Biological phosphorus removal in anoxic-aerobic sequencing batch reactor with starch as sole carbon source
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ABSTRACT
In traditional biological phosphorus removal (BPR), phosphorus release in anaerobic stage is the prerequisite of phosphorus excessive uptake in aerobic conditions. Moreover, when low molecular weight of the organic substance such as volatile fatty acids (VFAs) is scarce in bulk liquid or anaerobic condition does not exist, phosphate accumulating organisms (PAOs) have difficulty removing phosphorus. However, in this work, phosphorus removal in two anoxic-aerobic sequencing batch reactors (SBRs) was observed when starch was supplied as a sole carbon source. The relations of the BPR with idle period were investigated in the two identical SBRs; the idle times were set to 0.5 hr (R1) and 4 hr (R2), respectively. Results of the study showed that, in the two SBRs, phosphorus concentrations of 0.26–3.11 mg/L in effluent were obtained after aeration when phosphorus concentration in influent was about 8 mg/L. Moreover, lower accumulations/transformations of polyhydroxyalkanoates (PHAs) and higher transformation of glycogen occurred in the SBRs, indicating that glycogen was the main energy source that was different from the traditional mechanism of BPR. Under the different idle time, the phosphorus removal was a little different. In R2, which had a longer idle period, phosphorus release was very obvious just as occurs in a anaerobic–aerobic regime, but there was a special phenomenon of chemical oxygen demand increase, while VFAs had no notable change. It is speculated that PAOs can assimilate organic compounds in the mixed liquor, which were generated from glycolysis by fermentative organisms, coupled with phosphorus release. In R1, which had a very short idle period, anaerobic condition did not exist; phosphorus removal rate reached 63%. It is implied that a new metabolic pathway can occur even without anaerobic phosphorus release when starch is supplied as the sole carbon source.

Key words | anoxic-aerobic SBR, biological phosphorus removal, glycogen, PHAs, sequencing batch reactor, starch

INTRODUCTION
Nitrogen and phosphorus (P) are the two most important nutrients for organisms, while the excessive accumulation of nitrogen and phosphorus discharged into water can cause eutrophication, which has become a severe water pollution problem all around the world. Almost all wastewater treatment plants (WWTPs) worldwide achieve nitrogen removal by alternately exposing a population of bacteria including nitrifiers and denitrifiers to aerobic environments for nitrification and anoxic conditions for denitrification (Lee et al. 2010). Theories for biological phosphorus removal (BPR) have been established and developed since the phenomenon was first reported that activated sludge could take up excessive phosphorus required for normal biomass growth (Shrinath et al. 1959). Several empirical models have been described (Wentzel et al. 1986; Mino et al. 1998) to attempt to explain the enhanced biological phosphorus removal (EBPR) metabolism, mainly from data from studies analysing community chemistry in response to acetate as the sole carbon source, and these models have been critically examined in earlier reviews (Van Loosdrecht et al. 1997;
Mino et al. (1998). The models all explain successful EBPR as a consequence of the phosphate accumulating organisms (PAOs) achieving dominance under anaerobic/aerobic (A/O) recycling conditions by having a selective advantage over the other bacterial populations present in their abilities to synthesize intracellular storage compounds under the ‘feast-famine’ conditions which characterize EBPR systems. PAOs are enriched by recirculating the activated sludge through anaerobic and anoxic/aerobic conditions. Thus, under anaerobic conditions, the PAOs are thought to rapidly assimilate organic substrates like acetate and use these to synthesize polyhydroxyalkanoates (PHAs) using stored polyphosphate (poly-P) as an energy source, and the orthophosphate generated from poly-P degradation is released into the bulk liquid. Then in the absence of any organic compounds in the aerobic zone, organisms with stored PHA are able to use these as carbon and energy sources to grow and to assimilate phosphate to synthesize poly-P. Thus, PAOs achieve dominance under the prevailing anaerobic-aerobic conditions because they alone can grow aerobically in the absence of any exogenous source of carbon and energy, by using the PHA accumulated anaerobically.

However, the mechanism of phosphorus removal by PAOs is not fully established under conditions that differ from the classic anaerobic-aerobic conditions (Pijuan et al. 2005) and some phenomena cannot be explained by the existing mechanism. For example, Wang et al. demonstrated that the efficiency of phosphorus removal could be increased using glucose as the sole carbon source under single-stage oxic conditions (Wang et al. 2008), and the phenomenon was similar to that observed by other researchers (Wang et al. 2009; Xu et al. 2015; Chen et al. 2013a, 2013b, 2014; Li et al. 2014). Kapagianidis et al. (2012) reported that phosphorus removal also occurred in a continuous-flow anaerobic-anoxic activated sludge system. Jin et al. (2012) showed that phosphorus removal could be realized without significant anaerobic phosphorus release, indicating that there might be a special pattern for phosphorus removal.

As is known, carbon source plays an important role in BPR. The microbial-accumulated biopolymers (PHA, poly-P and glycogen) are vital for the metabolism of PAOs, and the formation of polymer composition is mainly dependent on the substrate (Ginige et al. 2009; Wang et al. 2012). Consequently, a mass of carbon sources in wastewater is a prerequisite for achieving EBPR process (Carvalho et al. 2007; Wang et al. 2008). However, the components of real wastewater are complex, including several organic carbon sources such as macromolecular organic compounds, and volatile fatty acids (VFAs). The available carbon source in municipal wastewater is mainly in the form of VFAs, which are limited and scarcely satisfy the demand of denitrification and EBPR simultaneously. As a result, the removal efficiency of the system cannot be improved.

Starch production plants in China generate more than 20 million tons of starch wastewater per year, which could cause serious pollution of the ecosystem (Xue et al. 2010; Vera et al. 2015). Several starch wastewater treatment methods have been developed including aerobic process, anaerobic process, hybrid process, gas flotation, chemical flocculation and biological ponding (Liu et al. 2013; Hinken et al. 2014). However, little information is available on utilization of starch wastewater for nitrogen and phosphorus removal. Tam et al. showed that the addition of readily biodegradable carbon significantly enhanced the nutrient removal, while starch supplementation was detrimental to phosphorus removal (Tam et al. 1992).

In this study, the BPRs in two alternating anoxic-aerobic sequencing batch reactors (SBRs), supplying starch as sole carbon source, were investigated. Its mechanism and relation with the idle time in the SBRs were explored.

MATERIALS AND METHODS

Experimental device and operational methods

This study was conducted using two identical SBR systems (R1 and R2), which were made of Lucite (barrel diameter 25 cm, working height 40 cm) and had a volume of 6 L. The running period was set at 6 hr for each cycle: 75 min anoxic period and 285 min aerobic period. The two reactors were run for three or two cycles each day, with idle times (including settling time 25 mins and decanting 5 mins) of 0.5 hr (R1) and 4 hr (R2). Synthetic wastewater (composition detailed below) was fed to reactor during the first 5 mins of the anoxic period. For each SBR cycle, about 3 L supernatant was drawn out. In aerobic phase, dissolved oxygen (DO) concentration was controlled at 2 ± 0.2 mg/L using an on/off control valve that was connected to a compressed air supply. The influent pH was not controlled and varied between 7.0 and 7.5. The reactor was inoculated with sludge from the secondary clarifier of a local municipal WWTP in Xi’an, China, was seeded and acclimated according to the method described above, the sludge retention time was controlled at about 20 d and the initial concentration of mixed liquor suspended solids (MLSS) in reactor was set around 3,000 mg/L.
Synthetic media

Soluble corn starch was used as the sole carbon source. The solution was made through mixing starch with an appropriate amount of warm water. The detailed compositions of synthetic wastewater are indicated below: chemical oxygen demand (COD) 400 mg/L, NH₄-N (NH₄Cl) 10 mg/L, NO₃-N (KNO₃) 20 mg/L, P (KH₂PO₄) 8 mg/L, MgSO₄·7H₂O 20 mg/L, alkalinity (CaCl₂) 20 mg/L. The trace metals solution has been also described in mg/L: 180 KI, 150 H₃BO₃, 30 CuSO₄·5H₂O, 120 ZnSO₄·7H₂O, 1.54 FeSO₄·7H₂O, 100 MnCl₂·4H₂O, 150 CoCl₂·6H₂O, 60 Na₂MoO₄·2H₂O, 120 NH₄HCO₃, 37 H₂BO₃, 180 KI, 150 H₃BO₃, 30 CuSO₄·5H₂O, 120 ZnSO₄·7H₂O, 1.54 FeSO₄·7H₂O, 10 ethylene diamine tetraacetic acid (EDTA).

Analytical methods

Water samples were collected daily. Sludge samples from the reactors were immediately filtered through a 0.45 μm filter. The filtrate was analysed for COD, total nitrogen (TN), NO₂-N, NO₃-N, NH₄-N, PO₄³⁻-P, MLSS and mixed liquor volatile suspended solids (MLVSS) according to Standard Methods (APHA 1998). Glycogen was measured by the anthrone method (Liu et al. 2007). Briefly, sludge samples were sonified using the method described by Dignac et al. (1998) with an ultrasonic probe (Bioblock Scientific, 37 W, 20 kHz, 1 min). Sonicated sludge was centrifuged (5,000 rpm for 5 min, 4°C) and washed three times with ultrapure water removal of the supernatant; a volume of 5 mL of 0.6 mol/L HCl was added into a known weight of freeze-dried biomass in screw-topped glass tubes, and then heated at 105°C for 6 hr. After cooling and centrifugation, 1 mL of the supernatant was analysed for glucose using ultraviolet spectrophotometry.

PHA was analysed by the gas chromatography method (Agilent 6890N, USA). Total PHA was calculated as the sum of measured poly-β-hydroxybutyrate (PHB) and poly-β-hydroxyvalerate (PHV) (Oehmen et al. 2005). About 50 mL sludge samples were mixed with formaldehyde at a ratio of approximately 1% formaldehyde per sample volume in order to inhibit biomass activity in the sludge. The samples were centrifuged and the supernatant removed, and then they were washed with a phosphate buffer solution, re-centrifuged, and the supernatant was decanted once more. All samples were then lyophilized through a freeze drying unit operated at −54°C and 0.1 mbar for at least 20 h. 2 mL of chloroform and 2 mL of an acidified methanol solution were added to approximately 20 mg of lyophilized sludge, and then the samples were digested at 100°C in an oven for 7 h and cooled to room temperature. Distilled water (2 mL) was then added and mixed vigorously with each sample to remove particulate debris from the chloroform phase and prevent degradation of the gas chromatography (GC) column. After mixing, 1 h of settling time was allowed to achieve phase separation. The chloroform (bottom) phase was then injected into the GC column. The chromatography was operated with a DB-5 column (30 m length × 0.25 mm inner diameter × 0.25 μm film thickness), a split injection ratio of 1:15 and helium as the carrier gas (1.5 mL/min). A flame ionization detection (FID) unit was operated at 500°C with an injection port temperature of 250°C. The oven temperature was set to 80°C for 1 min, increased at 10°C/min to 120°C, and then to 270°C at 45°C/min and held for 3 min. The glycogen, MLSS and MLVSS concentrations are the averages of duplicate measurements. Average values are presented in the text with standard deviation. Total phosphorus (TP) was converted to orthophosphate through digestion with potassium persulfate; then, the method for orthophosphate determination was applied. The sludge phosphorus content is calculated by (TP−Pₜ), where Pₜ is the phosphate in the effluent in mg/L. Samples for the determination of dissolved iron were immediately filtered using 0.22 μm filter membrane and then acidified by hydrochloric acid (1 M) before atomic absorption spectroscopy (GBC Avanta PM-GF3000, Australia).

VFAs were measured with GC, and the analysis of acetic acid, propanoic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, n-butyric acid, caproic acid, was conducted according to the method of Julák et al. (2000). The chromatography was operated with a DB-5 column (30 m length × 0.25 mm inner diameter × 0.25 μm film thickness), a split injection ratio of 1:15 and helium as the carrier gas (1.5 mL/min). A FID unit was operated at 200°C with an injection port temperature of 250°C. The oven temperature was set to 100°C for 1 min, and increased at 3°C/min to 160°C.

RESULTS AND DISCUSSION

Start-up and performance of the SBRs

The domestication of the sludge in the two SBRs for TN and soluble orthophosphate (SOP) removal was finished after about 20 days, and then experiments for TN and SOP removal were conducted and lasted for about 180 days. In the initial stage of the SBRs, the two systems showed no significant difference in their performance over the first 4 days of acclimation (Figure 1). This implies that a period of ‘accumulation time’ is required when starting SBR phosphorus removal systems (Jeon et al. 2003; Seviour et al.
2003; Wang et al. 2008). In the later stages of the start-up period, phosphorus removal of the two SBR systems was substantially higher. The two SBR systems showed similar TN removal efficiency in the start-up period during the first 7 days; TN removal efficiency was 73.3 ± 1.2% and 76.5 ± 1.3% for R1 and R2, respectively.

Comparison of SBR performance

Table 1 summarizes the main reactor performance of two reactors during a 50-day period after reaching steady-state operation. High levels of COD reduction were achieved in all reactors, with effluent COD levels consistently less than 40 mg/L. By comparing effluent NH₄⁺-N, NO₂⁻-N and NO₃⁻-N, it can be found that the difference in nitrogen removal performance in the two reactors was negligible. Effluent phosphorus concentration in R2, however, was lower than that in R1, which resulted in a higher phosphorus removal efficiency. Phosphorus removal efficiency of the two SBRs was 61.37 ± 2.54% and 94.47 ± 2.11%, respectively. Also, greater sludge TP content was measured in R2 compared with that in R1, which was in accordance with the TP removal efficiency data. The results show that the configuration and operation of SBRs could simultaneously improve TN and phosphorus removal and meanwhile have no detrimental effects on COD removal and nitrogen removal. In the following text, the reasons for the SBRs exhibiting a higher phosphorus removal will be explored.

Profile of phosphorus in SBRs during one cycle

In a traditional EBPR system, PAOs take up carbon sources, particularly VFAs anaerobically and store them as PHAs via poly-P hydrolysis and glycolysis. Subsequently, PAOs oxidize PHA via the tricarboxylic acid (TCA) cycle to provide energy for growth, glycogen replenishment, phosphorus uptake, and poly-P storage (Mino et al. 1998). Another group of microorganisms known as glycogen-accumulating organisms (GAOs) are usually found to have proliferated in these cases. GAOs are microorganisms that have a metabolism similar to PAO, i.e. taking up VFAs and accumulating them as PHA in the anaerobic phase, and oxidizing the stored PHA in the subsequent aerobic phase. Unlike PAO, the energy source and reducing power of GAOs are provided solely by glycogen degradation without poly-P involvement during anaerobic VFA uptake, and in addition no phosphorus is taken up by these species during the subsequent aerobic condition. Obviously, GAOs do not contribute to excess phosphorus

![Figure 1](https://iwaponline.com/wst/article-pdf/75/1/28/456378/wst075010028.pdf)
removal, although they compete with PAOs for the often limiting carbon sources in the wastewater (Bond et al. 1998; Thomas et al. 2003).

It has been well documented in the literature that BPR could be achieved in aerobic/extended-idle (AEI) process employing two typical substrates – glucose and acetate – as the carbon sources. Phosphorus release was observed at the initial aerobic stage where adequate carbon source was available and DO concentration was insufficient, which related the stage with anaerobic phase in traditional EBPR (Wang et al. 2009). Xu et al. (2015) deemed that an innovative static/oxic/anoxic activated sludge process characterized by static phase as a substitute for conventional anaerobic stage was proposed to enhance biological nutrient removal. Thus anaerobic conditions are necessary for EBPR. Unlike the classic anaerobic-aerobic process, in this research phosphorus removal was realized without experiencing specific anaerobic pools but extending the idle period to 30–240 mins, and the nitrate was the nitrogen source. Could phosphorus release occur under the long idle phase? Figure 2 shows some data about phosphorus, $\text{NH}_4^+$, $\text{NO}_3^-$, $\text{NO}_2^-$, COD, glycogen and PHAs in a typical cycle after about 80-day steady state operation.

The cyclic profiles of phosphorus, COD, glycogen and PHAs during the steady state operation are shown in Figure 2(a) and 2(b). In the initial part of the anoxic stage, the maximum phosphorus concentration in the mixed liquor reached 7.7 mg/L and 14 mg/L for R1 and R2 respectively. During the anoxic-aerobic period, phosphorus was taken up by microorganisms and effluent concentrations of 3.32 mg/L and 0.29 mg/L were obtained.

Starch is macromolecular organic matter. It is reported to be adsorbed, hydrolysed and then used for growth (or stored if excess amount is present) when available electron acceptors like DO and nitrate are present (Goel et al. 1998; Karahan et al. 2006). In R2, COD concentration rapidly decreased from 124 to 74 mg/L during the anoxic

![Figure 2](https://iwaponline.com/wst/article-pdf/75/1/28/456378/wst075010028.pdf)
first 30 min. It could be deduced that the COD reduction was mainly through initial physical adsorption (Jin et al. 2012). However, the value increased to 82 mg/L after the settle/decant/idle period. The increased COD concentration was quite different from the conventional EBPR, which will be discussed in the next part.

**Effect of idle time on phosphorus removal**

Two reactors were working in steady-state after approximately 80 days, displaying the effect of idle time on phosphorus removal.

It can be found that in R1, during the anoxic-aerobic period, amount of phosphorus uptake along with the carbon sources decreased, and then at the end of aerobic period, phosphorus concentration was 3.3 mg/L. Under the settle/decant/idle time (30 mins), phosphorus release was negligible at only 0.4 mg/L. In R1, anaerobic condition does not exist, and phosphorus absorption with glycogen synthesis occurred in both anoxic and subsequent aerobic period, whereas P uptake rate and glycogen synthesis rate declined. R1 phosphorus removal rate reached 63%. It is shown that phosphorus removal was achieved with no anaerobic condition, which was different from traditional phosphorus removal phenomenon. Janssen et al. (2002) showed that phosphorus/MLSS was 1–2% in activated sludge, which indicated that phosphorus absorption by microorganism growth and metabolism was finite. In R1 sludge, TP content was 42 mg P/g MLSS (shown in Table 1), showing that phosphorus removal was not only by microorganism growth and metabolism, but also by other phosphorus removal pathways.

As the extension of idle time, in R2 phosphorus release was obvious (the concentration of phosphorus increased from 0.28 mg/L to 11.25 mg/L). As a result, phosphorus concentration in R2 was much higher than that in R1 at the beginning of anoxic stage, which was the main reason for R2 showing higher P-up ratio. Also, it can be easily comprehended that phosphorus uptake ratio in R2 was greater than that in R1 (0.062 ± 0.003 versus 0.031 ± 0.002 mmol-phosphorus/g VSS h; VSS = volatile suspended solids). This behaviour was very similar to the classic phenotype of conventional PAOs. It can be seen in Figure 2(b) that glycogen content increased obviously during the whole anaerobic process. Similar to traditional anaerobic period, the settle/decant/idle phase also witnessed glycogen degradation with the phosphorus release. The experimental results also indicated that with the increase of idle time, phosphorus linearly increased.

**Effect of nitrate on phosphorus removal**

In the literature, nitrate has an obvious negative impact on phosphate release rate in the anaerobic stage. One of the possible reasons is that phosphorus can be used by denitrifying phosphorus removing bacteria (DPB) (Akin & Ugurlu 2004). The nitrate present is considered to provide denitrifying bacteria organic substrate by outcompeting PAOs; however, although nitrate is present, phosphorus release can proceed if the substrate is sufficient (Peng et al. 2010). In order to avoid an anaerobic period appearing in the system, the influent nitrogen source was NH4+ -N (NH4Cl) 10 mg/L, NO3- -N (KNO3) 20 mg/L.

As illustrated in Figure 2(c) and 2(d), during the anoxic period, almost all NO3 was denitrified to N2; almost all NH4 was oxidized to NO3 and NO2 in aerobic process. The changes of NH4-N, NO3-N and NO2-N in the anoxic-aerobic stage were similar in the two SBRs. Nitrate existed in the whole cycle of the two SBRs, and at the end of the aerobic period, nitrate concentration was 3.11 mg/L and 0.29 mg/L, respectively. In R1, effluent nitrate was 3.35 mg/L, and with a shorter idle time (30 min), phosphorus release under the idle period was only from 3.35 mg/L to 3.75 mg/L, so negligible variation of phosphorus release was observed during the idle period.

With an extension of idle time in R2, effluent nitrate was 0.29 mg/L, and P release during the idle time was obvious (the concentration of phosphorus was from 0.28 mg/L to 11.25 mg/L). Simultaneously, a large amount of glycogen degradation was found. This indicates that phosphorus release was affected by the nitrate concentration.

**Effect of external carbon source on phosphorus removal**

As is known, external carbon source plays an important role in BPR. The role of starch in phosphorus removal process is discussed as follows.

As can be seen from Figure 2, starch entered the reactor, COD concentration decreased rapidly, and at the same time poly-glucose concentration of the biomass (glycogen + adsorbed starch) increased. A high amount of phosphorus uptake along with the carbon sources decreased from 0 to 240 min, and then at the end of aerobic period, COD concentrations were under 40 mg/L. However, in the different idle period, COD concentrations increased linearly such as in R2 in which COD concentration increased from 32 mg/L to 82 mg/L. This was different from Gao et al.’s (2015) demonstration that the inclusion of a higher idle time caused an
extended anaerobic period in the system, which resulted from the endogenous respiration of bacteria, causing the hydrolysis of poly-Ps within their cells, and phosphorus release is not accompanied by the absorption of organic compounds. Montil et al. (2005) found that anaerobic phosphorus release rates were 5–30 mg-P/(g-VSS h). In contrast to the traditional activated sludge process, an idle starvation period was operated in this study. In R2, at the settle/decant/idle period, phosphorus release reached 11.89 mg-phosphorus/(g-VSS h); Phosphorus release rates in the settle/decant/idle periods were advantageous compared to the variation ranges of that in conventional anaerobic zone. This indicates that phosphorus release was not only intracellular poly-P hydrolysis, but also accompanied absorption of organic compounds.

In the traditional phosphorus removal process in sewage, PAOs assimilate VFAs under anaerobic conditions and store them mainly as PHB. Denitrifying bacteria use VFAs for denitrification under anoxic condition (van Loosdrecht et al. 1998). The sole carbon source starch belongs to macromolecular organic matter, which is not supposed to be utilized directly by PAOs and denitrifying bacteria traditionally. The starch needs to be transformed into small molecule organics by fermentative bacteria. In this study whether external carbon sources for PAOs were VFAs which were derived from starch hydrolysis was still unknown. Figure 3 shows some data about VFAs in a typical cycle of R2 after about 98-day steady-state operation.

As can be seen from Figure 3, the VFA concentration of R2 in the process of the whole operation had no obvious change, and was maintained at about 2.6 mg/L (VFAs were not detected in R1). It is speculated that the mechanism of EBPR in the SBRs was different from the traditional one.

**Effect of PHA and intracellular glycogen levels on phosphorus removal**

As is well known, PHA is the key storage product for conventional anaerobic/oxic process, and more anaerobic PHA accumulation will provide more energy for subsequent phosphorus uptake and poly-P synthesis, thus causing a higher phosphorus removal performance (Zhang et al. 2008). Wang et al. (2009) verified that the type of carbon sources could affect internal storage which provides power for the poly-P accumulation in the aerobic stage. Phosphate could be transformed to poly-P with single-stage oxic process with PHA accumulation fed acetate as carbon source; ATP provided for poly-P reserves was not supplied by PHA but glycogen fed glucose as carbon source. In this study, different to previous research with starch as the carbon source, the intracellular storage (PHA and glycogen) responsible for phosphorus removals during the entire operation cycle was also monitored in the two SBRs, which exhibited different variation characteristics. It can be seen in Figure 2 that the energy storage PHA increased not obviously in the two SBRs; this was similar to Wang et al. (2012) who showed a low-idle PHA production during the idle time. It has been reported that biological phosphorus removal is related to the transformation of intracellular PHA and glycogen; higher glycogen transformation suggests that the GAOs’ metabolism might be activated (Mino et al. 1998). Wang et al. (2012) proved that a long idle period with a low level of intracellular glycogen could significantly increase phosphorus release contents, thus remarkably enhancing phosphorus removal performance. In contrast, in this study there were conspicuous changes of glycogen during the whole process, denoting that glycogen was the main internal energy source for phosphorus removal and cell growth and maintenance, and two SBRs maintained higher phosphorus removal performance.

The settle/decant/idle phase witnessed glycogen degradation with phosphorus release (Figure 2(b)), implying that glycogen decomposed to provide energy through glycolysis. Two possible pathways were proposed to explain the source of the reducing power, i.e. the Comeau-Wentzel model suggested partial oxidation of acetyl-coenzyme A in the TCA cycle generated the reducing equivalents, and in the Mino model reducing power was derived from glycolysis of intracellular stored glycogen (Mino et al. 1998; Louie et al. 2000). In R2, PHA concentration remained almost
unchanged indicating the reducing power was derived from glycolysis of intracellular stored glycogen corresponding with the Mino model.

**Mechanisms of nutrient removal in the SBRs**

**Phosphorus removal mechanisms**

Since the first report of excess phosphate uptake by bacteria in the activated sludge process (Shrinath et al. 1979), several metabolic models have been described to attempt to explain the chemical changes (Wentzel et al. 1986; Mino et al. 1998). During the anaerobic stage, biomass PHA levels increase in parallel with the assimilation of short chain VFAs. Intracellular poly-P content falls, and phosphate levels in the bulk liquid increase. In the subsequent aerobic stage, PHA levels in the biomass fall concomitant with a decrease in phosphate levels in the bulk liquid and an increase in biomass phosphorus levels as poly-P (García et al. 2006). In recent years, biochemical models for EBPR with glucose have been supported by laboratory scale studies (Sudiana et al. 1999; Che & Jong 2000; Wang et al. 2002). For example, from 13C-NMR (nuclear magnetic resonance) data it has been suggested (Che & Jong 2000) that the lactic acid producing bacteria play a crucial role in the anaerobic zone.

In this study, however, starch which was thought to be not good enough for PAOs was only used as organic substrate. Additionally, conventional anaerobic period was not conducted and nitrate existed in the whole cycle of the SBRs. Two SBRs showed a completely different mechanism from the traditional mechanism of BPR (Mino et al. 1998) (PAOs take up carbon sources, particularly VFAs anaerobically and store them as PHAs via polyphosphate (poly-P) hydrolysis and glycolysis. Subsequently, PAOs oxidize PHA via the TCA cycle to provide energy for growth, glycogen replenishment, phosphorus uptake, and poly-P storage). Such as in R1, anaerobic condition does not exist, phosphorus removal rate reached 63%, and a higher level of phosphorus content in the sludge (Figure 4). As is well known, there are three forms of phosphorus in activated sludge: biological phosphorus (bio-P), metal phosphorus through physical chemistry process and poly-P. Bio-P is necessary for microorganism growth and metabolism; thus another possibility for phosphorus removal in this study was that phosphorus removal was the result of normal growth and metabolism by microorganisms. Janssen et al. (2002) showed that phosphorus/MLSS was 1–2% in activated sludge, which indicated that phosphorus absorption by microorganism growth and metabolism was finite. Furthermore, bio-P in activated sludge was associated with COD concentration, and previous investigations have shown that the COD/phosphorus rate by normal microorganism growth and metabolism was 200 mg COD/1 mg phosphorus. The COD/phosphorus rate in this study was 50 mg COD/1 mg phosphorus, which was much lower than the normal COD/phosphorus rate (200:1). As a result, it is inferred that phosphorus removal is achieved more by poly-P storage than by microorganism growth and metabolism. PO$_4^{3-}$ can form metal sediments like MgHPO$_4$, MgNH$_4$PO$_4$, Ca$_5$(PO$_4$)$_3$OH, FePO$_4$ etc. Could phosphorus removal in this study be through this approach? The Mg$^{2+}$, Ca$^{2+}$ and Fe$^{2+}$ concentrations in this study were very low (concentrations detailed above) and the sediments must be formed under the condition of pH > 8.0, but pH values in this study were controlled at 7 ± 0.5. Therefore, the hypothesis was also excluded. It is speculated that phosphorus removal can

**Figure 4** MLVSS/MLSS and sludge phosphorus (P) content transition during long-term operation in R1 and R2. The samples were taken from the solution at the end of aeration.
be achieved without an anaerobic phase, which is different from the traditional mechanism of BPR.

In R2, in contrast, phosphate release was observed during the long idle phase after 18 days, suggesting an increase in PAO activity in the systems; on the other hand, the lower MLVSS/MLSS ratio implied that there was a higher level of phosphorus content in the sludge (Figure 4). In the long idle time, however, COD increased obviously. It can thus be deduced that phosphorus removal in this research was not mainly performed by traditional PAOs but likely by other poly-P organisms. Che & Jong (2000) showed that the other poly-P organisms cooperated with lactic acid producing organisms (LPOs) to complete phosphorus release.

So with different idle time, the phosphorus removal pathway was different. It was shown that in R1 process with starch as sole carbon source, phosphorus removal was not only by microorganism growth and metabolism, but there were also other phosphorus metabolic pathways. A hypothesis that may explain this phenomenon is that some special PAOs in other phosphorus metabolic pathways. A hypothesis that may explain this phenomenon is that some special PAOs in other phosphorus metabolic pathways could be deduced that phosphorus removal in this research was not mainly performed by traditional PAOs but likely by other poly-P organisms. Che & Jong (2000) showed that the other poly-P organisms cooperated with lactic acid producing organisms (LPOs) to complete phosphorus release.

The phosphorus removal of R2 process may be described as follows: In anoxic period, starch was adsorbed as the sole carbon source, some fermentative bacteria utilized starch for glycogen synthesis and produced small molecule organic compounds at the same time; denitrifying phosphorus accumulating bacteria used nitrate as electron acceptor for phosphorus uptake. In the following aerobic period, PHAs decreased not only for energy storage, but also for electron acceptor for aerobic uptake. During settle/decant/idle period, glycogen was the primary energy source and rapidly decomposed to provide maintenance energy when the added external substrates exhausted, then the fermentative bacteria began to ferment, and at the same time, phosphorus accumulating bacteria used the fermented product for anaerobic metabolism with phosphorus release. So, it can be concluded that this SBR process was a typical EBPR system. Besides, phosphorus was removed through aerobic and anoxic process, with ratios of 75.5% and 24.5%, respectively.

Nitrogen removal mechanisms

In the anoxic reactors, phosphorus and nitrate were removed simultaneously, such as in R2, while the concentration of phosphate decreased by 5.50 ± 1.35 mg/L and that of nitrate decreased by 10.29 ± 0.64 mg/L, with the ratio of phosphorus/nitrogen of 0.55 ± 0.21. So it is inferred that part of nitrate was removed together with phosphate through denitrifying phosphorus removal. But Kuba et al. (1994) reported that the ratio of phosphorus/nitrogen removed through denitrifying phosphorus removal was 2.10, which was higher than the value in this study. In other words, the nitrate removed in the anoxic reactors was more than that of an ordinary denitrifying phosphorus removal process. So it is speculated that part of nitrate was removed through traditional denitrification pathway.

In R2, excessive phosphorus removal could be achieved without specific anaerobic period in activated sludge system. Compared with conventional anaerobic/oxic techniques, this regime has some merits, such as tolerance of higher nitrate level, achieving nitrogen and phosphorus removal simultaneously, and reduced dependence upon wastewater VFA content (Wang et al. 2008). Especially in idle phase, simpler operation control, absence of mechanical agitation, thus reduce running cost; also can avoid competition for carbon source between denitrifying bacteria and phosphorus accumulating bacteria in the subsequent anoxic period. In the future, further investigations are necessary to explore how to achieve phosphorus removal and what the metabolic process is when anaerobic phosphorus release does not exist; also what kind of carbon source is involved in the release of phosphorus in the long idle time.

CONCLUSIONS

This study showed that phosphorus and nitrogen removal could be achieved in the SBR with anoxic-aerobic process supplied with soluble starch as sole carbon source. It has been shown the energy storage PHAs decreased not obviously along with anoxic-aeration process; on the contrary, there were conspicuous changes of glycogen during the whole anoxic-aeration process, denoting that glycogen was the main internal energy source for phosphorus removal and cell growth and maintenance. Furthermore, different preference for phosphorus removal was achieved under two different idle times. Under the short idle time (30 min), phosphorus removal could be achieved in an anoxic-aerobic process with no anaerobic condition, and the phosphorus removal rate reached 63%. This is a new dephosphorization process. It was found that with the longer idle time of SBR with anoxic-aerobic process, phosphorus release was observed during long idle phase, but a special phenomenon of COD increased, while VFA had no notable change. Thus it can be deduced that phosphorus removal in this SBR was not mainly performed by traditional PAOs but likely by other poly-P organisms such as previous reports showed that the other poly-P organisms...
cooperated with another kind of microorganism hydrolysis fermentation to complete phosphorus release.

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