a speed of 2500–3000 r/min for 30 min and 1 ml distilled water was added in the centrifugal sludge, then 1 ml 5% phenol and 5 ml thick sulfuric acid were added in the mixture. After cooling, the samples were measured by spectrophotometry method at 490 nm.

The analyses of poly-3-hydroxybutyrate (PHB), poly-3hydroxyvalerate (PHV), and poly-3-hydroxy-2-methylvalerate (PH2MV) were conducted according to the method of Randall and Liu (2002) and Oehmen et al. (2005a) and described in our previous publication (Wang et al., 2008). Lyophilized sludge samples were digested, methylated and extracted with chloroform. The extracted methyl esters were analyzed using gas chromatography (GC) equipped with a DB-5 column (30 m length \times 0.25 mm $LD \times 0.25 \,\mu m$ film). A calibration curve using a PHB/PHV (88%/ 12%) standard and another calibration curve using 2-hydroxy-caproic acid purchased from Sigma-Aldrich Chemical Co. were obtained. It should be noted here that no sample was available for the direct measurement of PH2MV. In addition, 2-hydroxy-caproic acid and PH2MV are isomers, therefore, the former compound was utilized for the standard curve of PH2MV (Oehmen et al., 2005a). The total PHA was calculated as the sum of measured PHB. PHV and PH2MV.

TP, MLVSS and MLSS were measured according to Standard Methods (APHA, 1995), and TOC was determined using a TOC analyzer (ShimadzuTOC-500, Japan). Sludge TP content was measured by the method described in the Industry Standards of Town Construction of PRC (ISTCPRC, 2005). Dried sludge samples of 10–25 mg were put on the bottom of crucibles, 2 g of NaOH were tiled on the sludge samples after adding four drops of C_2H_5OH to the samples, and then dried in an oven. Shut off the switch for 15 min when the temperature was up to 400 °C. After that, dried at 650 °C for 15 min and cooled to room temperature. Approximately 80 °C of distilled water (10 ml) was added and mixed vigorously with each sample, and then the solution was filtered through a Whatmann GF/C glass microfiber filter (1.2 μ m). The filtrate was analyzed for TP according to Standard Methods.

3. Results

3.1. Summary of performances of TP removal in two SBRs during steady-state operation

Performances of TP removal in two SBRs during steady-state operation were summarized in Table 1. Besides Pi uptake for cell normal assimilation, e.g. C:N:P = 100:10:1 for wastewater (Yu et al., 2007), excess TP removal was observed in both G-SBR and A-SBR, but they showed quite different capabilities of TP removal (32.82 vs 9.55 mg/l on the average). Effluent TP concentration in G-SBR was much lower than in A-SBR (2.18 vs 25.45 mg/l on the average), and a much higher efficiency of TP removal in the G-SBR was obtained (93.8 vs 27.3% on the average). Unexpectedly, MLSS and MLVSS in G-SBR were also much higher than in A-SBR (4824 vs 2616 mg/l and 2991 vs 2114 mg/l on the average, respectively) when operated approximately as the same SRT (12 d), possibly because carbon source of acetate fed to A-SBR was shortchain fatty acids and was easier to be oxidized to CO₂ than that of glucose fed to G-SBR. To eliminate any possible effects of MLVSS concentrations on microbial ecosystem capabilities of TP removal, TP removal was also expressed relative to MLVSS present in two SBRs (Fig. 1). On a unit biomass basis there was an approximate 2.4-fold increase in the level of removal in G-SBR during steadystate operation, compared to that in A-SBR, which was in corre-

Table 1
Summary of performance parameters in two SBRs during steady-state operation.

Item	G-SBR			A-SBR		
	Max	Min	Avg	Max	Min	Avg
TP in effluent (mg/l)	2.87	1.95	2.18	26.84	25.02	25.45
TP removed (mg/l)	33.05	32.13	32.82	9.98	8.16	9.55
Efficiency of TP removal (%)	94.4	91.8	93.8	28.5	23.3	27.3
MLSS (mg/l)	5107	4561	4824	2831	2429	2616
MLVSS (mg/l)	3243	2785	2991	2386	1897	2114
Glycogen accumulation (mmol-C/VSS)	3.15	2.82	3.06	1.63	1.46	1.57
PHA accumulation (mmol-C/VSS)	0.28	0.13	0.23	2.94	2.68	2.75

Data were obtained over a 100-day steady-state operation period after stabilization for 40 days (more than 3 times of SRT).



Fig. 1. Comparisons of phosphorus removal performances of two SBRs over the first 100 day test period (filled symbols refer to changes in G-SBR, and open symbols refer to changes in A-SBR).

spondence with a greater TP content in the sludge of G-SBR than that of A-SBR (Fig. 1). Furthermore, the lower VSS/SS ratio (Fig. 1) showed in G-SBR also implied that there was a higher level of poly-P accumulated in the sludge (Oehmen et al., 2005b). All above facts exhibited that G-SBR showed a much better performance of TP removal than that of A-SBR.

3.2. Changes of TP, TOC, glycogen and PHAs during one cycle in two SBRs

From Figs. 2 and 3, it can be indicated that external substrates were quickly depleted in two SBRs (about 45 and 30 min of aeration in G-SBR and A-SBR, respectively), thus we divided one cycle into three periods: a feast period defined as the time when external substrates were consumed and a famine period when the added external substrates had been exhausted in aerobic zone as well as an idle starvation period (settling-decanting-idle periods).

Along with rapid decreases of TOC during the feast period, significant internal storage compounds were accumulated in two SBRs, and the phenomenon of aerobic storage was similar to that observed by other researchers (Carta et al., 2001; Dirck et al., 2001; Carucci et al., 2001). Nevertheless, different storage polymers were observed in two SBRs. Except for substantial glycogen accumulation (3.09 mmol of C/g VSS), unconspicuous increases of PHAs were observed in G-SBR, and similar changes of PHAs have been reported in our previous publication (Wang et al., 2008). In contrast to G-SBR, significant glycogen and PHAs were simultaneously synthesized in A-SBR (0.39 and 2.77 mmol of C/g VSS, respectively), moreover, Fig. 3 also showed that the storage polymer of PHB gave the main contribution to the calculation of PHAs accumulation (2.19 mmol-C of PHB vs 0.37 mmol-C of PHV vs 0.21 mmol-C of PH2MV/g VSS, respectively). However, just a slight TP removal was observed in the feast period of both SBRs (2.01 and 1.01 mg of TP/l in G-SBR and A-SBR, respectively).

During the famine period of aeration (from min 45 and 30 to min 240 in G-SBR and A-SBR, respectively), glycogen and PHAs were, respectively, degraded to provide carbon and energy sources for cell growth and maintenance in G-SBR and A-SBR, and glycogen accumulated in G-SBR and PHAs synthesized in A-SBR during the feast period were almost depleted at the end of aeration, but glycogen in A-SBR was continued synthesizing to a high level (from 3.25 to 4.36 mmol of C/g VSS). Surprisingly, obvious TP removal was observed in the famine period in both SBRs (24.10 and 7.71 mg of TP/I in G-SBR and A-SBR, respectively). The behavior of the current period in A-SBR was similar to that observed in aerobic zone of conventional EBPR by other researchers (Oehmen et al., 2005b; Tong and Chen, 2007), but the action in G-SBR was peculiar.

In contrast to the traditional activated sludge process, an idle starvation period was operated in this study. During this period, unconspicuous changes of glycogen and PHAs were observed other than a high level of Pi release (13.56 mg of Pi/l) in G-SBR. On the contrary, A-SBR had a lower release of Pi (6.19 mg of Pi/l), and had an obvious decrease of glycogen (1.54 mmol of C/g VSS) coupled with a substantial increase of PHAs (1.13 mmol of C/g VSS). Furthermore, the increase of PHAs was completely due to the significant increase of PHV.

3.3. Pi removal under fully aerobic conditions and the energy storages of conventional EBPR paradox

Since the first report of organisms capable of accumulating excess amounts of poly-P by alternating anaerobic and aerobic conditions (Shrinath et al., 1959), numerous studies have been conducted in order to understand the EBPR mechanisms and to accomplish stable and efficient biological phosphorus removal processes. Although several disputes still exist, the essential biochemical metabolisms of EBPR are well understood and accepted by most researchers. It is generally accepted that alternating anaerobic and aerobic conditions is necessary for EBPR and Pi is removed from wastewater by uptake into poly-P organisms and conversion into poly-P during the aerobic period. These poly-P organisms then break the phosphodiester bonds of the stored poly-P to provide an energy source for taking up and storing available VFAs (mostly acetate and propionate) as PHAs during the anaerobic period (Martín et al., 2006). Efficiently sequestering VFAs during the anaerobic



Fig. 2. Variations of TP, TOC and sludge glycogen as well as sludge PHAs during one cycle in G-SBR. MLSS and MLVSS were 4972 and 3047 mg/l. Supernatants of 7.8 l were discharged during min 268–270, and therefore, aqueous volume calculated for the release of TP concentrations during idle period was 4.2 l. In addition, 7.8 l synthetic wastewaters were fed during min 0–2, and data measured at min 0 represented the values which were obtained at the end of the idle period of last cycle.



Fig. 3. Variations of TP, TOC and sludge glycogen as well as sludge PHAs during one cycle in A-SBR. MLSS and MLVSS were 2836 and 2184 mg/l. Supernatants of 7.8 l were discharged during min 268–270, and therefore, aqueous volume calculated for the release of TP concentrations during idle period was 4.2 l. In addition, 7.8 l synthetic wastewaters were fed during min 0–2, and data measured at min 0 represented the values which were obtained at the end of the idle period of last cycle.

period is thought to give the poly-P organisms a selective advantage over other members of the community for subsequent growth and replication in the aerobic period, allowing them to dominate EBPR sludge. Moreover, PHB (PHA) is widely considered as a key storage for EBPR, because a high level of PHB (PHA) accumulation in the anaerobic period can insure the energy production required for Pi uptake in the subsequent aerobic period. Furthermore, glycogen just plays a role in providing NAD(P)H and extra energy for PHAs synthesis in the anaerobic zone (Mino et al., 1998). However, in G-SBR of this study, the anaerobic period was not conducted and no change of PHB (PHA) was observed during the whole cycle other than a substantial accumulation/transformation of aerobic storage of glycogen (Fig. 2), in addition, high efficiency of Pi removal was achieved (Table 1). Although significant accumulations of PHAs and glycogen were measured in A-SBR (Fig. 3), it showed the much lower efficiency of Pi removal (Table 1). The performances of Pi removals of two SBRs and the energy storages of conventional EBPR paradox strongly indicated that an entirely different energy metabolism of Pi removal did exist in such a single-stage oxic process.

4. Discussion

4.1. Energy metabolism of Pi removal with single-stage oxic process in this study

This research showed a completely different behavior of Pi removal from conventional EBPR, and the energy metabolisms we proposed were summarized in Fig. 4.

Since the bacteria in this study is to encounter external substrates feast and famine regime, and the regime can induce the bacteria to store external substrates as internal storage compounds in the feast period which hereby can take up available substrate very fast and utilize it to gain a more balanced growth (Dirck et al., 2001; Carucci et al., 2001). Glycogen and PHB were the dominant aerobic storages when glucose and acetate were fed to SBRs as the sole carbon source, respectively. Besides the reserves of PHAs, significant glycogen was synthesized in A-SBR (Fig. 4a), and the metabolic activities of the glycogen and PHA formations are summarized in Eqs. ((1)-(7)). Glucose is phosphorylated during the transportation into the cytoplasm Eq. (1), and is synthesized as the internal storage of glycogen (Wang et al., 2002) Eq. (2).

$$ATP + glucose \rightarrow glucose-6-phosphate + ADP$$
 (1)

glucose-6-phosphate + ATP + $(C_6H_{10}O_5)_n$ + H_2O

$$\rightarrow (C_6 H_{10} O_5)_{n+1} + ADP + 2H_3 PO_4$$
⁽²⁾

It is assumed that part of the end product of glycolysis, pyruvate, was utilized for PHB synthesis (Wang et al., 2002). The precursor for PHB formation is 2 acetyl-CoA, and the pathway of PHB synthesis is summarized in Eq. (3).



Fig. 4. The possible energy metabolism of poly-P organisms in the single-stage oxic process. (a) In the feast period external substrates are consumed and internal storages are synthesized. Furthermore, glucose is stored as glycogen (I), and acetate is store as PHB (the main storage), PHV, PH2MV and glycogen (II). (b) In the famine period the mass synthesis of poly-P via ATP depletes the wastewater of Pi, thus giving rise to EBPR. ATP is supplied by PHAs degradation in A-SBR (II), however, glycogen provides ATP for poly-P synthesis in G-SBR (I). (c) In the idle starvation period ATP required for bacteria maintenance is supplied by poly-P degradation and thus the poly-P of poly-P organisms reserves ensures its dominance in the SBR microbial ecosystem (I). Since a lower level of poly-P accumulation results in deficient ATP supply in A-SBR, extra ATP is produced through the conversion of glycogen to PHV (1 or 2 moles ATP are produced per mol of glycogen converted to PHV) (II).

(4)

 $2CH_3COCOOH + NAD + (C_4H_6O_2)_n$

$$\rightarrow (C_4 H_6 O_2)_{n+1} + 2CO_2 + NADH_2 \tag{3}$$

Additionally, PHV formation requires two precursors in equal amounts: acetyl-CoA and propionyl-CoA, and PH2MV formation requires 2 propionyl-CoA (Mino et al., 1998). The formations of acetyl-CoA and propionyl-CoA are summarized in Eqs. (4) and (5) (Wang et al., 2002; Oehmen et al., 2007).

$$CH_{3}COCOOH + NAD + CoASH$$

$$\rightarrow C_{3}COSCoA + CO_{2} + NADH_{2}$$

 $CH_3COCOOH + 2NADH_2 + CoASH + ATP$

 $\rightarrow C_3 CH_2 COSCoA + H_2 O + 2NAD + ADP + 2H_3 PO_4$ (5)

Therefore, PHV and PH2MV formations can be summarized in Eqs. (6) and (7).

$$\begin{array}{l} 2CH_{3}COCOOH + NADH_{2} + (C_{5}H_{8}O_{2})_{n} + ATP \\ \rightarrow \ (C_{5}H_{8}O_{2})_{n+1} + H_{2}O + NAD + CO_{2} + ADP + 2H_{3}PO_{4} \end{array} \tag{6}$$

 $2CH_3COCOOH + 4NADH_2 + (C_6H_{10}O_2)_n + 2ATP$

$$\rightarrow (C_6H_{10}O_2)_{n+1} + 2H_2O + 4NAD + 2ADP + 4H_3PO_4$$
(7)

The aerobic storages in the feast period are very important to bacteria growth under dynamic conditions, which can give an advantage over other bacteria in microbial ecosystem. The more the microorganisms are able to store during the feast period and subsequently use it for growth, the more they have a competitive advantage (Beccari et al., 1998).

In the famine period, Pi was incepted into cell and the Pi transported into the cell could be synthesized into poly-P via ATP (1.2 mole ATP per mole poly-P accumulation) (Maurer et al., 1997). Since the external substrates have been exhausted in the famine period, one might want to know where ATP required for poly-P accumulation comes from. From Figs. 2 and 3, glycogen/PHA accumulated in the feast period was obviously degraded, and the degradation of glycogen/PHA would generate ATP (Martín et al., 2006). The ATP generated from glycogen/PHA degradation just provided for the poly-P synthesis (Fig. 4b), thus the metabolic relation between Pi uptake and storage product formation was established. Moreover, the surplus ATP generated from glycogen oxidation was provided for cell growth and maintenance in G-SBR. However, in A-SBR, part of energy via PHAs degradation was utilized for glycogen synthesis other than cell growth and maintenance, which could provide an alternative explanation for lower MLVSS in A-SBR (Table 1). The PHAs degradation and glycogen accumulation in A-SBR could be explained by the reason that PHAs were the primary energy sources during the famine period. Lopez et al. (2006) observed that a rapid utilization of PHAs and a slower utilization of glycogen and poly-P to generate maintenance energy under aerobic starvation conditions. It should be noted that ATP provided for poly-P reserves was not supplied by PHAs but glycogen in G-SBR, and this behavior was absolutely different from the conventional energy metabolism of EBPR which proved that poly-P organisms utilized ATP produced through PHAs degradation only and dominant storage of glycogen would result in the breakdown of EBPR due to the outgrowth of glycogen accumulating bacteria (Cech and Hartman, 1993; Mino et al., 1998).

During the idle starvation period when the external substrate and aerobic storage of glycogen were exhausted in G-SBR, poly-P was hydrolyzed to provide energy for poly-P organisms maintenance and the organism shuttled out Pi across its plasma membrane, and therefore, poly-P synthesis and degradation in the famine and idle starvation periods respectively could give poly-P organisms an obvious advantage over other organisms of the community and ensure its dominance in the microbial ecosystem. In contrast, energy required for bacteria maintenance in A-SBR was supplied by both poly-P degradation and the conversion of glycogen to PHV (Fig. 4c), for neither poly-P degradation nor glycogen conversion could not provide enough ATP for bacteria maintenance during such a long idle starvation period. Lu et al. (2007) still observed that poly-P and glycogen were utilized simultaneously under anaerobic and anoxic conditions for maintenance energy production, and they explained that glycogen would be the primary energy source until the glycogen content reached very low levels. The possible biochemical pathway for the conversion of glycogen to PHV during the idle starvation period was summarized in Fig. 4c, 2 or 3 moles of ATP are produced in the glycolysis step, depending on the pathway considered (ED or EM), and propionyl-CoA must be produced in order to maintain the redox balance (Mino et al., 1998; Lopez et al., 2006). Overall, 1 or 2 moles ATP are produced per mol of glycogen converted to PHV. Another noticeable point was the essential difference between the idle starvation period in this research and the anaerobic period in conventional EBPR, though they had a similar behavior of Pi release. In anaerobic period of conventional EBPR, since external substrate was present, ATP generated from poly-P degradation was provided for PHAs synthesis rather than bacteria maintenance, but in the absence of external substrate of idle starvation period, ATP production was just provided for bacteria maintenance in this study.

4.2. The possible reason for significant Pi removal under single-stage oxic condition

This study allowed us to confirm the surprising results of Pi uptake/poly-P accumulation with single-stage oxic process and to investigate the energy storages. One might want to know why such a single-stage oxic process can achieve significant Pi removal. One possible reason is that Pi removal/poly-P accumulation is induced by exposure to nutrient limitation resulted from the operation of "feast-famine-idle starvation" periods in this study. Mullan et al. (2006) reported that microbial poly-P accumulation might be enhanced by exposure to such environmental conditions as nutrient limitation, osmotic stress, stationary phase stress, or, as was the case for EBPR, by alternating aerobic and anaerobic conditions. Moreover, from the view of biochemical pathway, the capability of Pi removal/poly-P accumulation by microbial ecosystem is depended on the role of poly-P playing in its biochemical metabolism (de-Bashan and Bashan, 2004). If ATP produced by poly-P degradation is required to maintain the microbial growth balance, good performance of Pi removal will be achieved such as traditional EBPR, or else, Pi removal will fail such as EBPR feeding with glucose as the sole carbon source, because glycogen substitutes for poly-P to provide energy for PHAs synthesis. This point can be supported by the publication reported by Wang et al. (2002), which showed a surprising performance of good EBPR, still using glucose as the sole substrate, and explained that the ED pathway was used for anaerobic glucose metabolism, because this pathway required poly-P as an energy source. In this study, external substrates and most of other aerobic storages are depleted during the feast and famine periods, in addition, poly-P don't seem to provide as an energy source for cell growth and maintenance during aerobic starvation (Lu et al., 2007), the facts thus insure poly-P playing an essential role in microbial energy metabolism because of its ATP production for bacteria maintenance during the long-term idle starvation period.

Furthermore, another question as to why the efficiency of Pi removal is much lower in A-SBR than that in G-SBR. The exact reason is still unclear so far and is the focus of our ongoing research program. Since glycogen is provided as the primary energy source for bacteria maintenance until the content of glycogen reaches very low levels (Lu et al., 2007), and a significant decrease of glycogen does occur during the idle period in A-SBR (1.54 mmol C/g VSS, Fig. 3), one probable reason is that glycogen serves as an energy source rather than poly-P during the idle starvation period (Fig. 4c), as a result, poly-P plays a subsidiary role in microbial maintenance, resulting in lower efficiency of Pi removal in A-SBR. Additionally, the additional energy is necessary to synthesize poly-P (1.2 mole ATP per mole poly-P accumulation) (Maurer et al., 1997), thus an alternative explanation is that ATP produced during the famine period in A-SBR is less than that in G-SBR, if a lower energy production occurred, then less ATP could be supplied for poly-P accumulation other than cell growth and maintenance. The degradations of total aerobic storages (mostly PHAs and glycogen) during a famine period in G-SBR and A-SBR were 3.07 and 2.65 mmol of C/g VSS, respectively (Figs. 2 and 3), thus less energy was produced on a unit biomass basis in A-SBR, resulting in less Pi uptake.

This paper suggested the existence of a significant, yet previously unrecognized, microbial response to nutrient limitation which may be of importance for phosphate-cycling in the biosphere and could also form the basis of a potentially novel strategy for the 'one-step' removal of Pi from effluents.

5. Conclusions

The enhanced aerobic uptake of Pi and its intracellular accumulation by poly-P organisms could be induced by exposure to nutrient limitation. G-SBR showed a much better performance of TP removal (91.8–94.4%) than that of A-SBR (23.3–28.5%). Additionally, different aerobic storages accumulations/transformations were observed in two SBRs, glycogen and PHA were main storages in G-SBR and A-SBR, respectively. The results indicated both glycogen and PHA would supply as energy source for Pi uptake under single-stage oxic process, and the possible biochemical metabolisms were proposed.

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