In order to alleviate water pollution, nitrogen and phosphorus removal is required in the process of municipal sewage treatment, but most of the time, the ratio of carbon—nitrogen and carbon—phosphorus ratio in municipal sewage are gradually decreasing, thereby making the removal of nitrogen and phosphorus difficult.\(^{1,2}\) Generally, in the synchronous biological nitrogen and phosphorus removal system of sewage, phosphorus-accumulating bacteria, nitrifying bacteria, and denitrifying bacteria coexist to complete for the process of phosphorus release, phosphorus absorption, nitrification, denitrification, and other biochemical reactions. However, the growth environment of each strain is quite different, resulting in a variety of contradictory relations.\(^{3–10}\)

In recent years, autotrophic denitrification technology has gradually gained massive attention for its efficiency in the treatment of low carbon—nitrogen ratio sewage. However, because hydrogen production bacteria, sulfur bacteria, and iron bacteria are autotrophic bacteria, the growth and reproduction rate is relatively low, and they often need a large volume; thus, they have not been applied in urban sewage treatment plants.\(^{11,12}\) In the 1990s, researchers discovered that denitrifying phosphorus-accumulating bacteria exists in the anoxic stage of the sewage treatment plant. The stable metabolic process of the bacteria could alleviate the above contradiction to a certain extent.\(^{13–20}\)

The electron acceptor of denitrifying phosphorus-accumulating bacteria is nitrate—nitrogen, and its metabolic characteristics are the same as those of common phosphorus-accumulating bacteria.\(^{21–23}\) However, after nearly 20 years of development, the process of denitrifying phosphorus uptake has not completely solved the problem of poor effect of simultaneous nitrogen and phosphorus removal in low carbon/nitrogen (C/
N) ratio wastewater, mainly because the quality and the quantity of influent water fluctuate greatly and the operating parameters of the sewage plant cannot respond immediately, and the denitrifying phosphorus-accumulating bacteria cannot reach the optimum state. The above problems can be solved to a certain extent by constructing a denitrifying phosphorus absorption process control system and developing control parameters and optimum control strategy in the sewage treatment plant.24

In the construction of the control system, the real-time, stable, and accurate detection of control parameters are very important. Presently, the control parameters commonly used in sewage treatment plants include dissolved oxygen (DO), pH, and oxidation-reduction potential (ORP) values, among which DO is used to control the air supply of the blower in the aerobic stage, whereas the pH value is used to monitor the operation of the system, and ORP is used to monitor the REDOX environment in the nonaerated stage. The ORP online detector has the advantages of on-line detection, fast response, high control accuracy, and easy access to the computer.24−26 In the field of environmental engineering, many countries and regions in the world have taken ORP value as one of the important detection indicators for disinfection of swimming pool water, drinking water, and hot spring water, and some experts and scholars have applied ORP to industrial process control.29−31 Over 90% of wastewater treatment plants for nitrogen and phosphorus removal in China are equipped with ORP online measuring instruments in the nonaerated section, which is used to monitor the redox level of the treatment environment and avoid the high oxygen content affecting the phosphorus release and denitification effect. However, there are few reports on its regulation as a process control parameter of continuous sewage biological treatment system. Among them, Ma and Peng studied the feasibility of ORP as the fuzzy control parameter of denitrification process by using ORP as the fuzzy control parameter of denitrification reaction in anoxic/aerobic activated sludge process and obtained the conclusion. They showed that, when the internal circulation flow rate was controlled separately, the ORP value at the end of denitrification zone was (−86 ± 2) mV; and when the external carbon source was controlled alone, the ORP value was (−90 ± 2) mV.32 In preliminary studies, researchers took low carbon—nitrogen ratio sewage as the treatment object and studied the feasibility of ORP as the control parameter of the continuous flow single sludge sewage treatment system. It has been determined that regulating the parameter can change the total phosphorus (TP) and total nitrogen (TN) concentration of the system effluent and at an optimal value of −95 mV.25 On this basis, the metabolism of poly-hydroxy alkanoates (PHA) and TP under different ORP conditions was discussed.33 In order to meet the needs of constructing denitrifying phosphorus absorption process control system and strengthen and stabilize denitrifying phosphorus absorption performance, this paper traces the flow and distribution of carbon and nitrogen under different ORP conditions and evaluates the carbon source savings produced by ORP as the operation control parameter. Mainly, the research is based on the biochemical reaction process of common heterotrophic bacteria, denitrifying bacteria, nitrifying bacteria, and phosphorus accumulating bacteria, combined with the principle of material balance.

2. TEST MATERIALS AND METHODS

2.1. Sewage Water Quality Characteristics. The pollutant composition and concentration in the manually configured sewage to be treated in modified university of cape town (MUCT) process system were determined by referring to the water quality of 10 sewage treatment plants in Changchun City, northern China.32−37 The dosage of each substance was 50 mg/L of whole milk powder, 0.5 mL/L of brewery wastewater, 50 mg/L of NH4Cl, 3.1 mg/L of KH2PO4, 0.4 g/L of NaHCO3, 10 mg/L of CaCl2, and 50 mg/L of MgSO4, respectively. In order to meet the growing needs of activated sludge, 0.6 mL/L of micronutrient solution was added, including 0.9 g/L of FeCl3, 0.15 g/L of H3BO3 and CoCl2·7H2O 0.15 g/L, CuSO4·5H2O 0.03 g/L, KI 0.18 g/L, CoCl2·4H2O 0.06 g/L, Na2MoO4·2H2O 0.06 g/L, and zinc ZnSO4·7H2O 0.12 g/L.

2.2. Main Operating Parameters of MUCT Device. As shown in Figure 1, the MUCT test system consisted of a 252 L sewage tank, 90 L MUCT reactor, precipitator with a diameter of 50 cm, and automatic control system. The size of the sewage

Figure 1. MUCT process test device diagram. (1) Sewage tank; (2) anaerobic section; (3) preanoxic section; (4) main anoxic section; (5) aerobic section; (6) clarifier; (7) influent water; (8) effluent water; (9) excess sludge; (10) sludge reflux; (11) aerobic/main anoxic nitrifying fluid circulation; (12) preanoxic/anaerobic mixture circulation.
tank was 80 cm × 70 cm × 45 cm (L × B × H), and the super height is 5 cm. The MUCT reactor was 75 cm × 30 cm × 45 cm (L × B × H), with a 5 cm super height. This included the anaerobic stage of 18 L, preanoxic stage of 9 L, main anoxic stage of 18 L, and aerobic stage of 45 L. The reactor was designed as a double gallery; each gallery was divided into five compartments with partition plates. The effective volume of each compartment was 9 L, and the size was 15 cm × 15 cm × 45 cm (L × B × H). The stirrer was installed in the sewage tank and nonaerated section, and the air diffuser was arranged in the aerobic section. The mixed liquid of the reactor flowed into the precipitator for solid–liquid separation. The settling device was equipped with a scraper with a rotating speed of 5 rpm. The MUCT system established an automatic control system, which consisted of ORP on-line detector, DO on-line detector, the programmable logic controller (PLC), computer, and electric control valve.

2.3. Test Scheme. The daily water treatment capacity of the test system was 240 L/d. Water quality parameters were assessed according to German (ATV-DVWK Specification), the design of Municipal Wastewater Treatment Plants (Volume 2: Liquid Treatment Processes) of United States, the Dutch Biological Phosphorus Removal Manual for Design and Operation, and Chinese Outdoor Drainage Design Specification (GB50014) (2016 edition). According to the regulations, operation parameters such as sludge load, hydraulic retention time, and sludge age were determined. “Chinese Outdoor Drainage Design Specification” (GB50014) (2016 edition) takes hydraulics, microbiology, and so forth as the scientific support and combines with the engineering design and operation experience of China’s urban drainage system, to analyze the design parameters. It is the design standard of the urban drainage system in China.

During the experiment, ORP_in was controlled by the PLC automatic control system. The control system adopted a feedback control structure, the control parameters, controlled variables, and the actuators were ORP_in, the internal circulation flow rate of nitrate, and electric control valve, respectively. The control process was as follows: the ORP value was detected by an ORP online measuring instrument at the end of the main anoxic stage (the output signal was 4–20 mA analogue signal), transmitted to the data acquisition card of PLC system. After being converted into a digital signal by A/D converter and compared with ORP_in setting value of proportional–integral–derivative regulator, the deviation was determined. Next, after the control of proportional, integral, and differential calculation, the results were given and converted into analogue signals (4–20 mA) by a D/A converter. As the output value, the results were fed back to the internal circulation electric control valve of nitrate. The Scientific Apparatus Maker’s Association (SAMA) diagram and logic control diagram of the automatic control system are shown in Figure 2. The experiment was carried out in six stages with ORP set values of −140, −125, −110, −95, −75, and −60 mV. The other operating parameters remained unchanged except the internal circulation flow of nitrate. Three sludge retention time were operated in each stage for 250 days. See Table 1 for the test scheme. The sludge retention time (SRT) was controlled by the hydraulic method; a peristaltic pump was used for continuous discharge of excess sludge from the main anoxic section at a rate of 7.5 L/d.

2.4. Activated Sludge Inoculation and System Startup. During the system startup, see Table 1 for operation parameters of the MUCT system, the recirculated sludge from the secondary sedimentation tank of Changchun Beijiao sewage treatment plant was added to the reactor for continuous operation after inoculation, and the activated sludge was cultured and domesticated. The concentration of water quality indicators was tested every day.

2.5. Detection Indicators and Methods. During the experiment, the samples were taken from anaerobic, preanoxic, main anoxic, and aerobic stages, respectively. After water samples were taken out and centrifuged at 4500 rpm for 7 min, the concentration of chemical oxygen demand (COD), TP, TN, NH4+–N, and NO3−–N in the supernatant was determined. The content of PHA in sludge after freeze-drying was determined by gas chromatography. The DO concentration in the main anoxic section was measured using a WTW-pH/OXi340 portable DO detector. DO in the aerobic section was regulated by PLC automatic control system, and
the value of the DO detector was read as the test result. The data used in the analysis of the test results were taken from the indexes for 20 days during the stable operation period of each section. Chemical samples used in this study are shown in Table 2.

Table 2. Chemical Samples Used and the Suppliers

| no. | component                        | no. | component                        |
|-----|----------------------------------|-----|----------------------------------|
| 1   | potassium dichromate (guaranteed reagent) | 18  | sodium carbonate                |
| 2   | 1,10-phenanthroline              | 19  | sodium bicarbonate               |
| 3   | ferrous sulfate                  | 20  | sodium nitrate (guaranteed reagent) |
| 4   | ammonium ferrous sulfate         | 21  | methanol (chromatographic purity) |
| 5   | concentrated sulfuric acid       | 22  | carboxybenzene (chromatographic purity) |
| 6   | potassium persulfate             | 23  | potassium dihydrogen phosphate  |
| 7   | ascorbic acid                    | 24  | calcium chloride                 |
| 8   | ammonium molybdate               | 25  | magnesium sulfate                |
| 9   | potassium hydrogen phosphate     | 26  | iron chloride                    |
| 10  | sodium hydroxide                 | 27  | boric acid                       |
| 11  | hydrochloric acid                | 28  | cobalt chloride heptahydrate     |
| 12  | potassium nitrate (guaranteed reagent) | 29  | copper sulfate pentahydrate      |
| 13  | trichloromethane                 | 30  | cobalt chloride tetrahydrate     |
| 14  | potassium iodide                 | 31  | sodium molybdate                 |
| 15  | mercury iodide                   | 32  | zinc sulfate heptahydrate        |
| 16  | potassium sodium tartrate        | 33  | a mixture of PHB and PHA (mass ratio 9:1) |
| 17  | ammonium chloride (guaranteed reagent) | 34  | PH2MV                             |

*Except for a mixture of PHB and PHA (mass ratio 9:1) and PH2MV were supplied by Sigma Corporation of America, the other Chemical samples were all supplied by Tianjin Guangfu Fine Chemical Research Institute, China.

2.6. Material Balance Analysis. Based on the principle of material balance, each reaction section was taken as the boundary of the system, and the amount of material reaction was calculated using the substance detection concentration. The results were expressed as mean ± error bar.

Assuming that the amount of material accumulated during the stable operation of the system was 0, the formula is as follows;

**Anaerobic stage**

\[
\Delta S_1 = \left( \frac{dS_1}{dt} \right) 
\cdot V_1 = (1 + r) \cdot Q \cdot S_0 - r \cdot Q \cdot S_2
\]

(1)

**Preanoxic stage**

\[
\Delta S_2 = \left( \frac{dS_2}{dt} \right) 
\cdot V_2
= (1 + r) \cdot Q \cdot S_2 - s \cdot Q \cdot S_3 - (1 + r) \cdot Q \cdot S_1
\]

(2)

**Main anoxic stage**

\[
\Delta S_3 = \left( \frac{dS_3}{dt} \right) 
\cdot V_3
= (1 + s) \cdot Q \cdot S_2 + a \cdot Q \cdot S_4 - (1 + a + s) \cdot Q \cdot S_3
\]

(3)
Aerobic stage

\[
\Delta S_i = \left( \frac{dS}{dt} \right)_i = V_i \cdot (1 + a + s) \cdot Q \cdot (S_3 - S_4)
\]  

(4)

where subscripts 0, 1, 2, 3, 4, and 5 represent inlet water, anaerobic stage, preanoxic stage, main anoxic stage, aerobic stage, and outlet water, respectively; \( \Delta S \) denotes the amount of material reaction, g/d; \( Q \) represents inflow flow, L/d; \( V \) represents the effective volume of the reactor, L; \( S \) denotes the concentration of substance, mg/L; \( s \) represents sludge reflux ratio; \( a \) represents aerobic/main anoxic nitrifying fluid circulation ratio; and \( r \) denotes preanoxic/anaerobic mixture circulation ratio.

2.7. Chemometrics and Coefficient of Reaction Process. The biochemical reaction processes of MUCT process included COD aerobic degradation, anaerobic phosphorus release, denitrifying phosphorus absorption, aerobic phosphorus absorption, denitrification, and nitrification. COD in sewage is expressed by empirical molecular formula \( C_{18}H_{19}O_9N \).

The empirical molecular formula of cell material is \( C_5H_7NO_2 \), and the empirical molecular formula of PHA is \( CH_{1.5}O_{0.5} \). The chemical equation for the oxidation of
C\textsubscript{18}H\textsubscript{19}O\textsubscript{9}N to CO\textsubscript{3} is shown in eq 5. \textsuperscript{54} Formula 5 shows that the oxygen equivalent of C\textsubscript{18}H\textsubscript{19}O\textsubscript{9}N was 1.42 gCOD/gC\textsubscript{18}H\textsubscript{19}O\textsubscript{9}N.

$$\text{C}_{18}\text{H}_{19}\text{O}_9\text{N} + 17.5\text{O}_2 + \text{H}^+ \rightarrow 18\text{CO}_2 + 8\text{H}_2\text{O} + \text{NH}_4^+$$

(5) The chemical equation for the oxidation of PHA to CO\textsubscript{2} is shown in eq 6. Formula 6 shows that the COD equivalent of PHA was 1.67 gCOD/gCH\textsubscript{1.5}O\textsubscript{0.5}.

$$\text{C}_3\text{H}_4\text{O}_3 + 1.125\text{O}_2 \rightarrow \text{CO}_2 + 0.75\text{H}_2\text{O}$$

(6) The biochemical reaction equation for aerobic degradation of COD is shown in eq 7. According to the formula, the stoichiometric coefficient of O\textsubscript{2}–C\textsubscript{18}H\textsubscript{19}O\textsubscript{9}N–COD was 2.19 gCOD/gO\textsubscript{2}.

$$\text{C}_{18}\text{H}_{19}\text{O}_9\text{N} + 0.74\text{NH}_3 + 8.0\text{O}_2$$

$$\rightarrow 1.74\text{C}_3\text{H}_4\text{NO}_2 + 9.3\text{CO}_2 + 4.52\text{H}_2\text{O}$$

(7) The denitrifying biochemical reaction equation using COD as a carbon source is shown in eq 8. It can be seen from the formula that the stoichiometric coefficient of NO\textsubscript{3}–C\textsubscript{18}H\textsubscript{19}O\textsubscript{9}N–COD was 0.18 gNO\textsubscript{3}/gCOD, and the stoichiometric coefficient of NO\textsubscript{3}–COD–C\textsubscript{18}H\textsubscript{19}O\textsubscript{9}N–NO\textsubscript{3} was 5.35 gCOD/gNO\textsubscript{3}.

$$0.61\text{C}_{18}\text{H}_{19}\text{O}_9\text{N} + 4.54\text{NO}_3^- + 0.39\text{NH}_4^- - \text{N}$$

$$+ 4.15\text{H}^+ \rightarrow \text{C}_3\text{H}_7\text{O}_2\text{N} + 2.27\text{N}_2 + 5.98\text{CO}_2$$

$$+ 5.15\text{H}_2\text{O}$$

(8) The biochemical reaction equation for aerobic degradation of COD as an acetic acid as a carbon source is shown in eq 9. According to the formula, the stoichiometric coefficient of C\textsubscript{2}H\textsubscript{4}O\textsubscript{2}–NO\textsubscript{3} was 5.10 gC\textsubscript{2}H\textsubscript{4}O\textsubscript{2}/gNO\textsubscript{3}.

$$4.69\text{C}_2\text{H}_4\text{O}_2 + 3.94\text{NO}_3^- - \text{N} + \text{NH}_4^- - \text{N} + 2.94\text{H}^+$$

$$\rightarrow \text{C}_3\text{H}_7\text{O}_2\text{N} + 1.97\text{N}_2 + 4.92\text{CO}_2 + 9.9\text{H}_2\text{O}$$

(9) 3. TEST RESULTS

3.1. Start-Up. After 37 days of continuous operation, the nitrification effect of the system was good, the activated sludge showed a strong ability of phosphorus release and absorption, the effluent concentration of TN and TP were below 20 and 1 mg/L, respectively, and the activated sludge had been cultured and domesticated. The biological phase of the activated sludge sample was examined by a microscope, and it was found that the characteristics of the activated sludge bacterial micelles were good.

In a previous study (2015)\textsuperscript{65} based on the principle of chemometrics, the proportion of main microorganisms in the MUCT process was calculated by using material balance: 35.94% of common heterotrophic bacteria, 16.84% of denitrifying bacteria (with COD as carbon source), 41.54% of phosphorus accumulating bacteria (of which, denitrifying phosphorus accumulating bacteria accounted for 20.63%), and 5.78% of nitrifying bacteria. The operation parameters and influent water quality of the above study were similar to that of the start-up period (8–37 d), especially in the later period (25–37 d). In addition, because the two studies were carried out in the same set of MUCT system test device, the above calculation results can be used as the microbiological basis for qualitative analysis in this paper.

3.2. ORP\textsubscript{an} and System Performance. During the test, the change rule of COD, nitrogen, phosphorus and other pollution index concentration, and PHA content in sludge in each reaction section of the system are shown in Figure 3. The errors of the measured values were less than 2 mV. It can be seen from Figure 3 that when the carbon–nitrogen ratio of the influent water was low, the setting value of ORP\textsubscript{an} could be adjusted and controlled, which had little influence on COD, ammonia nitrogen, and nitrite–nitrogen. The effluent concentration changed in the range of 10.26–23.65, 1.16–4.54, and 0–0.03 mg/L, respectively. However, the effluent concentration of TN, TP and nitrate–nitrogen was greatly affected by the regulation of ORP\textsubscript{an} setting value. The effluent concentration was 12.06–24.58, 0.21–5.03, and 8.67–22.34 mg/L, respectively. When the ORP\textsubscript{an} set value was −95 mV, the effluent concentration of the three was the lowest. Therefore, it can be inferred that −95 mV was the best setting value.

In addition, the removal rates of three pollutants were determined based on the calculation results of the material balance of COD, TN, and TP. When the ORP setting value was controlled at −140, −125, −110, −95, −75, and −60 mV, the removal rates of COD were basically stable, 238.26 ± 24.95 mg COD/(gMLSS-d); the removal rates of TN were 28.45 ± 3.52, 29.99 ± 1.23, 31.05 ± 0.94, 35.02 ± 0.83, 34.52 ± 1.08, and 34.11 ± 2.04 mg TN/(gMLSS-d), TP were 3.26 ± 0.22, 3.98 ± 0.82, 4.83 ± 1.45, 5.48 ± 0.76, 5.38 ± 0.45, and 5.20 ± 0.63 g TP/(gMLSS-d), respectively. The results showed that the COD removal rate was not affected by ORP regulation value but TN and TP changed significantly. When ORP regulation value was −95 mV, both of them reached the highest values, which also confirmed that −95 mV was the best setting value.

During the test, with the main anoxic section as the system boundary, the TP concentration detection value was used to calculate the phosphorus absorption, and the results are shown in Figure 4. It can be seen from Figure 4 that with the increase of ORP\textsubscript{an}, the phosphorus absorption rate in the main anoxic stage increased from 2.57 ± 0.52 mg TP/(gMLSS·d) to 13.07 ± 0.52 mg TP/(gMLSS·d). This indicated that the regulation of ORP\textsubscript{an} could enhance the function of denitrifying phosphorus.
accumulating bacteria and obtained better denitrifying phosphorus absorbing effect.

3.3. ORP<sub>a</sub> and the Quality Distribution of COD and Nitrate–Nitrogen. 3.3.1. ORP<sub>a</sub> and COD Mass Distribution. Based on material balance analysis and stoichiometry, the mass distribution of COD in each stage was tracked. When ORP<sub>a</sub> was controlled as −140 mV, the mass distribution of carbon and nitrogen in each reaction section was calculated using the average value of the calculated results of material balance.

Anaerobic stage:
The amount of COD stored in the synthesized PHA is

\[
\text{COD}_{\text{ANS,PHA}} = \Delta\text{PHA}_1 \times 1.67 \text{ gCOD/gPHA} = 16.17 \\
g\text{PHA}/d \times 1.67 \text{ gCOD/gPHA} = 27.01 \text{ gCOD/d} \tag{10}
\]

COD consumption in the denitrification process is

\[
\text{COD}_{\text{ANS,DE}} = \Delta(\text{NO}_3^-)_2 \times 5.35 \text{ gCOD/gNO}_3 = 0.19 \\
g\text{NO}_3^-/d \times 5.35 \text{ gCOD/gNO}_3 = 0.12 \text{ gCOD/d} \tag{11}
\]

Preanoxic stage:
COD consumption in the denitrification process is

\[
\text{COD}_{\text{PAnS,DE}} = \Delta(\text{NO}_3^-)_2 \times 5.35 \text{ gCOD/gNO}_3 = 1.45 \\
g\text{NO}_3^-/d \times 5.35 \text{ gCOD/gNO}_3 = 7.75 \text{ gCOD/d} \tag{12}
\]

The amount of COD stored in synthetic PHA is

\[
\text{COD}_{\text{ANS,PHA}} = \Delta\text{PHA}_2 \times 1.67 \text{ gCOD/gPHA} = 0 \\
g\text{PHA}/d \times 1.67 \text{ gCOD/gPHA} = 0 \text{ gCOD/d} \tag{13}
\]

Main anoxic stage:
The amount of COD degraded by the aerobic method is

\[
\text{COD}_{\text{MANnS,O}} = \Delta\text{DO}_2 \times 2.19 \text{ gCOD/gO}_2 = 0.47 \\
g\text{O}_2/d \times 2.19 \text{ gCOD/gO}_2 = 1.03 \text{ gCOD/d} \tag{14}
\]

COD consumption in the denitrification process is

\[
\text{COD}_{\text{MANnS,DE}} = \Delta\text{COD}_1 - \text{COD}_{\text{MANnS,O}} = 7.94 \\
g\text{COD/d} = 1.03 \text{ gCOD/d} = 6.91 \text{ gCOD/d} \tag{15}
\]

The amount of nitrate–nitrogen (NO<sub>3</sub>−) in COD as a carbon source is

\[
(\text{NO}_3^-)_{\text{MANnS,COD}} = \text{COD}_{\text{MANnS,DE}} \times 0.19 \text{ gNO}_3^-/\text{gCOD} \\
= 6.91 - 0.18 \text{ gNO}_3^-/\text{gCOD} = 1.24 \text{ gNO}_3^-/\text{gCOD} \tag{16}
\]
(NO3)3/MANsP = 5.28 gNO3/d − 1.24 gNO3/d
= 4.04 gNO3/d   (17)

Using the same method and procedure, the material distribution of COD in a nonaerated section can be calculated when ORPm was controlled to be −125, −110, −95, −70, and −60, respectively. The results are shown in Figure 5. Because of the need to regulate ORPm in each test stage, the circulation ratio of nitrate was a variable, and its value was the average value of the output of the automatic control system, which was 1.23 ± 0.13, 1.51 ± 0.25, 2.34 ± 0.09, 3.23 ± 0.06, 3.37 ± 0.17, and 3.59 ± 0.10, respectively. It can be seen from Figure 5a that when ORPm was increased from −140 to −95 mV, the amount of COD in anaerobic section synthesized into PHA and stored in phosphorus-accumulating bacteria was increased from 27.01 to 35.57 gCOD/d, and the distribution rate was increased from 39.63 to 51.74%. When denitrifying bacteria were used to transform nitrate nitrogen, the amount of COD decreased to 0.75 g COD/d at −140 mV, and the flow rate was 1.10%. When the control value was −95 mV, the denitrifying bacteria did not obtain COD.

Then again, when ORPm was set at −140 and −125 mV, the COD allocated to phosphorus-accumulating bacteria was 0 (Figure 5b). Thereafter, with the increase in ORPm value, the amount of COD allocated to phosphorus-accumulating bacteria increased. When the set value was −95 mV, the storage capacity of PHA and the allocation rate of COD reached the highest values of 1.39 g/d and 2.51%. Correspondingly, the amount of COD allocated to denitrifying bacteria decreased with the increase of ORPm. When the set value was −95 mV, the consumption and distribution rate of denitrifying bacteria reached the lowest values of 5.29 g/d and 7.70%.

From Figure 5c, it can be deduced that the distribution ratio of COD between denitrifying bacteria and heterotrophic bacteria changes regularly with the increase of ORPm. When the control value increased from −140 to −60 mV, the distribution ratio of COD to common heterotrophic bacteria increased, and the distribution ratio was 1.52, 2.10, 2.15, 4.40, 4.13, and 4.60%. Correspondingly, the COD to denitrifying bacteria decreased. The distribution rates were 10.13, 9.65, 9.28, 7.93, 7.19, and 6.15%, respectively.

To sum up, the flow and distribution of COD in MUCT process mainly occur in anaerobic, preaerobic, and main anoxic stages, where the distribution of COD in anaerobic and preaerobic stages was between phosphorus-accumulating bacteria and denitrifying bacteria. The distribution of COD in the main anoxic stage was between the common heterotrophic bacteria and denitrifying bacteria. The distribution proportion varied regularly with the difference of ORPm.

3.3.2. ORPm and Nitrate—Nitrogen Mass Distribution.
When ORPm was set at −140 mV, the flow direction of nitrate—nitrogen in the main anoxic stage was calculated as Section 3.3.1. Using the same method and procedure, the distribution of nitrogen in the main anoxic stage was calculated when ORPm was controlled as −125, −105, −95, −70, and −60 mV, respectively. The results are shown in Figure 5d. With ORPm increasing from −140 to −60 mV, the amount of nitrate—nitrogen transformed by denitrifying bacteria gradually decreased, at distribution rates of 23.54, 20.86, 19.89, 16.76, 15.10, and 13.68%, respectively. Correspondingly, nitrate—nitrogen transformed by denitrifying phosphorus-accumulating bacteria was increased, at distribution rates of 76.46, 79.14, 80.11, 83.24, 84.90, and 86.32%, respectively. That is to say, when ORPm control was −95 mV, most of nitrate—nitrogen flowed to denitrifying phosphorus accumulating bacteria, and the system experienced the best effect of nitrogen and phosphorus removal.

3.4. ORPm and Carbon Source Savings. With the assumption of no denitrifying phosphorus uptake, when ORPm was controlled at −140 mV, the required amount of acetic acid \((C_2H_4O_2)_{ECS}\) could be calculated according to formula 9.

\[
(C_2H_4O_2)_{ECS} = 5.10 gC_2H_4O_2/gNO_3·3.88 gNO_3/d
= 19.79 gC_2H_4O_2/d   \]

Under similar method and procedure as previous, we can calculate the amount of acetic acid as external carbon sources required when ORPm was controlled as −125, −105, −95, −70, and −60 mV, respectively, without anoxic phosphorus absorption. The results are shown in Figure 6. From the results, the amount of acetic acid added increased from 20.33 to 24.76 g/d when ORPm was increased from −140 to −95 mV. In other words, carbon sources can be saved during the process of phosphorus absorption by denitrification, and the carbon source saving rate increased from 23.19 to 26.56%. Subsequently, the dosage of acetic acid remained stable or decreased slightly with the increase of ORPm.

4. DISCUSSION
4.1. Mass Distribution of ORP, COD, and Nitrate Nitrogen.
4.1.1. ORPm and the Mass Distribution of COD.
From the perspective of microbiology and metabolism, in the sewage biological treatment system, the dynamic changes of activated sludge microbiome structure and dominant microbiome were closely related to the ORP of their living environment, and the dominant reaction process was also quite different, as shown in Figure 7. In addition, the regulation of ORP altered the content of NADH/NAD+, NADPH/NADP+ in bacterial metabolism and activated some substances. Combined with the experimental results, when...
the Faraday constant; and $C_{\text{NO}_3}$, $C_{\text{O}_2}$, and $C_{\text{COD}}$ are standard electrode potential; calculation; 4.54, 0.61, 8, and 1 are the stoichiometric relative values of the three concentrations to the standard extent, which enhanced the function of denitrifying phosphatase, and PHA degrading enzyme in the bacteria to the greatest phosphorous accumulating bacteria, activated nitrate reducing, and PHA degrading enzyme in the bacteria to the greatest extent, which enhanced the function of denitrifying phosphorous-accumulating bacteria.

There are many kinds of redox potentials in the main anoxic section of MUCT system, which is a complex redox system. ORP is the comprehensive result of the redox reaction of many kinds of redox substances and redox substances. From the macroenvironment, the main oxidizing substances are oxygen and nitrate nitrogen, and the reducing substances are COD. Therefore, ignoring other redox processes, the ORP in the main anoxic section can be calculated by the Nernst equation, as shown in formulas 19 and 20

$$E_{\text{NO}_3/\text{COD}} = E^\theta_{\text{NO}_3/\text{COD}} + \frac{RT}{11.35F} \ln \frac{C_{\text{NO}_3}^{4.54}}{C_{\text{COD}}^{0.61}}$$

(19)

$$E_{\text{O}_2/\text{COD}} = E^\theta_{\text{O}_2/\text{COD}} + \frac{RT}{32F} \ln \frac{C_{\text{O}_2}^{1.6}}{C_{\text{COD}}^{1}}$$

(20)

where $E$ is the ORP value in the main anoxic section; $E^\theta$ is the standard electrode potential; $R$ is the general gas constant; $n$ is the number of transferred electrons; $T$ is the temperature; $F$ is the Faraday constant; and $C_{\text{NO}_3}$, $C_{\text{O}_2}$, and $C_{\text{COD}}$ are the relative values of the three concentrations to the standard concentrations. Generally, the concentration is taken for calculation; 4.54, 0.61, 8, and 1 are the stoichiometric coefficients of the reaction, respectively, as shown in formulas 7 and 8. It can be seen from the formula that ORP in the main anoxic section is closely related to DO concentration, nitrate concentration, COD concentration, and pH value in this section. During the test, the DO concentration in this section was stable between 0.08 and 0.09 mg/L, and the COD concentration was stable between 18.19 and 22.30 mg/L with little change; the pH value was stable between 7.17 and 7.71, and the variation pattern is shown in Table 3. Therefore, it can be inferred that the change of ORP value was mainly caused by the concentration of nitrate—nitrogen, reflecting its concentration level. Based on the activated sludge reaction kinetic model 2D (ASM2d), Wang et al.47 established the MUCT process reaction kinetic model and checked and verified the kinetic parameters and stoichiometric coefficients. They also determined that the values of kinetic parameters $q_{\text{PHAF}}, K_{\text{A}}, K_{\text{PP}}, Y_{\text{PO}_4}, \mu_{\text{AUT}}$, and $\eta_{\text{NO}}$ were 2.90 g (g·d)$^{-1}$, 3.85 g (g·d)$^{-1}$, 0.35, 1.6, and 0.8, respectively. Other kinetic parameters and stoichiometric coefficients adopt the default values recommended by IWA. According to the model, the denitrification phosphorus-absorption performance was determined by the nitrate—nitrogen concentration in this section, which was also proved by the research of Wang and Musvoto et al.24,25

In order to make the most effective use of the denitrifying phosphorus uptake potential in the main anoxic stage, it was necessary to ensure that the nitrate—nitrogen concentration in the main anoxic stage is not too low or zero and not be too high. Explicitly, there was an optimum concentration range of nitrate—nitrogen and a corresponding optimum range in ORP. When the concentration of nitrate—nitrogen in the main anoxic stage varied from 0.5 to 2.5 mg/L (ORP was between −140 and −95 mV), the process of denitrifying phosphorus uptake became more complete with the increase of the set value. Nevertheless, when the concentration of nitrate—nitrogen was higher, thus ORP was greater than −95 mV; the internal circulation flow rate of nitrate solution needed to control the value increased. Therefore, the amount of DO entering this section increased (even when DO concentration changed slightly), also the actual hydraulic retention time was shortened, resulting in the inadequate realization of denitrification and phosphorus uptake process.

In the main anoxic stage, COD was distributed between heterotrophic bacteria and denitrifying bacteria and the distribution proportion was mainly determined by the DO consumption amount. According to formula 3, when the ORP control value increased from −140 to −60 mV, the DO reactions were 0.47, 0.65, 0.67, 1.38, 1.27, and 1.44 g O₂/d, respectively. Therefore, with the increase of ORP control value, the COD flow to heterotrophic bacteria increased, whereas the COD flow to denitrifying bacteria decreased (see Figure 5). Figure 8 shows the flow direction of COD and nitrate nitrogen in the nonaerated reaction section when

| ORP (mV) | Process         | Electron acceptor                        | Condition          |
|----------|-----------------|------------------------------------------|--------------------|
| +300     | 1               | O₂                                       | Aerobic            |
| +200     | 2               |                                          |                    |
| +100     | 3               |                                          |                    |
| 0        | 4               | Anoxic                                   |                    |
| -100     | 5               | Anaerobic carbon source fermentation     |                    |
| -200     | 6               |                                           |                    |
| -300     | 7               |                                           |                    |
| -400     | 8               |                                           |                    |

Figure 7. Microbial metabolic processes and ORP ranges. (1) Organic matter oxidation; (2) phosphorus accumulation; (3) nitratification; (4) denitrification; (5) phosphorus release; (6) hydrogen sulfide production; (7) acid production; (8) methane production.

Table 3. Variation Rule of pH Value in Each Section during the Test

| Section   | experiment 1 | experiment 2 | experiment 3 | experiment 4 | experiment 5 | experiment 6 |
|-----------|--------------|--------------|--------------|--------------|--------------|--------------|
| influent  | 7.41 ± 0.12  | 7.05 ± 0.03  | 7.14 ± 0.07  | 6.88 ± 0.11  | 7.07 ± 0.12  | 7.21 ± 0.05  |
| ANA       | 7.05 ± 0.11  | 6.97 ± 0.09  | 7.02 ± 0.05  | 7.08 ± 0.04  | 6.87 ± 0.08  | 7.01 ± 0.07  |
| PAnS      | 7.21 ± 0.09  | 7.19 ± 0.10  | 7.10 ± 0.06  | 7.24 ± 0.06  | 7.14 ± 0.14  | 7.11 ± 0.05  |
| MAnS      | 7.33 ± 0.16  | 7.42 ± 0.03  | 7.54 ± 0.12  | 7.62 ± 0.09  | 7.55 ± 0.03  | 7.48 ± 0.04  |
| AS        | 6.95 ± 0.10  | 7.07 ± 0.04  | 6.82 ± 0.15  | 7.01 ± 0.05  | 7.17 ± 0.04  | 7.07 ± 0.09  |

The data in the table is the average value ± standard deviation of pH value in each stage ($n = 20, P = 68.3%$)
ORP_{an} was controlled at −140 and −95 mV, respectively, showing the distribution ratio of COD and nitrate nitrogen as well. The distribution ratio of COD was the ratio between the distribution amount and total COD reaction amount, and the proportion of nitrate nitrogen distribution was the ratio between the distribution amount and the reaction amount of nitrate nitrogen in the main anoxic stage.

In anaerobic and preanoxic stage, with nitrate−nitrogen present, COD would be distributed to denitrifying bacteria and phosphorus accumulating bacteria, respectively, and the distribution proportion was affected by the competition between denitrifying bacteria and phosphorus accumulating bacteria. It has been reported that denitrifying bacteria are dominant in this competition.\(^{45}\) When ORP_{an} was controlled at a low value, the denitrification and phosphorus absorption potential in the main anoxic section had not been fully developed, and the nitrate−nitrogen concentration in the effluent was high. This caused the nitrate−nitrogen amount in the pre anoxic section to increase, while more COD flowed to denitrifying bacteria and few or almost no phosphorus accumulating bacteria. For example, when ORP_{an} control was −140, −125, −110 mV, there was no PHA synthesis and storage in the pre hypoxia section, and the distribution proportion of phosphorus accumulating bacteria was 0; but when increased to −95, −70, and −60 mV, some COD flowed to phosphorus accumulating bacteria to synthesize PHA (see Figure 5). It can be seen from Figure 8 that when ORP was −140 mV, the COD allocation rate of phosphorus accumulating bacteria in the preanoxic stage was 0%, and denitrifying bacteria was 11.37%; when ORP was −95 mV, the COD allocation rate of phosphorus accumulating bacteria in this stage was increased to 2.52%, and denitrifying bacteria was reduced to 7.70%.

4.1.2. ORP_{an} and Mass Distribution of Nitrate−Nitrogen. According to the principle of stoichiometric reaction, the COD flowing to denitrifying bacteria decreased and the consumption of nitrate−nitrogen decreased under conditions of increased ORP_{an} control value. In addition, the performance of denitrifying phosphorus uptake in the main anoxic zone was enhanced with the increase in ORP_{an} control value. Hence, the amount of nitrate−nitrogen transformed by denitrifying phosphorus-accumulating bacteria increased as well. The distribution and proportion (distribution ratio) increased similarly, as shown in Figure 5. The nitrate−nitrogen distribution ratio of denitrifying bacteria was 23.54% and that of denitrifying phosphorus-accumulating bacteria was 76.46% when the ORP_{an} control value was −140 mV. Similarly, when the ORP_{an} control value was −95 mV, the denitrifying bacteria distribution ratio decreased to 16.76% and that of denitrifying phosphorus-accumulating bacteria increased to 83.24%, as shown in Figure 8.

On the other hand, the competition between denitrifying bacteria and phosphorus-accumulating bacteria was enriched when nitrate−nitrogen was circulated to the anaerobic stage with mixed liquor. Compared with the situation without nitrate, the amount of COD consumed by denitrifying bacteria and the amount of phosphorus-accumulating bacteria absorbed were both transformed as shown in Figure 4. Therefore, the COD distribution ratio was also changed, as shown in Figure 8.

4.2. ORP_{an} and Carbon saving. Assuming that the absence of denitrifying phosphorus uptake in the main anoxic stage of MUCT process, the nitrate−nitrogen consumed by the anaerobic growth of phosphorus-accumulating bacteria needed to be transformed by denitrifying bacteria, which required the addition of external carbon sources to achieve the same treatment effect (denitrifying phosphorus uptake took place). Specifically, there was a denitrifying phosphorus absorption process, which reduced the number of external carbon sources. In the wastewater treatment process, the common external carbon sources are methanol and acetic acid, which are simple, economical, and practical organic substances. The dosage of acetic acid as a carbon source was determined by stoichiometry (see Figure 7). In the MUCT process, with the increase of ORP_{an} control value, the denitrification and phosphorus absorption performance could be enhanced, and more nitrate−nitrogen would be removed in this process. Therefore, more external carbon sources were saved. When ORP_{an} was controlled to −95 mV, the highest saving rate was 26.56%. This result was lower than the 30% saving rate obtained by Kuba et al.\(^{48}\) but close to 24.29% of Wang when the internal circulation ratio of nitration liquid was 3.0 and the internal circulation ratio of mixed liquid was 1.5.\(^{49}\) Kuba et al. run the double sludge A2NSBR system in the laboratory: one was A/ASBR operated by anaerobic/anoxic mode to achieve denitrification and phosphorus removal; the other was NSBR operated by aerobic mode to carry out nitrification. At the end of nitrification, the effluent entered into anoxic SBR. In the A2NSBR system, denitrifying phosphorus-accumulating bacteria and nitrifying bacteria were completely separated, which had the best denitrifying phosphorus absorption performance. Therefore, the carbon source saving rate was also very high. In the MUCT process operated by Wang et al., the internal circulation ratio of nitration liquid was constant at 3.0, which was basically close.
5. CONCLUSIONS

In summary, this study assessed the distribution rate of COD between denitrifying bacteria and phosphorus-accumulating bacteria in anaerobic and preaerobic stages. The results proved that when the ORP \textsubscript{an} set value was \(-95\) mV, the distribution rate reached the highest, 51.74 and 7.70\%, respectively. COD in the main anoxic segment was distributed between ordinary heterotrophic bacteria and denitrifying bacteria. When ORPan in the main anoxic segment was distributed between ordinary heterotrophic bacteria and denitrifying bacteria, the acetic acid dosage increased from 20.33 g/d at 24.76 g/d at \(-140\) to \(-95\) mV. Meanwhile, the amount of nitrate–nitrogen transformed by denitrifying phosphorus-accumulating bacteria increased from 4.03 to 5.18 g/d under similar conditions. Again, with the increase in ORP \textsubscript{an} control value, the acetic acid dosage increased from 20.33 g/d at \(-140\) to \(-95\) mV, and the carbon source saving rate increased from 23.19 to 26.56\%, and then remained stable.

From this study, the MUCT process can achieve good operation status and better carbon saving effect by regulating ORP \textsubscript{an}. Future studies should consider building a control system of the denitrification phosphorus absorption process to examine and realize the regulation function of this parameter.

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Notes
The authors declare no competing financial interest.

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