RESEARCH ON THE CHARACTERISTICS OF SLUDGE PROCESS ACTIVATED BY SIDESTREAM SEQUENCING BATCH REACTOR

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Abstract. With the biological phosphorus removal process of activated sludge by side flow sequencing batch as the research object, the hydrolysis and acidification effects of sludge in the anaerobic fermentation tank and the pollutant removal effects under different operating conditions were preliminarily analyzed. Subsequently, the relationship between activated sludge characteristics and the pollutant removal mechanism was explored from the perspective of extracellular polymeric substances and microorganisms. The composition and quantity of extracellular polymers excreted by activated sludge were analyzed, together with the population density of phosphorus-accumulating microorganisms. Results showed that extracellular polymers consist of three components: tryptophan-like substances, humus-like substances, and protein-like substances. *Proteobacteria* were identified as the predominant group of phosphorus-accumulating bacteria, accounting for up to 52.6%. *Gammaproteobacteria*, *Betaproteobacteria*, and *Rhodocyclaceae* were also identified as Important phosphorus-accumulating microorganisms, accounting for up to 22% to 50%.
Keywords: *side flow SBR, biological phosphorus removal, hydrolysis acidification, pollutant removal, phosphorus-accumulating microorganisms*

Introduction

The exponential advancement of China's economy and population, coupled with the simultaneous increase in industrial and domestic wastewater, has led to a serious deterioration of the nation's surface water environment. This includes, but is not limited to, unsightly water blooms and damaging red tides, both of which trace back to extensive eutrophication. Phosphorus, however, has been identified as a key contributor to this phenomenon (Liu et al., 2020). The traditional biological phosphorus removal process, while effective, is limited by low efficiency, intense competition for carbon sources, and the potential for cross-contamination of intermediate products. Therefore, there is an urgent need to develop an enhanced biological phosphorus removal process that exhibits superior phosphorus removal efficiency and minimal energy consumption, ensuring that the effluent phosphorus concentration meets the required standards.

The SBR enhanced biophosphorus removal process can provide a unique growth environment, which is conducive to the growth and enrichment of polyphosphate-accumulating bacterium, thus effectively avoiding the competition of denitrifying bacteria and polyphosphate-accumulating bacterium for carbon sources and the conflict

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between nitrifying bacteria and phosphorus-accumulating bacteria on sludge age. However, at present, there are relatively few reports on the characteristics of the biophosphorus removal process of SBR lateral flow. Li et al. (2001) studied the characteristics of submerged sequential batch biofilm phosphorus removal process, Kang et al. (2016) studied the characteristics of nitrite denitrification phosphorus removal process, Wang et al. (2018). studied the characteristics of electroadsorption phosphorus removal process, and Liu et al. (2014). studied the characteristics of catalytic iron and biological coupled phosphorus removal process. Therefore, it is necessary to reveal the mechanism of pollutant removal by exploring the pollutant removal effect of SBR side flow biological phosphorus removal process and the distribution characteristics of activated sludge microorganisms.

This study used a side stream Sequencing Batch Reactor (SBBR) biological phosphorus removal process as the primary treatment method, using prepared wastewater as a nutrient source to simulate the treatment process. The study also cultivated activated sludge with superior phosphorus removal efficiency. The biological phosphorus removal performance under various operating conditions was investigated, and the microbial community structure of activated sludge and extracellular polymeric substances (EPS) produced by activated sludge were analyzed. This study aimed to elucidate the biological characteristics of activated sludge and the mechanism of pollutant removal.

Materials and methods

Test water quality and inoculation mud

The experimental water quality of this study was designed to simulate the characteristics of general wastewater. Specifically, the content of key nutrients, namely carbon (C), nitrogen (N), and phosphorus (P), was prepared. The carbon source was sucrose, the nitrogen source was ammonium chloride, and the phosphorus source was calcium phosphate. The inoculated activated sludge was obtained from the sludge concentration tank of Xi’an No. 3 Wastewater Treatment Plant. Detailed experimental water quality and sludge parameter values are outlined in *Table 1*.

Table 1. *The experimental water quality and sludge parameters*

Category		Parameter conditions
Test water distribution	Sludge acclimation, stable operation stage	C:N:P = 60:8:1, COD: 300 mg/L, NH4 + -N: 40 mg/L, TP: 5 mg/L C:N:P = 80:6:1, COD: 400 mg/L, NH4 + -N: 30 mg/L, TP: 5 mg/L
Inoculation sludge		SV: 98%, MLSS: 12.88 g/L, SVI: 73.8 mL/g, VSS: 7.17 g/L COD: 34 mg/L, NH4 + -N: 12.9 mg/L, TN: 15.8 mg/L, TP: 25.8 mg/L

Index measurement and methods

Instrumental analysis was conducted to determine the following parameters: TN refers to total nitrogen, representing the sum of various forms of inorganic and organic nitrogen in water, and is determined by basic potassium persulfate digestion ultraviolet spectrophotometry; TP refers to total phosphorus, indicating that after digestion of water samples, various forms of phosphorus are converted into orthophosphate.

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Measured in milligrams of phosphorus per liter of water sample, the determination is made by potassium persulfate digestion of ammonium molybdate spectrophotometry; NH4 + -N, also known as ammonia nitrogen, refers to nitrogen in two specific forms of ammonium ions and ammonia, and is determined by Noller's reagent spectrophotometry; CODCr refers to under certain conditions, the reducing substances in water are oxidized and decomposed under the action of an applied strong oxidant The amount of oxidizing agent consumed, expressed in mg/L of oxygen, is determined by rapid digestion spectrophotometry; SCOD refers to the amount of organic matter that can be dissolved in water and participate in chemical reactions during water treatment, and is determined by potassium dichromate method; TOC refers to total organic carbon, indicating the total amount of organic matter in water, usually expressed in terms of carbon content. The result is expressed in terms of the mass concentration of carbon (mg/L), and the measurement is carried out by non-dispersive infrared absorption method.

EPS is a polymer substance mainly produced by the secretion of microbial cells and exists outside the cell. The main components of EPS are protein, polysaccharide, and humic acid. Protein is determined by [specific method name]. Polysaccharide and humic acid are determined by the anthrone method. VFAs, also known as volatile fatty acids, are usually determined by liquid chromatography. All index determination methods are derived from the fourth edition of the Water and Wastewater Monitoring and Analysis Method.

Fluorescence spectroscopy: Three-dimensional fluorescence spectroscopy can semi-quantitatively analyze substances such as proteins and humic acids. In this study, parallel factor model (EEM-PARAFAC) was used to analyze fluorescence data, specifically using Dreem 3D fluorescence parallel factor analysis toolkit (DOMFlour) on the Matlab software platform and Openfluor fluorescence database. Test parameters: excitation wavelength scanning range 240-600 nm, emission wavelength scanning range 290-600 nm, step size 5 nm, slit width 3 nm.

Data Statistics Method: SPSS 25 software was used to analyze the experimental data, and Origin 2022 software was used to plot and analyze all the experimental data.

Experimental device

The main equipment of this experimental apparatus is a SBR reactor. In addition, the apparatus includes a flow meter, water pump, stirring mechanism, aeration mechanism, controller unit, and an anaerobic fermentation tank. The main equipment SBR reactor has an inner diameter of 24 cm, a wall thickness of 1 cm, and a height of 38.5 cm. The water outlet is located 15 cm from the reactor base, and the effective volume is 17.5 L. The inner diameter of the anaerobic tank is 16 cm, the wall thickness is 1 cm, the height is 25 cm, and the effective volume is 5.0 L. A schematic diagram of the experimental apparatus is shown in *Figure 1*.

Experimental condition setting

This experiment primarily investigated the influence of hydraulic residence time, lateral flow ratio, and various aeration methods on the composition and content of extracellular polymeric substances (EPS) (Ma et al., 2017; Yang et al., 2020). The objective is to further explore the biological characteristics of sludge and the mechanism of pollutant removal. The operating parameters are presented in *Table 2*.

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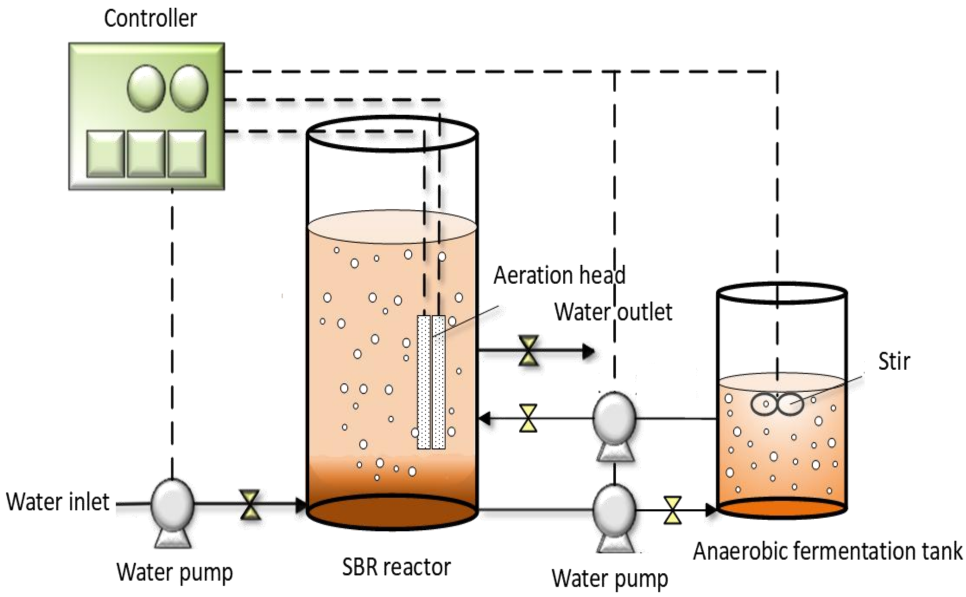

Figure 1. *Experimental device schematic*

Table 2. *The working condition parameter setting*

Main influencing factors	Parameter setting
HRT	4 h, 6 h, 8 h
Lateral flow ratio	10%, 15%, 20%
Aeration method	Exposure 3 h stop 3 h, Exposure 4 h stop 2 h, Exposure 5 h stop 1 h

Domestication of inoculated sludge

At the initial stage of the experiment, the residual sludge was procured from the sludge concentration tank of the residual sludge of Xi'an No. 3 Wastewater Plant. Initial domestication of residual sludge was carried out as follows:

The initial sludge is first filtered through a screen, then passed through static sedimentation and the supernatant is removed, followed by 24 h Stuffing treatment, and finally wastewater is added (that is, the experimental water distribution in the sludge acclimation stage (COD 300 mg/L, ammonia nitrogen 40 mg/L, total phosphorus 5 mg/L), followed by aeration treatment, aeration for 9 h per day, standing for 3 h, aeration amount of 35 L/h, supernatant removal, continuous treatment for 48 h.

After initial culture, the concentration of activated sludge (MLSS) is adjusted to 3200 mg/L, which is then transferred to the SBR. From the early stage, a small amount of continuous wastewater (i.e. experimental water for the reactor operation stage) is added. The wastewater flow is initially maintained at a low level to avoid excessive loading and unsuitable conditions for microbial growth. Therefore, it is recommended to start with a flow rate of 6 L/h for the initial phase, gradually increasing this rate by 1 L/h per day. It is anticipated that the required flow rate of 12 L/h will be achieved after six days. Continuous cultivation is carried out until the effluent quality remains stable.

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Results and discussion

Analysis of hydrolysis and acidification effect of sludge under different working conditions

SCOD refers to the amount of oxidant consumed when reducing substances in water are oxidized and decomposed under certain conditions, and TOC refers to the index of the total amount of organic matter in the system expressed in C content. Therefore, the ratio of TOC to SCOD may reflect the change in the effective carbon source in the system. As can be seen from *Figure 2*, with the increase of the lateral flow ratio, the TOC/SCOD in the system shows a trend of first decreasing and then increasing, from 0.3 to 0.12 and then rising to about 0.16. When the lateral flow ratio is 10%, the TOC/SCOD overall shows a downward trend, indicating that the organic matter in the system has a high dissolution rate. When the lateral flow ratio is 15%, the TOC/SCOD tends to be stable, indicating that the dissolution rate of organic matter in the system remains essentially the same as the sum of conversion and consumption rates. When the lateral flow ratio is 20%, TOC/SCOD increases slightly, indicating a small accumulation of carbon sources in the system. As can be seen from *Figure 2*, the HRT and TOC/SCOD in the system show an inverse relationship. When the hydraulic residence time gradually increases, the TOC/SCOD shows a gradual downward trend, falling from 0.3 to about 0.2. When the hydraulic residence time is 4 h, the TOC/SCOD rises slightly and then maintains a downward trend, indicating that a small amount of organic carbon accumulates in the system. When the hydraulic residence time is 6 h, the TOC/SCOD ratio increases slightly, indicating that a small amount of organic carbon accumulates in the system. When the hydraulic residence time is 8 h, the TOC/SCOD tends to stabilize, indicating that the dissolution and consumption rates of organic matter in the system are basically the same. The accumulation and consumption of organic matter at this stage are basically the same. It can be seen from *Figure 2* that when the aeration time in the aeration method gradually increases, the TOC/SCOD in the system also shows a trend of first decreasing and then increasing, from 0.37 to 0.22 and then rising to about 0.23. When the aeration method is aeration for 3 h and stops for 3 h, the TOC/SCOD in the system shows a downward trend, indicating that there is a higher organic dissolution rate in the system. When the aeration method is aeration for 4 h and stops for 2 h, the TOC/SCOD ratio increases slightly, indicating that a small amount of organic carbon accumulates in the system. When the aeration method is aeration for 5 h and stops for 1 h, the TOC/SCOD tends to stabilize, indicating that the dissolution and consumption rates of organic matter in the system are basically the same, and the accumulation and consumption of organic matter in this stage are basically the same (Zhao, 2022; Xu, 2010).

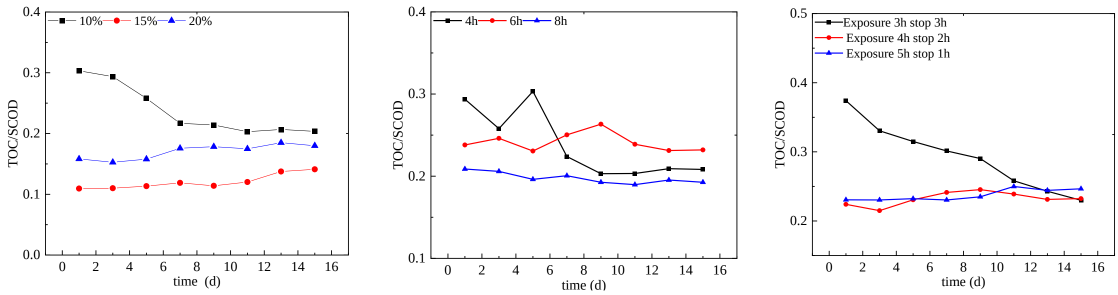

Figure 2. *Changes of TOC/SCOD under different working conditions*

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Influence of different working conditions on pollutant removal effect

As can be seen from *Figure 3*, the removal rate of COD fluctuates slightly under different lateral flow ratio conditions, and the overall trend is upward, and the removal rate is stable above 90%. The removal effect of COD is good, and it can meet the "Pollutant Discharge Standards for Urban Wastewater Treatment Plants" (GB18918-2002) first-class A standard (COD ≤ 50 mg/L), indicating that each stage of the system needs to consume COD to supplement itself and losses, so COD is easily degraded. As can be seen from *Figure 3*, with the increase of the lateral flow ratio, the removal rate of TP also shows an upward trend. When the side flow ratio is 20%, the TP removal rate is 88%, and the effluent concentration is about 0.6 mg/L. When the side flow ratio is 15%, the TP removal rate is 85%, and the effluent concentration is about 0.75 mg/L. When the side flow ratio is 10%, the TP removal rate is 83%, and the effluent concentration is about 0.9 mg/L. The removal effect is still better than before the side flow ratio is not added. This is because with the continuous improvement of the side flow ratio, the sludge content in the reactor continues to decrease, and the discharge rate of TP becomes larger, which in turn leads to an increase in the removal rate (Zhang et al., 2014).

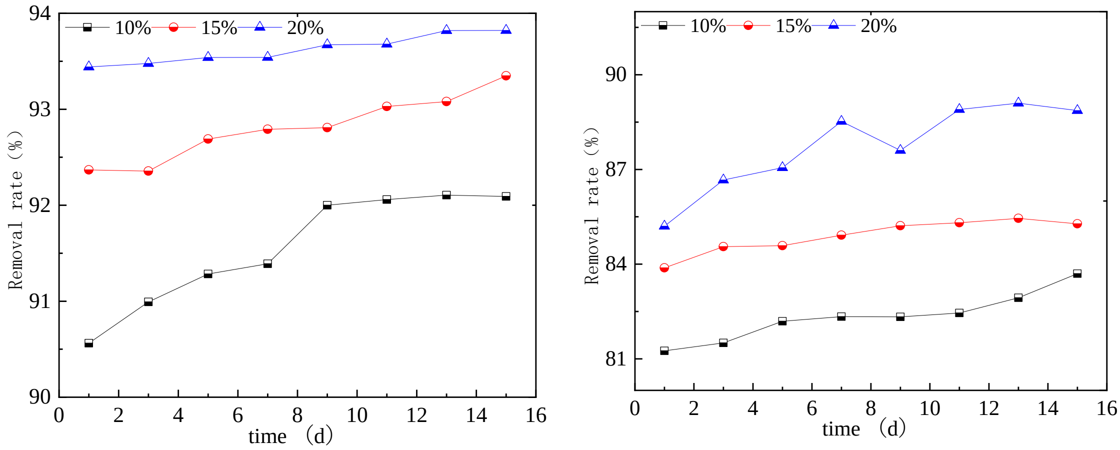

Figure 3. *The removal effect of COD and TP with different side flow ratios*

It can be seen from *Figure 4* that under different hydraulic residence time conditions, the COD removal rate fluctuates little, and with the increase of hydraulic residence time, the COD removal rate reaches 90%, and the average COD concentration in the effluent is about 30 mg/L, which can meet the "Urban Wastewater Treatment Plant Pollutant Discharge Standard" (GB18918-2002) first-class A standard (COD ≤ 50 mg/L), indicating that the change process of poly-β-hydroxybutyrate in polyphosphorus bacteria may consume part of the carbon source in the sewage, resulting in better COD removal. As can be seen from *Figure 4*, under different water flow residence time conditions, the TP removal rate fluctuates greatly. With the increase of hydraulic residence time, the TP removal rate also rises again. When the hydraulic residence time is 4 h, the TP removal rate is 85%. The concentration of TP in the effluent is about 0.75 mg/L, which can meet the first-class B standard (TP ≤ 1.0 mg/L) of the "Urban Sewage Treatment Plant Pollutant Discharge Standard" (GB18918-2002). When the hydraulic residence time is 6,8 h, the removal rate of TP is 91% and 87.5%.

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The concentration of TP in the effluent is about 0.4-0.5 mg/L, which can meet the "Urban Sewage Treatment Plant Pollutant Discharge Standard" (GB18918-2002) First Class A standard (TP ≤ 0.5 mg/L) (He, 2019).

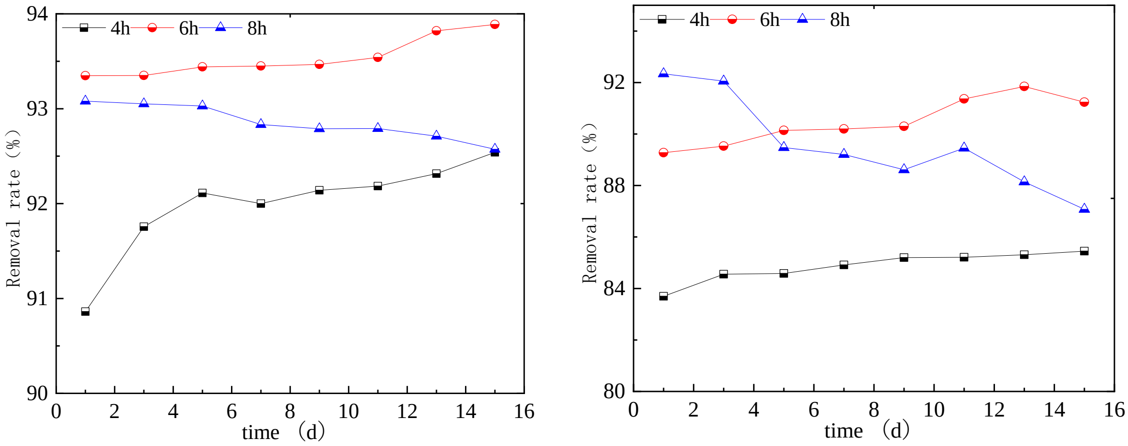

Figure 4. *The removal effect of COD and TP under different HRT*

It can be seen from *Figure 5*, under different aeration conditions, the COD removal effect is significantly different. The COD removal rate is above 90%, and the COD concentration in the effluent is ≤ 40 mg/L, which can meet the "Urban Wastewater Treatment Plant Pollutant Discharge Standard" (GB18918-2002) First Class A standard (COD ≤ 50 mg/L), indicating that due to the increase of aeration time, the amount of carbon source in the wastewater consumed by nitrifying bacteria and phosphorus-accumulating bacteria in the system becomes larger, resulting in a higher COD removal rate. As can be seen from *Figure 5*, with the increase of aeration time, the removal rate of TP shows a trend of first increasing and then decreasing. When the aeration method is aerated for 3 h and stopped for 3 h and aerated for 4 h and stopped for 2 h, the removal rate is 91% and above. The average TP concentration in the effluent is about 0.47 mg/L, which can meet the first-class A standard (TP ≤ 0.5 mg/L) of the "Pollutant Discharge Standards for Urban Sewage Treatment Plants" (GB18918-2002), and when the aeration method is aeration for 5 h and stopping for 1 h, the TP removal rate is 88.5%, and the average TP effluent concentration is 0.55 mg/L, which cannot meet the first-class A emission standard (TP ≤ 0.5 mg/L), indicating that the aeration time is too long, which breaks the anaerobic conditions, resulting in the inability to release phosphate or release under anaerobic conditions Small, which in turn makes the amount of phosphorus absorbed under aerobic conditions small, and the TP content of the effluent is high, which cannot meet the first-class A discharge standard (Ji et al., 2010).

Study on biological characteristics of side flow SBR process

Using a comprehensive analysis of the hydrolytic and acidification conditions of sludge in the anaerobic fermentation tank, together with the parameter variations of the processing systems under various operations, a comprehensive examination of the activated sludge microstructure, as well as the dynamic changes in the composition and content of the secreted extracellular polymer (EPS), and the succession of microbial flora were carried out.

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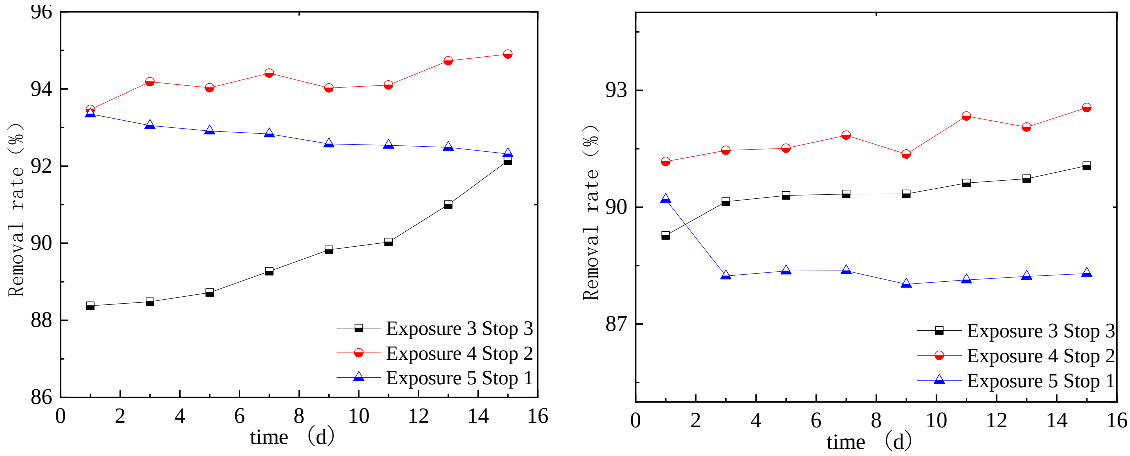

Figure 5. *Removal effect of COD and TP under different aeration modes*

Qualitative analysis of EPS

In the context of biological phosphorus removal, EPS is a complex high molecular weight polymer mixture consisting mainly of proteins, polysaccharides, humic-like substances, uric acid, nucleic acids, and lipids (Shi et al., 2017). The predominant constituents of EPS are proteins and polysaccharides, which can be further classified as combined EPS (coated polymer) and soluble EPS (also known as soluble microbial product SMP). In addition, combined EPS can be further divided into tightly combined EPS (TB-EPS) and dispersed loose EPS (LB-EPS) present on the outer layer (Li et al., 2019). As a significant bacterial species in the phosphorus removal process, phosphorus-accumulating bacteria release phosphorus in an anaerobic environment, with EPS acting as a phosphorus repository. Due to its physical properties (Cloete, 2001), EPS is capable of storing approximately 27% to 30% of the phosphorus content (Long et al., 2017), subsequently releasing it in an anaerobic environment, thus contributing to enhanced phosphorus removal efficiency (Yang et al., 2017). The effect of temperature on phosphorus removal efficiency of EPS has been reported (Li et al., 2010), who observed that at low temperatures, the phosphorus removal amount of EPS can account for about 13% of the total removal amount.

Because EPS contains certain fluorescent substances, these fluorescent substances can be detected by three-dimensional fluorescence spectroscopy. Therefore, EPS can be analyzed qualitatively and quantitatively. Gong et al. (2023) studied the use of different extraction methods to detect EPS characteristics in the sludge granulation process, and extracted EPS by ultrasonic heat extraction method, and used EEM to conduct qualitative and quantitative analysis of its main components. Luo et al. (2021) used EEM technology to study changes in EPS and SMP in MBR sludge under salinity effects.

An analytical approach using parallel factor analysis (PARAFAC) was employed to decipher EPS EEM data. The specific method was to use Matlab’s DOMFluor toolkit to evaluate EPS EEM data under different side flow ratios, HRT, and aeration modes by PARAFAC to determine the visible fluorescent components of EPS. As shown in *Figure 6*, three discernible fluorescent components were identified, specifically C1 (275 nm/340 nm), C2-A (240 nm/430 nm), C2-B (270 nm/430 nm), C2-C (350 nm/430 nm), and C3 (285 nm/350 nm). Among them, C1 is the tryptophan-like peak (Wu et al., 2001), C2 is the humic acid-like peak (Fu et al., 2004), C2-A is the

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fulvic acid-like peak, C2-B, C2-C are unidentified humic substances, and C3 is the protein-like peak (Jin et al., 2015). Since tryptophan is a type of amino acid, C1 and C3 can be classified as protein-like substances, while C2 is a humic acid-like substance.

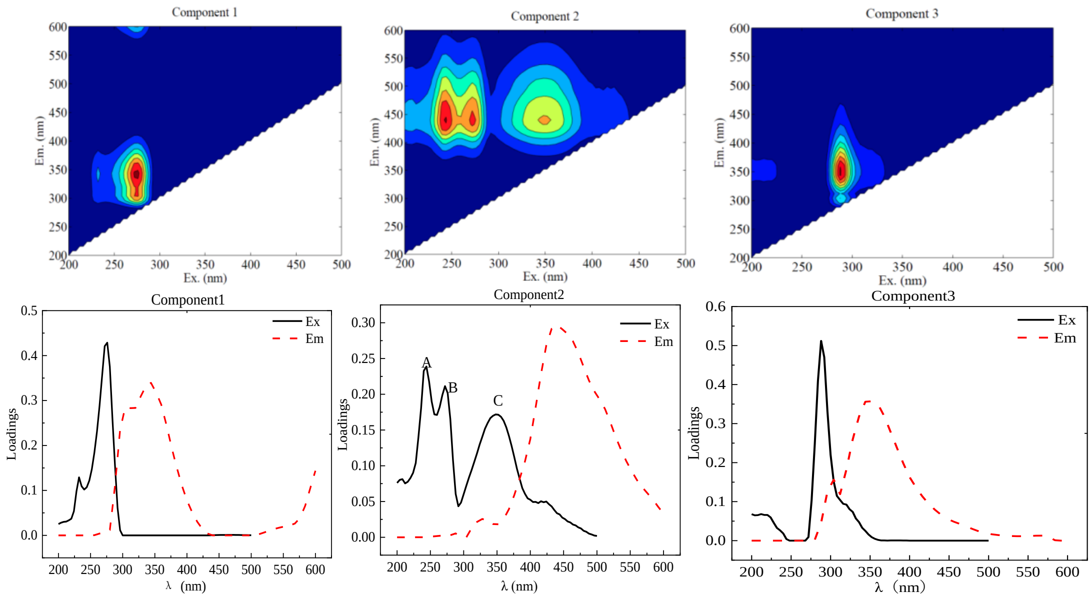

Figure 6. *The fluorescence components and their positions determined by EEMs-PARAFAC*

In summary, the nature of organic matter in wastewater is mainly humic acid-like substances and protein-like substances. DOM components typically present in municipal wastewater include humic acid, fulvic acid, various hydrophilic organic acids, carboxylic acids, amino acids, carbohydrates, etc. (Yuan et al., 2015) whereas the typical organic pollutants in domestic wastewater are protein-like substances (Le Moullec et al., 2008; Zhang et al., 2012; Le Moullec et al., 2010). The findings of this chapter are largely consistent with other research literature.

Effect of EPS fluorescence component distribution under different working conditions

It is evident that varying lateral flow ratios, hydraulic retention times, and aeration modes significantly affect activated sludge activity, thus indirectly affecting EPS component distribution. Therefore, it is essential to investigate the distribution of fluorescent components of EPS under different operating conditions to establish a better understanding of the relationship between the properties of activated sludge and the mechanisms involved in pollutant removal.

Effect of side flow ratio on the distribution of EPS fluorescence components

Since C1 and C3 are related to protein-like substances, C2 is related to humic acid substances, and the protein content in EPS is usually much larger than the humic acid content (Jin et al., 2015), it can be seen from *Figure 7* that the maximum fluorescence peak intensity of C2 is in the order of SMP > LB-EPS > TB-EPS, and the maximum peak fluorescence intensity of C2 in LB-EPS and TB-EPS is less than C3 < C1. The results are largely consistent with other studies. Because C1 and C3 are proteins, it can be seen from the figure that the maximum fluorescence peak intensity of C1 in SMP, LB-EPS, and TB-

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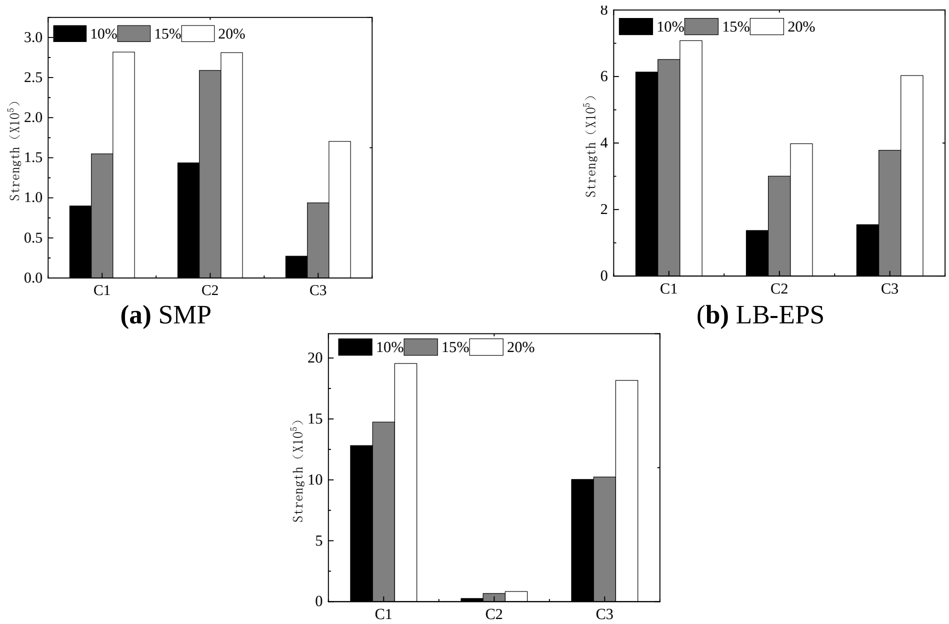
EPS is always greater than C3, and the current lateral flow ratio is 20%. The maximum fluorescence peak intensity of C1, C2, and C3 is always greater than the maximum fluorescence peak intensity under other operating conditions (side flow ratio is 10%, 15%). This is because the better the activated sludge grows in the anaerobic fermentation tank, the more abundant the microorganisms in the sludge, the more P content is released, and the better the denitrification and phosphorus removal effect is ultimately achieved. With the secretion of activated sludge, EPS content naturally increases, resulting in more fluorescent substances being dissolved by EPS, and the higher the peak of the maximum fluorescence peak exhibited (Lv, 2014; Gresch et al., 2011).



(a) SMP (**b)** LB-EPS

(c) TB-EPS

Figure 7. *The maximum peak fluorescence intensity of fluorescent components in EPS with different by-flow ratios*

Effect of HRT on the distribution of fluorescence components in EPS

From *Figure 8*, it can be observed that the maximum fluorescence peak intensity of C2 in SMP, LB-EPS, and TB-EPS showed a first increasing and subsequently decreasing trend, and the maximum fluorescence peak intensity of C2 was in the TB-EPS < SMP < LB-EPS order. Similarly, the maximum fluorescence peak intensities of C1 and C3 in SMP, LB-EPS, and TB-EPS showed a similar trend. The maximum fluorescence peak intensities of C1 and C3 were arranged in the SMP < LB-EPS < TB-EPS order with the highest observed intensity for C1. When the HRT was 6 h, C1, C2, and C3 reached the maximum fluorescence peak intensity in SMP, LB-EPS, and TB-EPS, indicating that the activated sludge was in an optimal growth state and demonstrated the best nitrogen and phosphorus removal efficiency. The content of EPS secreted during this period was relatively high. Importantly, the amount of fluorescent substances that can be detected using EEM was the greatest during this period, with the highest peak value also. The lowest fluorescence peak intensity observed for SMP was attributed to its predominantly dissolved state, representing dissolved organic matter, and the SMP content decreased as organic pollutants were transported away. The

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centrally located fluorescence peak observed for LB-EPS was due to EPS's loose outer location, which resulted in reduced adhesion, whereas the highest fluorescence peak intensity for TB-EPS was due to its location within the inner layer of the cell wall, where it was tightly bound to the cell wall and was affected by slight bubble shear force and turbulence (Shen, 2010; Wang, 2014).

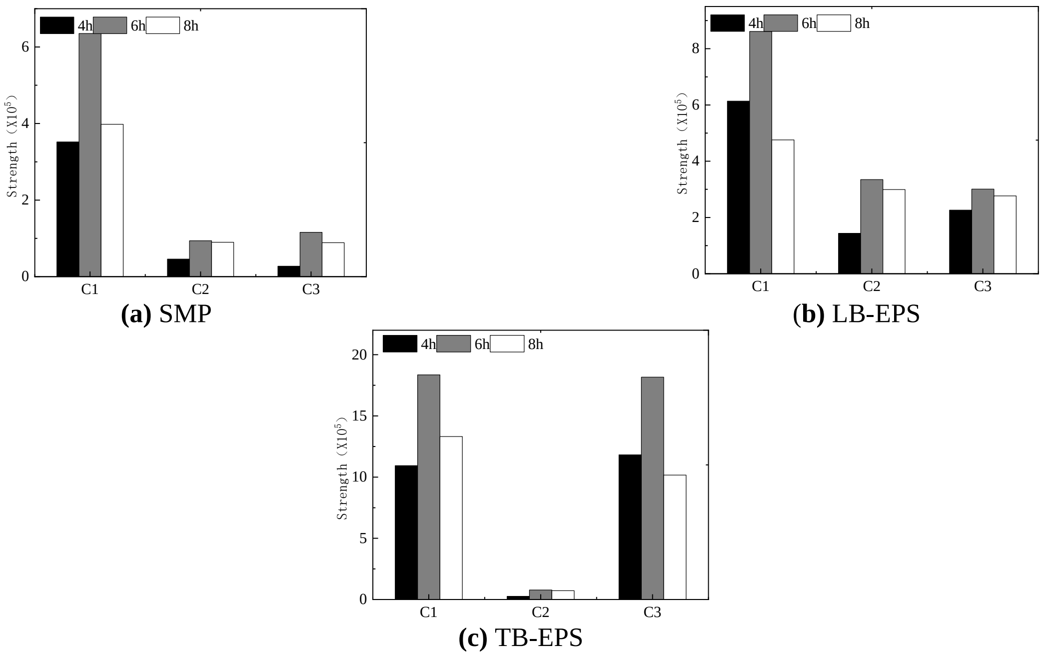

Figure 8. *The maximum peak fluorescence intensity of fluorescent components in EPS under different HRT*

Effect of aeration mode on EPS fluorescence component distribution

As shown in *Figure 9*, the maximum fluorescence peak intensities of C1, C2, and C3 in SMP, LB-EPS, and TB-EPS showed an initial increase, followed by a subsequent decrease, and the maximum fluorescence peak intensities of C1, C2, and C3 showed minimal fluctuations under various aeration methods. This observation suggests that activated sludge maintained high levels of activity and was minimally affected by the aeration method. The highest peak intensities of C1, C2, and C3 were observed when the aeration method was aerated for 4 h and then stopped for 2 h. This finding indicates that at this specific time point, activated sludge exhibited optimal growth, denitrification, and phosphorus removal efficiency, and the extracellular polymer content was at its highest, indicating maximum solute binding capacity.

Assessment of microbial community biodiversity

Four different working conditions of activated sludge were selected for sequencing analysis. The four working conditions were the original stage, the by-flow ratio was 20%, the HRT was 6 h, the aeration method is aeration for 4 h and stop for 2 h. The conditions were used to analyze the structural characteristics of microbial communities and community changes of Phosphorus-accumulating bacteria under different working conditions.

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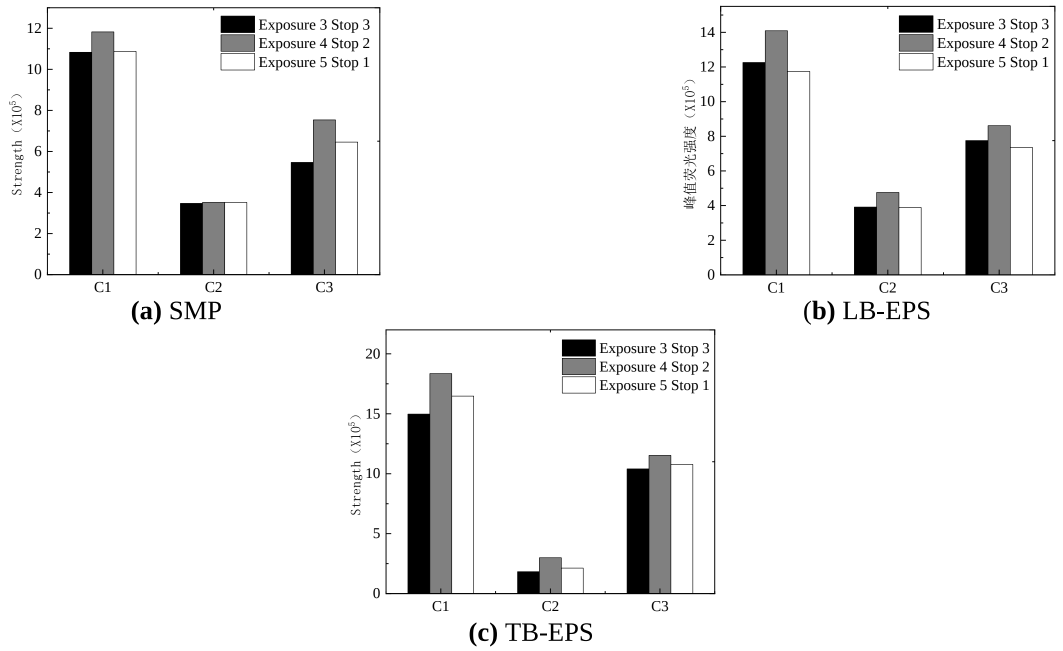

Figure 9. *The maximum peak fluorescence intensity of fluorescent components in EPS under different aeration modes*

Alpha diversity index analysis

The extent of diversity within a microbial community is typically assessed using the Alpha Diversity Index. This index includes four key metrics: Chao1, ACE, Shannon, and Simpson. In general, Chao1 and ACE indices are used to assess microbial community richness, with higher values indicating greater richness. Conversely, Shannon and Simpson indices are used to assess microbial community diversity, with larger values indicating higher diversity. As shown in *Table 3*, Sample No. 2 exhibits the highest diversity of microbial communities, indicating that the aeration method of aeration for 4 h and subsequent cessation for 2 h is optimal. Conversely, sample No. 1 exhibits the lowest microbial community diversity, indicating that the original sludge has relatively poor microbial diversity.

Table 3. *Sample diversity index table*

Number	Simpson index	Chaos1 index	ACE index	Shannon index
1	0.984071	1594.14	1600.02	8.34
2	0.973601	1842.49	1910.64	8.37
3	0.976893	1618.00	1618.00	8.35
4	0.980837	1745.00	1745.00	8.35

Biodiversity of the microbiome

The composition and structure of the microbial flora of each sample were further delineated by analyzing the structure of the microbial community at the phylum, class, order, family, and genus levels. A total of 32 phyla, 86 classes, 208 orders, 315 families, and 532 genera of microorganisms were identified within the microbial community.

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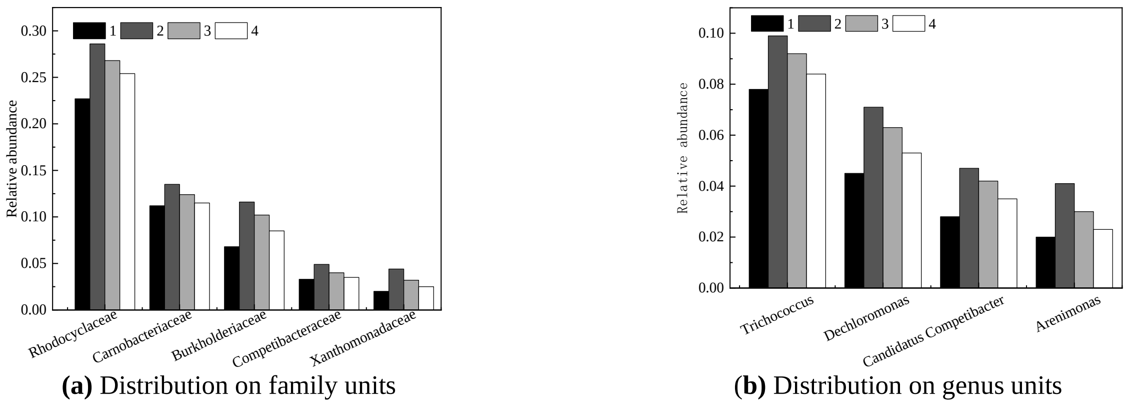
The results show that at the phylum level, *Proteobacteria* exhibit a substantial distribution across all samples, ranging from 47.1% to 57.5%. The fluctuations within each sample are minimal, indicating that the abundance of *Proteobacteria* within each sample is relatively consistent at the sampling stage. *Proteobacteria* are also classified as a dominant phosphorus-accumulating bacterial group. *Firmicutes* followed, accounting for about 11.1% to 15.2%. *Chloroflexi* and *Bacteroidetes* each contribute about 11.5% to 13% and 7.5% to 12.8%, respectively. *Patescibacterium* contributes about 3% to 4% of total abundance. *Actinobacteria* and *Acidobacteria* contribute between 2.4% to 3.5% and 1% to 2% of total abundance, respectively (Zhao, 2022).

The findings indicate that, at the taxonomic level of class, *Gammaproteobacteria* are the prevalent phosphorus-accumulating bacteria, present in high abundance in all analyzed samples (ranging from 42% to 50%), followed by *Anaerolineae* (ranging from 11.1% to 12.5%), *Bacilli* (ranging from 9.8% to 13.0%), *Bacteroidia* (ranging from 6.5% to 11.2%), *Alphaproteobacteria* (ranging from 2.4% to 6%), *Saccharimonadia* (ranging from 1.9% to 3.1%), and *Deltaproteobacteria* (ranging from 0.5% to 2.2%).

The findings suggest that at the order level, *Betaproteobacteriales*, as typical phosphorus-accumulating microorganisms, are prominently present within each examined sample, accounting for a distribution range of approximately 31.2%-37.6%. Subsequently, *Lactobacillales*, representing about 9.6% to 13%, are also present. *Competibacterales*, comprising about 2.8% to 4.8%, and *Xanthomonadales*, comprising about 2.6% to 6.3%, are also observed. Finally, *Flavobacteriales*, comprising approximately 2.6% to 5.1%, are also identified.

From the results of the distribution of flora in *Figure 10a*, it can be seen that *RHodocyclaceae* accounts for about 21.9%-28.8%, *Carnobacteriaceae* accounts for about 7.3%-11.6%, *Burkholderiaceae* accounts for about 5.9%-10%, *Competibacteraceae* accounts for about 2.9%-4.8%, and *Xanthomonadaceae* accounts for about 2.3%-6.1%.

From the distribution results of the flora in *Figure 10b*, it can be seen that *Trichococcus* accounts for about 6.8%-9.9%, *Dechloromonas* for about 4.2%-6%, *Candidatus Competibacter* for about 2.9%-4.8%, and *Arenimonas* for about 2.1%-6.1%.



(a) Distribution on family units

(**b)** Distribution on genus units

Figure 10. *Distribution of microbial communities in each sample*

In summary, *Proteobacteria* in activated sludge belong to the representative phosphorus-accumulating bacteria, and also belong to the dominant bacteria group, accounting for up to 52.3%. *Gammaproteobacteria*, *Betaproteobacteria*, and

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RHodocyclaceae also belong to phosphorus-accumulating microorganisms. Under the condition of aeration for 4 h and stopping for 2 h, the number of phosphorus-accumulating microorganisms reached the maximum, indicating that this condition is most suitable for the growth and enrichment of phosphorus-accumulating microorganisms, and the phosphorus removal effect is the best.

Conclusion

In this paper, by exploring the hydrolysis and acidification of sludge in anaerobic fermentation tanks under different working conditions, the removal effect of pollutants under different working conditions, and the biological characteristics of activated sludge, the following results were obtained:

Under the condition that the lateral flow ratio is 20%, HRT is 6 h, and the aeration mode is aeration for 4 h and stops for 2 h, the acid production effect of the sludge is good, there is organic carbon accumulation, and the removal effect of COD and TP is good.

The main components of EPS are C1 as tryptophan, C2 as humus, and C3 as protein. The order of maximum peak fluorescence intensity of C1 and C3 is TB-EPS > LB-EPS > SMP, and the order of maximum peak fluorescence intensity of C2 is TB-EPS < LB-EPS < SMP.

Proteobacteria in activated sludge belong to the representative phosphorus-accumulating bacteria, and also belong to the dominant bacteria group, accounting for up to 52.3%. *Gammaproteobacteria*, *Betaproteobacteria*, and *RHodocyclaceae* also belong to phosphorus-accumulating microorganisms. Under the condition of aeration for 4 h and stopping for 2 h, the number of phosphorus-accumulating microorganisms reached the maximum, indicating that this condition is most suitable for the growth and enrichment of phosphorus-accumulating microorganisms, and the phosphorus removal effect is the best.

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