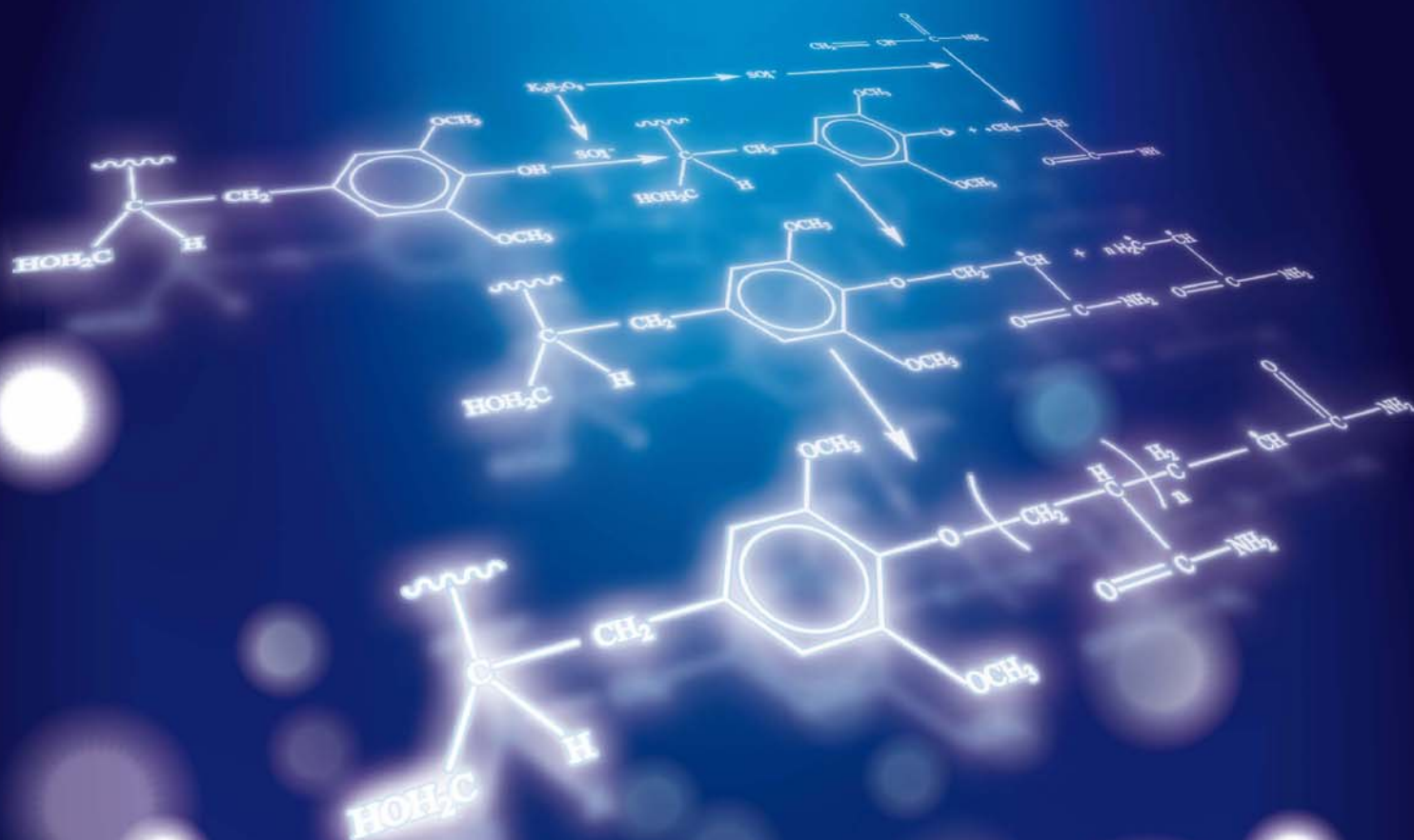


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## Effects of idle time on biological phosphorus removal by sequencing batch reactors

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### Abstract

Three identical sequencing batch reactors (SBRs) were operated to investigate the effects of various idle times on the biological phosphorus (P) removal. The idle times were set to 3 hr (R1), 10 hr (R2) and 17 hr (R3). The results showed that the idle time of a SBR had potential impact on biological phosphorus removal, especially when the influent phosphorus concentration increased. The phosphorus removal efficiencies of the R2 and R3 systems declined dramatically compared with the stable R1 system, and the P-release and P-uptake rates of the R3 system in particular decreased dramatically. The PCR-DGGE analysis showed that uncultured *Pseudomonas* sp. (GQ183242.1) and  $\beta$ -Proteobacteria (AY823971) were the dominant phosphorus removal bacteria for the R1 and R2 systems, while uncultured  $\gamma$ -Proteobacteria were the dominant phosphorus removal bacteria for the R3 system. Glycogen-accumulating organisms (GAOs), such as uncultured *Sphingomonas* sp. (AM889077), were found in the R2 and R3 systems. Overall, the R1 system was the most stable and exhibited the best phosphorus removal efficiency. It was found that although the idle time can be prolonged to allow the formation of intracellular polymers when the phosphorus concentration of the influent is low, systems with a long idle time can become unstable when the influent phosphorus concentration is increased.

**Key words:** biological phosphorus removal; polyphosphate-accumulating organisms; idle time; microbial community

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### Introduction

The discharge standards for nitrogen and phosphorus are becoming increasingly stringent, due to the role of these chemicals in causing the worsening problem of eutrophication. High phosphorus levels are a major contributing factor to algal growth (Sibrell et al., 2009), and reducing its content in water bodies is an important aspect of preventing eutrophication. Currently, the process of biological phosphorus removal using polyphosphate-accumulating organisms (PAOs) to remove phosphorus from wastewater is becoming widely applied. However, glycogen-accumulating organisms (GAOs) become a limiting factor to the accumulation of PAOs, because GAOs increase the volatile fatty acids (VFA) requirement but do not contribute to phosphorus removal (Saunders et al., 2003). The cultivation and enrichment of PAOs can be promoted by facilities with flexible and reliable controls for alternating between the anaerobic and aerobic operation stages.

Nutrients, such as VFA, are stored as polymers within bacterial cells. It is likely that the formation of intracellular polymers occurs only when the steady state is disrupted (Van Loosdrecht et al., 1997). Under anaerobic conditions, the mechanism of biological phosphorus removal by PAOs leads to the breakdown of intracellular polyphosphate, to produce energy and to synthesize intracellular polymers (such as poly- $\beta$ -hydroxybutyrate, PHB). Alternatively, under aerobic conditions, PAOs degrade the PHB to obtain energy for cell growth, glycogen synthesis and the uptake of excess phosphorus. Phosphorus removal from the system can therefore be achieved by the discharge of the excess aerobic sludge. Sequencing batch reactors (SBRs) can achieve alternating anaerobic and aerobic conditions by controlling the operational process, and consequently biological phosphorus removal using SBRs has drawn increasing attention worldwide (Cassidy and Belia, 2005; Pierson et al., 2000; Lin et al., 2003).

However, there have been few detailed or convincing studies regarding the ideal idle time of a SBR for efficient phosphorus removal or the effects of idle times on the

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growth of PAOs, which impacts the efficiency of the biological phosphorus removal process. In particular there have been no reports regarding the effect of idle time on the composition of the microbial communities within the system.

The purpose of the research was to investigate the effect of idle time on biological phosphorus removal systems. The combined technique of PCR-DGGE was used to analyze the microbial communities of the different systems to provide a theoretical basis for the initiation and stable operation of SBR biological phosphorus removal systems.

## 1 Experimental materials and methods

### 1.1 Operating conditions of the SBR systems

This study was conducted using three identical SBR systems (R1, R2 and R3), which were made of Plexiglas and had a volume of 12 L. The entire process, including water feeding (10 L), anaerobic phase, aeration (0.2 m<sup>3</sup>/hr), settling and decanting (10 L) was controlled by a timer. The running period was set at 7 hr for each cycle: 3 hr anaerobic and 4 hr aerobic. The three reactors R1, R2 and R3 were run for three, two or one cycle every day, with idle times (include settling time, feeding, and decanting) of 3, 10 and 17 hr, respectively. The concentration of the inoculated sludge was 3–3.5 g/L mixed liquor suspended solids (MLSS), and the sludge retention time was set to 8–10 days. This study was mainly divided into three phases, namely phase I (low phosphorus concentration start-up), phase II (stable phase) and phase III (high phosphorus concentration). In phase I and phase II, the phosphorus concentration of the influent was 8 mg/L. In phase III, phosphorus concentration of the influent was increased to 16 mg/L.

### 1.2 Sewage and sludge

The seeding sludge was taken from the secondary settling tank of the Harbin Wenchang Wastewater Treatment Plant, China, and was fed into the SBR reactors after a period of acclimation. A synthetic wastewater with the following composition was used: chemical oxygen demand (COD) (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>) 200–300 mg/L, NH<sub>4</sub><sup>+</sup>-N (NH<sub>4</sub>Cl) 15 mg/L, PO<sub>4</sub><sup>3-</sup>-P (KH<sub>2</sub>PO<sub>4</sub>) 8–16 mg/L, MgSO<sub>4</sub>·7H<sub>2</sub>O 50 mg/L, KCl 18 mg/L, alkalinity (CaCO<sub>3</sub>) 60 mg/L; trace element solution 1.0 mL/L. The composition of the trace element solution was FeCl<sub>3</sub> 1.5 g/L, CuSO<sub>4</sub> 0.03 g/L, MnSO<sub>4</sub> 0.16 g/L, KI 0.18 g/L, ZnSO<sub>4</sub>·7H<sub>2</sub>O 0.12 g/L, CoSO<sub>4</sub> 0.32 g/L, and EDTA 10 g/L. The temperature of the reactor was maintained at (24 ± 1)°C. The dissolved oxygen (DO) and pH were continuously monitored.

### 1.3 Analytical methods

Water samples were collected daily. All samples were filtered through qualitative filter paper. The COD PO<sub>4</sub><sup>3-</sup>-P, MLSS, mixed liquid volatile suspended solids (MLVSS),

and sludge volume (SV) were measured according to the standard methods (APHA, 2005).

### 1.4 Molecular biology methods

The biomass for bacterial population analysis was sampled from the reactor on the 120th cycle. Specific bacterial primer GC-338 (primer 338 plus a GC clamp attached at its 5' end, underlined below) and a reverse universal primer 518 supplied by Shanghai Songon Biology Engineering Technology & Services Co. Ltd. (China), were used in this study to amplify bacterial 16S rDNA. The nucleotide sequence of the primers was as follows: Primer GC-BSF: 5'-CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCGCTACGGGAGGCAGCAG-3'; primer 518: 5'-ATTACCGCGGCTGCTGG-3'. Genomic DNA extraction and PCR conditions were the same as described previously (Ovres et al., 1997) except that the annealing temperature of the touchdown PCR was 55°C. The PCR products were verified in 1% agarose gel. DGGE analysis of PCR products was performed with a Bio-Rad D-Code System (Bio-Rad Laboratories, Mississauga, Ontario, Canada). PCR samples were concentrated and 300 ng were loaded onto a 8% (W/V) polyacrylamide gel containing a 30%–60% gradient of denaturant (80% denaturant corresponding to 5.6 mol/L urea and 32% (V/V) deionized formamide). Bands of interest were reamplified, purified and sequenced using the Gel Recovery Purification Kit (Watson Biotechnologies Inc., Shanghai, China) according to the manufacturer's instruction. The DNA sequences were determined using the chain termination method in an ABI 3730 stretch sequencing system by a commercial service (Sangon, China), and submitted for comparison to the GenBank database using BLAST algorithms.

## 2 Results and discussion

### 2.1 Effect of idle time on the start-up

When the phosphorus concentration of the influent was low, 8 mg/L, the three systems showed no significant difference in their performance over the first four days of acclimation, in terms of anaerobic phosphorus release or aerobic phosphorus removal (Fig. 1). This implies that a period of “accumulation time” is required when starting SBR phosphorus removal systems (Wang et al., 2008; Seviour et al., 2003; Jeon, 2003). By the fifth day a low level (1.14 mg/L) of anaerobic phosphorus release was detected in R1. However by the tenth day, the phosphorus removal efficiency of R1 had gradually increased from 26.2% to 53.28%. A low level (less than 1 mg/L) of anaerobic phosphorus release was also detected for R2 by the fifth day. However, in the later stages of the start-up period, the phosphorus release and phosphorus removal of R2 was substantially higher. In contrast, almost no anaerobic phosphorus release was observed for R3 within the

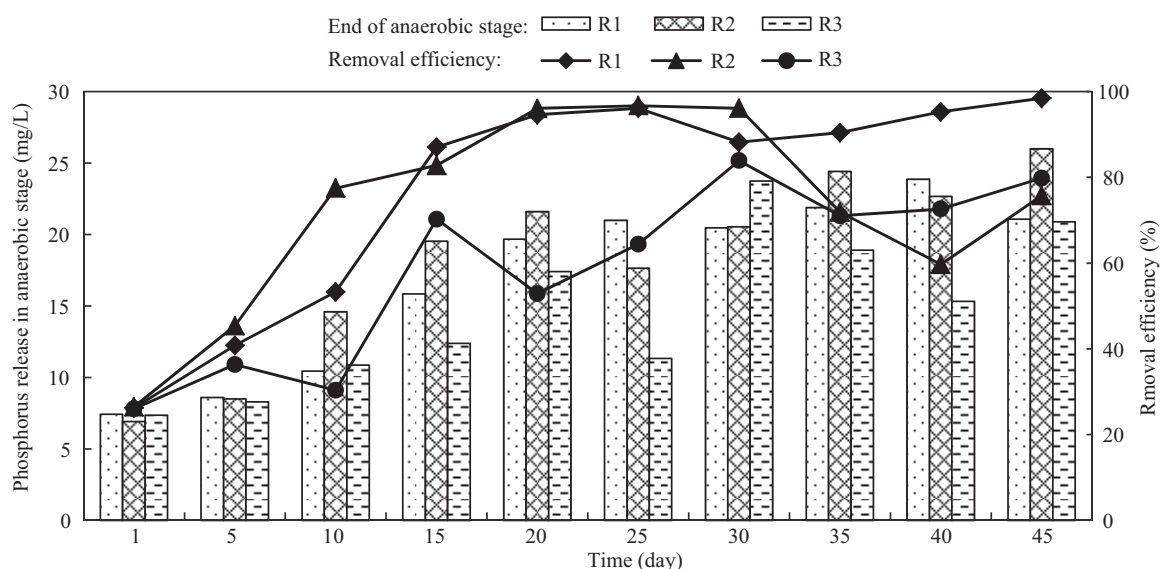


Fig. 1 Phosphorus removal under low phosphorus influent.

first ten days, although the system's phosphorus removal efficiency was consistently around 30%. After fifteen days of operation, anaerobic phosphorus release began to be detected in R3 (2.18 mg/L). **Figure 1** shows that the changes in the phosphorus removal efficiency of the R1 system were relatively stable, with a gradual increase in phosphorus release. This was because R1 had less idle time than either R2 or R3, which had a corresponding effect on the PAOs in the system. However the changes for R2 and R3 exhibited greater fluctuation. The specific phosphorus release rate (SPRR) and the specific phosphorus uptake rate (SPUR) are fully accepted methods for determining PAO mass in sludge (Panswad et al., 2003). The SPRR were 9.92, 7.74 and 4.29 mg/(g VSS·hr) in R1, R2 and R3, respectively. Meanwhile, the SPUR were 7.79, 4.19 and 2.18 mg/(g VSS·hr) in R1, R2 and R3, respectively. In the later stages of the start-up period, the phosphorus removal

efficiency and the amount of phosphorus released by all three systems showed an overall increase.

## 2.2 Effect of idle time on the stable phase of SBR biological phosphorus removal systems

### 2.2.1 Anaerobic phosphorus release

All the SBR systems considered in the study achieved a good level of phosphorus removal during the stable phase. In terms of phosphorus release, the amount for each of the three systems was maintained at a stable level for R1, R2, and R3 respectively (**Fig. 2**). This was because the bacteria at this stage are engaged in an adaptive process. The PAOs with poor adaptability are eliminated, while the concentration of the organic matter available for microbial growth remains constant (Wang et al., 2008). However, the three different operation cycles resulted in different populations of PAOs in the three systems and accounted

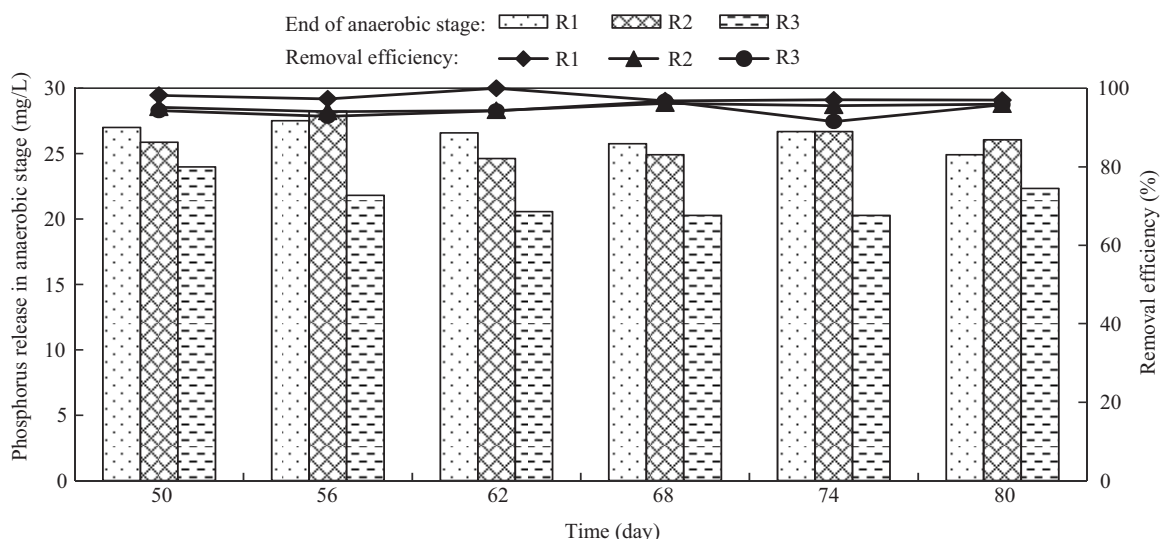


Fig. 2 Phosphorus removal during the stable phase.



for the different amounts of phosphorus released.

### 2.2.2 Aerobic phosphorus uptake

During the aerobic phase of stable operation, the effluent phosphorus concentrations of the three SBR systems were maintained at 1 mg/L or less. The phosphorus removal efficiencies of the three systems all reached 90% and the three systems facilitated a stable removal of phosphorus (Fig. 2).

### 2.2.3 Secondary phosphorus release

Secondary phosphorus release occurred regardless of low or high phosphorus concentration in the influent, or whether the start-up phase or the stable phase of the process was considered. Based on instantaneous sampling after feeding water to the reactor, the phosphorus concentration of mixed liquid in R1 was almost the same as the phosphorus concentration of the influent, whereas for R2 and R3 the phosphorus concentrations of mixed liquid were higher than the influent. This indicates that the R2 and R3 systems exhibited secondary phosphorus release. The secondary phosphorus release is not accompanied by the absorption of organic compounds. This indicates that the inclusion of a higher idle time caused an extended anaerobic period in the system, which results from the endogenous respiration of bacteria, causing the hydrolysis of polyphosphates within their cells.

During the start-up phase of the reactors, the secondary phosphorus release from R2 and R3 was 1 and 2 mg/L, respectively (Fig. 3). In the stable phase, the secondary release of phosphorus for R2 and R3 stabilized at 2 and 3 mg/L, respectively. When the concentration of phosphorus in the influent was high, the secondary phosphorus release of R2 and R3 also increased to 2.5 and 4 mg/L, respectively. The amount of phosphorus released, and the rate of phosphorus release and uptake for R2 and R3 were lower than those of R1, due to the secondary phosphorus release in the stable phase. When the phosphorus concentration of the influent was high, the slow growth of PAOs could not meet the demands of phosphorus removal. However with a progressively stronger release of secondary phosphorus, more intracellular polyphosphate hydrolysis occurs.

The alternation of an anaerobic/aerobic cycle stimulates the growth of PAOs in SBRs. However, the inclusion of an idle time prevents the PAOs from entering an aerobic state for long periods, forcing the PAOs into a depressed state caused by an extended PolyP decomposition phase, in the absence of a synthesis phase. This ultimately results in the reduction of the diversity of the PAO population and inevitably leads to a deterioration of the phosphorus removal performance.

### 2.3 Effect of changing phosphorus concentration on the performance of systems

When the phosphorus concentration of the influent was increased two-fold, to 16 mg/L, with no additional COD,

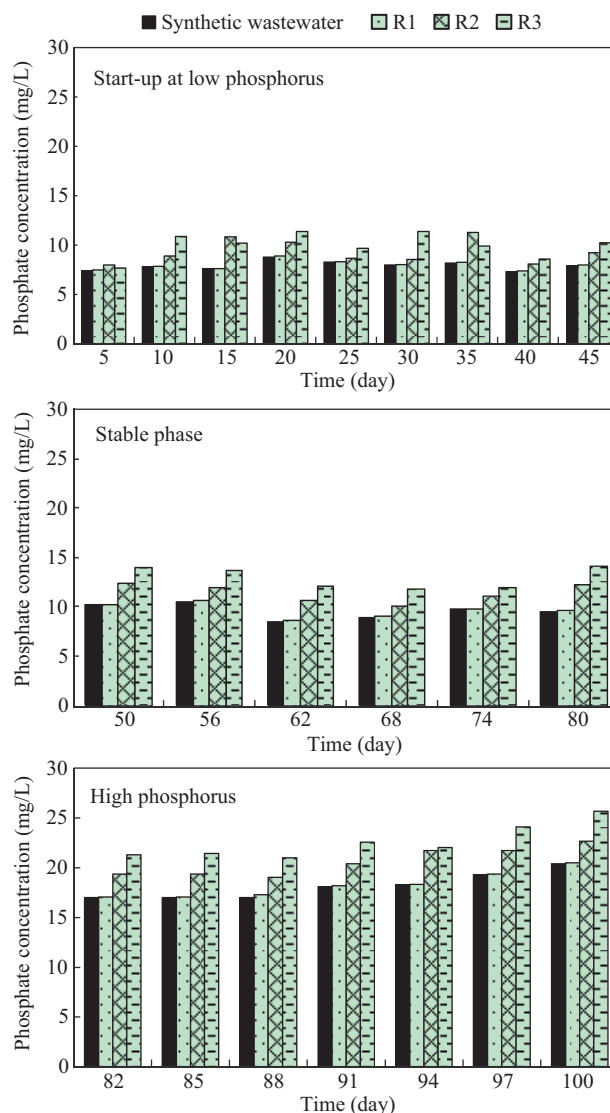


Fig. 3 Secondary phosphorus release at different stages.

the three systems responded differently, as shown in Fig. 4. It was found that R1 was still able to operate stably, and produced an accompanying increase in its release of phosphorus, from 16.5 mg/L under low phosphorus conditions up to 36.44 mg/L when phosphorus was high. Furthermore, at the end of the aerobic stage, the  $\text{PO}_4^{3-}\text{-P}$  remaining was less than 1 mg/L, correlating to a removal efficiency > 90%. In contrast, the R2 and R3 systems were negatively affected. By the eighth day, the anaerobic phosphorus released for R2 decreased to 6.52 mg/L, producing a corresponding drop in the phosphorus removal efficiency from 90% to 66.38%. R2 and R3 also showed signs of deterioration and the inability of their bacterial populations to absorb the anaerobic phosphorus released during the aerobic stage, resulting in a decrease in their overall phosphorus removal capacity. The SPRR were 9.1, 3.48 and 2.09 mg/(g VSS·hr) in R1, R2 and R3, respectively. Meanwhile, the SPUR were 8.11, 2.56 and 1.83 mg/(g VSS·hr), in R1, R2 and R3, respectively.

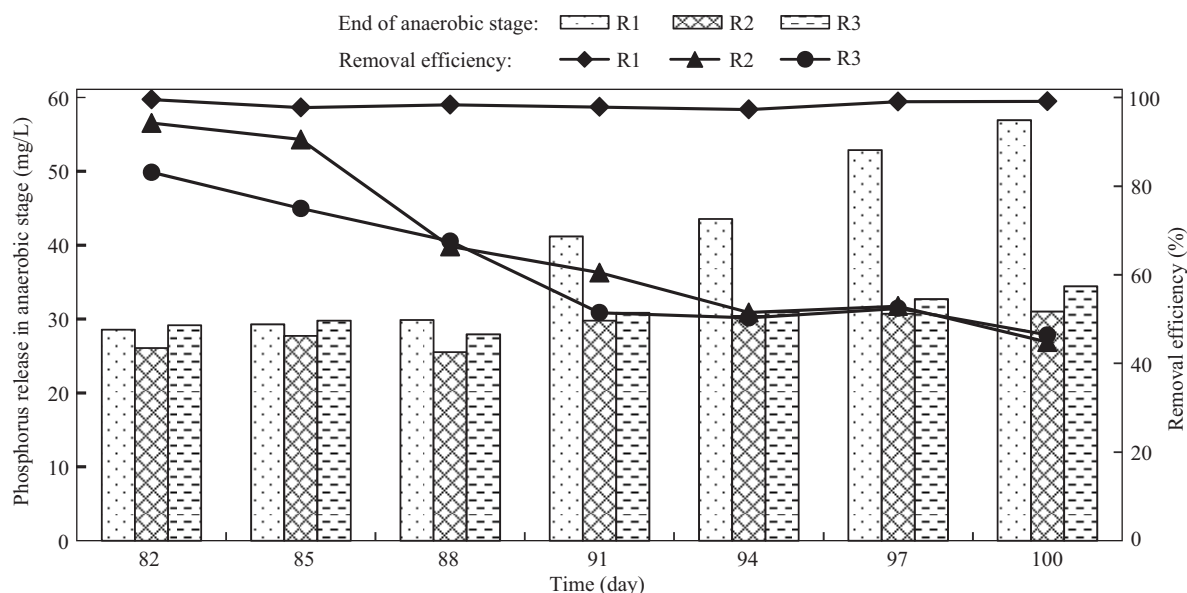


Fig. 4 Phosphorus removal under high phosphorus influent.

The PHB synthesized in the cells of the bacteria was insufficient to take up the excess phosphorus and resulted in an increase in the concentration of phosphorus in the effluent. The anaerobic COD conversion was partially generated by the PAOs, in order to form intracellular storage materials related to phosphorus removal. However, the remaining portion was consumed by other bacteria for the storage of materials unrelated to phosphorus removal. Alternatively, COD might become attached to the outside of the cells and used for cell growth in the aerobic phase (Morgenroth et al., 2000).

Under aerobic conditions, the entire microbial community is engaged in cell synthesis, so the phosphorus uptake rate is generally higher than the phosphorus release rate (Thongchai et al., 2003). The further illustrates that an increase in the idle time reduced the number of PAOs at higher influent phosphorus, creating a disadvantage for the PAOs and favoring the survival of other bacteria unrelated to phosphorus removal, which disrupted the process of phosphorus removal.

#### 2.4 Composition of microbial communities in SBRs with different idle times

The diversity of the microbial community differed between the three SBR systems. The PCR-DGGE analysis (Fig. 5) revealed that the three systems shared some common species (bands 2, 3, 9, 13, 14, 17, 18), but also had system-specific species (bands 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 16). The R2 and R3 systems shared several species, including band 6 and band 15, which were not present in R1. This observation indicates that these two strains were anaerobic bacteria, and are a result of the longer idle time. In the R3 system, the dominant PAO was uncultured  $\gamma$ -Proteobacteria. In contrast, the dominant PAOs in systems R1 and R2 were *Pseudomonas* sp.

and uncultured  $\beta$ -Proteobacteria (AY823971). In addition to these PAO species, an uncultured *Sphingomonas* sp. (AM889077), known to be a GAO (Beer et al., 2004; Oehmen et al., 2006), was also found in both R2 and R3 systems. Similar to PAOs, GAOs absorb carbon in the anaerobic stage, obtaining energy for PHA synthesis by the decomposition of glycogen, while the energy for glycogen synthesis is obtained in the aerobic stage by the decomposition of PHA. However, no phosphorus release or uptake occurs during the metabolic process of GAOs. It has been reported previously that stable EBPR systems can contain large populations of GAOs, which can become strong competitors of the PAOs if the water quality or operating conditions change, and may cause the failure of the phosphorus removal function (Wang et al., 2002; Jeon et al., 2003; Whang and Park, 2002). In addition, the competition of GAOs can result in damage to the growth environment of PAOs, causing the PAOs to become weak competitors within the system, and leading to a deterioration of the phosphorus removal capacity.

### 3 Conclusions

The idle time of SBRs impacts the biological phosphorus removal increasingly with rising phosphorus concentration in the influent. The systems that had a long idle time (R2 and R3) exhibited slow bacterial growth, which was insufficient to remove the high concentrations of phosphorus. The hydrolysis of polyphosphates due to the secondary phosphorus release contributed to the deterioration of the phosphorus removal capacity of the R2 and R3 systems. Based on the PCR-DGGE analysis, the presence of GAOs in systems R2 and R3 and the change of the PAO population were important reasons for the decrease in their phosphorus removal efficiency.

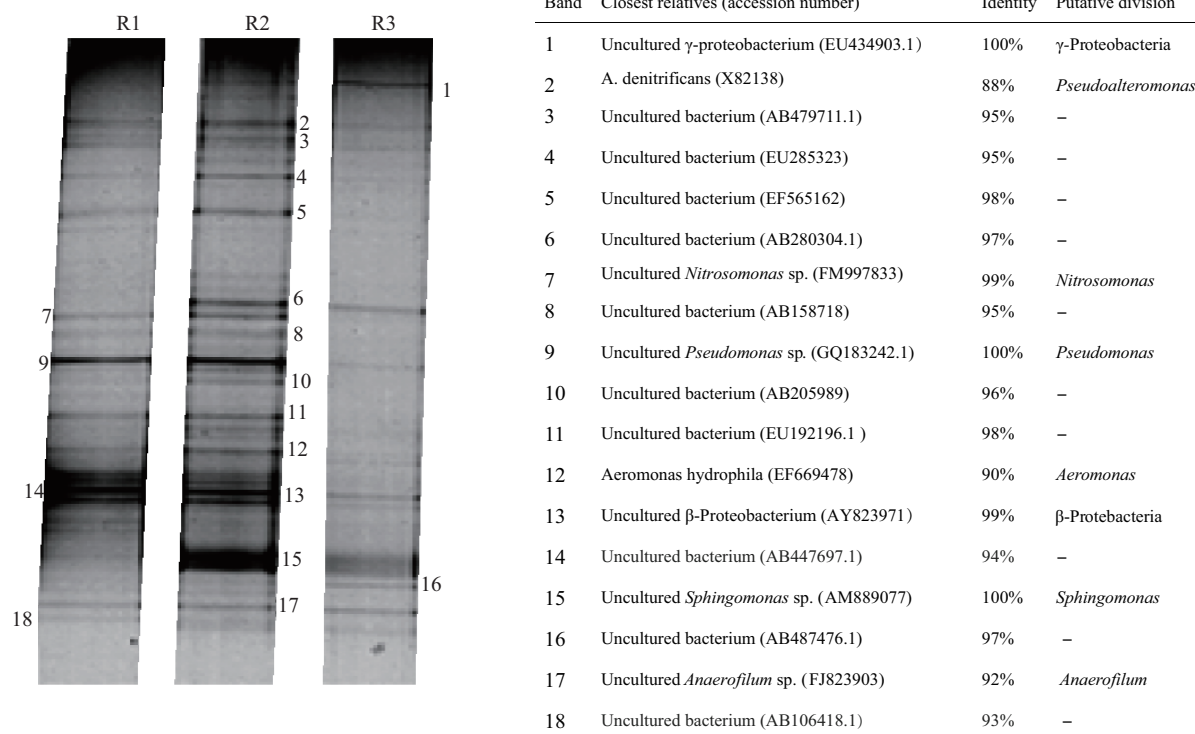


Fig. 5 Sequence analysis and identification of bacteria from DGGE bands. “–”: unknown bacteria.

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