The effects of main anoxic section oxidation-reduction potential on the metabolism of PHA and TP in continuous-flow single-sludge treatment system

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Abstract−The experimental results and material balance analysis in this paper revealed the regularity of poly-hydroxy alkanoates (PHA) and total phosphorus (TP) metabolism in a continuous-flow single-sludge wastewater treatment system under different main anoxic section oxidation-reduction potential (ORP_{an}) conditions. We also evaluated the effectiveness of the operation control parameters of ORP_{an} as the continuous-flow single-sludge sewage treatment system from the aspect of the reaction mechanism. Using a programmable logic controller (PLC) automatic control system to take the circulating flow in nitrification as the controlled variable based on the feedback control structure, an experimental study was carried out under the condition of ORP_{an} setting value of −143 mV, −123 mV, −105 mV, −95 mV, −72 mV and −57 mV, respectively, with other operational design parameters remaining unchanged. Influent water quality of chemical oxygen demand/total nitrogen (COD/TN) was 5.0±0.6. The results showed that when ORP_{an} was set at −95 mV, the maximum values of PHA synthesis and storage rate, PHA degradation rate, phosphorus release rate and phosphorus absorption rate in anaerobic and pre-anoxic segments were 82.34, 7.90, 47.31, 14.27, 1.50 and 8.52 mg/(L·h), respectively. According to the metabolic mechanism of PHA and TP, ORP_{an} was further proved to be the operation control parameter of the continuous-flow single-sludge sewage treatment system, and when the COD/TN value was 5.0±0.6, the optimal setting value was −95 mV.

Keywords: Continuous-flow, Main Anoxic Section ORP, PHA, Phosphorus, Material Balance

INTRODUCTION

With the improvement of living standards, the discharge of urban sewage is increasing, sewage water quality has changed, and organic pollutant content is gradually being reduced. Nitrogen, phosphorus, and other substances are gradually increasing, that is, the carbon and nitrogen ratio, as well as carbon and phosphorus ratio of current urban sewage, have seen significant declination [1,2]. It is well known that biological nitrogen and phosphorus removal processes in sewage are complex because of the various biochemical processes involved, such as phosphorus release, phosphorus uptake, nitrification and denitrification. These processes are completed by phosphorus-accumulating bacteria, nitrifying bacteria, denitrifying bacteria, respectively, but the growth environment of each strain is very different, which results in the contradiction between nitrogen removal and phosphorus removal in continuous-flow single-sludge sewage treatment system [3,4]. Numerous studies and practical operation results prove that, the contradiction can be alleviated to a certain extent with the existence of denitrifying phosphorus-accumulating bacteria and the occurrence of its metabolic process in activated sludge of sewage treatment system, which is often the key to guaranteeing the effect of nitrogen and phosphorus removal in a continuous-flow single-sludge sewage treatment system [5-12].

The electron acceptor of denitrifying phosphate-accumulating bacteria is nitrate nitrogen, and its metabolic characteristics are basically the same as that of ordinary phosphorus-accumulating bacteria [13-15]. However, some studies have demonstrated that in the continuous-flow single-sludge sewage treatment system, the performance of denitrifying phosphorus uptake and its contribution to the system of nitrogen and phosphorus removal are not stable, so even the existence of denitrifying phosphorus-accumulating bacteria cannot guarantee the nitrogen and phosphorus meet the standards in sewage treatment plant with low carbon-nitrogen ratio. This may be because there is no immediate response of operation parameters of sewage treatment plant to the greatly fluctuating water quality and water quantity, and the biochemical process is not in the best operation state [16]. Establishing a denitrification and phosphorus-absorbing process control system in a sewage treatment plant and developing control parameters and optimal control strategies can solve this problem to some extent.

According to the kinetic model of activated sludge reaction 2d, $(K_{O_2}/K_{O_2}+S_{O_2})·(S_{NO_3}/K_{NO_3}+S_{NO_3})$ is the switching function of denitrification rate and phosphorus uptake rate equation in the anoxic section. In the formula, S_{O_2} is the dissolved oxygen concentration (DO) in main anoxic section, while S_{NO_3} is the nitrate-nitrogen concentration in main anoxic section. K_{O_2} is the DO concentration semi-

saturation constant (IWA expert group recommends this value to be 0.2 mg·L^{-1}); and K$_{NO_3}$ is the nitrate nitrogen semi-saturation constant (IWA expert group recommends this value to be 0.5 mg·L^{-1}) [12]. According to the switch function, denitrification and phosphorus uptake rate of the main anoxic section are affected by the concentration of DO and nitrate nitrogen.

In the construction of sewage biological treatment process control system, it is very important to detect control parameters in real time, stably and accurately. Although the concentration of nitrate nitrogen in the main anoxic section can be used as the operating control parameter of the synchronous nitrogen and phosphorus removal system [13,14], it is difficult to maintain the on-line nitrate analyzer and the detection accuracy needs to be improved. Whether the DO concentration can be used as the control parameter of the anoxic section has not been reported so far, and the DO online analyzers have poor sensitivity and accuracy under low oxygen concentration conditions. Therefore, both nitrate online analyzer and DO online analyzer are not suitable as instruments for the control of anoxic section process. Some studies have shown that the concentration of DO and nitrate nitrogen in the main anoxic section could be represented indirectly by oxidation-reduction potential (ORP), which is closely related to the concentration of nitrate and DO [15]. ORP on-line detector has many advantages, such as on-line detection, fast response, high control accuracy and easy access to computers. It has been widely used in the batch activated sludge process, especially in the batch process test materials and methods [16-19]. In the environment field, many countries and regions in the world have used ORP as one of the important indicators for disinfecting swimming pool water, drinking water and hot spring water. ORP has been widely applied in industrial process control [20]. In wastewater treatment, after many years of theoretical research and practical application, ORP has gradually become a recognized economic and effective process control parameter, especially in low oxidizing reaction systems [21]. In the process of simultaneous nitrogen and phosphorus removal in wastewater, Ruano et al. replaced the traditional online ammonia nitrogen sensor with pH, DO, ORP and other indicators, established a control system, and applied it in the factory comprehensively. It practically proved that the system was effective. The control strategy and index investment were at a low operating cost with easier maintenance [22]. Peng et al. in the anoxic/aerobic activated sludge process with ORP as the denitrification reaction fuzzy control parameter

achieved optimal control of the denitrification process. In that, when the internal circulation return flow was controlled separately, the end ORP value of the denitrification zone was (−86±2) mV; the ORP value of the externally controlled external carbon source was (−90±2) mV [23]. Kim et al. established ORP and pH as the control strategy for online control parameters of the phosphorus removal process in SBR phosphorus removal process. ORP curve information provided a theoretical basis for real-time control of anaerobic phosphorus release process [24]. However, up to now, there are few reports on the use of ORP to control the process of denitrifying phosphorus uptake. In the previous research work, we studied the feasibility of the main anoxic section ORP (ORP$_{an}$) as the control parameter of the continuous-flow single-sludge sewage treatment system. It has been determined that the regulation of this parameter can change the concentration of TP and TN in the effluent of the system, and the establishment of a process control system with this control variable can promote and stabilize the denitrification and phosphorus absorption performance of the system [17]. Based on the analysis of material balance, this paper probes the metabolic regularity of PHA and TP in the continuous-flow single-sludge sewage treatment system operating under different ORP$_{an}$ conditions, aiming at evaluating the effectiveness of ORP$_{an}$ as the operating control parameter of a continuous-flow single-sludge sewage treatment system.

TEST MATERIALS AND METHODS

1. Characteristics of Sewage Water Quality

The experiment took municipal sewage as the object of investigation, and the composition and concentration of pollutants were determined with reference to the water intake in the biochemical tank of the Changchun municipal sewage treatment system. Pure milk powder and brewery wastewater were used as organic carbon source, which is commonly used as carbon sources in artificially configured sewage [1,2,18,19,25,26]. Using a mixture of them as a carbon source, the organic matter composition, organic nitrogen and biochemical oxygen demand BOD$_5$/COD values were more similar to the sewage treatment plant biochemical tank water (by hydrolysis acidification tank pre-treatment). Ammonium chloride was used as nitrogen source, potassium di-hydrogen phosphate as the source of phosphorus, and sodium bicarbonate for the acidity released by the nitrification reaction, the dosing quantity was auto-

Table 1. Experimental simulation of municipal sewage reagents and water quality characteristics

Formulation	Dosage/ g·L^{-1}	Micronutrient composition	Fluid concentration/ g·L^{-1}	Water quality index	Concentration/ mg·L^{-1}
Brewery wastewater	0.5 mL·L^{-1}	Ferric chloride	0.9	COD$_{cr}$	290±10
Pure milk powder	0.5	Boric acid	0.15	BOD$_5$	172±8
Ammonium chloride	0.05	Cobalt chloride heptahydrate	0.15	Ammonia nitrogen	53.9±4.10
Potassium dihydrogen phosphate	0.031	Copper sulfate pentahydrate	0.03	Nitrate Nitrogen	<1
Calcium chloride	0.01	Potassium iodide	0.18	Total nitrogen	58.13±3.79
Magnesium sulfate	0.05	Manganese tetrahydrate	0.06	Total phosphorus	7.14±0.51
Micronutrient solution	0.6 mL·L^{-1}	Sodium molybdate dihydrate	0.06		
		Zinc sulfate heptahydrate	0.12		

matically regulated by a PLC control system, and magnesium sulphate and calcium chloride were added in order to satisfy the demand of phosphorus metabolism of phosphate-accumulating bacteria for magnesium ion and calcium ion. To meet the nutritional needs of microbial growth and reproduction of activated sludge, trace element solution was added. The concentration of each agent and the characteristics of sewage water quality are shown in Table 1.

The inoculated sludge was taken from the Changchun Beijiao Wastewater Treatment Plant. The plant was built on a modified anaerobic-anoxic-oxic (A^2/O) process and was put into operation in 2006. The inoculated sludge was placed in a continuous-flow single-sludge sewage treatment reactor to start the system. After 20 days, the mixed liquor suspended solids (MLSS) in the system
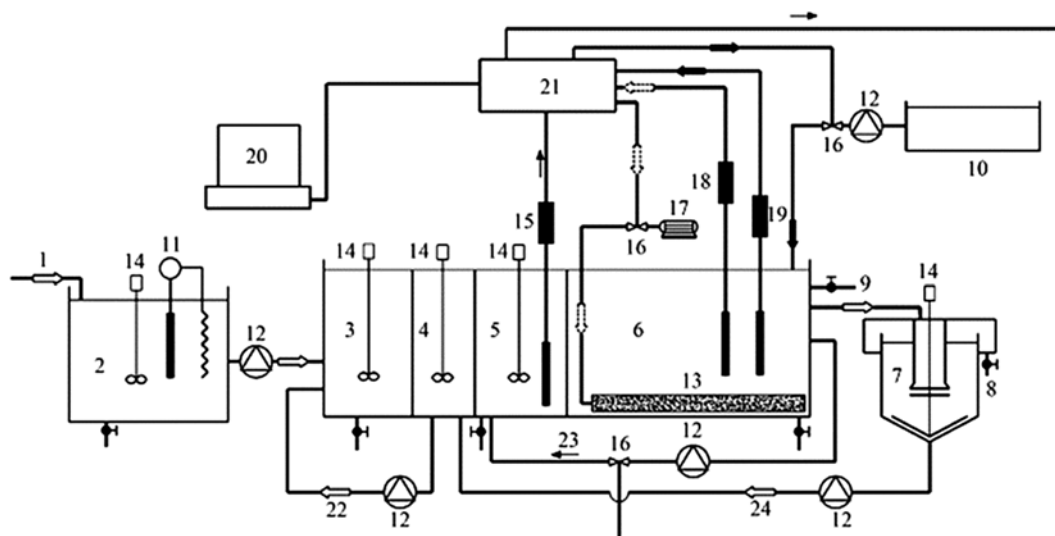

Fig. 1. Schematic diagram of test system flow.

1. Simulated municipal sewage	9. Excess sludge	17. Air compressors
2. Sewage tank	10. NaHCO₃ solution tank	18. DO online analyzer
3. Anaerobic section	11. Automatic temperature control device	19. pH online tester
4. Pre-anoxic section	12. Peristaltic pump	20. Computer
5. Main anoxic section	13. Air diffusers	21. Programmable logic controllers
6. Aerobic section	14. Electric magnetic stirrer	22. Anoxic/anaerobic mixture circulation
7. Clarifier	15. ORP online analyzer	23. Aerobic/anoxic nitrification solution circulation
8. Effluent	16. Electric regulating valve	24. Sludge reflux

Table 2. Water quantity, design operation parameters and reactor volume

Water quantity and design parameters of sewage treatment			
Treatment volume (L·d⁻¹)	240 L·d⁻¹	Absence/abuse mixture circulation ratio	1
The retention time of sewage tank	24 h	Anaerobic DO concentration	0.08-0.09 mg·L⁻¹
COD sludge load	0.253 kg·(kg·d)⁻¹	Pre-anoxic DO concentration	0.08-0.09 mg·L⁻¹
Aerobic section TN load	0.049 kg·(kg·d)⁻¹	Main anoxic DO concentration	0.08-0.09 mg·L⁻¹
Anaerobic section TP load	0.006 kg·(kg·d)⁻¹	Aerobic DO concentration	2 mg·L⁻¹
Total hydraulic retention time	9 h	Reaction temperature	20-25 °C
HRT ratio in each section	2:1:2:5	pH value of aerobic section	7
Sludge age	12 d	Clarifier settling time	3.6 h
Sludge reflux ratio	0.5	Clarifier surface load	0.5 m³·(m²·h)⁻¹

Reactor volume			
Sewage tank	280 L, L×B×H=80 cm×70 cm×50 cm, ultrahigh 5 cm	Main anoxic section	20.25 L, L×B×H=30 cm×15 cm×45 cm, ultrahigh 5 cm
Anaerobic section	20.25 L, L×B×H=30 cm×15 cm×45 cm, ultrahigh 5 cm	Aerobic section	50.625 L, L×B×H=75 cm×15 cm×40 cm, ultrahigh 5 cm
Pre-anoxic section	10.125 L, L×B×H=150 cm×15 cm×45 cm, ultrahigh 5 cm	Clarifier diameter	50 cm

basically remained unchanged, the system denitrification and phosphorus removal were good and stable. This indicated that activated sludge had been cultured and matured, sampling and tracking of the experiment study could be carried out.

2. Continuous-flow-single Sludge Sewage Treatment System and its Main Operating Parameters

The continuous-flow single-sludge sewage treatment test system is shown in Fig. 1. The reactor was composed of the anaerobic section, pre-anoxic section, main anoxic section and aerobic section. Agitators were installed in the sewage tank, anaerobic section, pre-anoxic section, and main anoxic section to ensure uniform mixing of sewage or mixed liquor. An air diffuser was installed in the aerobic section to provide both dissolved oxygen and activated sludge in suspension. The reactor was designed as a double corridor, and each corridor was divided into five compartments by a partition, thus a total of ten compartments. The reactor mixture flowed into the clarifier for solid-liquid separation. The amount of sewage treatment water, design and operation parameters, and reactor volume are shown in Table 2. Operating parameters such as hydraulic retention time, sludge load, and sludge age of the continuous-flow single-sludge sewage treatment system were in accordance with the German ATV-DVWK Code [27], the US Design of Municipal Wastewater Treatment Plants (Volume 2: Liquid Treatment Processes) [28] and China's Outdoor Drainage Design Code (GB50014) (2016 version) [29] specified range selection. The sludge discharge from the reactor was controlled continuously by hydraulic method [18], and the sludge discharge was 7.50 L/d. The sewage temperature was controlled by an automatic temperature control device installed in the sewage tank. The clarifier was provided with a scraper plate with a rotation speed of 5 rpm/h.

3. Experimental Scheme

To identify the intrinsic relationship between ORP_{an} and PHA and phosphorus metabolism in the continuous-flow single-sludge sewage treatment system, experimental research was carried out under different ORP_{an} set values. According to the research reports, the ORP in the anoxic section was closely related to the concentration of DO and nitrate nitrogen, and the DO and nitrate were circulated from the nitrification solution to the anoxic section. During the test, ORP_{an} was controlled by PLC automatic control system. The control system adopted a feedback control structure, with ORP_{an} as a control parameter, the circulation flow in nitrification liquid as a controlled variable, and the electric regulating valve as an actuator. The SAMA diagram and logical control diagram of the automatic control system are shown in Fig. 2. The control process was as follows: the ORP online measuring instrument installed at the end of the main anoxic section detects the ORP value (the output signal had an analogue signal of 4-20 mA), transmits it to the PLC data acquisition system. It then converts it into a digital signal by analog-digital converter (A/D converter), then compares it with an ORP_{an} setting value of proportional-integral-derivative controller (PID controller), calculates deviation, and carries out proportion, and scales. After the integral and differential calculation control, the result is given and converted into an analogue signal (4-20 mA) by the D/A converter. As an output
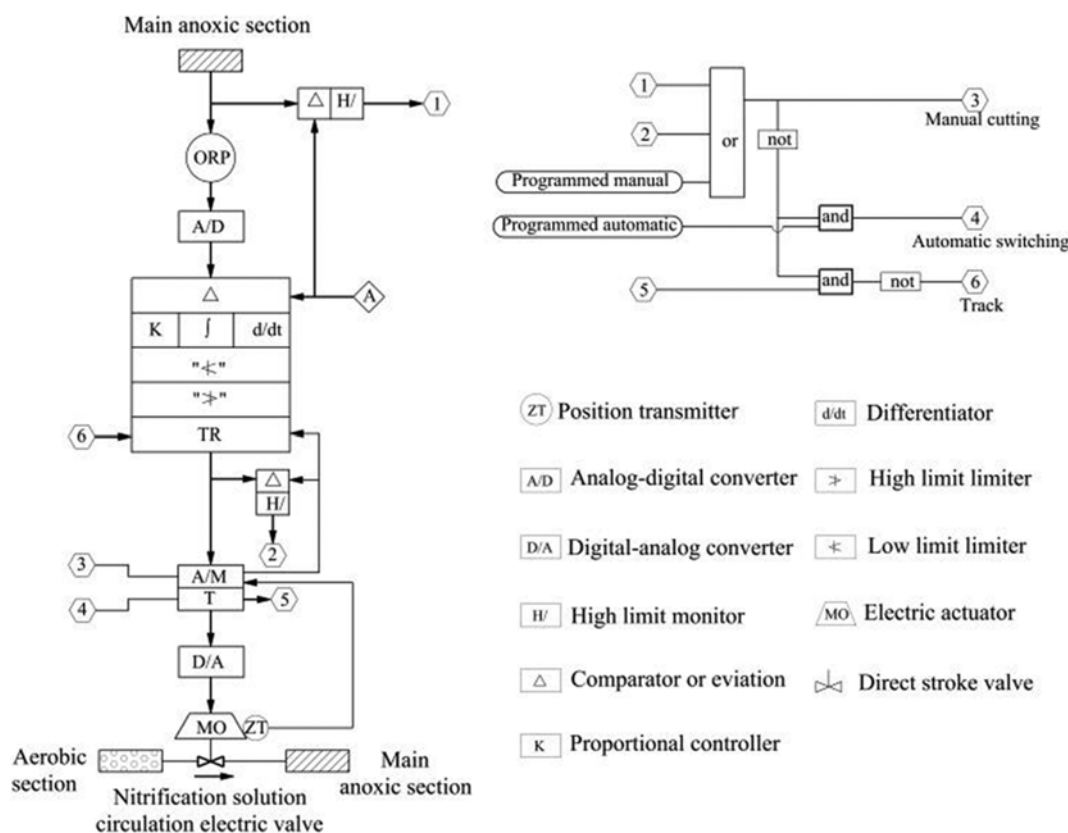

Fig. 2. SAMA diagram and logic control diagram of an automatic control system.

value, the internal electric control valve of the nitrifying liquid was adjusted to achieve the purpose of regulating ORP_{an}. The experiment was carried out in six stages, with ORP_{an} set values of −143 mV, −123 mV, −105 mV, −95 mV, −72 mV and −57 mV, respectively. During the test, except for the aerobic/anoxic nitrification liquid circulation ratio, other operating parameters remained unchanged. Two to three sludge retention times (SRT) were run at each stage, and the test was carried out for 200 days. Each test phase was operated under the set value conditions, and the error range of the ORP_{an} measured values was within 2 mV.

4. Test Index and Method

During the test, the indicators detected of sewage were COD, TP, TN, NH_4^+-N, NO_3^--N, etc. The national standard method was used for detection after the water sample was centrifuged [30].

The indexes of sludge were MLSS and PHA. MLSS was deter-

mined by the national standard method, and PHA was determined by improved gas chromatography [31].

The ORP analyzer of PLC automatic control system used WTW Sen Tix Ro, the pH value online analyzer was WTW ph296/170, and the DO online analyzer was WTW oxi170.

To reveal the process of PHA and P metabolism when ORP_{an} was used as operation control parameter, the anaerobic section, the pre-anoxic section, the main anoxic section, and the aerobic section were used as the system boundaries, respectively. Based on the principle of material balance, the rate of synthesis or degradation of each section was calculated by the concentration of COD, PHA, TP and NO_3^--N in each section of measurement. When the system was in stable operation, the amount of matter accumulated in each reaction section was 0, so the rate of reaction of the substance in each section can be calculated as follows:
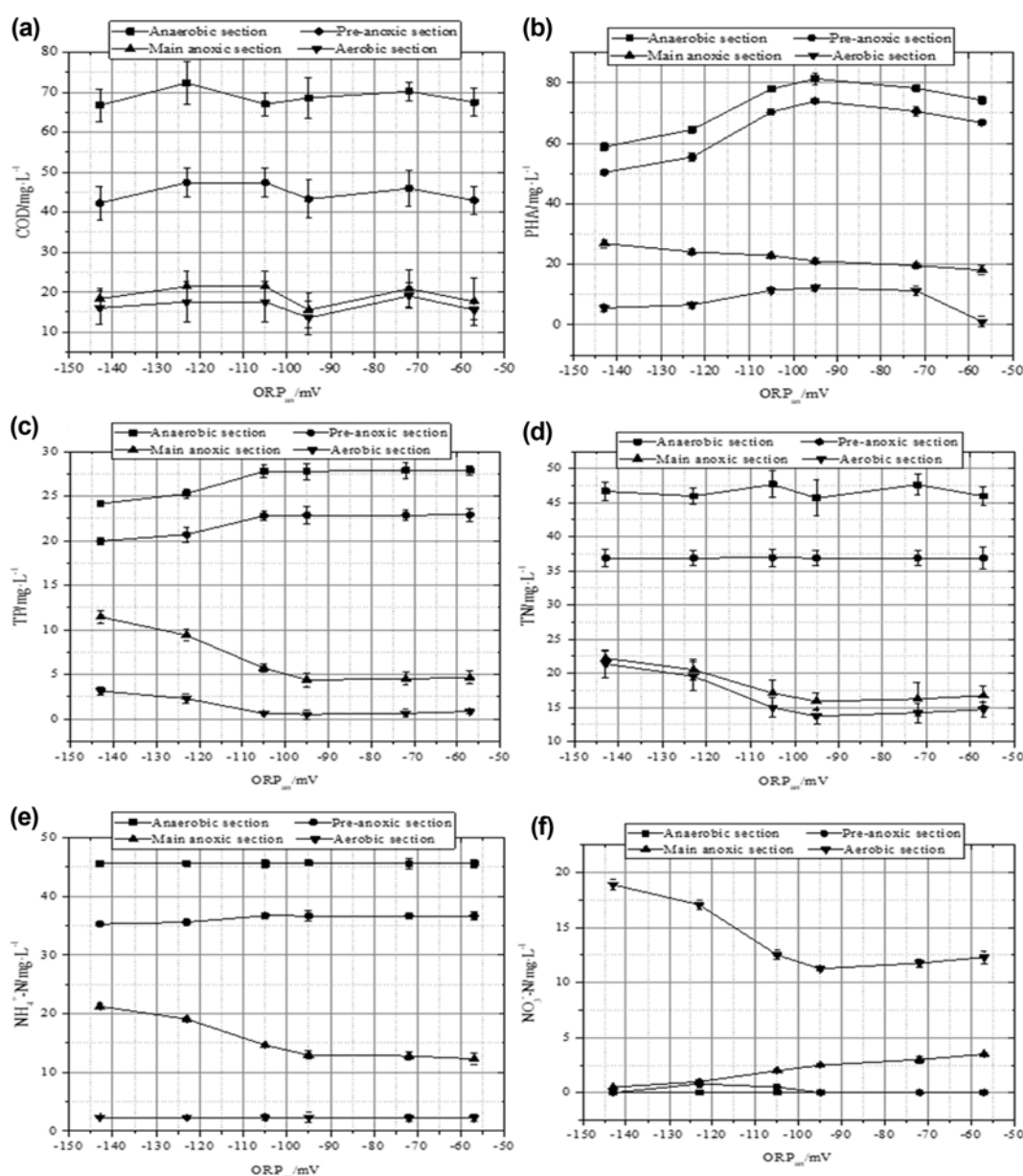

Fig. 3. COD, PHA, TN, NH_4^+-N, NO_3^--N, TP concentration in each reaction section.

Anaerobic section:

$$\left(\frac{dS}{dt}\right)_1 = \frac{(1+r)\cdot Q\cdot S_1 - Q\cdot S_0 - r\cdot Q\cdot S_2}{V_1} \tag{1}$$

Pre-anoxic section:

$$\left(\frac{dS}{dt}\right)_2 = \frac{(1+r+s)\cdot Q\cdot S_2 - s\cdot Q\cdot S_5 - (1+r)\cdot Q\cdot S_1}{V_2} \tag{2}$$

Main anoxic section:

$$\left(\frac{dS}{dt}\right)_3 = \frac{(1+s)\cdot Q\cdot S_2 + a\cdot Q\cdot S_4 - (1+a+s)\cdot Q\cdot S_3}{V_3} \tag{3}$$

Aerobic section: $\left(\dfrac{dS}{dt}\right)_4 = \dfrac{(1+a+s)\cdot Q\cdot(S_3 - S_4)}{V_4}$ (4)

The amount of material reaction in each reaction section can be further calculated as:

Anaerobic period:

$$\Delta S_1 = \left(\frac{dS}{dt}\right)_1 \cdot V_1 = (1+r)\cdot Q\cdot S_1 - Q\cdot S_0 - r\cdot Q\cdot S_2 \tag{5}$$

Pre-anoxic section:

$$\Delta S_2 = \left(\frac{dS}{dt}\right)_2 \cdot V_2 = (1+r+s)\cdot Q\cdot S_2 - s\cdot Q\cdot S_5 - (1+r)\cdot Q\cdot S_1 \tag{6}$$

Main anoxic section:

$$\Delta S_3 = \left(\frac{dS}{dt}\right)_3 \cdot V_3 = (1+s)\cdot Q\cdot S_2 + a\cdot Q\cdot S_4 - (1+a+s)\cdot Q\cdot S_3 \tag{7}$$

Aerobic period: $\Delta S_4 = \left(\dfrac{dS}{dt}\right)_4 \cdot V_4 = (1+a+s)\cdot Q\cdot(S_3 - S_4)$ (8)

In the formula, the subscripts 0, 1, 2, 3, 4, and 5 indicate the inlet, anaerobic, pre-anoxic, main anoxic, aerobic, and effluent concentration, respectively, mg/L; dS/dt indicates the rate of material reaction, mg/(L·h); ΔS indicates the amount of material reaction, g/h; Q represents the flow rate of inflow, L/h, V represents the effective volume of the reactor, L; S denotes the concentration of the substance, mg/L; s is sludge reflux ratio; a is aerobic/anoxic nitrification fluid internal circulation ratio; r is the cycle ratio of the anoxic/anaerobic mixture. After the water samples were centrifuged, the effluent quality of aerobic section was basically the same as that of the clarifier: $S_4 = S_5$.

TEST RESULTS AND DISCUSSION

1. Test Results

1-1. ORP$_{an}$ and System Performance

During the test period, the changes of COD, nitrogen, phosphorus and other pollutants in the reaction sections of the system and the PHA content in the sludge are shown in Fig. 3. It can be seen that, the variation in the ORP$_{an}$ set value had less effect on COD and ammonia nitrogen removal, the effluent COD concentration was in the range of 15-20 mg·L^{-1}, and the effluent ammonia nitrogen concentration was about 1.9-3.0 mg·L^{-1}. However, these changes had a rather great influence on TP and TN concentration, both of which were in ORP$_{an}$ set to −95 mV, so when the water COD/TN was low, −95 mV was the ORP$_{an}$ optimal setting value.

1-2. ORP$_{an}$ and PHA Metabolism

1-2-1. Synthesis of ORP$_{an}$ and PHA

Based on the material balance analysis, according to formulas (5)-(8), using the COD concentration measurement results for each section, the COD removal rates of the anaerobic section, the pre-anoxic section, the main anoxic section, and the aerobic section were calculated. When ORP$_{an}$ was controlled to −143 mV, the calculation procedure of COD removal for each section was as follows:

$$\Delta COD_1 = -((1+r)\cdot Q\cdot S_1 - Q\cdot S_0 - r\cdot Q\cdot S_2)$$
$$= -(2\cdot10\ L/h\cdot66.70\ mg/L - 10\ L/h\cdot300\ mg/L$$
$$-10\ L/h\cdot42.26\ mg/L = 2.09\ g/h$$

$$\Delta COD_2 = -((1+r+s)\cdot Q\cdot S_2 - s\cdot Q\cdot S_5 - (1+r)\cdot Q\cdot S_1)$$
$$= -(2.5\cdot10\ L/h\cdot42.26\ mg/L - 10\ L/h\cdot16.05\ mg/L$$
$$-2\cdot10\ L/h\cdot66.70\ mg/L = 0.36\ g/h$$

$$\Delta COD_3 = (1+s)\cdot Q\cdot S_2 - a\cdot Q\cdot S_4 - (1+a+s)\cdot Q\cdot S_3$$
$$= 1.5\cdot10\ L/h\cdot42.26\ mg/L + 1.17\cdot10\ L/h\cdot16.05\ mg/L$$
$$-2.67\cdot10\ L/h\cdot18.38\ mg/L = 0.33\ g/h$$

$$\Delta COD_4 = (1+a+s)\cdot Q\cdot(S_3 - S_4)$$
$$= 2.67\cdot10\ L/h\cdot(18.38 - 16.05)mg/L = 0.06\ g/h$$

Using the same method and procedure, the COD removal amount of each reaction section was calculated where the ORP$_{an}$ value was controlled at −123 mV, −105 mV, −95 mV, −72 mV and −57 mV, respectively. Meanwhile, the anaerobic section, as well as the pre-anoxic section COD removal rate, was calculated based on the result, as shown in Fig. 4. Due to the need to regulate ORP$_{an}$ in each test stage, the nitrification cycle ratio "a" was a variable, and the value was the average value of the output value of the automatic control system at each test stage, which was 1.17, 1.51, 2.34, 3.20, 3.43, 3.59. It can be seen from Fig. 4 that about 80% of COD was removed in the anaerobic section and the pre-anoxic section, and the ORP$_{an}$ set value variation had little effect on the COD removal rate of each section.

Taking the anaerobic section and the pre-anoxic section as the system boundary, according to the formulas (1) and (2), the material balance analysis was performed based on the average PHA con-
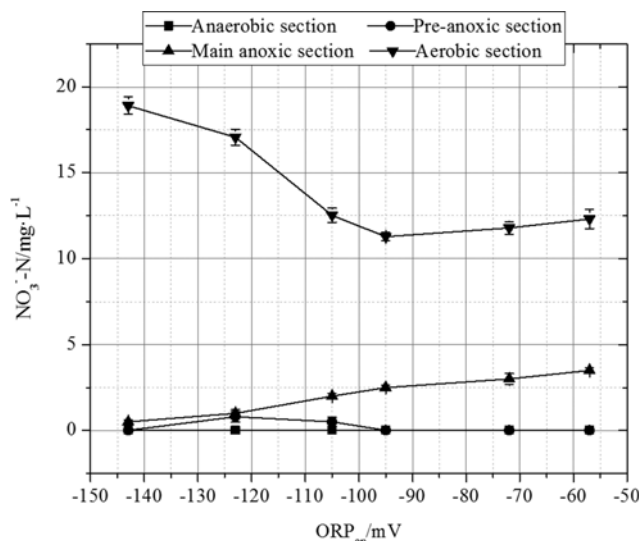

Fig. 4. COD removal rates in each reaction section.

tent determined. PHA rates stored in the anaerobic section and the pre-anoxic section were calculated (COD indicated that the COD stoichiometric constants of PHA were 1.67 gCOD/gPHA [31]). When the ORP_{an} was controlled at -143 mV, the PHA storage rate calculation process was as follows:

$$\left(\frac{dPHA}{dt}\right)_1 = \frac{(1+r)\cdot Q\cdot S_1 - Q\cdot S_0 - r\cdot Q\cdot S_2}{V_1}$$
$$= \frac{2\cdot 10 \text{ L/h}\cdot 58.87 \text{ mg/L} - 10 \text{ L/h}\cdot 50.36 \text{ mg/L}}{18 \text{ L}}$$
$$= 65.61 \text{ mg/(L}\cdot\text{h)}$$

$$\left(\frac{dPHA}{dt}\right)_2 = \frac{(1+r+s)\cdot Q\cdot S_2 - s\cdot Q\cdot S_5 - (1+r)\cdot Q\cdot S_1}{V_2}$$
$$= \frac{\begin{array}{c}2.5\cdot 10 \text{ L/h}\cdot 50.36 \text{ mg/L} - 0.5\cdot 10 \text{ L/h}\cdot 5.54 \text{ mg/L}\\ -2\cdot 10 \text{ L/h}\cdot 58.87 \text{ mg/L}\end{array}}{9 \text{ L}}$$
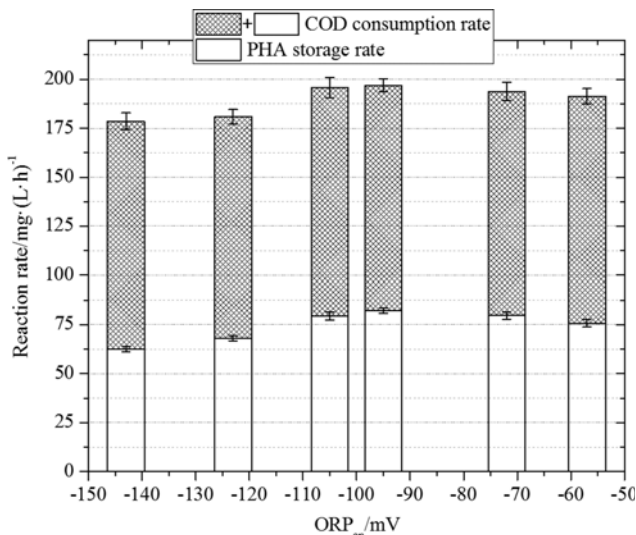
Fig. 5. Reaction rate of COD and PHA in the anaerobic section during the test.
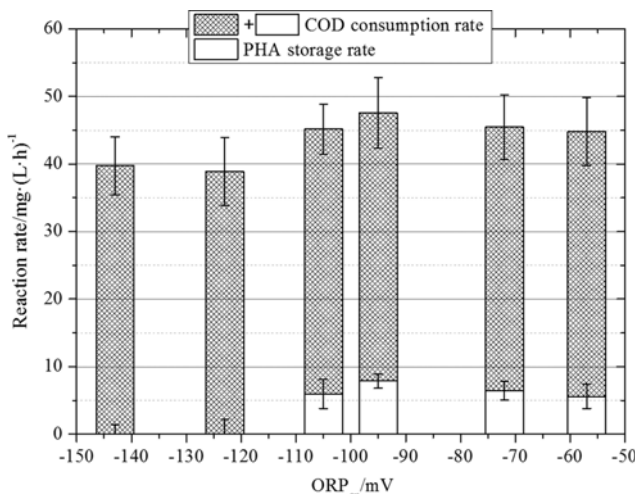
Fig. 6. The reaction rate of COD and PHA in the pre-anoxic section during the test.

$$= 0.22 \text{ mg/(L}\cdot\text{h)}$$

Under similar method and procedure, the PHA synthesis rate was determined at ORP_{an} controlled values of -123 mV, -105 mV, -95 mV, -72 mV, and -57 mV, respectively. The results are shown in Figs. 5 and 6. From Fig. 5, as ORP_{an} increased from -143 mV to -95 mV, while PHA synthesis and storage rate increased from 62.513 mg/(L·h) to 82.343 mg/(L·h). Thereafter, with the increase of ORP_{an}, the storage rate of PHA remained stable or decreased slightly, that is, ORP_{an} affected the storage rate of PHA in the anaerobic segment.

In addition, according to formula (5), the synthetic amount of PHA of the anaerobic segment was calculated, and the calculation process was the same as COD. Combined with the COD material balance calculation results, it was determined that 54%-71% of COD in the anaerobic section was synthesized as PHA stored in the polyphosphate bacteria; thus, the degradation of COD in this section was mainly based on the storage of PHA, and the storage proportion reached the highest value when ORP was at -95 mV.

Fig. 6 shows that when the ORP_{an} setting values were -143 and -123 mV, the PHA storage rate in the pre-anoxic section was 0. Subsequently, with the increase in ORP_{an} value, the storage rate of PHA increased. When the set value of ORP_{an} was -95 mV, the maximum value of PHA synthesis and storage reached 7.898 mg/ (L·h), and the storage amount accounted for 20% of the COD removal rate of the section.

1-2-2. ORP_{an} and PHA Degradation

Taking the main anoxic and aerobic sections as the system boundary, based on the determined PHA content, a material balance analysis was performed according to formulas (3) and (4). The calculation process when ORP_{an} was set at -143 mV was as follows;

$$\left(\frac{dPHA}{dt}\right)_3 = \frac{(1+s)\cdot Q\cdot S_2 + a\cdot Q\cdot S_4 - (1+a+s)\cdot Q\cdot S_3}{V_3}$$
$$= \frac{\begin{array}{c}1.5\cdot 10 \text{ L/h}\cdot 50.36 \text{ mg/L} + 1.17\cdot 10 \text{ L/h}\cdot 5.54 \text{ mg/L}\\ -2.67\cdot 10 \text{ L/h}\cdot 26.88 \text{ mg/L}\end{array}}{18 \text{ L}}$$
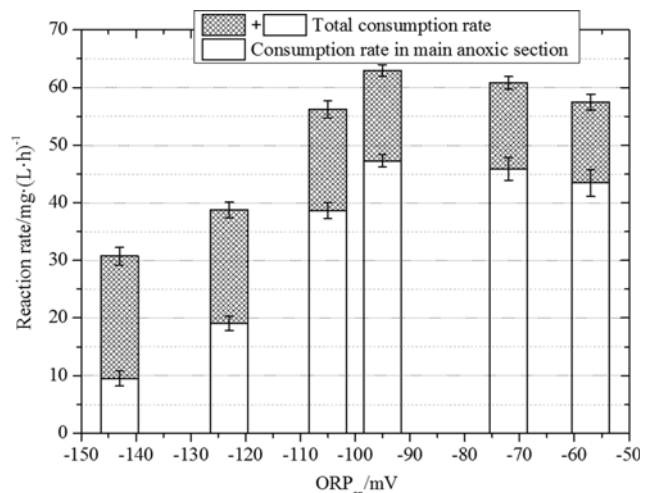$$= 9.50 \text{ mg/(L}\cdot\text{h)}$$

Fig. 7. PHA consumption rate during the test.

$$\left(\frac{dPHA}{dt}\right)_4 = \frac{(1+a+s)\cdot Q\cdot(S_3-S_4)}{V_4}$$

$$= \frac{2.67\cdot 10\ L/h\cdot(26.88-5.54)mg/L}{45\ L}$$

$$= 21.24\ mg/(L\cdot h)$$

The PHA consumption rate was calculated when the ORP_{an} settings were −123 mV, −105 mV, −95 mV, −72 mV, and −57 mV, respectively, following similar steps as previous. The results are shown in Fig. 7.

It can be seen from the diagram that PHA was consumed in the main anoxic and aerobic sections. When ORP_{an} increased from −143 mV to −95 mV, the consumption rate of the main anoxic section increased from 9.502 mg/(L·h) to 47.313 mg/(L·h), and subsequently, the value remained stable with the increase of ORP_{an}.

Additionally, using the material balance principle, with formulas (7) and (8), the PHA consumption of the main anoxic section and the PHA consumption of the aerobic section were calculated, and then the percentage of the main anoxic section consumption was also determined. The calculation process was the same as the amount of COD reaction. The results showed that when ORP_{an} increased from −143 mV to −95 mV, the value increased from 15% to 55%. Subsequently, with the increase of ORP_{an}, the percentage of consumption in the main anoxic section stabilized at about 55%.

1-3. ORP_{an} and TP Metabolism
1-3-1. ORP_{an} and TP Release

Based on the TP concentration determined in each section, a material balance analysis calculation was carried out according to the formulas (1)-(8) to determine the TP reaction amount and reaction rate of each section. The calculation process and steps were the same as used previously in COD reaction amount and the PHA reaction rate. The results are shown in Fig. 8. The figure indicates the mass fraction of total phosphorus uptake by the anoxic phosphorus uptake. According to the empirical molecular formula $C_{60}H_{87}N_{12}O_{23}P$ for microbial cell material in activated sludge, the phosphorus content of the cell material was 2.3% [32]. The assimilated phosphorus content was low as compared to the amount of

Fig. 8. Phosphorus reaction rate in each reaction section: Phosphorus uptake (+ve), and phosphorus release (−ve).

phosphorus released and absorbed, so it was ignored in the material balance analysis process. As can be seen from Fig. 8, the rate of phosphorus release in the anaerobic section increased gradually with the increase of ORP_{an} during the test period. When ORP_{an} increased from −143 mV to −95 mV, the rate of phosphorus released in anaerobic section increased from 11.878 mg/(L·h) to 14.272 mg/(L·h) After that, with the increase of ORP_{an}, the rate of phosphorus released from anaerobic section remained basically unchanged.

From the figure above, it can be deduced that when the ORP_{an} setting value was −143 and −123 mV, there was no phosphate released in the pre-anoxic section until the ORP_{an} set value was −105 mV, the rate of phosphorus release was 1.217 mg/(L·h). When ORP_{an} set value was controlled at −95 mV, the rate of phosphorus release reached a maximum of 1.500 mg/(L·h) in the pre-anoxic section. After that, with the increase of ORP_{an}, the rate of phosphorus released in the pre-anoxic section also remained stable.

1-3-2. ORP_{an} and TP Absorption

Fig. 8 shows that the phosphorus uptake rate in the main anoxic section increased with the increasing ORP_{an} setting value. When ORP_{an} increased from −143 mV to −53 mV, the phosphorus uptake rates in the main anoxic section were 1.682, 3.452, 6.921, 8.516, 8.274 and 7.822 mg/(L·h), respectively.

In addition, according to the material balance principle, the percentage of phosphorus uptake in the main anoxic section to the total phosphorus uptake was calculated. The results showed that the percentages were 12, 22, 37, 43, 44 and 42%, respectively.

2. Discussion
2-1. ORP_{an} and Metabolism of PHA
2-1-1. ORP_{an} and Synthesis of PHA

As can be seen from Fig. 4, the degradation of COD mainly occurred in the anaerobic and pre-anoxic sections, which was conducive to the subsequent denitrification phosphorus uptake and nitrification reaction and also reduced the oxygen supply in the aerobic section. The removal of COD in the anaerobic section was mainly due to the following possible causes: biological adsorption, absorption and synthesis of phosphorus-accumulating bacteria into PHA storage, dilution of circulating flow in mixed liquid, hydrolysis and acidification of anaerobic bacteria.

Fig. 5 shows that COD synthesis in the anaerobic section was the main reason for the reduction of COD concentration in PHA stored in the organism. But the storage ratio of PHA synthesis was influenced by ORP_{an} since ORP is a parameter that characterizes the environmental conditions of the sewage treatment system. From a microscopic point of view, the dynamic changes of the microbial community structure and dominant flora of activated sludge were closely related to the ORP of its living environment [33]. Therefore, there were differences in the dominant reaction processes performed under different ORP values. The relationship between the ORP value range and the reaction process is shown in Fig. 9 [34]. That is, certain dominant microorganisms in activated sludge have a suitable ORP range. Other studies have shown that regulating ORP values can alter the levels of nicotinamide adenine dinucleotide $NADH/NAD^+$ and nicotinamide adenine dinucleotide phosphate $NADPH/NADP^+$ in the metabolic processes of microorganisms, activate certain key enzymes, change the metabolic network of substances, and promote the assimilation and dissimilation reaction of
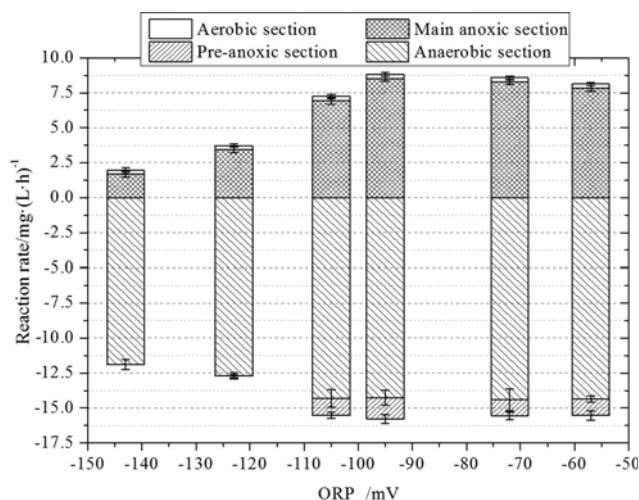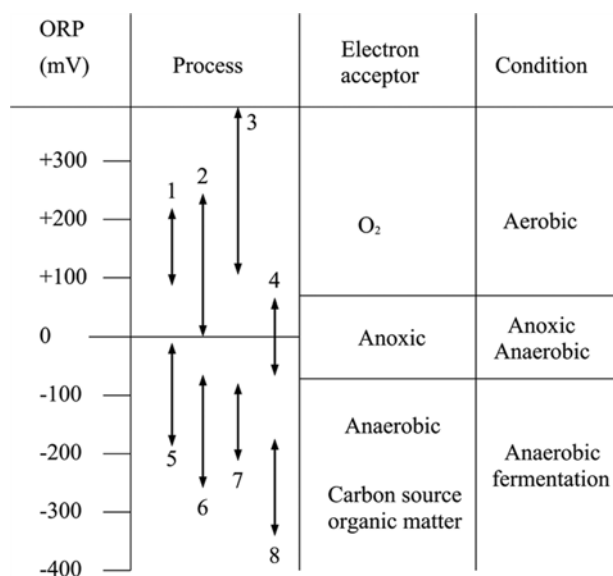
Fig. 9. Microbial metabolic process and ORP range.
1. Organic matter oxidation
2. Phosphorus accumulation
3. Nitrification
4. Denitrification
5. Phosphorus release
6. Hydrogen sulfide production
7. Acid production
8. Methane production

microbes along the target metabolic pathway [33]. Combined with the test results, when the ORP_{an} set value was adjusted to -95 mV, the nitrate-nitrogen reaction rate and phosphorus uptake rate were highest in the main anoxic section, indicating that the ORP_{an} was within the suitable ORP range of the denitrifying phosphorus accumulating bacteria. Under this condition, the nitrate reductase, PHA degrading enzymes, etc. have been maximally activated, with the lowest concentration of nitrate in the effluent.

From a macro perspective, ORP_{an} in the main anoxic section was closely related to DO concentration and nitrate concentration [15]. The DO concentration in the main anoxic section was within the range of 0.08-0.09 mg·L^{-1}, and the change was relatively small during the test. Therefore, the ORP_{an} value reflects the level of nitrate nitrogen to a certain extent; thus, the higher the nitrate concentration was, and the higher the ORP_{an} was, which can be seen from Fig. 5. According to the kinetic model of activated sludge reaction, denitrification and phosphorus uptake of continuous-flow single-sludge activated sludge system are affected by nitrate-nitrogen concentration which has been confirmed by Wang and Cardete et al. [16,17]. To maximize the potential of denitrification and phosphorus uptake by the main anoxic section, it was necessary to ensure that the section contains sufficient nitrate nitrogen, but the nitrate concentration should not be too low or too high, that is, there was the best value of nitrate nitrogen, and the optimal value of ORP_{an} was also present. If the concentration of nitrate in this section changed within a relatively low-value range greater than zero, that is, when the ORP_{an} was between -140 mV and -95 mV, the process of denitrifying phosphorus uptake in the main anoxic section became more and more sufficient as the ORP_{an} increased. However, when the nitrate-nitrogen concentration was high, that is, ORP_{an} was greater than -95 mV, the internal circulation flow of the nitrification solution required

to control this value increased. The results are as follows:

1. The amount of DO entering the main anoxic section increased and consumed more carbon source (even if the concentration of DO in the anoxic section did not change).

2. The actual hydraulic residence time of the mixed solution in the main anoxic section was shortened, which eventually led to the denitrification and phosphorus absorption not being fully realized.

In the continuous-flow single-sludge wastewater treatment system, whether the denitrifying phosphorus uptake reaction in the main anoxic section was adequate or not, it would directly affect the nitrate concentration in the effluent. When the ORP_{an} was low, the nitrate-nitrogen concentration in the effluent was higher, and the nitrate nitrogen content was higher with the reflux sludge entering the pre-anoxic section, even more than the denitrification potential of the section, which led to the nitrate nitrogen with the mixture circulating flow to the anaerobic section. It has been reported that the existence of nitrate nitrogen in the anaerobic section of a continuous-flow single-sludge sewage treatment system can lead to a contradiction between denitrifying bacteria and polyphosphate bacteria competing for COD [35]. In the process of competition, denitrifying bacteria predominated, to be exact, the feed COD was first absorbed and utilized by denitrifying bacteria, which reduced the amount of PHA absorbed and synthesized by polyphosphate bacteria. With reference to Fig. 10, it can be seen that as the ORP_{an} increased, the amount of denitrifying nitrate in the anaerobic section decreased as well. When ORP_{an} was set to -143 and -123 mV, the nitrate-nitrogen reaction rate in the anaerobic section was 0.444 and 0.278 mg/(L·h), respectively. When the set value was greater than or equal to -105 mV, the reaction rate of nitrate nitrogen in the anaerobic section was 0, indicating that no denitrification process occurred in this section. At this time, most of the degraded COD was absorbed and synthesized by polyphosphate bacteria and synthesized PHA. It makes the PHA synthesis rate up to the highest and keeps stable. The reaction process in the pre-anoxic section may include anaerobic metabolism of phosphorus accumulation bacteria, nitrate reduction of denitrifying bacteria, and so on; the removal of COD in the pre-anoxic section and the rate of PHA synthesis was more closely related to the reaction rate of nitrate nitrogen in this section. Nitrate nitrogen in the pre-anoxic section came from the reflux sludge. The reaction rate was directly related to the nitrate concentration in the effluent. That is, the nitrate-nitrogen reaction rate decreased as the ORP_{an} raised. When the ORP_{an} setting value was at -95 mV, the concentration of nitrate nitrogen in the effluent was the lowest, and the reaction rate of nitrate nitrogen in the pre-anoxic section was the lowest. The rate of PHA synthesis under this condition was the highest.

In conclusion, there must be suitable ORP_{an} in the continuous-flow single-sludge denitrification and phosphorus removal system to have the highest PHA synthetic storage rate; the value was -95 mV when the COD/TN value was 5.0±0.6.

2-1-2. OPR_{an} and Degradation of PHA

According to the material balance calculation results, when the ORP_{an} increased from -143 mV to -53 mV, the rate of nitrate nitrogen reaction in the main anoxic section was 12.231, 13.088, 13.400, 13.532, 13.402 and 12.815 mg/(L·h), respectively, and the removal rates of COD were 18.384, 18.431, 16.174, 19.619, 17.675 and 17.005

mg/(L·h). The calculation showed that the COD removal/nitrate nitrogen reaction amount was 1.50, 1.41, 1.21, 1.45, 1.32, 1.33, respectively, much less than 2.86. Based on the measurement method of COD concentration, the conversion indicated that the electronic equivalent was 8 g COD. When nitrate nitrogen is an electron acceptor, the oxidation state of nitrogen is reduced from the +5 valence to the 0 valences and the electron equivalent of 1 g of nitrate nitrogen is 2.86 g COD according to the electronic equivalent. Thus, theoretically, 1 g of nitrate nitrogen is needed to consume 2.86 g of COD, and the complexity of the sewage treatment system is generally considered to ensure the smooth progress of the denitrification reaction when the value of the sewage COD/NO_3^- is greater than 4. In this study, COD/NO_3^- was less than 1.5, which is still less than the theoretical value of 2.86. Therefore, COD in the main anoxic section was insufficient for the denitrification process. We can speculate that the reaction process of denitrification and absorption of phosphorus in the main anoxic section was the dominant reaction process, so the main carbon source of the denitrifying bacteria was PHA. Under the condition of hypoxia, phosphorus accumulation bacteria degraded the stored PHA and used the energy generated to complete the process of absorbing phosphorus from sewage. According to the kinetic model of activated sludge reaction, the consumption rate of PHA was related to the concentration of nitrate nitrogen and PHA in the main anoxic section. Correspondingly, the rate of PHA consumption was related to the rate of nitrate nitrogen reaction in the main anoxic section and the PHA storage rate in the anaerobic section (the higher the storage rate, the higher the content of the main anoxic section). As can be seen from Fig. 5, the PHA consumption rate was 9.502, 19.073, 38.665, 47.313, 45.892, and 43.457 mg/(L·h) when the ORP_{an} was increased from −143 mV to −53 mV. The degradation of PHA in the main anoxic section is beneficial to save the energy consumption of oxygen section aeration. This indicates that the −95 mV value was also in a suitable range of ORP range for denitrifying phosphorus accumulation bacteria. Under this condition, the activity of PHA degrading enzyme was activated to the maximum extent, the effect of denitrification was the best and the nitrate concentration in the effluent was the lowest.

2-2. ORP_{an} and TP Metabolism

2-2-1. ORP_{an} and TP Release

The results showed that the anaerobic phosphorus release reaction and the phosphorus release were mainly affected by the nitrate nitrogen carried by the reflux sludge in the system of nitrogen and phosphorus removal from the continuous-flow single-sludge wastewater [37-40]. In this system, the sludge was refluxed into the pre-anoxic section, where nitrate nitrogen in the reflux sludge was the first denitrification. If the denitrification process was completed, no nitrate entered the anaerobic section and the denitrifying bacteria would not compete with the carbon source of the polyphosphate bacteria. However, if the amount of nitrate was more than the denitrification potential of the pre-anoxic section, the remaining nitrate nitrogen would enter the anaerobic section and affect the anaerobic metabolism of the phosphate accumulating bacteria. When the ORP_{an} settings were greater than or equal to −105 mV, as shown in Fig. 10, the rate of nitrate nitrogen reaction in the anaerobic section was 0; consequently, there was no denitrifica-
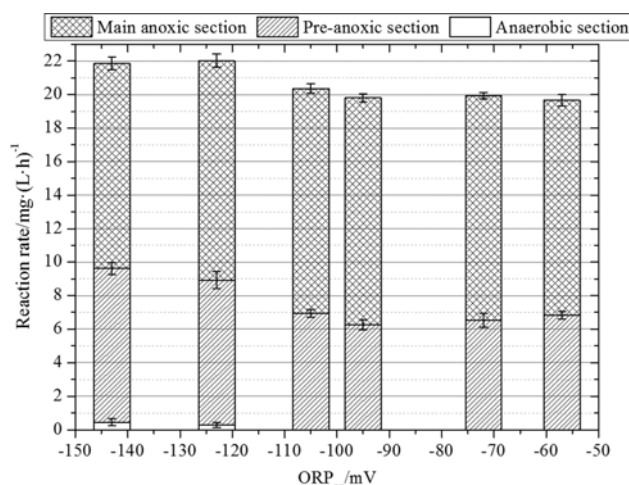

Fig. 10. Consumption rate of nitrate nitrogen in anaerobic, pre-anoxic and main anoxic sections during the test period.

tion process in the anaerobic section, so the release of phosphorus reached the maximum value and remained stable at this time.

According to the results of the material balance, when the ORP_{an} was low, the nitrate nitrogen of the effluent was higher than that in the pre-anoxic section due to the high concentration of nitrate nitrogen in the effluent, which exceeded the denitrification potential of the section. All of the COD in this section was used in the denitrification process, without the occurrence of phosphorus release reaction. With the increase of ORP_{an} value, the nitrate-nitrogen concentration in effluent decreased, and the nitrate nitrogen content entering the pre-anoxic section was lower than that in the denitrification potential. Therefore, when the nitrate nitrogen reaction was completed, the remaining COD was absorbed by the polyphosphate accumulating PHA and stored in the polyphosphate bacteria. The energy required for the synthesis process came from the hydrolysis of phosphorus. So, when the ORP_{an} value was high, phosphorus release occurred in the pre-anoxic section, but the amount of phosphorus released only accounted for about 4% of the total phosphorus released, which was much lower than the anaerobic section.

2-2-2. ORP_{an} and TP Absorption

According to the basic principle of biological phosphorus removal, the anaerobic phosphorus release process was the key to biological phosphorus removal system. The amount of phosphorus released directly affected phosphorus uptake, and there was a good correlation between the two. It can be seen from Fig. 8 that the variation trend of phosphorus uptake and phosphorus release was the same.

In the main anoxic section of the continuous-flow single-sludge sewage denitrification and phosphorus removal system, the amount of carbon source COD was low due to the low availability of external carbon source. As a result, the main carbon source of denitrifying bacteria was PHA. The denitrification of denitrifying polyphosphate bacteria was dominant; thus, while reducing nitrate nitrogen, a large amount of phosphorus was absorbed. Yin et al. pointed out that the main factor affecting the rate of denitrification and phosphorus uptake was the reaction rate of nitrate in the main anoxic section in the synchronous nitrogen and phosphorus removal activated sludge system [31]. According to the relationship between

ORP_{an} and nitrate nitrogen reaction rate in the main anoxic section, as shown in both Figs. 8 and 10, with an increase in ORP_{an} value, both nitrate-nitrogen reaction rate and phosphorus uptake rate increased in the main anoxic section, and both reached their peak values in the environment with ORP_{an} set at −95 mV. From the figures, it can also be seen that when the ORP_{an} was greater than −95 mV, the rate of phosphorus uptake was reduced. This was due to the need for greater internal circulation flow in the nitrifying liquid to maintain a higher ORP_{an}, which led to a decrease in the actual hydraulic retention time of the section. As the amount of DO entering the main anoxic section increased with the nitrification solution, the metabolic process of the denitrifying phosphate accumulating bacteria was affected, resulting in a decrease in the rate amount of anoxic phosphorus uptake and in the anoxic phosphorus uptake mass fraction.

CONCLUSIONS

1. ORP_{an} has a significant effect on the PHA synthesis and storage rate in the anaerobic section. When the influent COD/TN was 5.0±0.6, the PHA synthesis and storage rate was the fastest when the set value was −95 mV. The storage volume accounted for more than 70% of the COD removal volume in this section and reached the highest value. The PHA synthesis and storage rate in the pre-anoxic section was also mainly affected by ORP_{an}, and when the ORP_{an} setting value was −95 mV, the PHA synthesis and storage rate also reached the highest.

2. ORP_{an} had a great effect on the degradation rate of PHA in the main anoxic section. When ORP_{an} increased to −95 mV, the degradation rate of the main anoxic section reached the highest value.

3. The release amount of phosphorus in the anaerobic section changed with the increase of ORP_{an} value in that when the set value was −95 mV, the phosphorus release rate reached the highest value of 14.27 mg/(L·h). In addition, ORP_{an} had a significant effect on phosphorus release reaction and process in the pre-anoxic section. When the set value was −95 mV, the maximum rate of phosphorus release was 1.50 mg/(L·h) in the pre-anoxic section.

4. The phosphorus uptake effect of the main anoxic section in the continuous-flow single-sludge wastewater treatment system was affected by ORP_{an}. Under the condition that the set point was controlled at −95 mV, the rate of phosphorus uptake reached the maximum value of 8.52 mg/(L·h).

5. Based on the analysis of the metabolic mechanism of PHA and TP, the validity of ORP_{an} as a continuous-flow single-sludge sewage treatment system was confirmed, and the optimum setting value was −95 mV when the influent COD/TN value was 5±0.6.

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