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Phosphate removal mechanism in an airlift-loop reactor under limited filamentous bulking conditions

Yiming Su[a], Xuefei Zhou[a,*], Chaomeng Dai[a,b], Ming Jiang[a], Yalei Zhang[a,*]

[a]*State Key Laboratory of Pollution Control and Resources Reuse, Tongji University, Shanghai 200092, China, Tel. +86 21 65982503; Fax: +86 21 65988885; emails: zhangyalei@tongji.edu.cn (Y. Zhang), zhouxuefei@tongji.edu.cn (X. Zhou), su_yi_ming@126.com (Y. Su), daichaomeng@163.com (C. Dai), andyjiangming@126.com (M. Jiang)*
[b]*College of Civil Engineering, Tongji University, Shanghai 200092, China*

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ABSTRACT

This study presents detailed evaluations of nutrients removal efficiencies, micro-organism community structure, and phosphate removal mechanism in a specific airlift-loop reactor (ALR) under limited filamentous bulking (LFB) state. The removal efficiencies of chemical oxygen demand (COD), total phosphate (TP), total nitrogen (TN), and ammonium (NH_4^+–N) were approximately 90, 93, 86, and 93%, respectively. The result of polymerase chain reaction–denaturing gradient gel electrophoresis analysis suggested that *Rhodocyclaceae* and *Thiothrix* sp. were the main polyphosphate-accumulating micro-organisms (PAOs) and filaments, respectively, under LFB state. Batch tests indicated that PAOs included 38% denitrifying PAOs (DPAOs). Under anaerobic condition, when COD was below 24.1 mg O_2/L, PAOs would stop phosphate (P) release; when COD was below 21.3 mg O_2/L in anoxic condition or below 60.8 mg O_2/L in aerobic condition, PAOs began to recover the storage pool of polyphosphate. In the ALR, P-uptake occurred in the aeration zone while P-release happened at the bottom of the riser. Hence, high and stable P removal efficiency was observed.

Keywords: Airlift-loop reactor; Limited filamentous bulking; Polyphosphate-accumulating micro-organisms; Polyphosphate metabolism

1. Introduction

Many middle-sized cities and towns in China are faced with a specific problem of the unevenly distributed sewage water, which leads to difficulties in collection and treatment. To achieve simultaneous economic and environmental effectiveness, integrated bioreactors have attracted substantial attention [1–7]. Among them, the airlift-loop reactor (ALR) (a type of integrated bioreactor), which incorporates anaerobic, anoxic, and aerobic conditions in a single reactor, is a valuable alternative for the simultaneous removal of nitrogen (N) and phosphate (P) [7].

Unlike conventional methods, such as adding membrane system [2,5] or granular sludge system [6] to ALR, Jiang et al. [7] successfully introduced limited filamentous bulking (LFB) to a specific ALR through reducing dissolved oxygen (DO) concentrations in the aeration zone, realizing simultaneous removal of N and P. The specific structure of ALR enables different kinds of functional micro-organisms, such as nitrifiers, denitrifiers, and polyphosphate-accumulating

*Corresponding authors.

micro-organisms (PAOs), to grow together, and therefore simultaneous N and P removal are achieved. With regard to LFB, filamentous micro-organisms should be ascertained. Hence, to obtain a deep insight into nutrients removal mechanism in the specific ALR under LFB state, an analysis of micro-organism structure, polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE) [8], is necessary.

In order to formulate P removal mechanism clearly under LFB state in ALR, PAOs' metabolism should be studied. LFB was firstly advocated by Peng et al. [9] and had been corroborated as an energy-saving way to remove nutrients. While much attention has been paid to the removal mechanism of N [2,4–6], little has been placed on the removal mechanism of P under LFB state.

It is widely acknowledged that PAOs are able to transform volatile fatty acids (VFAs) into polyhydroxyalkanoates and release orthophosphate under anaerobic phase, and then absorb orthophosphate excessively under following anoxic or oxic phase [10]. But how PAOs switch between different biochemical pathways as a response to the variation of the quantity of available carbon sources under LFB state has not received sufficient attention. Indeed, understanding how the energy state of the PAOs and the levels of the environmental carbon source affect polyphosphate (polyP) metabolism are crucial to improve P removal performance and understand the reason why PAOs can coexist with filaments. PolyP metabolism might be one way through which the PAOs maintain energy charge because polyP accumulates under energy-rich growth conditions and is degraded under energy-poor conditions [11,12]. Despite the fact that the response of polyP metabolism to various stressed conditions, including amino acid starvation, inorganic phosphate limitation and low pH, has been intensively investigated [13–15], further investigation concerning the effect of the levels of environmental carbon source on polyP metabolism of PAOs under LFB condition is still necessary for exploring the reason why high and stable P removal efficiency can be maintained.

Hence, the objectives of this study are to investigate the micro-organism structure, to determine the relative population of different types of PAOs, to evaluate the polyP metabolism of PAOs, and to study the P removal process in the ALR under LFB state.

2. Materials and methods

2.1. Reactor configuration

This investigation involved the use of a laboratory-scale ALR with a reaction volume of 22 L illustrated schematically in Fig. 1. The ALR was inoculated with mixed liquor which was collected from a secondary clarifier from the Quyang Wastewater Treatment Plant (Shanghai, China). The running conditions of the reactor were described previously [7]. Under the typical running conditions, the influent was introduced into the ALR through the upper part of annulus and the flow rates were controlled by a peristaltic pump; the effluent was withdrawn from the liquid surface in the settling zone. The feed flow rates were controlled to achieve a hydraulic retention time of 9 h. The aeration rate was controlled by an airflow meter to maintain DO concentration of 0.8 ± 0.1 mg/L. The sludge retention time was maintained at 12–15 d by wasting an appropriate amount of sludge in the aeration zone. The mixed liquor suspended solid (MLSS) concentration was approximately 3,000 mg/L, and the value of food/micro-organism was maintained at 0.13 g COD/g SS.

2.2. Batch tests for exploring the mechanism of filamentous bulking

The sludge used in the batch tests was collected from the parent reactor. First, a 1.5 L mixture was collected from the aerobic zone and then was centrifuged; the supernatant fluid was discarded before adding 1.5 L synthetic wastewater that did not contain any C, N, P or DO. Then, this new mixture was immediately transferred to an airtight column container. To determine the preferred carbon source, a certain amount of sodium acetate or propionate was added to provide a shock concentration of carbon source (the theoretical values

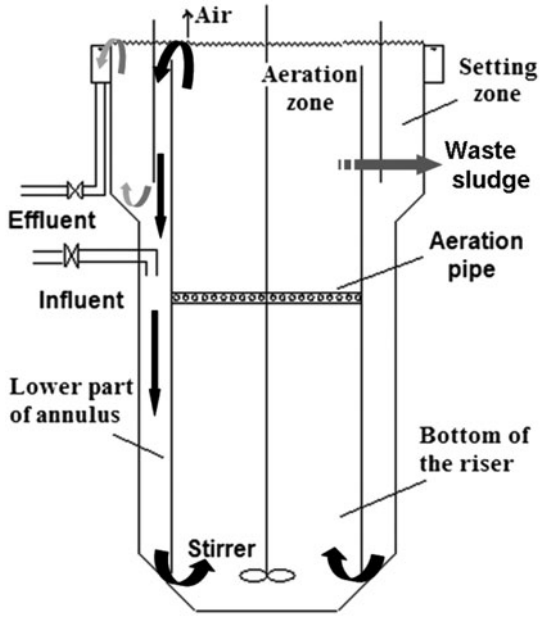

Fig. 1. Schematic structure of the ALR.

were 98 mg acetate/L and 63 mg propionate/L). To evaluate the effect of different carbon levels on anaerobic P release, different amount of acetate was added at the beginning of anaerobic phase (initial acetate sodium concentrations were 0.2, 0.3, 0.4, and 0.6 g/g, SS respectively). During the 2 h anaerobic stage, samples were collected every 15 min during the first hour, and then every 30 min until the end. In the batch tests for calculating the ratio of DPAOs in all PAOs, when anaerobic phase was over, the mixture was divided into two parts, one was given a plus of nitrate to achieve a load of 12 mg NO_3^--N/g SS; the other one not only received nitrate addition but also was aerated to create an oxic condition. Samples were taken every 15 min during the first 30 min of the anoxic or oxic stage, followed by every 30 min until the third hour. The concentrations of HAc, propionate, phosphate, nitrate, nitrite, and MLSS were monitored. This method was modified from the method of Hu et al. [16], who successfully characterized denitrifying phosphorus removal bacteria by using three different electron acceptors.

2.3. Synthetic wastewater composition

The novel reactor was supplied with synthetic wastewater containing sodium acetate (300 mg/L), glucose (125 mg/L), ammonium chloride (40 mg NH_4^+−N/L), and KH_2PO_4 (5 mg PO_4^{3-}−P/L). Other components consisted of $MgSO_4 \cdot 7H_2O$ (150 mg/L), $CaCl_2 \cdot 2H_2O$ (35 mg/L), EDTA (5 mg/L) and trace elements (1 mL/5 L). The composition of the trace element solution was composed as that described in Martins et al. [17].

2.4. Analytical methods

The DO was measured by a WTW oxi/340i oxygen probe (Germany). Chemical oxygen demand (COD), MLSSs, and mixed liquor volatile suspended solids were measured according to standard methods [18]. Sludge volume index (SVI) was measured according to Martins et al. [17]. All nitrogen-containing compounds were assessed spectrophotometrically according to Third et al. [19], and PO_4^{3-}−P concentrations were analyzed according to Guo et al. [20]. Acetate content was measured by gas chromatography according to Smolders et al. [21].

2.5. PCR-DGGE analysis of the filamentous bacteria in activated sludge

Bacterial genomic DNA of the activated sludge was first extracted as per the following steps: first, samples collected from the reactor were centrifuged, and the supernatant was removed. Second, the settling was washed with sterile water, and the mixture was recentrifuged. Third, the Fast DNA® Spin Kit for soil (QBIOgene, Carlsbad, CA, USA) was used to extract total DNA from approximately 0.3 g settling. DNA was stored at −20˚C.

The 16S rDNA genes from the mixed bacterial DNA were PCR-amplified with the primer set of 8f and 1492r as described by Bosshard et al. [22]. PCR amplification was carried out in a total volume of 50 μL containing approximately 50 ng template DNA, 1 U Taq DNA polymerase, 40 μM dNTP, 0.2 μM each primer, 5 μL 10× PCR buffer (15 mM $MgCl_2$, 500 mM KCl, 100 mM Tris-HCl; pH 8.3), and 2 μL bovine serum albumin. The PCR conditions used for general bacteria was as follows: an initial denaturation of 5 min at 94˚C; 30 cycles of 1 min denaturing at 94˚C, 1 min annealing at 40˚C and, 3 min extension at 72˚C; and a final extension of 7 min at 72˚C.

The 16S rDNA variable V3 region of extracted DNA was amplified with the primers 341f with a GC-clamp and 534r according to the literature [8]. The amplification program was as follows: an initial denaturation step of 5 min at 94˚C; 30 cycles of 30 s denaturation at 94˚C, 30 s annealing at 58˚C, and 30 s extension at 72˚C; and a final extension of 6 min at 72˚C.

PCR products were electrophoresed on an 8% polyacrylamide gel with gradients ranging from 40 to 60% denaturant in 1× TAE buffer at a constant voltage of 100 V for 10 h at 60˚C using a Dcode Universal Mutation Detection System (BioRad). After electrophoresis, DNA was stained with ethidium bromide and viewed with a BioRad Gel Documentation system (BioRad). Bright bands were then excised from the gel and cleaned. The DNA was reamplified, purified, cloned into the top MD19-T vector (TaKaRa, Japan) and sequenced via an ABI PRISM 3730 automated DNA sequencer (Applied Biosystems, USA). The closest matching sequences were searched using the BLAST program, and the identified sequences from this study had been submitted to the GenBank database under accession numbers **JX178701** to **JX178717**.

2.6. Statistical analysis

All batch tests were performed at least three times, and an analysis of variance was used to test the significance of results and $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Integrated nutrient removal performance of the limited bulking system

During the operation period of more than two months, the values of SVI and the removal rates of COD, total nitrogen (TN), total phosphate (TP), and NH_4^+–N were recorded. The low DO concentration stimulated the growth of filaments, yet the substrate concentration gradient formed by the specific feeding model restricted the proliferation of filaments [7]. Hence, a stable LFB state was achieved. Fig. 2 illustrates the maintenance of the relatively high nutrients removal rates under LFB conditions. The removal rates of COD, TN, TP, and NH_4^+–N were approximately 90, 86, 93, and 93%, respectively. In addition, the turbidity of the effluent was maintained at approximately 0.5 NTU throughout the long-term running experiment. COD removal efficiency was even enhanced under LFB state while NH_4^+–N removal rate declined slightly. Due to the contribution of SND, ideal TN removal efficiency could be kept and TN in effluent could meet China's first class A standard. Further analysis revealed that nitrogen removed by SND was 32% under LFB state [7]. With regard to P removal, the previous study [7] indicated that PAOs had played a critical role in removal process. However, the detail P removal process had not been fully explored.

3.2. Bacterial community in activated sludge during the entire operation time

To investigate the bacterial community structure in activated sludge during the entire operation period, PCR-DGGE technique was applied. The result of PCR-DGGE is shown in Fig. 3 and all identified bacteria are illustrated in Table 1. The most important information given by PCR-DGGE analysis is that *Rhodocyclaceae* and *Thiothrix* sp. are found to be the main PAOs and filaments, respectively, in the limited bulking system. Thus, it is likely that the proliferation of *Thiothrix* sp. causes the increase of SVI, resulting in LFB. This result is consistent with our previous study [7] and the report of Martins et al. [17]. Among the other bacteria, *Desulfovibrio* is known for its flexibility in response to the extended amount of electron acceptors; it utilizes sulfate, sulfur, nitrate, and nitrite among others [23]. The growth of *Sulfurovum* occurs chemolithoautotrophically with the elemental sulfur or thiosulfate as an

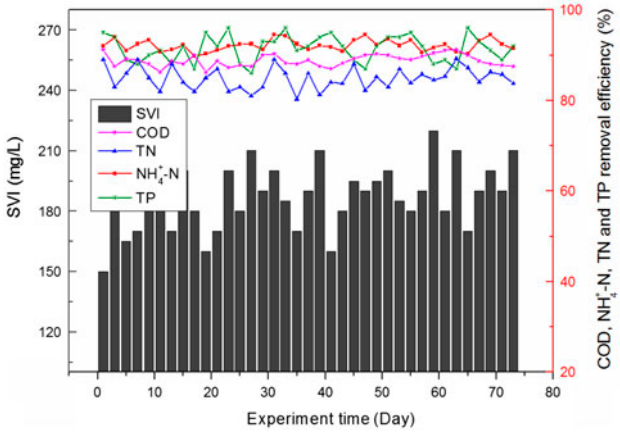

Fig. 2. Removal efficiency of TN, TP, COD, and NH_4^+–N under LFB state.

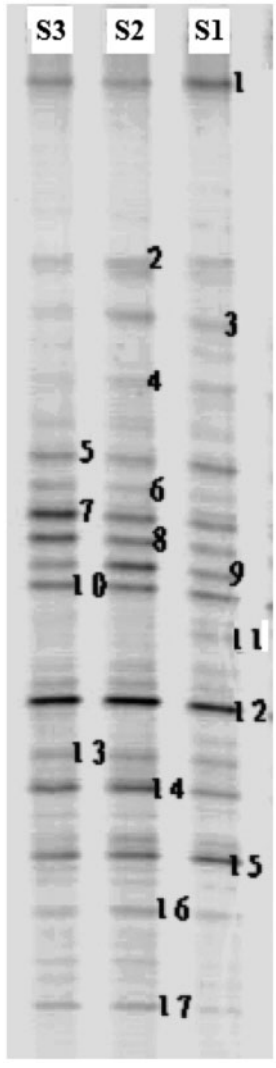

Fig. 3. DGGE profile of bacterial communities during the different operation time (S1, S2, and S3 represent activated sludge on 10th, 30th, and 60th day, respectively).

Table 1
DGGE bands and their closely related sequences

Band ID	Closely related sequence from GenBank	Accession No.	Identity (%)
1	Uncultured *Desulfovibrio* sp. clone BES-NB-13 16S ribosomal RNA gene, partial sequence	JX178701	99
2	Uncultured gamma proteobacterium isolate DGGE gel band hrb-240 16S ribosomal RNA gene, partial sequence	JX178702	100
3	Uncultured bacterium clone F5K2Q4C04IVXJ7 16S ribosomal RNA gene, partial sequence	JX178703	100
4	Uncultured *Sulfurovum* sp. clone ZLL-D22 16S ribosomal RNA gene, partial sequence	JX178704	97
5	*Limnobacter* sp. Aph2 16S ribosomal RNA gene, partial sequence	JX178705	92
6	Uncultured gamma proteobacterium gene for 16S rRNA, partial sequence, clone: 094-Cadma	JX178706	100
7	Uncultured *Rhodocyclaceae* bacterium clone MBfR_NS-150 16S ribosomal RNA gene, partial sequence	JX178707	99
8	Uncultured *Klebsiella* sp. clone S1-5 16S ribosomal RNA gene, partial sequence	JX178708	93
9	Uncultured *Thiothrix* sp. isolate DGGE gel band B12 16S ribosomal RNA gene, partial sequence	JX178709	100
10	Uncultured *Tolumonas* sp. clone R40-93 16S ribosomal RNA gene, partial sequence	JX178710	100
11	Uncultured *Trichococcus* sp. isolate DGGE gel band HRB9 16S ribosomal RNA gene, partial sequence	JX178711	99
12	Uncultured *Trichococcus* sp. gene for 16S rRNA, partial sequence, clone: TCE-109	JX178712	99
13	*Azonexus* sp. HME6654 16S ribosomal RNA gene, partial sequence	JX178713	97
14	Uncultured *Dokdonella* sp. clone R15-51 16S ribosomal RNA gene, partial sequence	JX178714	100
15	Uncultured *Rhodocyclaceae* bacterium partial 16S rRNA gene, clone VidyA-27	JX178715	91
16	Uncultured *Variovorax* sp. clone MR97_dry_d08 16S ribosomal RNA gene, partial sequence	JX178716	100
17	Uncultured *Rhodocyclaceae* bacterium clone eub62B1 16S ribosomal RNA gene, partial sequence	JX178717	100

electron donor, oxygen and nitrate as an electron acceptor and CO_2 as the carbon source [24]; however, this bacteria disappeared under limited bulking conditions and it was probably replaced by another thiosulfate-oxidizing bacteria (*Limnobacter*). The genus *Limnobacter* is composed of two species, *L. thiooxidans* [25] and *L. litoralis* [26], and its presence might be due to the low food/micro-organism value. Given that the carbon source in the influent includes glucose, the presence of the glucose fermentative bacteria, such as *Trichococcus* [27], *Tolumonas* [28], and *Variovorax* [29], is reasonable. However, it is slightly surprising to detect the presence of *Dokdonella*, which belongs to the family Xanthomonadaceae and may be an aerobic denitrifier [30].

3.3. Relative population and polyP metabolism of PAOs in the system

3.3.1. Acetate preference of PAOs

Before identifying the relative population of different types of PAOs, batch tests fed with acetate or propionate was employed to ascertain the suitable carbon source for PAOs. Fig. 4(a) shows that acetate or propionate is absorbed to a substantial extent immediately after the dosing. The theoretical values were 98 mg HAc/L and 63 mg propionate/L, but the actual values were 56 mg HAc/L and 40 mg propionate/L. However, compared with PAOs in acetate system, PAOs in the propionate system neither could completely release P under anaerobic conditions, nor could they absorb as much P as those in the acetate system under anoxic conditions. This result suggested that PAOs in this type of activated sludge were capable of using both acetate and propionate as a carbon source, but acetate was more suitable for the batch tests to identify the relative population of different types of PAOs.

3.3.2. Effect of different carbon concentrations on anaerobic P release

As acetate was determined to be the optimal carbon source, we then evaluated the exact quantity of acetate consumption during the anaerobic phase. As

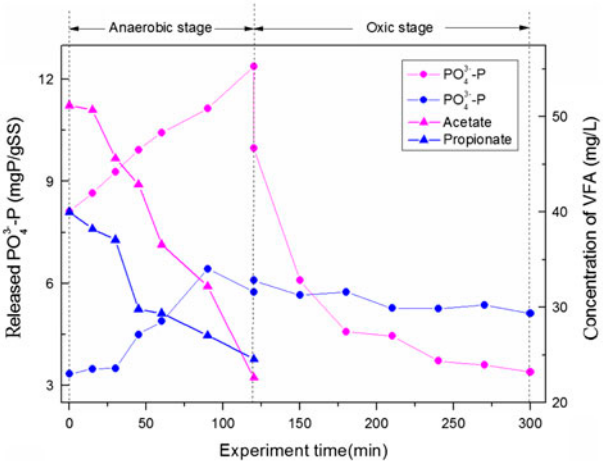

Fig. 4(a). Preferential carbon source for PAOs. –▲– and –●– represent the changes of acetate and P concentration in batch test fed with acetate as solo carbon source (initial acetate concentration, 98 mg/L); –▲– and –●– represent the changes of propionate and P concentration in batch test fed with propionate as solo carbon source (initial propionate concentration, 63 mg/L).

shown in Fig. 4(b), the four different initial acetate sodium concentrations were 0.2, 0.3, 0.4, and 0.6 g/g SS, respectively for test 1, 2, 3, and 4; when the 2 h anaerobic phase was complete, the final VFA concentrations were 25.95, 67.39, 110.63, and 200.56 mg/L, respectively. The total acetate consumption was approximately 80 mg/g SS within the 2 h anaerobic phase. Therefore, the concentration of 0.2 g/g SS

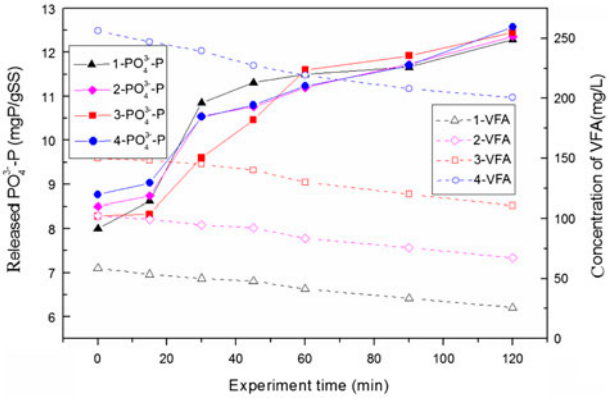

Fig. 4(b). Optimal dosage for anaerobic P release. –△– and –▲– represent the changes of acetate and P concentration in test 1 (initial acetate concentration, 98 mg/L); –◇– and –◆– represent the changes of acetate and P concentration in test 2 (initial acetate concentration, 146 mg/L); –□– and –■– represent the changes of acetate and P concentration in test 3 (initial acetate concentration, 196 mg/L); –○– and –●– represent the changes of acetate and P concentration in test 4 (initial acetate concentration, 292 mg/L).

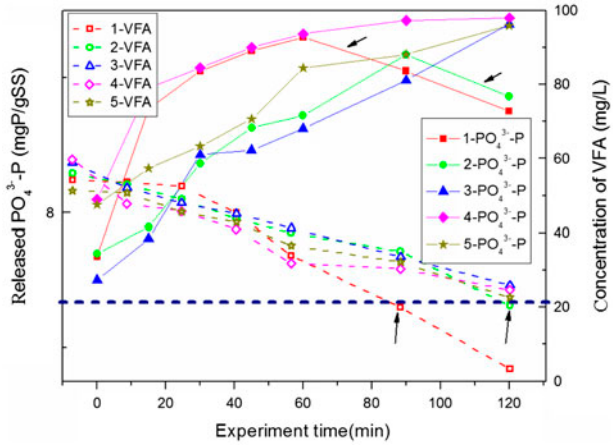

Fig. 4(c). Minimum concentration of acetate for anaerobic P release (initial acetate concentration, 98 mg/L; symbols with the same shape mean that the parameters are calculated from the same test).

acetate sodium was sufficient for anaerobic P-release; otherwise extra carbon would suppress P uptake in the subsequent anoxic or oxic stage and intensify the competition between PAOs and the other organisms. Furthermore, under the dosage of 0.2 g/g SS, another five batch tests (Fig. 4(c)) indicated that if acetate concentration was below 22.6 mg/L in anaerobic phase, PAOs would stop P release. Hence, it was necessary to maintain the acetate concentration above 22.6 mg/L for stable P release.

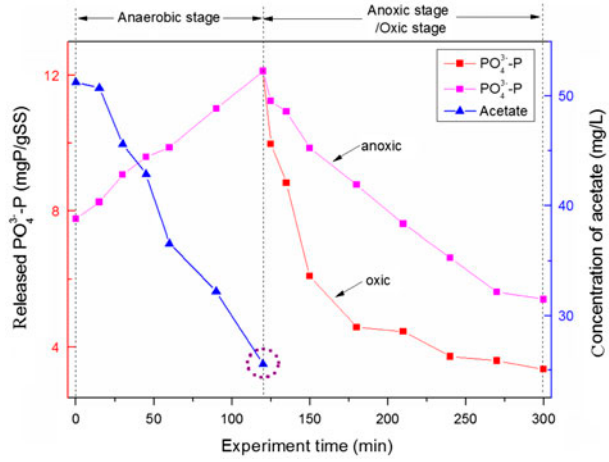

Fig. 4(d). Influence of the residual carbon from the anaerobic phase (25.9 mg/L acetate) on the anoxic or aerobic P uptake: –▲– represents the trend of acetate concentration under anaerobic condition; –■– represents the changes of P concentration under anaerobic or aerobic condition; –■– represents the changes of P concentration under anoxic condition.

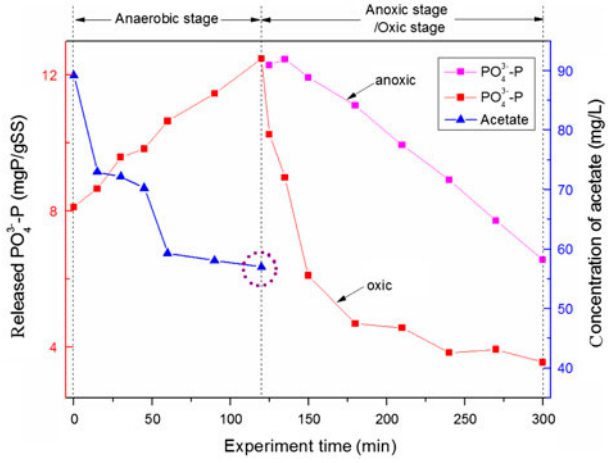

Fig. 4(e). Influence of the residual carbon from the anaerobic phase (57 mg/L acetate) on the anoxic or aerobic P uptake: –▲– represents the trend of acetate concentration under anaerobic condition; –■– represents the changes of P concentration under anaerobic or aerobic condition; –■– represents the changes of P concentration under anoxic condition.

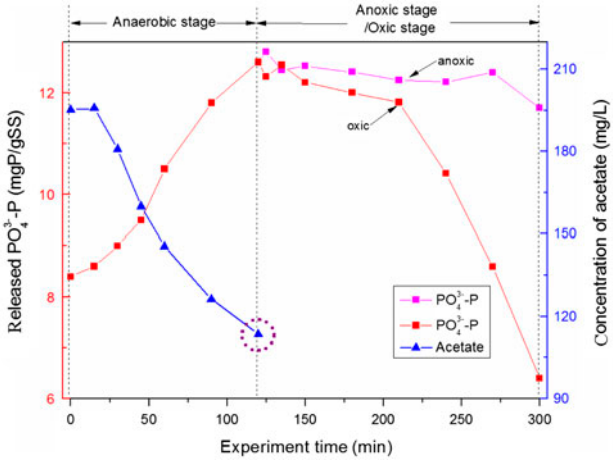

Fig. 4(f). Influence of the residual carbon from the anaerobic phase (113.5 mg/L acetate) on the anoxic or aerobic P uptake: –▲– represents the trend of acetate concentration under anaerobic condition; –■– represents the changes of P concentration under anaerobic or aerobic condition; –■– represents the changes of P concentration under anoxic condition.

3.3.3. Inhibition of the high residual carbon concentrations from the anaerobic phase on the anoxic or aerobic P uptake

The residual carbon from the anaerobic phase has some influence on anoxic or aerobic P uptake. It can delay the P uptake process to some extent. Fig. 4(d) illustrated that acetate concentrations below 20 mg/L almost had no negative effect on anoxic P uptake, whereas concentrations lower than 57 mg/L were suitable for aerobic P uptake (in Fig. 4(e)). If the final concentration at the last moment of the anaerobic stage was higher than 20 mg/L but lower than 57 mg/L, then it had a substantial negative influence on the anoxic P uptake but almost no influence on the aerobic P uptake. However, when the concentration surpassed 57 mg/L in aerobic phase, it took time to consume the extra carbon and then P uptake occurred (Fig. 4(f)). It seemed that both in aerobic and anoxic P uptake, the residual high amount of carbon was consumed first and then P uptake occurred.

3.3.4. Calculating the ratio of DPAOs in PAOs

Previously, Hu et al. [16] classified PAOs into three groups according to their electron acceptor: P_O (can use oxygen), P_{ON} (can use oxygen and nitrate), and P_{ONn} (can use oxygen, nitrate, or nitrite). In this study, when nitrate was added into the mixture, nitrite appeared firstly and disappeared subsequently (data not shown), resulting in the difficulty to separate P_{ON} and P_{ONn}. Therefore, PAOs were classified into two groups: P_{de} (can use nitrite or nitrate as the electron acceptor) and P_{ox} (can use oxygen, nitrate, or nitrite as the electron acceptor). The calculation formulas were as follows:

$$P_{de}/P(\%) = (M_{de})_{max}/(M_{ox})_{max} \times 100\%; \quad (1)$$

$$P_{ox}/P(\%) = [(M_{ox})_{max} - (M_{de})_{max}]/(M_{ox})_{max} \times 100\%; \quad (2)$$

where P is the total population of PAOs, $(M_{de})_{max}$ is the maximum rate of phosphorus uptake with nitrate (mg P/(g VSS min)), and $(M_{ox})_{max}$ is the maximum rate of phosphorus uptake with oxygen and nitrate (mg P/(g VSS min)). Hence, as shown in Fig. 4(d) and Fig. 4(e), on the basis of formulas (1) and (2), the calculated percentage of DPAOs in PAOs was approximately 38.7%. However, as shown in Fig. 4(f), the residual carbon had a crucial influence on the calculation of $(M_{de})_{max}$, so it was not used to calculate the relative population of DPAOs.

4. Discussion

4.1. The integrated nutrient removal efficiencies and the microbial community under LFB conditions

The novel reactor could run stably when the value of C/N was approximately 10 and LFB could be

achieved and maintained under low DO concentration. Under LFB state, the integrated nutrients removal efficiencies were slightly higher than those under normal operating conditions, because filamentous bacteria could provide more binding sites for the attachment of free functional bacteria or smaller aggregates by EPS [31,32]. This result was in line with the research of Guo et al. [20] and Tian et al. [33]. The result of PCR-DGGE indicated that *Rhodocyclaceae* and *Thiothrix* sp. were the main PAOs and filaments, respectively, in the limited bulking system. In this study, the carbon sources in the influent included a large amount of acetate (2/3 total COD), which favored the growth of PAOs [34]. Moreover, the prevailing oxygen-deficient environment not only contributed the SND phenomenon, but the appearance of DPAOs as well (38% of PAOs were DPAOs indicating from the result of batch tests).

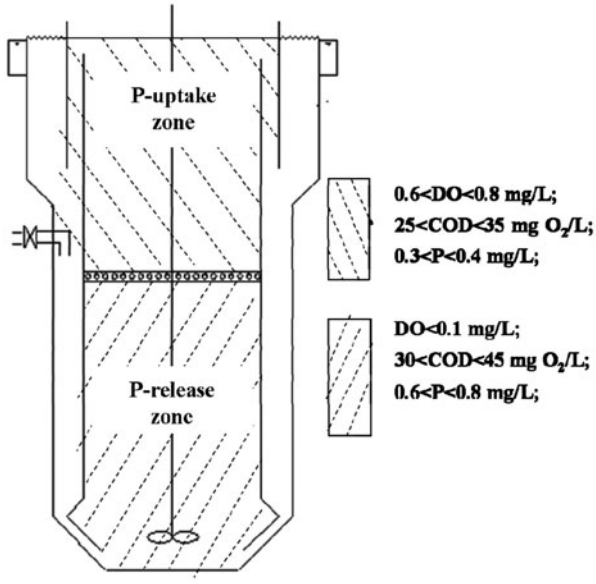

Fig. 5. P removal process in the ALR.

4.2. PAOs' PolyP metabolism and P removal process in the ALR

With regard to the quantity of acetate given at the beginning of the anaerobic stage, the total amount of anaerobic P released increased with the rise of the C/P ratio when it was lower than 30 [35]. In this study, although the C/P ratio increased from 9 to 27, the total amount of anaerobic phosphorus release did not change significantly, which demonstrated, to a certain extent, that PAOs could gain sufficient carbon to survive when the C/P ratio was 9. Moreover, one of the necessary conditions for maintaining P release confirmed by the batch experiment was that the concentration of acetate in the anaerobic stage had to be higher than 22.6 mg/L, which was consistent with the lowest COD level of 25 mg O_2/L needed to maintain phosphorus release in the anaerobic stage [36]. This phenomenon suggests that PAOs are able to detect the quantity of carbon sources and assess whether they are available for use.

When the anaerobic stage is complete, the residual carbon source in the system has different effects on anoxic or aerobic P uptake. However, it seems that in both the anoxic or aerobic stage, the synthesis of polyP could not occur until the excess carbon source is consumed. While a concentration of residual carbon above 20 mg/L is enough to delay the synthesis of polyP in anoxic conditions, a residual carbon concentration above 57 mg/L starts to affect polyP synthesis in oxic conditions. Although the discrepancy between the two data is quite large, it reflects the fact that PAOs are skillful in regulating their essential metabolism according to different quantities of the available carbon source. Under anoxic conditions, PAOs are able to grow/reproduce, or they only simply synthesize important substrates (such as polyP) to prepare themselves for difficult environments. When the acetate concentration is above 20 mg/L, PAOs prefer to grow and they do not recover their storage of polyP. However, when the acetate concentration is below 20 mg/L, they rebuild the storage pool of polyP. The same situation exists for PAOs in oxic conditions. The difference between the concentration values indicates that PAOs require more carbon to support their growth and reproduction under oxic conditions. This is consistent with the result of Smolders et al. [21], showing that under aerobic conditions, carbon was consumed at a higher rate than that in anoxic conditions.

Consequently, the capacity to regulate essential functions according to the different quantities of the available carbon source enables PAOs to remain in the LFB state. On the basis of the polyP metabolism of PAOs and the data collected from the reactor, P removal process in the ALR could be well illustrated by Fig. 5, in which P-uptake occurs in the aeration zone while P-release happens at the bottom of the riser.

5. Conclusions

This study presented detailed evaluations of nutrients removal efficiencies, the micro-organism community structure, and the polyP metabolism of PAOs in the ALR under LFB state. The result of PCR-DGGE analysis suggested *Rhodocyclaceae* and *Thiothrix* sp. were the main PAOs and filaments, respectively,

under LFB state. PAOs, including 38% denitrifying PAOs, could regulate their life activities depending on the quantity of available carbon sources. When COD was below 24.1 mg O_2/L, PAOs would stop anaerobic polyP release; when COD was below 21.3 (in anoxic condition) or 60.8 (in oxic condition) mg O_2/L, PAOs began to recover the storage pool of polyP. In the ALR, P uptake occurred in the aeration zone while P release happened at the bottom of the riser.

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