Environmental Science and Pollution Research (2020) 27:37877–37886
https://doi.org/10.1007/s11356-020-09912-9

RESEARCH ARTICLE

Investigation of the polyphosphate-accumulating organism population in the full-scale simultaneous chemical phosphorus removal system

Baogui Wang[1] · Erlong Jiao[2] · Yu Guo[1] · Lifang Zhang[2] · Qingan Meng[1] · Wei Zeng[1] · Yongzhen Peng[1]

Received: 17 January 2020 / Accepted: 26 June 2020 / Published online: 2 July 2020

Abstract

The simultaneous chemical phosphorus removal (SCPR) process has been widely applied in wastewater treatment plants (WWTPs) due to the high phosphorus removal efficiency through the synergy of biological and chemical phosphorus removal (BPR and CPR). However, phosphorus removal reagents could affect the bacterial community structure in the SCPR system and further affect the BPR process. The BPR phenotypes and community structures in the SCPR system, especially the population of polyphosphate-accumulating organisms (PAOs), are not completely clear. In order to clarify these problems, the phosphorus removal performance and the PAO population in a full-scale SCPR system were investigated. Results showed that diverse PAOs still existed in the SCPR system though the BPR phenotypes were not observed. However, the relative abundances of Accumulibacter and *Tetrasphaera*, the two most important genera of PAOs, were only 0.59% and 0.20%, respectively, while the relative abundances of Competibacter and *Defluviicoccus*, two genera of glycogen-accumulating organisms (GAOs), were as high as 5.77% and 1.28%, respectively. Batch tests showed that PAOs in the SCPR system still had a certain polyphosphate accumulating metabolic activity, which could gradually recover after stopping the addition of chemical reagents. This study provided a microbiological basis for the SCPR system to recover the enhanced biological phosphorus removal (EBPR) performance under suitable conditions, which could reduce the dosage of chemical reagents and the operational cost.

Keywords Wastewater treatment plants (WWTPs) · *Candidatus* Accumulibacter · Polyphosphate-accumulating metabolism (PAM) · Glycogen-accumulating organisms (GAOs) · *Candidatus* Competibacter · Aluminum sulfate

Introduction

The excessive discharge of nitrogen and phosphorus is the main reason leading to water eutrophication. Quantities of wastewater treatment plants (WWTPs) had been established to prevent nitrogen and phosphorus from entering aquatic water systems (Zhang et al. 2016). For these two nutrients, reducing the phosphorus input into lakes possibly played a more important role in the eutrophication control (Schindler et al. 2008).

As an economical process for phosphorus removal, an enhanced biological phosphorus removal (EBPR) system had been widely applied in WWTPs. However, the performance of EBPR was vulnerable to the insufficient carbon source (Oehmen et al. 2007), high temperature (Panswad et al. 2003), and other adverse environmental factors (Nielsen et al. 2019). Thus, the simultaneous chemical phosphorus removal (SCPR) process was increasingly adopted in WWTPs. In the SCPR system, phosphorus removal reagent was dosed at the end of the aerobic zone, which could coprecipitate with the residual phosphorus and further decrease the phosphorus concentration in effluent. However, the chemical reagent could flow back into the biological zone along with the sludge reflux, which would reversely affect the operational

Responsible editor: Ta Yeong Wu

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11356-020-09912-9) contains supplementary material, which is available to authorized users.

✉ Wei Zeng
zengwei@bjut.edu.cn

[1] National Engineering Laboratory for Advanced Municipal Wastewater Treatment and Reuse Technology, Department of Environmental Engineering, Beijing University of Technology, No.100 Pingleyuan, Chaoyang District, Beijing 100124, China

[2] Beijing Drainage Group Co., Ltd., Beijing 100037, China

characteristics and the bacterial community in the system, especially for those related to phosphorus removal (Auger et al. 2013; Zheng et al. 2018).

Polyphosphate-accumulating organisms (PAOs) and glycogen-accumulating organisms (GAOs) were the two common bacterial groups related to the biological phosphorus removal (BPR). For PAOs, *Candidatus* Accumulibacter (hereafter referred to as Accumulibacter) was considered to be the most significant genus which had been extensively examined (Li et al. 2019; Nielsen et al. 2019; Zhang et al. 2018). Accumulibacter could take up volatile fatty acids (VFAs) to synthesize poly-β-hydroxyalkanoates (PHAs) in the anaerobic zone accompanied by the phosphorus release and excessively absorb phosphorus in the aerobic zone using the energy from the PHA decomposition (Oehmen et al. 2007). In recent years, *Tetrasphaera* was also found to be an important PAO group, which widely existed in WWTPs around the world (Muszynski and Zaleska-Radziwill 2015; Nielsen et al. 2019; Stokholm-Bjerregaard et al. 2017). *Tetrasphaera* could obtain energy from fermenting glucose and proteins (Liu et al. 2019). Several other putative PAOs were also identified, such as *Comamonadaceae* (Ge et al. 2015), *Dechloromonas* (Gunther et al. 2009; Terashima et al. 2016), *Thiothrix* (Rey-Martínez et al. 2019), and *Microlunatus* (Kawakoshi et al. 2012; Zhong et al. 2018). GAOs always coexisted with PAOs in the WWTPs. The common GAOs included *Candidatus* Competibacter (hereafter referred to as Competibacter) and *Defluviicoccus* (Coats et al. 2017; Xia et al. 2018). It is generally accepted that PAOs decomposed polyphosphate to obtain energy, while GAOs needed to decompose glycogen. Polyphosphate hydrolysis was much faster than glycogen decomposition; thus, PAOs took up VFAs more quickly and possessed a competitive advantage. But the PAO abundance declined under some adverse conditions, and then, the abundance of GAOs increased (Tu and Schuler 2013). PAOs could survive by changing metabolic pathways from polyphosphate accumulating metabolism (PAM) to glycogen accumulation metabolism (GAM) under adverse conditions according to Acevedo et al. (2012, 2017). In the SCPR system, the addition of phosphorus removal reagent reduced the amount of phosphorus available to PAOs, which was disadvantageous to PAOs. At present, there are few studies on the community structures of PAOs and GAOs in the SCPR system.

In this study, the phosphorus removal performance in a full-scale SCPR system operated for a long term was investigated. And the bacterial community structures, particularly the populations of PAOs and GAOs, were analyzed using quantitative polymerase chain reaction (qPCR) and high-throughput sequencing.

Materials and methods

Description of the SCPR system

The modified anaerobic-anoxic-aerobic (A^2O) process was applied in the SCPR system (Fig. 1). In this system, the reflux sludge mixed with 10% of the influent flowed into the preanaerobic zone to deplete the oxygen and subsequently entered into the anaerobic zone, where it mixed with the remaining 90% of the inflow. The internal recycle from the end of the aerobic zone to the anoxic zone was adopted. The ratios of sludge reflux and internal reflux were 100% and 300%, respectively. The municipal wastewater was treated in this system, without industrial wastewater. The influent flow rate was 2.0×10^5 t/day. The influent chemical oxygen demand (COD) and total phosphorus concentrations were 180–400 mg/L and 3–6 mg/L, respectively. The hydraulic retention time (HRT) and the sludge retention time (SRT) were 10 h and 16 days, respectively. Aluminum sulfate ($Al_2(SO_4)_3$) was added at the end of the aerobic zone (that is the end of the O3 zone) to ensure that the total phosphorus concentrations in effluent stably met the discharge standard of the total phosphorus (0.5 mg/L). The dosage of $Al_2(SO_4)_3$ was 33 g/m^3, which was excessive compared with the value from the theoretical calculation. Samples were collected along the wastewater flow. The COD, *ortho*-phosphate, mixed liquor suspended solid (MLSS), mixed liquor volatile suspended solids (MLVSS), and some other parameters were detected. Moreover, activated sludge samples were lyophilized using the freeze dryer system (FreeZone®, Labconco Co., USA) and then stored at − 20 °C for subsequent DNA extraction.

Restorability tests for the BPR performance

Batch tests were carried out to verify the BPR performance of the activated sludge in the SCPR system. The activated sludge was collected at the end of the aerobic zone, which was upstream of the $Al_2(SO_4)_3$ addition point. Three conical bottles of 1000 mL were used in the tests, and the real domestic sewage was used as the influent. Tests were operated under alternating anaerobic/aerobic mode for three cycles without the addition of $Al_2(SO_4)_3$. The anaerobic phase was 1.5 h, and the aerobic one was 3 h. The mixed liquid was sampled regularly during the cycles, and the VFAs and phosphorus concentrations were determined.

Analytical methods

MLVSS, MLSS, sludge volume index (SVI), phosphorus, and COD were measured as described by the APHA standard methods (2005). VFAs and PHAs were analyzed by gas chromatography (Agilent 6890A, USA) according to Zeng et al. (2016). A PerkinElmer fluorescence spectrometer (LS55,

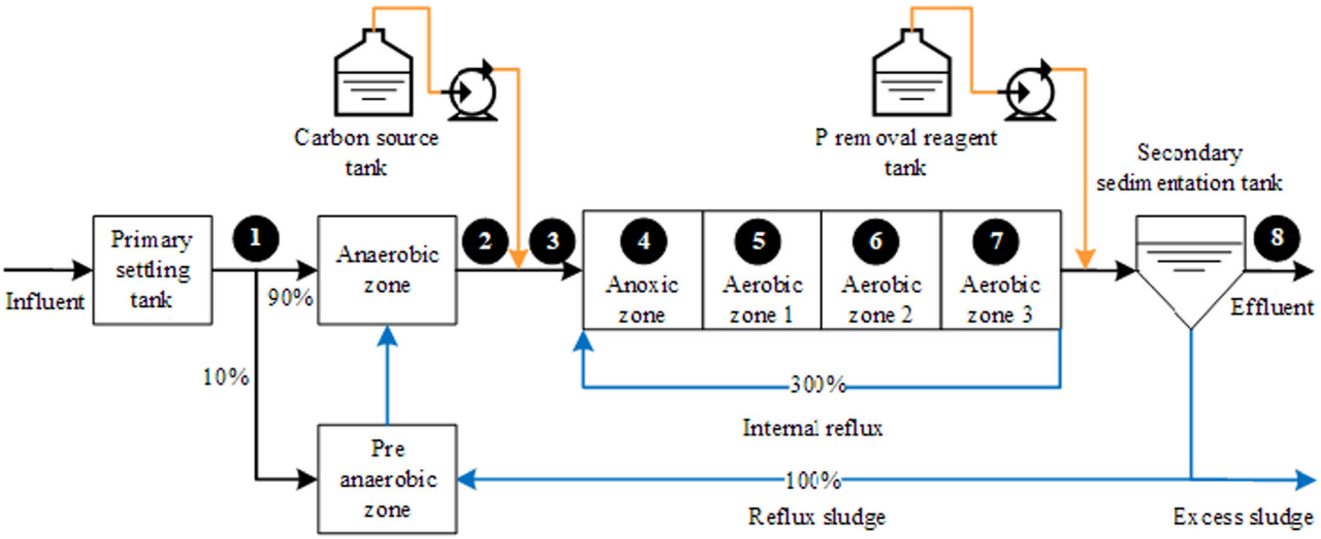

Fig. 1 Schematic of the full-scale SCPR system. The numbers represent the manual sampling points (1, effluent of the primary sedimentation tank; 2, effluent of the anaerobic zone; 3, effluent of the anaerobic zone after HAc addition; 4, effluent of the anoxic zone; 5, effluent of the aerobic zone 1; 6, effluent of the aerobic zone 2; 7, effluent of the aerobic zone 3; 8, effluent of the secondary sedimentation tank)

USA) was adopted to analyze the chemical compositions in the aquatic water from the SCPR system, and the results were presented and analyzed as described by Chen et al. (2003). The Standards of Measurements and Testing (SMT) protocol was used to analyze the phosphorus fractions in the activated sludge (Pardo et al. 2004). According to the SMT protocol, the total phosphorus was fractionated into organic phosphorus (OP) and inorganic phosphorus (IP), and IP was further fractionated into apatite phosphorus (AP) and non-apatite inorganic phosphorus (NAIP).

Quantitative PCR

Genomic DNA of the activated sludge in the SCPR system was extracted using the FastDNA™ SPIN Kit for Soil (MP Biomedicals, USA). The 16S rRNA genes of bacterial and Accumulibacter were quantified by Mx3005P (Agilent Technologies, USA). The *ppk1* genes of Accumulibacter (clades IA, IIA, IIB, IIC, IID, and IIF) were quantified to reveal its clade-level structures. The programs and primers referred to the previous research (Fan et al. 2019).

High-throughput sequencing

The high-throughput sequencing of the genomic DNA was conducted by the Illumina MiSeq PE3000 platform in Majorbio Co., Ltd. (Shanghai, China). The 338F and 806R primers were used. The sequences were quality-filtered and analyzed according to Guo et al. (2018). The raw sequences had been submitted to Sequence Read Archive (SRA) at NCBI with PRJNA595869.

Results and discussions

Characteristics of activated sludge

The MLSS and MLVSS maintained at 4500 ± 235 mg/L and 2900 ± 190 mg/L, respectively. The ratio of MLVSS/MLSS was about 64%, which indicated a considerable proportion of inorganic components in the activated sludge. Inorganic components are favorable to improve sludge compactness and settleability, which signified the low SVI of 83 mL/g MLSS. However, there are some disadvantages with the low MLVSS/MLSS, such as the high sludge yield and low sludge fermentation efficiency.

$Al_2(SO_4)_3$ altered the phosphorus fractions in the activated sludge through coprecipitating with phosphorus. Thus, the phosphorus fractions in the sewage sludge were analyzed using the SMT protocol, and the results are shown in Table 1. Based on the results, the TP content reached up to 37.8 ± 1.8 g/kg MLSS. The IP fraction was the dominant constituent, accounting for 84.7 ± 1.3% of TP. Among IP, NAIP was the major fraction with a percentage of 75.8 ± 4%. AP represents phosphorus combined with calcium ions, and NAIP represents those coprecipitated with aluminum ions, iron ions, and other ions. Results indicated that the activated sludge contained higher TP content than that described by Wang et al. (2018). Meanwhile, the NAIP proportion was also much higher than that found by Wang et al. (2018), which was due to the addition of $Al_2(SO_4)_3$. Moreover, the sum of OP and NAIP was as high as 86.7% of the TP, indicating the high proportion of releasable and bioavailable phosphorus, which should be considered in the subsequent sludge treatment (Ruban et al. 2001).

Table 1 Phosphorus fractions in the activated sludge

Content (g/kg SS)				Percentage (%)			Reference
TP	OP	NAIP	AP	OP	NAIP	AP	
37.8 ± 1.8	4.6 ± 0.1	24.3 ± 0.7	8.5 ± 2.0	12.3	64.2	22.5	This study
7.1–27.6	1.7–8.1	3.1–16.3	1–4.3	26.8 ± 7.9	52.0 ± 10.4	18.1 ± 8.4	Wang et al. (2018)[a]

[a] Sampled from forty-six WWTPs located in 30 provinces of China

Carbon and nutrient conversions along the wastewater flow

The SCPR system presented stable removal performance of organic matter and nutrients. The TP removal efficiencies were as high as 93 ± 3% according to long-term monitoring data. Figure 2 shows the conversions of the organic matter and *ortho*-phosphate concentration along the wastewater flow at 2018-09-26 and 2018-10-24. In the anaerobic zone, most of the VFAs from the influent wastewater were consumed, accompanied by the increase of PHA content in the sludge, while the phosphorus release was not observed. In the anoxic zone, the phosphorus concentration kept stable and the PHA content slightly decreased. In O1 zone, the phosphorus concentration decreased by 36% and 67%, respectively. In O2 zone, the phosphorus concentration rose back, which was probably due to the phosphorus release from sludge. After $Al_2(SO_4)_3$ addition at the end of the O3 zone, the phosphorus concentration declined. The PHA content in the oxic zones further decreased. In the secondary sedimentation tank, phosphorus concentration continued to descend to 0 mg/L at the lowest.

In the EBPR system, PAOs release phosphorus into bulk liquid in the anaerobic stage and excessively absorb phosphorus in the aerobic zone (Oehmen et al. 2007). In this SCPR system, these phenotypes were not observed because $Al_2(SO_4)_3$ was overdosed. The excess $Al_2(SO_4)_3$ could recirculate into the anaerobic zone along with the reflux sludge and coprecipitate with the phosphate, which caused a rapid decline of the phosphorus concentration in the anaerobic zone. PAOs

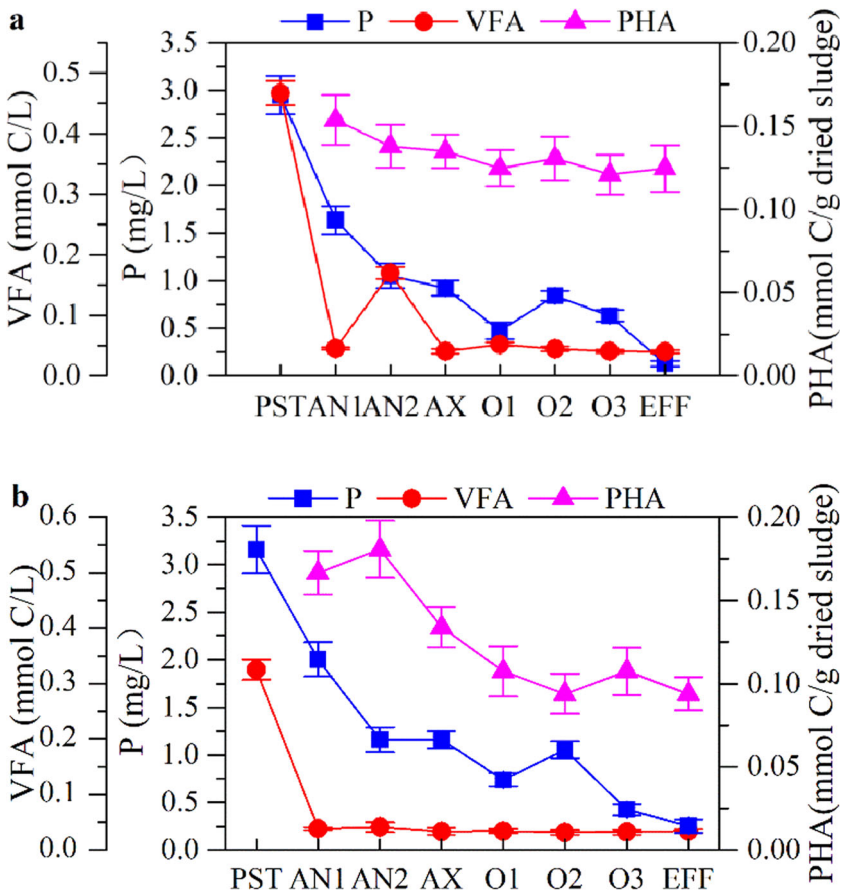

Fig. 2 Variations of the phosphate, VFAs, and PHAs along the wastewater flow sampled at **a** 2018-09-26 and **b** 2018-10-24 (PST, effluent of the primary sedimentation; AN1, effluent of the anaerobic zone; AN2, effluent of the anaerobic zone after HAc addition; AX, anoxic zone; O1, aerobic zone 1; O2, aerobic zone 2; O3, aerobic zone 3; EFF, effluent of the secondary sedimentation tank)

could not obtain enough phosphorus, leading to the decline of PAM activity. PAOs obtained energy more quickly through PAM, which ensured the preferential absorption of VFAs. Therefore, the decrease of PAM activity made it difficult for PAOs to obtain enough VFAs and accumulate sufficient polyphosphate, which decreased the proliferation rate of PAOs and finally caused the reduction of their relative abundances. Partial PAOs might survive and proliferate through altering their metabolic mode from PAM to GAM under the low content of polyphosphate (Acevedo et al. 2017; Welles et al. 2015). Besides, some groups of PAOs were possibly alive through PAM, but the *ortho*-phosphates released by them in the anaerobic zone were immediately precipitated with $Al_2(SO_4)_3$. All these three situations weakened the phenotype of phosphorus release in the anaerobic zone and absorption in the aerobic zone. The community structures should be investigated to deepen the understanding of this SCPR system.

Organic dynamics along the wastewater flow

The organic compositions in the bulk liquid were analyzed and presented using the excitation-emission matrix (EEM) (Fig. 3). As described by Chen et al. (2003), the EEM could be divided into five regions, i.e., tyrosine-like region (I), tryptophan-like region (II), fulvic acid–like region (III), the soluble microbial by-product-like (SMP) region (IV), and humic acid–like region (V). As shown in Fig. 3a, the effluent of the primary settling tank (PST) contained abundant organics, which presented high fluorescence intensity. The excitation-emission area volume of tryptophan-like organics (Φ_{II}) was 3.1×10^6 Au nm^2, lower than those of SMPs (6.6×10^6 Au nm^2) and humic acid–like organics (8.7×10^6 Au nm^2), but its normalized percent of fluorescence response ($P_{II,n}$) was the highest of 35% (Table 2).

In the anaerobic zone, the fluorescence intensity of the five regions decreased (Fig. 3b). The excitation-emission area volume of regions I–V, respectively, decreased by 75%, 63%, 43%, 45%, and 20% (Table 2), indicating the higher biodegradability of aromatic proteins (regions I and II). After the acetate addition, the fluorescence intensity was unchanged (Fig. 3c). In the anoxic zone, the fluorescence intensity of these five compositions kept declining. Compared with the influent, the excitation-emission area volume decreased by 95%, 87%, 75%, 54%, and 20%, respectively (Table 2). In

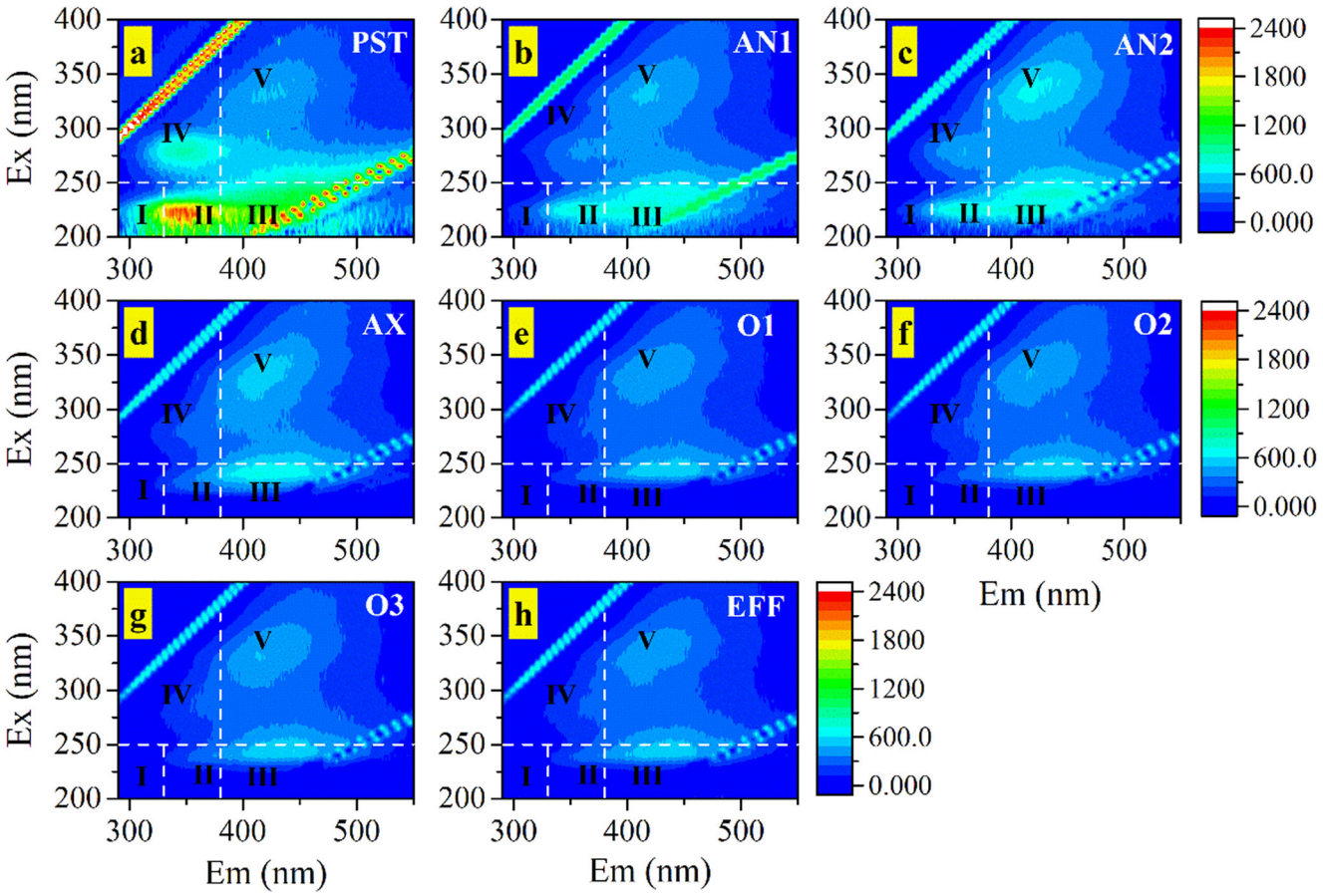

Fig. 3 Three-dimensional fluorescence spectra of soluble organics in the bulk liquid along the wastewater flow (PST, effluent of the primary sedimentation; AN1, effluent of the anaerobic zone; AN2, effluent of the anaerobic zone after HAc addition; AX, anoxic zone; O1, aerobic zone 1; O2, aerobic zone 2; O3, aerobic zone 3; EFF, effluent of the secondary sedimentation tank)

Table 2 Excitation-emission area volume and the percent of fluorescence response following the wastewater flow

Sample points		Φ_i[a] ($\times 10^{-6}$, Au nm^2)					$\Phi_{i,n}$[b] ($\times 10^{-6}$, Au nm^2)					$P_{i,n}$[c]				
		I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
1	PST	1.30	3.14	6.64	2.43	8.76	26.54	51.28	31.94	21.30	15.42	18%	35%	22%	15%	11%
2	AN1	0.32	1.16	3.78	1.34	7.02	6.47	18.91	18.19	11.72	12.35	10%	28%	27%	17%	18%
3	AN2	0.26	1.04	3.22	1.50	8.10	5.33	16.92	15.48	13.11	14.26	8%	26%	24%	20%	22%
4	AX	0.07	0.40	1.66	1.12	7.05	1.46	6.50	7.97	9.77	12.40	4%	17%	21%	26%	33%
5	O1	0.03	0.20	1.00	0.88	6.14	0.59	3.31	4.79	7.71	10.80	2%	12%	18%	28%	40%
6	O2	0.03	0.21	1.04	0.90	6.30	0.59	3.38	5.00	7.91	11.10	2%	12%	18%	28%	40%
7	O3	0.03	0.22	1.06	0.93	6.26	0.66	3.58	5.08	8.11	11.01	2%	13%	18%	29%	39%
8	EFF	0.03	0.21	1.01	0.91	5.85	0.66	3.46	4.85	7.99	10.29	2%	13%	18%	29%	38%

PST effluent of the primary sedimentation, *AN1* effluent of the anaerobic zone, *AN2* effluent of the anaerobic zone after HAc addition, *AX* anoxic zone, *O1* aerobic zone 1, *O2* aerobic zone 2, *O3* aerobic zone 3, *EFF* effluent of the secondary sedimentation tank

[a] The excitation-emission area volume for each region

[b] The normalized excitation-emission area volume for each region

[c] The normalized percent of fluorescence response

the O1 zone, the fluorescence intensity further decreased (Fig. 3e). The excitation-emission area volume of regions I–V decreased by 98%, 94%, 85%, 64%, and 30%, respectively. However, the fluorescence intensity and the normalized percent of fluorescence response kept stable in all the three aerobic zones (Fig. 3e–g), suggesting that aeration had a weak effect on the removal of fluorescent organics. In the effluent, SMPs and humic acid–like compositions became the major fluorescent substances with their normalized fluorescence response of 29% and 38%, respectively (Fig. 3h, Table 2). EEM results showed that there were diverse organic matters in the wastewater, which provided the material basis for microbial diversity.

Bacterial community structure in the SCPR system

The community structure in the SCPR system was investigated using high-throughput sequencing. The bacterial population exhibited high richness and diversity, with the Chao1 and Shannon indexes as high as 1498 and 5.46. In the phylum level, *Proteobacteria* was the dominant phylum with a relative abundance of 52%, and the *Gammaproteobacteria* (18.6%), *Betaproteobacteria* (15.4%), and *Alphaproteobacteria* (11.1%) were the major classes which affiliated to *Proteobacteria*. *Chloroflexi*, *Bacteroidetes*, *Actinobacteria*, and *Nitrospirae* were also the major bacterial phyla in this SCPR system, and the relative abundances were 13.7%, 9.51%, 8.45%, and 4.2%, respectively. The relative abundances of *Firmicutes*, *Acidobacteria*, *Planctomycetes*, and *Saccharibacteria* were all below 3%.

In this study, the community structures of PAOs and GAOs were the focuses. The putative populations of PAOs are shown in Fig. 4a, including *Ca*. Accumulibacter, *Tetrasphaera*, *Dechloromonas*, *Microlunatus*, *Tessaracoccus*, *Thiothrix*, and *Comamonadaceae*. In this study, the relative abundance of Accumulibacter was only 0.59%. Five Accumulibacter OTUs were observed, among which OTU1816 (41.8%) and OTU1602 (37.8%) were dominant. The relative abundance of Accumulibacter was also quantified using qPCR (Fig. 5a). The copy numbers of the Accumulibacter 16S rRNA gene and bacterial 16S rRNA gene were 2.40×10^{10} and 3.24×10^{12} copies/g sludge, respectively. According to Harms et al. (2003) and He et al. (2007), the average copy numbers of bacterial and Accumulibacter 16S rRNA gene were 3.6 and 2. Therefore, the relative abundance of Accumulibacter was 1.33% according to qPCR data, slightly higher than the ratio got by high-throughput sequencing. The clade-level population of Accumulibacter was investigated using *ppk1* genes (Fig. 5b). Based on the qPCR results, clades IIC and IID were the dominant clades in this SCPR system with ratios of 39.3% and 37.6%, respectively. Different Accumulibacter clades had different metabolic characteristics. Clade IIC was observed as a dominant clade in diverse wastewater treatment systems (He et al. 2007; Mao et al. 2015; Mielczarek et al. 2013; Muszynski et al. 2018; Ong et al. 2014; Qiu et al. 2019). Particularly, the dominant clades in this SCPR system were consistent with those in 18 WWTPs from six countries (Mao et al. 2015). These suggested that the fine-scale population of Accumulibacter in this SCPR system was not affected by the addition of $Al_2(SO_4)_3$ although the relative abundance of Accumulibacter was low. Results both from high-throughput sequencing and qPCR indicated that Accumulibacter exhibited relatively lower abundance in the SCPR system than that observed in EBPR plants (Stokholm-Bjerregaard et al. 2017).

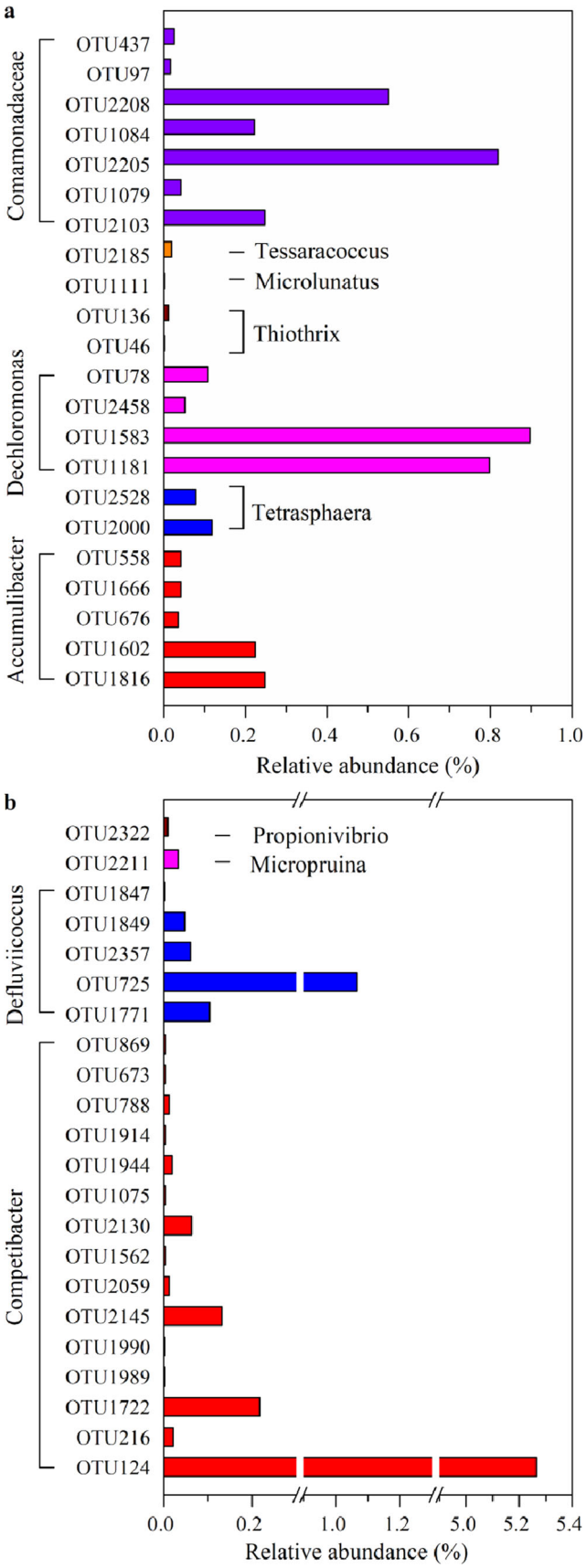

Fig. 4 The populations and relative abundance of **a** PAOs and **b** GAOs in the SCPR system

Tetrasphaera was another significant genus of PAOs (Fernando et al. 2019; Liu et al. 2019). In this study, the relative abundance of *Tetrasphaera* was only 0.20%, and only two OTUs were observed. *Tetrasphaera* was a fermentative bacterial group, which obtained energy from the fermentation of proteins and polysaccharides. In a WWTP in Denmark, the relative abundance of *Tetrasphaera* reached up to 21.2% (Stokholm-Bjerregaard et al. 2017). Similarly, in 4 Polish full-scale WWTPs, *Tetrasphaera* constituted 11–25% of all bacteria (Muszynski and Zaleska-Radziwill 2015). The diet structure with abundant milk and meat in Denmark and Poland possibly caused the high content of protein compositions in the sewage, which was conducive to *Tetrasphaera* proliferation. However, the $Al_2(SO_4)_3$ was added in the SCPR system, which consumed the phosphorus source and limited the proliferation of *Tetrasphaera*.

Dechloromonas was affiliated to the *Rhodocyclaceae* family, the same as Accumulibacter (Terashima et al. 2016). In this study, the relative abundance of *Dechloromonas* was 1.85%, which was higher than Accumulibacter and *Tetrasphaera*. *Dechloromonas* was subdivided into four taxa with OTU1181 (43.0%) and OTU2458 (48.3%). *Comamonadaceae* had a high growth rate in the activated sludge ecosystems (Ge et al. 2015; Saunders et al. 2016). In this SCPR system, *Comamonadaceae* was divided into seven OTUs with a relative abundance of 1.92%, among which OTU2205 and OTU2208 exhibited higher proportions of 42.6% and 28.6%.

In the SCPR system, Competibacter and *Defluviicoccus* were the predominant genera of GAOs (Fig. 4b). The relative abundances of Competibacter and *Defluviicoccus* were 5.77% and 1.28%, respectively. For Competibacter, sixteen OTUs were observed, among which OTU124 was the dominant one with a ratio of 91.2%. For *Defluviicoccus*, five OTUs were found with OTU725 (83.2%) as the dominant one. Results showed that the relative abundances of Competibacter and *Defluviicoccus* were both higher than previous findings (Stokholm-Bjerregaard et al. 2017). According to Nielsen et al. (2019), Competibacter and *Defluviicoccus* were GAOs possessing the canonical GAM mode. In this mode, VFAs were taken up and transformed into glycogen at anaerobic conditions. At aerobic conditions, glycogen was degraded to supply the energy and substrate for their proliferation, and the remaining glycogen was transformed into PHAs for storage. In the EBPR system, PAOs used the energy from the hydrolysis of polyphosphate to preferentially absorb VFAs. In the SCPR system, $Al_2(SO_4)_3$ coprecipitated with the phosphorus in the bulk liquid, which created an environment with a high ratio of carbon to phosphorus. These conditions limited the metabolism and proliferation of PAOs, whereas GAOs got more VFAs and grew better.

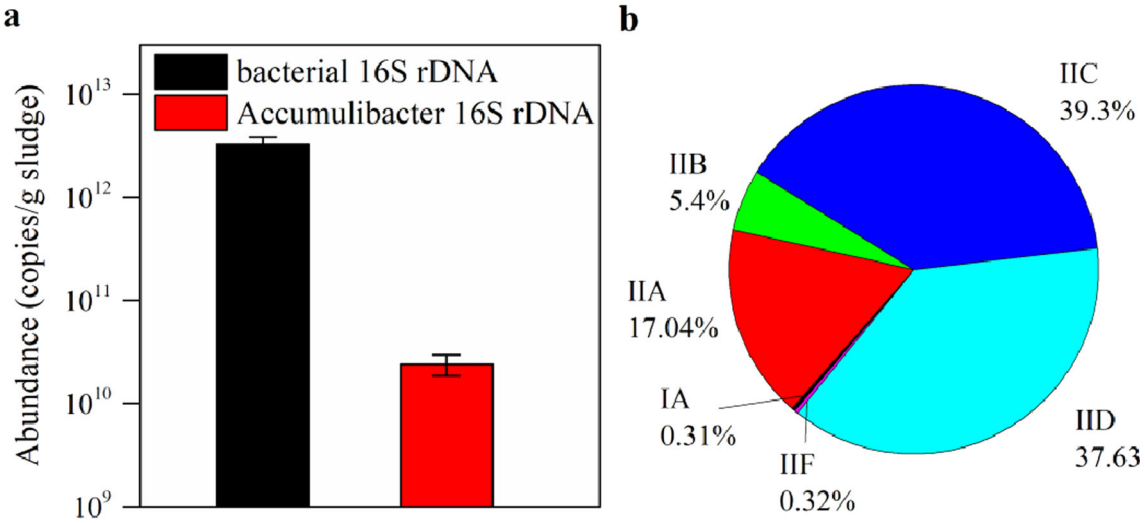

Fig. 5 The abundances of 16S rRNA genes of bacteria and Accumulibacter in the SCPR system

BPR restorability batch tests

To verify the BPR performance of the activated sludge in this SCPR system, the alternating anaerobic/aerobic operation was carried out (Fig. 6). Chemical reagent was not added in the tests. The phosphorus and VFA concentrations were regularly detected, and the ratios of phosphorus release to VFA uptake (P_{rel}/VFA_{upt}) were calculated. In the first cycle, the activated sludge showed slight BPR performance. The phosphorus concentration increased from 2.6 to 6.4 mg/L during the anaerobic stage and rapidly declined to 0.2 mg/L after 1 h of aerobic reaction. The P_{rel}/VFA_{upt} was 0.13 mmol-P/mmol-C. In the second cycle, the phosphorus concentration at the end of the anaerobic stage increased to 10.0 mg/L, and the P_{rel}/VFA_{upt} increased to 0.19 mmol-P/mmol-C. In the third cycle, the maximum phosphorus concentration and P_{rel}/VFA_{upt} increased to 12.2 mg/L and 0.20 mmol-P/mmol-C, respectively. The P_{rel}/VFA_{upt} had been used as an indicator of the PAM activity, which was generally higher than 0.5 mmol-P/mmol-C in EBPR systems (Acevedo et al. 2017; Oehmen et al. 2007; Schuler and Jenkins 2003). Results showed that although no typical characteristics of PAM were observed in the SCPR system, the PAM activity of the activated sludge gradually recovered after the $Al_2(SO_4)_3$ addition was stopped, which corresponded to the existence of diverse PAOs.

The addition of chemical reagents in the SCPR system increased the operation cost of sewage treatment. This study demonstrated that PAOs still existed in the SCPR system and could recover the PAM activity after stopping the addition of the chemicals. Therefore, it is possible to enhance the BPR performance by gradually reducing or even stopping the addition of chemical reagents in the SCPR systems, so as to reduce the operating costs.

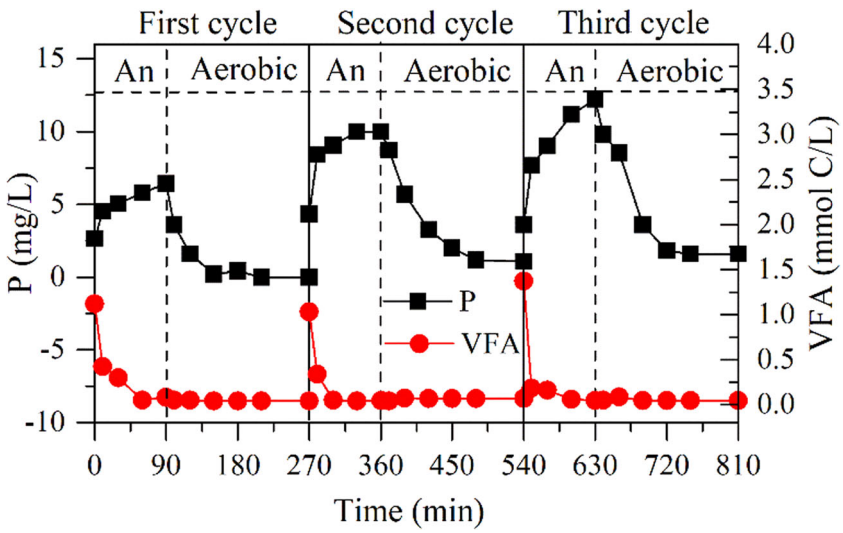

Fig. 6 The variations of phosphorus and VFA concentration during the anaerobic/aerobic cycles after stopping dosing $Al_2(SO_4)_3$

Conclusion

In the long-term SCPR system with $Al_2(SO_4)_3$ addition, the PAM phenotype disappeared. PAOs were still present in the system with relatively low abundances, while GAOs of Competibacter and *Defluviicoccus* possessed relatively high abundances. However, the PAOs in the system showed high diversity and maintained the PAM ability. The activated sludge could recover the BPR performance after the $Al_2(SO_4)_3$ addition was stopped. These results proved the potential of the SCPR system to resume EBPR performance.

Acknowledgments This work was supported by the National Key Research and Development Programme of China (2016YFC0401103) and Beijing Excellent Talents Project (2017A36).

References

Acevedo B, Oehmen A, Carvalho G, Seco A, Borras L, Barat R (2012) Metabolic shift of polyphosphate-accumulating organisms with different levels of polyphosphate storage. Water Res 46(6):1889–1900

Acevedo B, Murgui M, Borras L, Barat R (2017) New insights in the metabolic behaviour of PAO under negligible poly-P reserves. Chem Eng J 311:82–90

Auger C, Han S, Appanna VP, Thomas SC, Ulibarri G, Appanna VD (2013) Metabolic reengineering invoked by microbial systems to decontaminate aluminum: implications for bioremediation technologies. Biotechnol Adv 31(2):266–273

Chen W, Westerhoff P, Leenheer JA, Booksh K (2003) Fluorescence excitation−emission matrix regional integration to quantify spectra for dissolved organic matter. Environ Sci Technol 37(24):5701–5710

Coats ER, Brinkman CK, Lee S (2017) Characterizing and contrasting the microbial ecology of laboratory and full-scale EBPR systems cultured on synthetic and real wastewaters. Water Res 108:124–136

Fan Z, Zeng W, Wang B, Chang S, Peng Y (2019) Analysis of microbial community in a continuous flow process at gene and transcription level to enhance biological nutrients removal from municipal wastewater. Bioresour Technol 286:121374

Fernando EY, McIlroy SJ, Nierychlo M, Herbst F, Petriglieri F, Schmid MC, Wagner M, Nielsen JL, Nielsen PH (2019) Resolving the individual contribution of key microbial populations to enhanced biological phosphorus removal with Raman–FISH. ISME J

Ge H, Batstone DJ, Keller J (2015) Biological phosphorus removal from abattoir wastewater at very short sludge ages mediated by novel PAO clade Comamonadaceae. Water Res 69:173–182

Gunther S, Trutnau M, Kleinsteuber S, Hause G, Bley T, Roske I, Harms H, Muller S (2009) Dynamics of polyphosphate-accumulating bacteria in wastewater treatment plant microbial communities detected via DAPI (4′,6′-diamidino-2-phenylindole) and tetracycline labeling. Appl Environ Microbiol 75(7):2111–2121

Guo Y, Zeng W, Li N, Peng Y (2018) Effect of electron acceptor on community structures of denitrifying polyphosphate accumulating organisms in anaerobic-anoxic-oxic (A^2O) process using DNA based stable-isotope probing (DNA-SIP). Chem Eng J 334:2039–2049

Harms G, Layton AC, Dionisi HM, Gregory IR, Garrett VM, Hawkins SA, Robinson KG, Sayler GS (2003) Real-time PCR quantification of nitrifying bacteria in a municipal wastewater treatment plant. Environ Sci Technol 37(2):343–351

He S, Gall DL, McMahon KD (2007) "Candidatus accumulibacter" population structure in enhanced biological phosphorus removal sludges as revealed by polyphosphate kinase genes. Appl Environ Microbiol 73(18):5865–5874

Kawakoshi A, Nakazawa H, Fukada J, Sasagawa M, Katano Y, Nakamura S, Hosoyama A, Sasaki H, Ichikawa N, Hanada S, Kamagata Y, Nakamura K, Yamazaki S, Fujita N (2012) Deciphering the genome of polyphosphate accumulating Actinobacterium Microlunatus phosphovorus. DNA Res 19(5):383–394

Li C, Zeng W, Li N, Guo Y, Peng Y (2019) Population structure and morphotype analysis of "*Candidatus* Accumulibacter" using fluorescence in situ hybridization-staining-flow Cytometry. Appl Environ Microbiol 85(9):1–13

Liu R, Hao X, Chen Q, Li J (2019) Research advances of Tetrasphaera in enhanced biological phosphorus removal: a review. Water Res 115003

Mao YP, Graham DW, Tamaki H, Zhang T (2015) Dominant and novel clades of *Candidatus* Accumulibacter phosphatis in 18 globally distributed full-scale wastewater treatment plants. Sci Rep 5(11857)

Mielczarek AT, Nguyen HTT, Nielsen JL, Nielsen PH (2013) Population dynamics of bacteria involved in enhanced biological phosphorus removal in Danish wastewater treatment plants. Water Res 47(4):1529–1544

Muszynski A, Zaleska-Radziwill M (2015) Polyphosphate accumulating organisms in treatment plants with different wastewater composition. Architect Civil Eng Environ 8:99–105

Muszynski A, Zaleska-Radziwill M, Doskocz N (2018) Factors affecting Accumulibacter population structure in full- and laboratory-scale biological reactors with nutrients removal. Water Sci Technol 77(12):2794–2802

Nielsen PH, McIlroy SJ, Albertsen M, Nierychlo M (2019) Re-evaluating the microbiology of the enhanced biological phosphorus removal process. Curr Opin Biotechnol 57:111–118

No author (2005) Standard methods for the examination of water and waste water, 21st edn. American Public Health Association, Washington, DC

Oehmen A, Lemos PC, Carvalho G, Yuan Z, Keller J, Blackall LL, Reis MAM (2007) Advances in enhanced biological phosphorus removal: from micro to macro scale. Water Res 41(11):2271–2300

Ong YH, Chua A, Fukushima T, Ngoh GC, Shoji T, Michinaka A (2014) High-temperature EBPR process: the performance, analysis of PAOs and GAOs and the fine-scale population study of *Candidatus* "Accumulibacter phosphatis". Water Res 64:102–112

Panswad T, Doungchai A, Anotai J (2003) Temperature effect on microbial community of enhanced biological phosphorus removal system. Water Res 37(2):409–415

Pardo P, Rauret G, Lopez-Sanchez JF (2004) Shortened screening method for phosphorus fractionation in sediments - a complementary approach to the standards, measurements and testing harmonised protocol. Anal Chim Acta 508(2):201–206

Qiu G, Zuniga-Montanez R, Law Y, Thi SS, Nguyen TQN, Eganathan K, Liu X, Nielsen PH, Williams RBH, Wuertz S (2019) Polyphosphate-accumulating organisms in full-scale tropical wastewater treatment plants use diverse carbon sources. Water Res 149:496–510

Rey-Martínez N, Badia-Fabregat M, Guisasola A, Baeza JA (2019) Glutamate as sole carbon source for enhanced biological phosphorus removal. Sci Total Environ 657:1398–1408

Ruban V, Lopez-Sanchez JF, Pardo P, Rauret G, Muntau H, Quevauviller P (2001) Harmonized protocol and certified reference material for the determination of extractable contents of phosphorus in freshwater sediments - a synthesis of recent works. Fresenius J Anal Chem 370(2–3):224–228

Saunders AM, Albertsen M, Vollertsen J, Nielsen PH (2016) The activated sludge ecosystem contains a core community of abundant organisms. ISME J 10(1):11–20

Schindler DW, Hecky RE, Findlay DL, Stainton MP, Parker BR, Paterson MJ, Beaty KG, Lyng M, Kasian SE (2008) Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. Proc Natl Acad Sci U S A 105(32):11254–11258

Schuler AJ, Jenkins D (2003) Enhanced biological phosphorus removal from wastewater by biomass with different phosphorus contents, part I:experimental results and comparison with metabolic models. Water Environ Res 75(6):499–511

Stokholm-Bjerregaard M, McIlroy SJ, Nierychlo M, Karst SM, Albertsen M, Nielsen PH (2017) A critical assessment of the microorganisms proposed to be important to enhanced biological phosphorus removal in full-scale wastewater treatment systems. Front Microbiol 8:718

Terashima M, Yama A, Sato M, Yumoto I, Kamagata Y, Kato S (2016) Culture-dependent and -independent identification of polyphosphate-accumulating Dechloromonas spp. predominating in a full-scale oxidation ditch wastewater treatment plant. Microbes Environ 31(4):449–455

Tu Y, Schuler AJ (2013) Low acetate concentrations favor polyphosphate accumulating organisms over glycogen-accumulating organisms in enhanced biological phosphorus removal from wastewater. Environ Sci Technol 47(8):3816–3824

Wang C, Geng Y, Cheng L, Mao Y (2018) Speciation, mass loadings, and fate of phosphorus in the sewage sludge of China. Environ Sci Pollut Res 25(35):35531–35537

Welles L, Tian WD, Saad S, Abbas B, Lopez-Vazquez CM, Hooijmans CM, van Loosdrecht M, Brdjanovic D (2015) Accumulibacter clades type I and II performing kinetically different glycogen-accumulating organisms metabolisms for anaerobic substrate uptake. Water Res 83:354–366

Xia Y, Wen XH, Zhang B, Yang YF (2018) Diversity and assembly patterns of activated sludge microbial communities: a review. Biotechnol Adv 36(4):1038–1047

Zeng W, Zhang J, Wang AQ, Peng YZ (2016) Denitrifying phosphorus removal from municipal wastewater and dynamics of "*Candidatus* Accumulibacter" and denitrifying bacteria based on genes of ppk1, narG, nirS and nirK. Bioresour Technol 207:322–331

Zhang QH, Yang WN, Ngo HH, Guo WS, Jin PK, Dzakpasu M, Yang SJ, Wang Q, Wang XC, Ao D (2016) Current status of urban wastewater treatment plants in China. Environ Int 92-93:11–22

Zhang C, Sun G, Zhao K, Zou S, Yuan L (2018) Performance of A2NO-MBR process in treating synthetic and municipal wastewater. Environ Sci Pollut Res 25(11):10782–10791

Zheng WL, Yu ZY, Xia Y, Wen XH (2018) Influence of polyaluminum chloride on microbial characteristics in anaerobic membrane bioreactors for sludge digestion. Appl Microbiol Biotechnol 102(2): 1005–1017

Zhong CQ, Fu JF, Jiang TY, Zhang CM, Cao GX (2018) Polyphosphate metabolic gene expression analyses reveal mechanisms of phosphorus accumulation and release in Microlunatus phosphovorus strain JN459. FEMS Microbiol Lett 365(6)