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Effect of organic loading on extracellular polymer secretion and phosphorus forms transformation in sludge

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ABSTRACT

The contribution of extracellular polymeric substances (EPS) to phosphorus uptake and the forms of accumulated extracellular phosphorus were investigated under three organic loading conditions (0.29, 0.58, 1.16 kg COD $(m^3 \cdot d)^{-1}$) in continuous flow A^2O system. The results showed that activated sludge flocs contained more EPS per unit mass under high organic load, which could store more phosphorus. EPS accounted for up to 32.57% of the total phosphorus accumulation in sludge floc with the increasing of organic load, which indicated that the phosphorus accumulation in EPS was non-negligible in the system. High organic load promoted the production of organic phosphorus and non-apatite inorganic phosphorus (NAIP) in EPS, increased the ratio of bioavailable phosphorus, which was affected by the microbial population structure and metabolic mode. The influence of organic load on intracellular and extracellular phosphorus removal performance was compared, NAIP in bacterial cells played an important role in intracellular phosphorus removal, while apatite phosphorus in EPS played an important role in extracellular phosphorus removal.

Keywords: Continuous-flow process; Organic loading; Extracellular polymer secretion; Phosphorus forms transformation; Phosphorus removal performance

1. Introduction

Urban domestic sewage in China has the characteristics of large quantity and wide range of pollutant sources, leading to significant water quality fluctuation [1–3]. The contribution of extracellular polymeric substance (EPS) to phosphorus uptake and the forms of accumulated extracellular phosphorus under different organic loads vary substantially in different studies [4–9]. Furthermore, the biological phosphorus research for migration between various forms of phosphorus and its transformation rule was almost discussed in enhanced biological phosphorus removal system operated by sequencing batch reactor (SBRs) [10–13]. However, the distribution of phosphorus forms in the continuous-flow simultaneous denitrification and dephosphorization system is still unclear.

In practical application, continuous-flow system is the most commonly used system in sewage treatment plants, and the growth environment of sludge strains is very different from that of SBRs. It is necessary to analyze the relationship between the form of phosphorus and EPS secretion under different organic loads in the continuous-flow simultaneous nitrogen and phosphorus removal system, evaluate the effect of EPS on phosphorus removal, and clarify the mechanism of enhanced phosphorus removal by EPS.

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According to the above practical problems and technical background, a continuous-flow A^2/O reactor was operated with different organic loads for 6 months in this study. The change of phosphorus form components and sludge characteristics was compared and analyzed. The influence of organic loads on intracellular and extracellular phosphorus removal performance and the correlation between EPS and phosphorus forms were discussed. Combined with the role of EPS in biological phosphorus removal, the adsorption of phosphorus by EPS can be maximized in practical engineering according to the wastewater properties and operation process, so as to effectively and rapidly remove phosphorus.

2. Materials and methods

2.1. Setup of continuous-flow reactor and operation

A detailed description of this experimental facility has been published by the study of Li et al. [14]. The operating conditions were as follows: the water inflow was 188.0 L/d, the hydraulic retention times of anaerobic tank, anoxic tank and aerobic tank were 1.6, 2.5 and 8.3 h, respectively. The return sludge ratio was 100%, and the nitrate recycling ratio was 250%. Sludge retention time was controlled at 15 d and sludge concentration was about 3,000 mg/L. DO was controlled at about 3.0 mg/L by aeration.

2.2. Synthetic wastewater

The activated sludge used was taken from A^2/O process of a WWTP in Tianjin, which possessed dense bacterial micelle and good settling property. The synthetic wastewater, with sodium acetate as carbon source, KH_2PO_4 and NH_4Cl as phosphorus source and nitrogen source. The experimental period included three different organic load phases by adjusting influent chemical oxygen demand (COD) concentration, which named Phase I (0.29 kg COD $(m^3 \cdot d)^{-1}$), Phase II (0.58 kg COD $(m^3 \cdot d)^{-1}$) and Phase III (1.16 kg COD $(m^3 \cdot d)^{-1}$), respectively. In order to ensure sufficient supply of nitrogen and phosphorus in the system, the initial concentration of influent ammonia nitrogen and total phosphorus were set as 40 and 10 mg/L, respectively. An additional amount of $CaCl_2$, $MgSO_4$, $NaHCO_3$ and trace element solution was added in the synthetic wastewater, and the content composition was the same as described by Li [15].

2.3. Method for determination of morphological phosphorus

The phosphorus forms in sludge and bacterial cells were measured by European Standard SMT Protocol. This method divided total phosphorus (TP) into inorganic phosphorus (IP) and organic phosphorus (OP), IP was further divided into non-apatite inorganic phosphorus (NAIP) and apatite phosphorus (AP). NAIP referred to the unstable and weakly bonded phosphorus adsorbed on the surface of iron, manganese, aluminum oxides and their hydroxides. AP referred to various inert phosphorus bound to calcium ion; NAIP and OP were collectively known as bioavailable phosphorus, which can be directly utilized by microorganisms. The specific extraction method was referred to Guo et al. [16].

2.4. High-throughput sequencing

16S rRNA high-throughput sequencing was carried out using the Illumina MiSeq System (Illumina MiSeq, USA) at Shanghai Sangon Biotech Co., Ltd. A detailed description of this experimental procedure can be seen in Li et al. [14].

2.5. Conventional analytical method

COD, NH_4^+–N, NO_3^-–N, PO_4^{3-}–P, total nitrogen, mixed liquor suspended solids (MLSS), SV30 and SVI were determined according to the Standard Method [17]. EPS extraction was carried out by ultrasonic-cation exchange resin method [18]. Polysaccharide (PS) were determined by anthrone colorimetry, while protein (PN) was determined by coomassie bright blue colorimetry [19,20]. Humic acid (HA) was determined by modified Folin–Lowry method [21]. The correlations between the content and composition of EPS and the forms of phosphorus were determined by Pearson correlation using IBM SPSS Statistics 25.

3. Results and discussion

3.1. Effect of organic load on EPS content and composition

As shown in Fig. 1, the total EPS in anaerobic, anoxic, and aerobic tanks increased from 43.65 to 85.60 mg/g MLSS with the increasing of organic load. It was also found that loosely-bound PN (LB-PN) content was significantly higher than that of loosely-bound PS (LB-PS) under three organic loads in this experiment. The loosely-bound PN/PS (LB-PN/PS) ratio increased from 4.09 to 14.02 with the increasing of organic load. However, the tight-bound PN/PS (TB-PN/PS) ratio was relatively stable. Compared with tightly-bound EPS (TB-EPS), loosely-bound EPS (LB-EPS) was more susceptible to influent organic load, and protease was mainly concentrated in LB-EPS, which promoted the production of LB-PN. Furthermore, EPS content in the aerobic tank decreased under medium and high organic load conditions (Phases II and III). The reason may be that high organic load lead to high bacterial activity, organic load in aerobic tank can't meet the biological growth, EPS was absorbed as organic matter. Too little organic matter in aerobic tank under low organic load condition (Phase I), poor microbial activity lead to the death of some microorganisms. In the process of extinction, humic acid, protein, polysaccharide, nucleic acid and other end products was produced, which increased the content of EPS.

3.2. Effect of organic load on phosphorus forms variation in EPS

The contents of TP, IP, OP, AP and NAIP all increased with organic load increasing in bacterial cells and in EPS (as shown in Fig. 2a and b). The main reason was that activated sludge flocs contained more EPS per unit mass under high organic load, which could store more phosphorus. As shown in Table 1, IP accounted for the largest

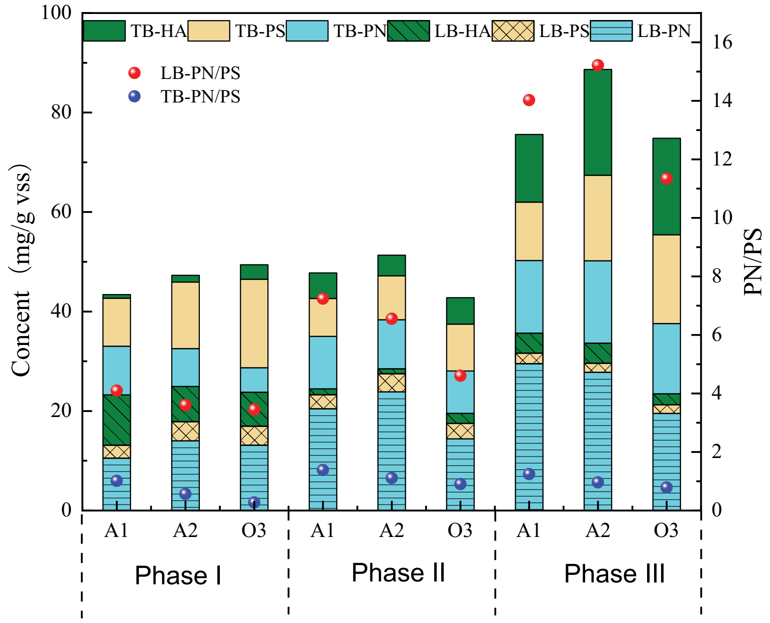

Fig. 1. Variation of EPS content and composition in anaerobic, anoxic, and aerobic tanks during different phases (A1, A2 and O3 indicate that the samples are taken from anaerobic, anoxic and aerobic tanks respectively).

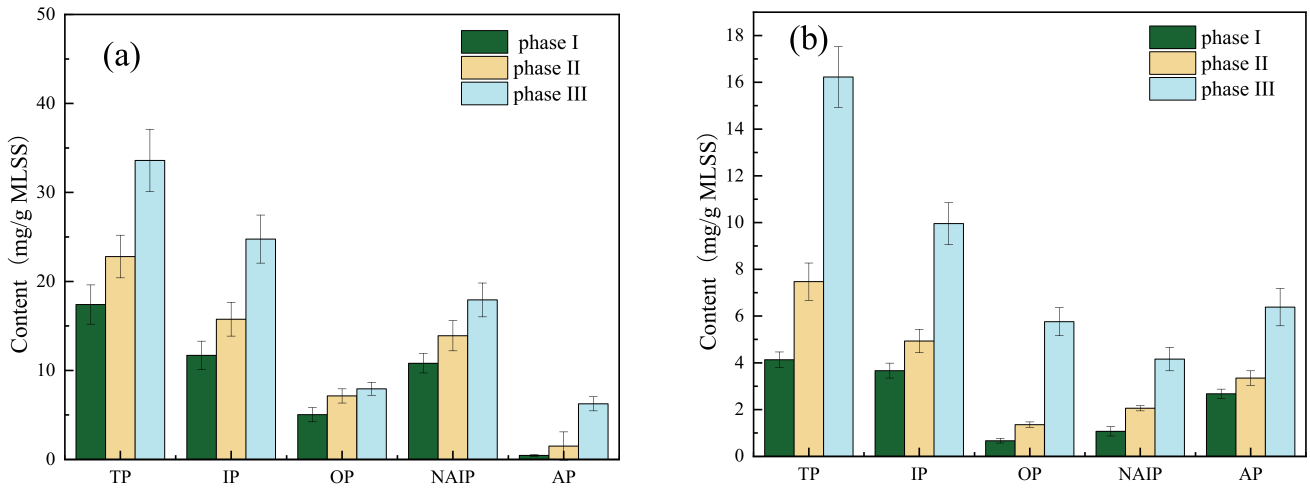

Fig. 2. Variation of phosphorus form and content of aerobic tanks in bacterial cells and in EPS under different organic loads (a) changes of phosphorus form in bacterial cells and (b) changes of phosphorus form in EPS.

Table 1
Percentages of various forms of phosphorus in extracellular polymers under different organic loads

Phases	IP_{EPS}/TP_{EPS}	OP_{EPS}/TP_{EPS}	$NAIP_{EPS}/IP_{EPS}$	AP_{EPS}/IP_{EPS}
Phase I	88.71%	16.17%	29.23%	72.94%
Phase II	66.04%	27.42%	41.68%	67.84%
Phase III	61.34%	35.50%	41.77%	64.10%

proportion of total extracellular phosphorus under different organic loads, and AP was the main component of IP in EPS. AP was Ca^{2+} bound phosphorus and strongly adsorbed phosphorus, which was more easily adsorbed by cations in EPS, formed precipitation and stored in EPS. With the increasing of organic load, the ratio of IP to TP in EPS decreased from 88.71% to 61.34%, the ratio of NAIP to IP increased from 29.23% to 41.77%, and the ratio of AP to IP decreased from 72.94% to 64.10%. High organic load also promoted the production of OP in EPS, the proportion of OP to TP content in EPS increased from 16.17% to 35.50%.

Table 2 shows the percentages of each form of extracellular phosphorus to that of in sludge floc under different organic loads. The proportion of TP increased from 19.19% to 32.57%, the proportion of IP increased from 23.88% to 28.68%, and the proportion of OP increased from 11.73% to 42.06%. It can be seen that the higher the organic load, the stronger the ability of EPS to bind phosphorus. The proportion of NAIP increased from 9.02% to 18.83%, and the proportion of AP decreased from 85.40% to 50.51%, indicating that high organic load was beneficial to the absorption and binding of NAIP by EPS, but unfavorable to the absorption of AP. This was affected by the microbial population structure and metabolic mode, which was explained in detail in the following chapters. In a word, about 19.19%~32.57% of TP in the sludge was reserved in the EPS at the end of the aerobic phase and could contribute to the phosphorus removal through sludge discharge. These results indicated that the contribution of EPS to the phosphorus removal in the A^2/O process could not be neglected.

3.3. Correlation between EPS component and different phosphorus forms

In order to explore the influence of EPS contents and components on different phosphorus forms in EPS, a correlation analysis was conducted between EPS and various phosphorus forms (Fig. 3). The results showed that TP content was positively correlated with LB-PN (r = 0.707, P = 0.033), TB-PN (r = 0.828, P = 0.006), TB-HA (r = 0.907, P = 0.001), LB-PN/PS (r = 0.872, P = 0.002), EPS (r = 0.924, P = 0.011), PN (r = 0.791, P = 0.011), HA (r = 0.876, P = 0.002), TB-EPS (r = 0.913, P = 0.001), and was negatively correlated with LB-PS (r = –0.748, P = 0.020), LB-HA (r = –0.671, P = 0.048). TP and EPS had the highest positive correlation, which indicated that the total EPS had the greatest influence on TP in the EPS. The TP content was mainly affected by TB-EPS, but had no significant correlation with LB-EPS content. According to the component analysis of EPS, proteins in LB-EPS and TB-EPS were favorable for the storage of TP, polysaccharides in LB-EPS were unfavorable for the storage of TP, polysaccharides in TB-EPS had no obvious effect on the storage of TP, humic acid in LB-EPS was unfavorable for the storage of TP, humic acid in TB-EPS was favorable for the storage of TP. The same correlation trend was found in IP, OP, NAIP, AP as that of TP.

The correlation analysis of different phosphorus forms in sludge was also investigated (as shown in Table 3). It can be seen that the content of TP in sludge was significantly correlated with the content of other phosphorus forms. The correlation between TP and IP was the strongest, followed by TP with NAIP and TP with AP. There was a significant positive correlation between TP, IP and bioavailable P (r_1 = 0.995, r_2 = 0.924, P < 0.01). The higher the content of TP and IP in sludge, the higher the content of bioavailable P, which consisted with the conclusion of Wang et al. [22]. There was a very significant positive correlation between NAIP and bioavailable P (R = 0.983, P < 0.01), which indicated that NAIP in sludge had a great impact on bioavailable phosphorus, and the potential agricultural value of sludge can be judged from the level of NAIP content in sludge.

Table 2
The proportion of each form of phosphorus in extracellular polymer to each form of phosphorus in sludge

Phases	IP_{EPS}/IP_{floc}	OP_{EPS}/OP_{floc}	$NAIP_{EPS}/NAIP_{floc}$	AP_{EPS}/AP_{floc}	TP_{EPS}/TP_{floc}
Phase I	23.88%	11.73%	9.02%	85.40%	19.19%
Phase II	23.85%	15.94%	12.89%	69.16%	24.68%
Phase III	28.68%	42.06%	18.83%	50.51%	32.57%

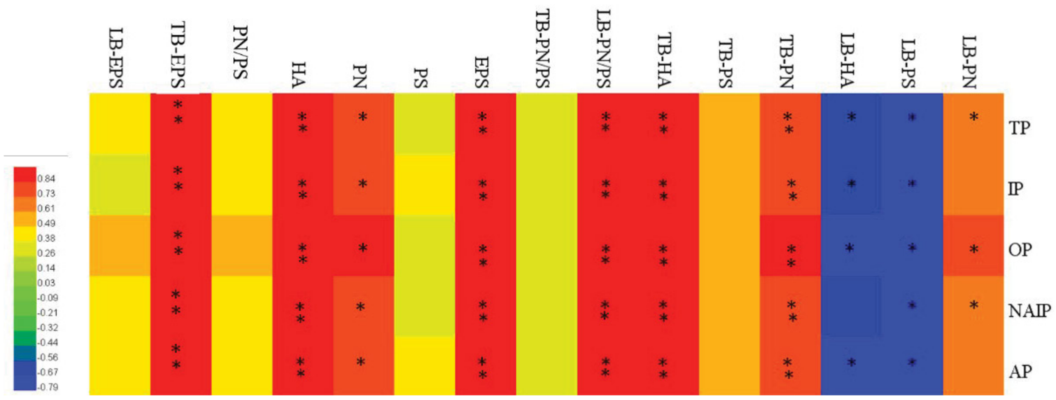

Fig. 3. Heat map of correlation between EPS components and phosphorus forms in EPS.

Table 3
Correlation coefficient matrix of the different forms of phosphorus in sludge

	TP	IP	OP	NAIP	AP	NAIP + OP
TP	1					
IP	0.995**	1				
OP	0.740*	0.692*	1			
NAIP	0.962**	0.954**	0.838**	1		
AP	0.931**	0.953**	0.501*	0.829**	1	
NAIP + OP	0.924**	0.900**	0.922**	0.983**	0.746*	1

**indicated significant correlation at 0.01 level (two-tailed test);
*indicated significant correlation at 0.05 level (two-tailed test).

Table 4
Different forms phosphorus uptake in bacterial cells and in EPS under different organic loads

Phases	Type	ΔTP_{uptake}	ΔIP_{uptake}	ΔOP_{uptake} (mg/g)	$\Delta NAIP_{uptake}$	ΔAP_{uptake}
Phase I	Intracellular	1.32 ± 0.05	0.89 ± 0.02	0.45 ± 0.02	0.73 ± 0.02	0.04 ± 0.01
	Extracellular	1.20 ± 0.04	0.79 ± 0.03	0.15 ± 0.01	0.27 ± 0.02	0.45 ± 0.02
Phase II	Intracellular	2.74 ± 0.05	1.30 ± 0.04	0.70 ± 0.02	1.02 ± 0.03	0.23 ± 0.01
	Extracellular	1.27 ± 0.05	0.94 ± 0.02	0.23 ± 0.01	0.58 ± 0.03	0.69 ± 0.03
Phase III	Intracellular	3.79 ± 0.08	2.52 ± 0.05	1.42 ± 0.04	1.62 ± 0.05	0.46 ± 0.02
	Extracellular	3.23 ± 0.12	1.70 ± 0.05	1.02 ± 0.05	0.82 ± 0.04	0.86 ± 0.04

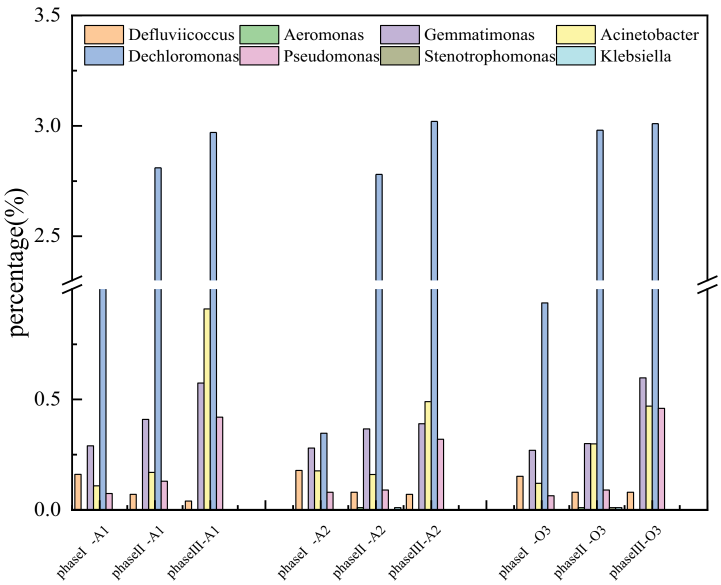

Fig. 4. Proportion of phosphorus accumulating bacteria in anaerobic, anoxic, and aerobic tanks during different phases (A1, A2 and O3 indicate that the samples are taken from anaerobic, anoxic and aerobic tanks respectively).

3.4. Influence of organic load on intracellular and extracellular phosphorus removal performance

As shown in Table 4, the phosphorus uptake in bacterial cells from anaerobic to aerobic stage increased from 1.32 mg/g MLSS to 3.79 mg/g MLSS with the increase of organic loads, indicating the bacterial cells with high organic load showed higher phosphorus uptake. ΔIP_{uptake} accounted for 55.6%~60.3% in ΔTP_{uptake}, and the greatest change in ΔIP_{uptake} was $\Delta NAIP_{uptake}$, which was 53.8%~82.0% of ΔIP_{uptake}. It was concluded that NAIP in bacterial cells played an important role in intracellular phosphorus removal. The phosphorus uptake in EPS increased from 1.20 mg/g MLSS to 3.23 mg/g MLSS from anaerobic to aerobic stage. The change of TP was dominated by IP increment, which was 52.6%~74.0%, and the greatest change in ΔIP_{uptake} was ΔAP_{uptake}, which was 50.6%~71.2% of ΔIP. Furthermore, it can be analyzed from the data in the last column of Table 4, ΔAP_{uptake} in EPS of three phases were much higher than that in bacterial cells. The ratio of ΔAP_{uptake} to ΔIP_{uptake} were 91.8%, 75.0% and 65.2% with the increase of organic loads, which had a distinct advantage in extracellular phosphorus removal. It was concluded that AP in EPS played an important role in extracellular phosphorus removal.

It can be seen from Table 4, high organic load promoted the production of OP and NAIP in EPS significantly, which was speculated that they were affected by the metabolism of functional flora. By using high-throughput sequencing, it was found that the abundance of phosphorus accumulating bacteria (PAOs) increased with the increase of organic load (as shown in Fig. 4). As denitrifying phosphorus removal bacteria, the abundance of *Dechloromonas* increased from 1.06% to 2.97%, and *Gemmatimonas* increased from 0.29% to 0.57%. As traditional phosphorus removal bacteria, *Acinetobacter* increased from 0.11% to 0.91%, and *Pseudomonas* increased from 0.07% to 0.42%.

4. Conclusion

This study revealed the characteristics of phosphorus accumulation in EPS under different loads in the continuous-flow A^2/O process. All forms of phosphorus showed higher content in the EPS with the increasing of organic load, IP was the main form of phosphorus, and AP is the main component of IP in EPS.

High organic load promoted the production of OP and NAIP in EPS significantly, meanwhile the abundance of PAOs increased with the increase of organic load, which mean that the formation and transformation of phosphorus in EPS were affected by the microbial population structure and metabolic mode, rather than a simple process of extracellular phosphate dissolution and precipitation.

The ratio of bioavailable P to TP in sludge significantly improved with the increase of organic load. In other words, more phosphorus in sludge under high load condition can be used biologically. It can be seen that mastering the change rule of phosphorus form can provide scientific reference and theoretical basis for subsequent sludge reasonable disposal and application management.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contributions

The corresponding author, Li Yajing is responsible for ensuring that the descriptions are accurate and agreed by all authors. Li Yajing and Wang Shaopo conceived and designed the study. Liu Lu and Li Weiquan performed the experiments. Wu Yaping investigated and verified research data. Wang Shaopo provided the study materials and other analysis tools. All authors read and approved the manuscript.

References

[1] S. Yu, P. Sun, W. Zheng, L. Chen, X. Zheng, J. Han, T. Yan, The effect of COD loading on the granule-based enhanced biological phosphorus removal system and the recoverability, Bioresour. Technol., 171 (2014) 80–87.
[2] Y. Liang, D. Li, H. Zeng, C. Zhang, J. Zhang, Rapid start-up and microbial characteristics of partial nitrification granular sludge treating domestic sewage at room temperature, Bioresour. Technol., 196 (2015) 741–745.
[3] D. Li, Y.F. Lv, H.P. Zeng, J. Zhang, Long term operation of continuous-flow system with enhanced biological phosphorus removal granules at different COD loading, Bioresour. Technol., 216 (2016) 761–767.
[4] A.G. Geyik, B. Kılıç, F. Çeçen, Extracellular polymeric substances (EPS) and surface properties of activated sludges: effect of organic carbon sources, Environ. Sci. Pollut. Res., 23 (2015) 1653–1663.
[5] D. Li, Y. Lv, H. Zeng, J. Zhang, Effect of sludge retention time on continuous-flow system with enhanced biological phosphorus removal granules at different COD loading, Bioresour. Technol., 219 (2016) 14–20.
[6] S.-S. Yang, J.-W. Pang, W.-Q. Guo, X.-Y. Yang, Z.-Y. Wu, N.-Q. Ren, Z.-Q. Zhao, Biological phosphorus removal in an extended ASM2 model: roles of extracellular polymeric substances and kinetic modeling, Bioresour. Technol., 232 (2017) 412–416.
[7] Y.H. Shi, J.H. Huang, G.M. Zeng, Y.L. Gu, Y.N. Chen, Y. Hu, B. Tang, J.X. Zhou, Y. Yang, L.X. Shi, Exploiting extracellular polymeric substances (EPS) controlling strategies for performance enhancement of biological wastewater treatments: an overview, Chemosphere, 180 (2017) 396–411.
[8] P. Rusanowska, A. Cydzik-Kwiatkowska, P. Świątczak, I. Wojnowska-Baryła, Changes in extracellular polymeric substances (EPS) content and composition in aerobic granule size-fractions during reactor cycles at different organic loads, Bioresour. Technol., 272 (2019) 188–193.
[9] M. Zhang, Y.X. Wang, Y.J. Fan, Y.Z. Liu, M. Yu, C.D. He, J. Wu, Bioaugmentation of low C/N ratio wastewater: effect of acetate and propionate on nutrient removal, substrate transformation, and microbial community behavior, Bioresour. Technol., 306 (2020) 1–10, doi: 10.1016/j.biortech.2019.122465.
[10] X.Y. Long, R. Tang, Z.D. Fang, C.X. Xie, Y.Q. Li, G. Xian, The roles of loosely-bound and tightly-bound extracellular

polymer substances in enhanced biological phosphorus removal, Chemosphere, 189 (2017) 679–688.

[11] S.P. Wang, Z. Li, D. Wang, Y.J. Li, L.P. Sun, Performance and population structure of two carbon sources granular enhanced biological phosphorus removal systems at low temperature, Bioresour. Technol., 300 (2020) 1–9, doi: 10.1016/j.biortech.2019.122683.

[12] Y.J. Li, S.P. Wang, Y.P. Wu, L.P. Sun, Characterization of extracellular phosphorus in enhanced biological phosphorus removal granular sludge, Desal. Water Treat., 218 (2021) 260–269.

[13] X.Y. Long, R. Tang, T. Wang, G.J. Tao, J.Y. Wang, H.W. Zhou, M. Xue, Y.P. Yu, Characteristics of enhanced biological phosphorus removal (EBPR) process under the combined actions of intracellular and extracellular polyphosphate, Chemosphere, 279 (2021) 1–9.

[14] Y.J. Li, Y.P. Wu, S.P. Wang, L.Y. Jia, Effect of organic loading on phosphorus forms transformation and microbial community in continuous-flow A^2/O process, Water Sci. Technol., 83 (2021) 2640–2651.

[15] Z. Li, Metabolism Characteristics of Polyphosphate Accumulating Organisms and Quorum Sensing of Granular Sludge in A/O-SBR System at Low Temperature, Dissertation, Tianjin Chengjian University, Tianjin, 2019.

[16] C. Guo, H.Y. Liu, Q. Wang, S.Q. Liu, Y.L. Luo, X.Y. Liu, Existing morphology of phosphorus in dephosphorization granular sludge and its content analysis, Saf. Environ. Eng., 22 (2015) 44–48.

[17] S.E.P.A. Chinese, Water and Wastewater Monitoring Methods (4th ed.), Chinese Environmental Science Publishing House, China, 2002.

[18] Y.J. Li, S.P. Wang, Z. Li, L.P. Sun, Extraction method and structural and composition characteristics of extracellular polymeric substances in granular sludge from an enhanced biological phosphorus removal system, Desal. Water Treat., 185 (2020) 41–50.

[19] J. Zhou, L.X. Zhou, H.Z. Huang, Optimization of extracellular polymeric substance extraction method and its role in the dewaterability of sludge, Environ. Sci., 34 (2013) 2752–2757.

[20] C.H. Tan, K.S. Kon, C. Xie, M. Tay, Y. Zhou, R. Williams, W.J. Ng, S.A. Rice, S. Kjelleberg, The role of quorum sensing signaling in EPS production and the assembly of a sludge community into aerobic granules, ISME J., 8 (2014) 1186–1197.

[21] J.J. Liu, Y. Yuan, X.Y. Li, Z.J. Xue, Y.Z. Peng, Effect of pretreatment with humic acids on the activity of nitrifying bacteria in activated sludge, J. Harbin Inst. Technol., 49 (2017) 15–19.

[22] C. Wang, Q.W. Liu, Y. Zhi, L. Cheng, N. Wang, C.D. Li, Y.X. Mao, Contents and forms of phosphorous in the municipal sewage sludge of China, Environ. Sci., 40 (2019) 1922–1930.