



Effect of hydraulic retention time on pollutant removal and sludge characteristics of multi-level A/O coupled biofilm reactor at low temperature

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ABSTRACT

The experiment used the three-level anoxic/oxic (A/O) coupled biofilm reactor as the object to investigate the effect of hydraulic retention time (HRT) on the removal of pollutants and sludge characteristics at low temperature. The low C/N value municipal wastewater was treated at the temperature was $10^{\circ}\text{C} \pm 1^{\circ}\text{C}$, the influent flow distribution ratio was 25:15:6, the total aeration was 6 L min^{-1} , the sludge residence time was $43 \pm 2 \text{ d}$ and the sludge return ratio was 50%. The reactor was operated under three operating conditions with HRT were 12, 8 and 6 h, respectively. The results show that the denitrification effect was the best when HRT was 8 h, and the average removal rates of chemical oxygen demand, $\text{NH}_4^+\text{-N}$ and total nitrogen were $85.08\% \pm 3.10\%$, $92.03\% \pm 4.51\%$ and $72.02\% \pm 3.03\%$, respectively. At the same time, with the decrease of HRT, the values of sludge volume index, the contents of extracellular polymeric substances (EPS) and the values of dehydrogenises activity of the activated sludge and the EPS contents of the biofilm were increased. When the HRT was 8 h, the aerobic denitrification and aerobic simultaneous nitrification and denitrification of the coupling process were rationally utilized, so that the denitrification effect was better. The results enrich the theory of denitrification in multi-stage A/O coupling biofilm reactor under low temperature, and provide reference for engineering design and application.

Keywords: Low temperature; Activated sludge; Biofilm; Hydraulic retention time; Biological nitrogen removal

1. Introduction

Municipal wastewater treatment facilities in China are facing increasingly stringent effluent quality standards, in response to the government's attempt to limit nutrient (particularly nitrogen) pollution of natural water bodies [1]. Thus, there is a pressing demand amongst new and existing wastewater treatment plants (WWTPs) to implement more efficient and cost-effective biological nutrient removal (BNR) technologies to meet effluent quality standards [2]. However, the influent temperature of the WWTPs in northern China is

about 10°C in winter [3]. Generally, low temperatures result in overall nitrogen elimination by reducing the growth and activity of biomass involved in ammonification [4]. At the same time, due to the increase of nitrogen content in municipal wastewater, the low ratio of chemical oxygen demand (COD) to total nitrogen (TN) have emerged [5]. When the low C/N value urban sewage is treated by traditional process, the denitrification efficiency is low due to the insufficient denitrification carbon source. Affected by low temperatures and low C/N values, many WWTPs have experienced $\text{NH}_4^+\text{-N}$ and TN exceeding the standard in winter. It is not only affected the

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normal operation of WWTPs, but also caused serious harm to the environment. Therefore, the research and development of low-temperature and high-efficiency denitrification process is of great significance.

It has been proposed to enhance the denitrification effect by adding floating or fixed carriers [6]. At the same time, the multi-level anoxic/oxic (A/O) process is a BNR treatment process that has flourished in recent years. It has the advantages of high sludge concentration, balanced load, low energy consumption and strong impact load resistance and so on [7,8]. Moreover, another important feature of the multi-level A/O process is dispersed the influent. The wastewater dispersion enter the reaction system from the anoxic zones of each level, so that it can fully utilize the carbon source of the sewage for denitrification which effectively reducing the adverse effects caused by low C/N values. Since both nitrification and denitrification require sufficient time, especially at low temperatures, hydraulic retention time (HRT) is considered to be one of the important control parameters for BNR process [9].

Refer to the above ideas and to strengthen the low temperature and low C/N value of urban sewage denitrification, this study used the three-level A/O coupled flow biochemical process to treat low temperature simulated urban sewage. The reactor was operated under three operating conditions with HRT were 12, 8 and 6 h, respectively. By comparing and analyzing the pollutant removal efficiency, nitrogen migration and transformation, sludge characteristics under three working conditions, to provide reference for the practical application of multi-level A/O coupled flow biochemical process.

2. Materials and methods

2.1. Description of the multi-level A/O coupled biofilm reactor

The main body of the experimental device was a three-level A/O reactor which is shown in Fig. 1. The reactor had a total operational volume of 126 L and was made of plexiglass. Each level of the reactor was made separately and was divided into four zones along the length of the pool. The four zones were named as Ax1, Ax2, Ox1 and Ox2 (x represents the number of levels, which is equal to 1, 2, 3)

and the ratio of the zone's operational volume was 3:3:4:4. The same amount of flow ball filler was added to each zone. Due to the difference in operational volume, the filling rates of aerobic zones and anoxic zones were 13.04% and 17.43%. The clarification unit of the reactor was a vertical sedimentation tank with an operational volume of 54 L. The raw influent, which came from the feeding pool, was divided into three streams to enter the first anoxic zone of each level. And the mixed liquor moved from the first anoxic zone of the first level to the second aerobic zone of the third level and finally to the clarification unit. The return sludge was returned from the bottom of the secondary settling tank to the first anoxic zone of the first level. The influent flow and the return sludge were controlled by four adjusting rotational speed of peristaltic pump (BT300-2J, Longer Pump, China). Six electric agitators (JJ-1A 160W, Zhengrong, China) were used to stir the anoxic zones to ensure that the filler and the mixed liquor mixed thoroughly. In aerobic zones, air was provided through sand bubble diffusers fixed at the bottom of the reactor and the amount of aeration in aerobic zones were controlled by using three flow meters (LZB-DK600-4F, Chengfeng, China). The two compartments of the aerobic zone at all levels use a three-way for air distribution. The amount of ventilation and the number of revolutions of the electric mixer at each level during normal operation are described in Table 1.

2.2. Flow ball filler

The flow ball is composite filler filled with Kaldnes ring and sponge filler. It is composed of a spherical mesh cover and fillers filled therein. The material of Kaldnes ring is high density polyethylene, the shape is cylindrical which 25 mm in diameter and 10 mm in height, the density is 0.95 g cm^{-3} , and the specific surface area is more than $560 \text{ m}^2 \text{ m}^{-3}$. It has good gas permeability and water permeability, and the inside of the filler is not easy to block. Meanwhile, the sponge filler is made of hydrophilic polyurethane. The shape of it is cube with side length of 30 mm, the density is $16\sim 17 \text{ kg m}^{-3}$, and the specific surface area is $3.8 \times 10^5 \text{ m}^2 \text{ m}^{-3}$. It has the advantage of high porosity and makes microbes adhere quickly and effectively. The net cover is a tennis ball with

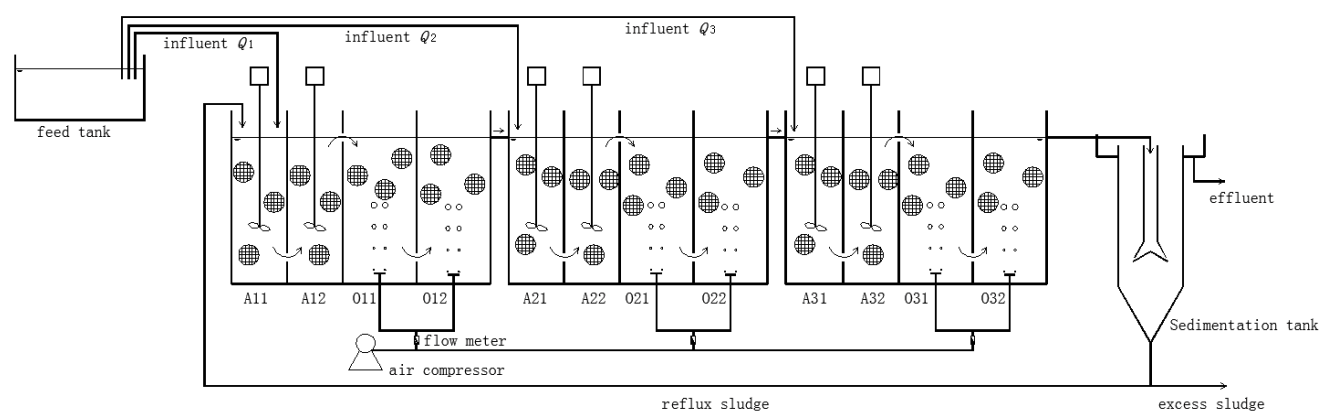


Fig. 1. Schematic diagram of test device.

Table 1
Operating parameters of various working conditions

| Operating condition | HRT (h) | Flow rate (mL min ⁻¹) | | | Aeration rate (L min ⁻¹) | | | Stirring intensity (r min ⁻¹) | | | Others |
|---------------------|---------|-----------------------------------|--------------|-------------|--------------------------------------|--------------|-------------|---|--------------|-------------|--|
| | | First level | Second level | Third level | First level | Second level | Third level | First level | Second level | Third level | |
| Run1 | 12 | 95.1 | 57.1 | 22.8 | 3.26 | 1.96 | 0.78 | 300 | 250 | 200 | Temperature was (10°C ± 1°C), sludge age was (43 ± 2 d) and sludge return ratio was 50%. |
| Run2 | 8 | 142.7 | 85.6 | 34.2 | 3.26 | 1.96 | 0.78 | 300 | 250 | 200 | |
| Run3 | 6 | 190.2 | 114.1 | 45.7 | 3.26 | 1.96 | 0.78 | 300 | 250 | 200 | |

a size of 100 mm in diameter. It is made of polypropylene and 5 sponge fillers and 5 Kaldnes rings are filled therein.

2.3. Seed sludge

The seed sludge was taken from the aerobic and anoxic pools of the sewage treatment plant with A/O process, where biological nitrogen removal and chemical phosphorus removal were successfully achieved. The sludge was screened to prevent large sand or particles from clogging the reactor. Then, the flow ball filler and seed sludge were added to the reactor and maintain the average sludge concentration in the reactor was 5,000 ± 100 mg L⁻¹.

2.4. Experiment water quality

In this work, synthetic wastewater was used to simulate domestic sewage in northeast of China. Due to the chlorine retention and water temperature, tap water for preparing sewage need to be placed in a low temperature controlled room for 1 d. The reagents used for the preparation of synthetic wastewater were soluble starch, beef extract, peptone, sodium acetate (CH₃COONa), ammonium chloride (NH₄Cl), potassium nitrate (KNO₃), potassium dihydrogen phosphate (KH₂PO₄), sodium hydrogen carbonate (NaHCO₃) and sodium hydroxide (NaOH). The purity of the chemicals was analytically pure and that were purchased from Tianjin Guangfu Technology Development Co. Ltd., (China). During the operation of our experiment which lasted for more than 300 d, the average influent wastewater characteristics are described in Table 2. Moreover, the ratio of C/N ranged from 5.79 to 8.67 and with an average was 6.58.

2.5. Startup and operation of the reactor

The reactor was operated in a temperature controlled room, and the water temperature was controlled to be 10°C ± 1°C by controlling the room temperature. That room was equipped with two refrigerating machines which could automatically control the room temperature to vary between 9.5°C and 11°C. After the reactor was filled with the filler and the seed sludge, the membrane culture was carried out by a closed loop method. Two weeks later, wastewater was initially introduced into the reactor and the flow distribution ratio of the three-level was 25:15:6. Then, the influent flow rate was gradually increased. When the influent load was reached the predetermined value and the removal rates of COD and NH₄⁺-N were above 80%, respectively. It was the end of the reactor to startup and cultured.

The experimental procedures and conditions for more than 300 d, defined as Run 1~Run 3, are summarized in Table 1. The influent flow distribution ratio was calculated based on the equivalent sludge load between each level. Moreover, the organic matter flowing from the upper level to the next was calculated as 20% of the influent of the upper level and the three levels activated sludge concentrations used for calculation were 5,000; 4,000 and 3,000 mg L⁻¹ which came from pre-cultured period. The total aeration of the reactor was 6 L min⁻¹ and the aeration ratio of the three aerobic zones was the same as the ratio of the influent water to ensure that the gas-water ratio at all levels was consistent. The sludge reflux ratio was 50% in Run 1~3, and the sludge age was controlled at 43 ± 2 d. The experiment selected three kinds of HRT of 12, 8 and 6 h for operation. Each working condition had a period of adaptation, and the water quality index of the mixed liquid was tested after the working conditions started. Considering that the experiment was carried out at low temperature, the biofilm culture was not as fast as the activated sludge, so the experiment was carried out with the order of low to high organic load.

2.6. Analysis methods

2.6.1. Membrane biomass detection

When performing membrane biomass testing, the flow ball filler was randomly selected in each zone. After removing the mesh cover, the sponges and the Kaldnes rings were separately immersed in ultrapure water. After washing away the reversible adhesive component, the two fillers were dried at 70°C~80°C for 6 h separately. Then, the filler was taken out to be weighed, recorded mass as M1. The dried filler was ultrasonically eluted in NaOH solution with concentration was 0.1 mol L⁻¹ for 30 min to mechanically peel the biofilm. Finally, the filler was repeatedly washed with ultrapure water, dried again at the same temperature for 6 h, weighed the filler again, and the weighing mass M2 was recorded. Subtract M1 from M2 and divide by the number of fillers that was the biomass on a single sponge or Kaldnes ring. Calculated the total biomass of each zone, and divided by the effective volume of the zone to obtain the concentration of the biofilm.

2.6.2. Other analysis methods

Temperature, DO and pH were monitored by WTW Multi 340i meter with DO and pH probes (WTW Company, Germany). COD was measured with a COD quick-analysis

Table 2
Characteristics of synthetic wastewater

| | COD | TN | NH ₄ ⁺ -N | NO ₃ ⁻ -N | TP |
|-------------------------------|-------------|-------------|---------------------------------|---------------------------------|-----------|
| Range (mg L ⁻¹) | 180.6~269.4 | 21.76~40.28 | 10.15~19.48 | 8.67~13.45 | 2.25~9.12 |
| Average (mg L ⁻¹) | 210.07 | 32.33 | 15.57 | 9.68 | 6.92 |

apparatus (Lian-hua Tech. Co. Ltd, 5B-1, China). TN, NH₄⁺-N, total phosphorus (TP), mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids were analyzed according to standard methods [10]. The extraction of tightly bound extracellular polymeric substances (TB-EPS) and loosely bound extracellular polymeric substances (LB-EPS) was in accordance with Li and Yang [11]. The protein (PN) and polysaccharides (PS) were measured using coomassie brilliant blue G-250 method and anthrone-sulfuric acid method [12] with bovine serum albumin and glucose as standard solution, respectively. The measurement method of dehydrogenases activity (DHA) was according to Yin et al. [13].

2.6.3. Calculation methods

2.6.3.1. Calculation of flow distribution ratio

$$\frac{Q_1 \times S}{5000} = \frac{0.2Q_1 \times S + Q_2 \times S}{4000} = \frac{0.2Q_1 \times S + 0.2Q_2 \times S + Q_3 \times S}{3000} \quad (1)$$

Q_1 , Q_2 , Q_3 are the influent flow rate of each level, L d⁻¹; S is the influent organic matter concentration, mg L⁻¹.

2.6.3.2. Calculate the total amount of contaminants (T_i) removed from each compartment

$$T_i = Q_{\text{infi}} \times S_1 - Q_{\text{effi}} \times S_2 \quad (2)$$

T_i is the total amount of pollutants removed in each compartment, g d⁻¹; Q_{infi} is the unit that flows into the compartment, L d⁻¹; Q_{effi} is the unit of the outflow pool, L d⁻¹; S_1 , S_2 are the concentration of pollutants flowing into and out of the compartment, respectively, g L⁻¹.

3. Results and discussions

3.1. Effect of HRT on pollutant removal

After the periods of startup and culture, the reactor was continuously operated for more than 300 d, and the pollutants removal performances are illustrated in Fig. 2. The three working conditions with HRT of 12, 8 and 6 h were named as Run 1, Run 2 and Run 3. Although the influent COD (210.69 ± 35.14 mg L⁻¹) was changed, Run 1~Run 3 were almost equally effective in COD removal (Fig. 2a). When the reactor was stable in each condition, the average COD concentrations in the effluent were 26.39 ± 5.62, 31.89 ± 6.03 and 24.64 ± 4.06 mg L⁻¹, respectively. It met the Class A standard of China's urban sewage pollutant discharge standard [14]. The COD removal results show that the coupling process had good effect on the removal of

organic matter and could be stable for a long time at the temperature of 10°C ± 1°C.

When it comes to NH₄⁺-N, we found that there were two obvious periods when the reactor changed its working condition to another. The removal efficiency of NH₄⁺-N had a gradual increase period and a stable period in Run 1~Run 3. Related studies have confirmed that nitrification takes more time to adapt to low temperatures than denitrification [15]. With influent NH₄⁺-N concentration of 16.33 ± 2.41 mg L⁻¹, the reactor could achieve high nitrification efficiency (>90%) with average NH₄⁺-N in the effluent below 1.5 mg L⁻¹ (Fig. 2b) when HRT was greater or equal to 8 h. When HRT was reduced to 6 h, the nitrification effect dropped sharply and the removal efficiency was just 39.74% ± 4.91%. Low removal efficiency of NH₄⁺-N had impact on TN removal. Fig. 2c illustrates variations of TN in the influent and effluent of Run 1~Run 3. The average TN in the influent was 29.28 ± 4.10 mg L⁻¹, the average TN in the effluent was 8.18 ± 1.13, 7.67 ± 0.88 and 12.56 ± 0.78 mg L⁻¹ in stable stage. The corresponding removal efficiency was 69.68% ± 5.95%, 72.02% ± 3.03% and 55.11% ± 4.20%. It is obviously that the nitrogen removal efficiency of Run 3 was lower than that in Run 1 and Run 2. In addition, more than 80% of the TN in the effluent of Run 3 was NH₄⁺-N. It can make a conclusion that the nitrification rather than the denitrification affected the removal of TN in Run 3. The removal rate of Run 1 was lower than that in Run 2, and the variance of the removal rate was larger. That means Run 1 was unstable to the removal of TN. Run 1 had lower influent because of longer HRT and the experiment water quality with a low C/N values, that made a difference when supplied carbon source which denitrification need. Moreover, there was no change in ventilation for Run 1~Run 3 during the experiment. Run 1 was more likely to place the aerobic zone in an excessive aerobic state, which not only lead to excessive consumption of carbon sources, but also weaken the simultaneous nitrification and denitrification (SND) in the aerobic zone.

Fig. 2d shows variations of TP concentrations in the influent and effluent of Run 1~Run 3. The average removal rates of TP in Run 1~Run 3 were 59.32% ± 37.00%, 60.73% ± 33.46% and 83.07% ± 23.87%, respectively. It can be found that Run 3 which had a lower HRT has the highest TP removal efficiency. The removal of TP mainly depends on sludge discharge and microbial assimilation [16]. The use of long sludge age may be the main reason for the poor TP removal effect in various working conditions.

The effect of the reactor on the treatment of contaminants is closely related to the microbial properties therein [17,18]. Therefore, in order to further verify the results obtained above, the extracellular polymeric substances (EPS), DHA, MLSS, sludge volume index (SVI) and biomass were examined in the stable stage of Run 1~Run 3, the results are shown as follows.

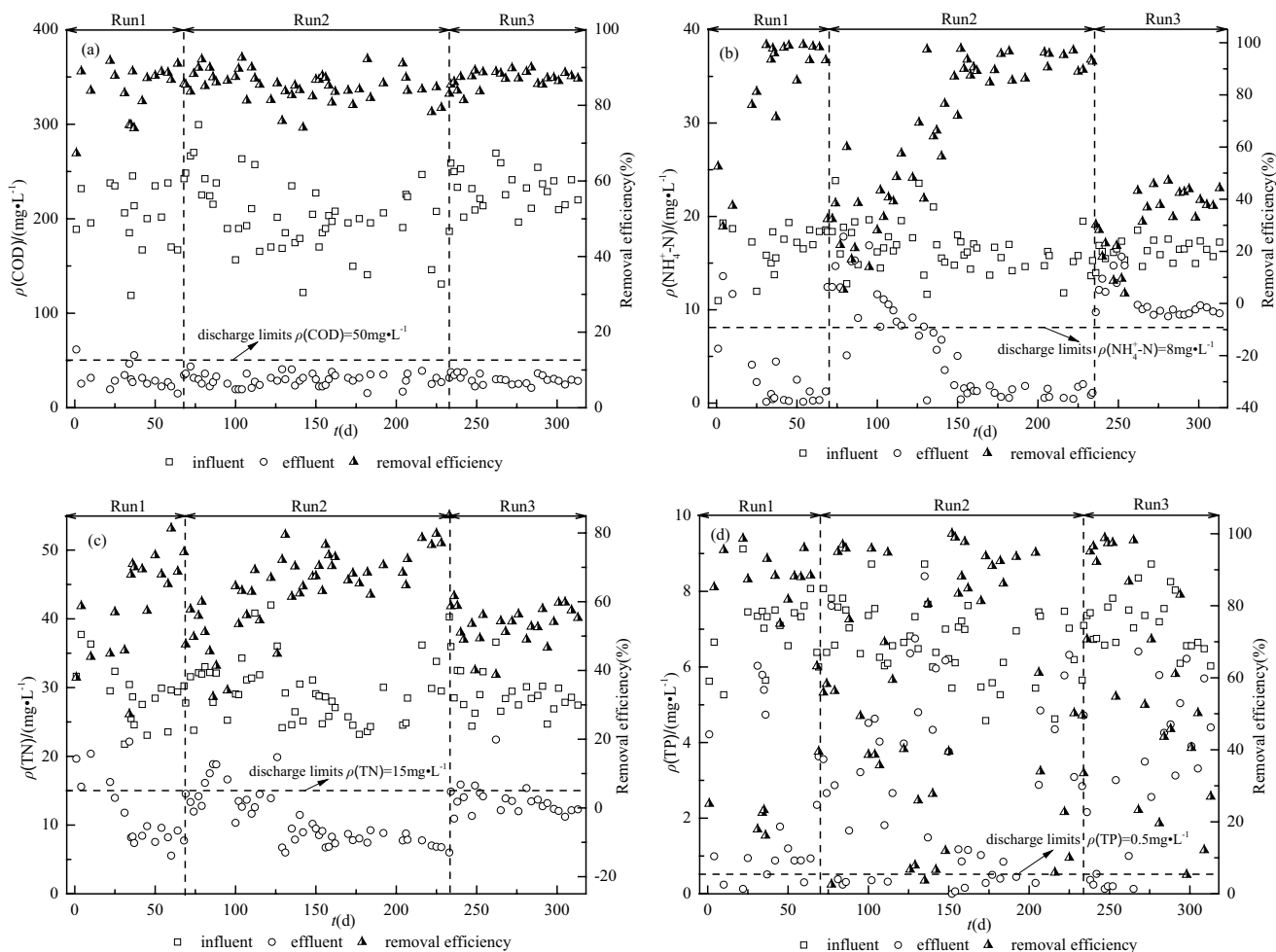


Fig. 2. Pollutant removal effect under various working conditions.

3.2. Effect of HRT on MLSS, SVI and biomass

Fig. 3a illustrates MLSS concentrations along the reactor in each working condition. The concentration of activated sludge gradually decreased along the reactor. Moreover, the average sludge concentration under Run 1~Run 3 of the reactor was 5,105; 4,627 and 3,500 mg L⁻¹. The average MLSS concentration of the three levels in Run 1 was 6,380; 4,492.5 and 4,440 mg L⁻¹; 5,277.5; 4,408.75 and 4,195 mg L⁻¹ in Run 2 while 3,885; 3,350 and 3,265 mg L⁻¹ in Run 3. It is indicated that the sludge concentration in the reactor was decrease and the difference in sludge concentration at different levels was also reduced when the HRT was shortened. Furthermore, the sludge interception capacity of the multi-level A/O process was weakened with the decrease of HRT. Due to the decrease in sludge concentration, the number of functional bacteria such as nitrifying bacteria in the reactor must be decrease relatively. It may be the main reason for the poor nitrification in Run 3 except for low temperature.

The SVI test results are shown in Fig. 3b. Normally, the SVI value is between 70 and 100 mL g⁻¹. In this study, the SVI values for all conditions exceeded 100 mL g⁻¹, and SVI values

would become higher when HRT reduced. At Run 3, the SVI value exceeded 200 mL g⁻¹ along the reactor. There was no significant sign of filamentous expansion from the microscopic results (not shown). The situation will be correlated with low temperature and EPS which discussed later. Under high organic load and low temperature conditions, sludge was highly susceptible to viscous expansion [19]. Since larger sludge sizes provide more oxygen-deficient environments, slight sludge expansion may be beneficial for nitrogen removal in this study. Moreover, sludge expansion also did not affect the removal of organic matter in this study.

Fig. 3c and d illustrate the biofilm content of the aerobic zones and anoxic zones under Run 1~Run 3. It can be seen from the figures that the membrane biomass content of the anoxic zone of Run 2 compartment was higher, which played an important role in promoting denitrification. From the perspective of membrane biomass at all levels, the biomass content increase along the three levels which was contrary to the change rule of activated sludge. It may be because the sessile phase and the suspended phase microorganisms compete with each other in the system. The activated sludge concentration of Run 3 was the lowest

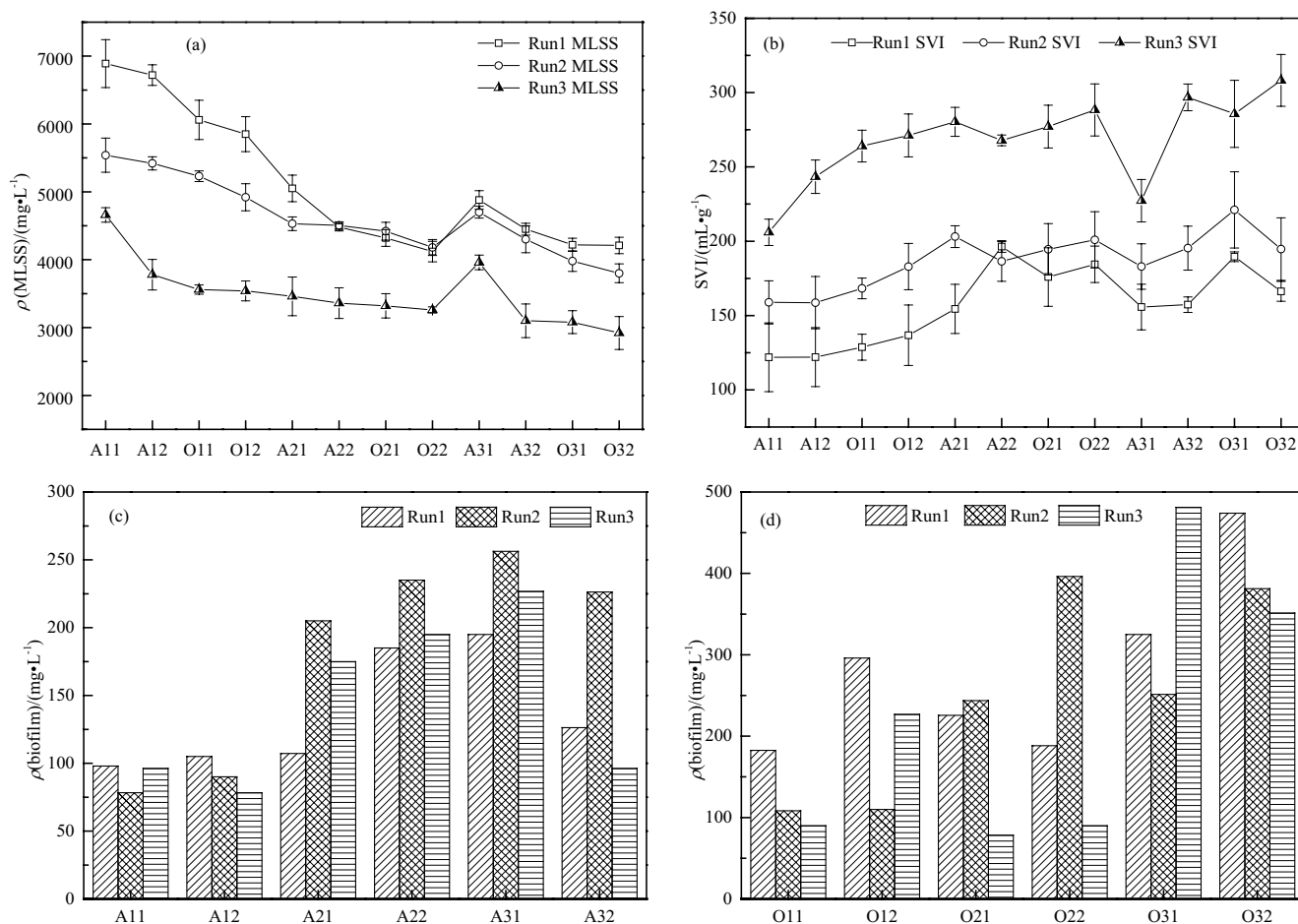


Fig. 3. MLSS, SVI, biomass under various working conditions along the reactor.

along the reactor. However, the membrane biomass was not the highest. The reason was that the total aeration volume was constant and the activated sludge concentration was reduced, which causes the biofilm to be shared by the water flow, which made the biofilm easy to fall off. Meanwhile, the reactor was operated at the temperature of $10^{\circ}\text{C} \pm 1^{\circ}\text{C}$ that made the rate of renewal of the biofilm decreased. At the same time, study [20] had shown that higher concentrations of free ammonia will inhibit the growth of nitrifying and nitrated bacteria, which may another reason for the low content of biofilm in Run 3.

3.3. Effect of HRT on EPS

EPS is produced by microbes during metabolism [21], and the main compositions of it were protein and polysaccharides, accounting for 70%–80%. For activated sludge, EPS plays an important role in physicochemical character, flocculation structure, surface charge, sedimentation and dewatering performance [22]. For biofilm, EPS is essential in membrane culture and capture of nutrients in water.

In this study, PN and PS in EPS (including TB-EPS and LB-EPS) were measured. Figs. 4a–c show changes in the EPS components of the activated sludge, while Figs. 4d–f show that in the biofilm. Whether in activated sludge or

in biofilm, the TB-EPS content was higher than the LB-EPS content, and the PN content was higher than the PS content in total EPS at all working conditions (Run 1–Run 3) in this study. Besides, the total EPS content increased with the decrease of HRT. The increase of total EPS may result from more organic matter entered in the reactor while the influent increased. In addition, the higher total EPS were tested than the similar experiment in higher temperature [16]. This verified that the EPS could increase the resistance of activated sludge to toxic substances, and protect the viability of cells in unfavorable condition such as low temperature environment [23]. Except for Run 3, there were lower total EPS were tested in biofilm than that in activity sludge. One function of the EPS was enrichment and capture nutrients in wastewater. When the amount of pollutants in the water was low, the activated sludge in the suspension was more likely to get nutrients than the attached biofilm. This situation was changed when the amount of contaminants in the water increases, as shown in (Figs. 4c and f). Furthermore, the ratio of LB/TB-EPS and PN/PS-EPS were calculated and show in Figures. The ratio of LB/TB-EPS increased and the ratio of PN/PS-EPS decreased when the conditions from Run 1 to Run 3. In Run 1, the average of the two ratios along the reactor was 0.14 and 2.18 in the activated sludge, and 0.31 and 2.35 in the biofilm. While in Run 3, it was

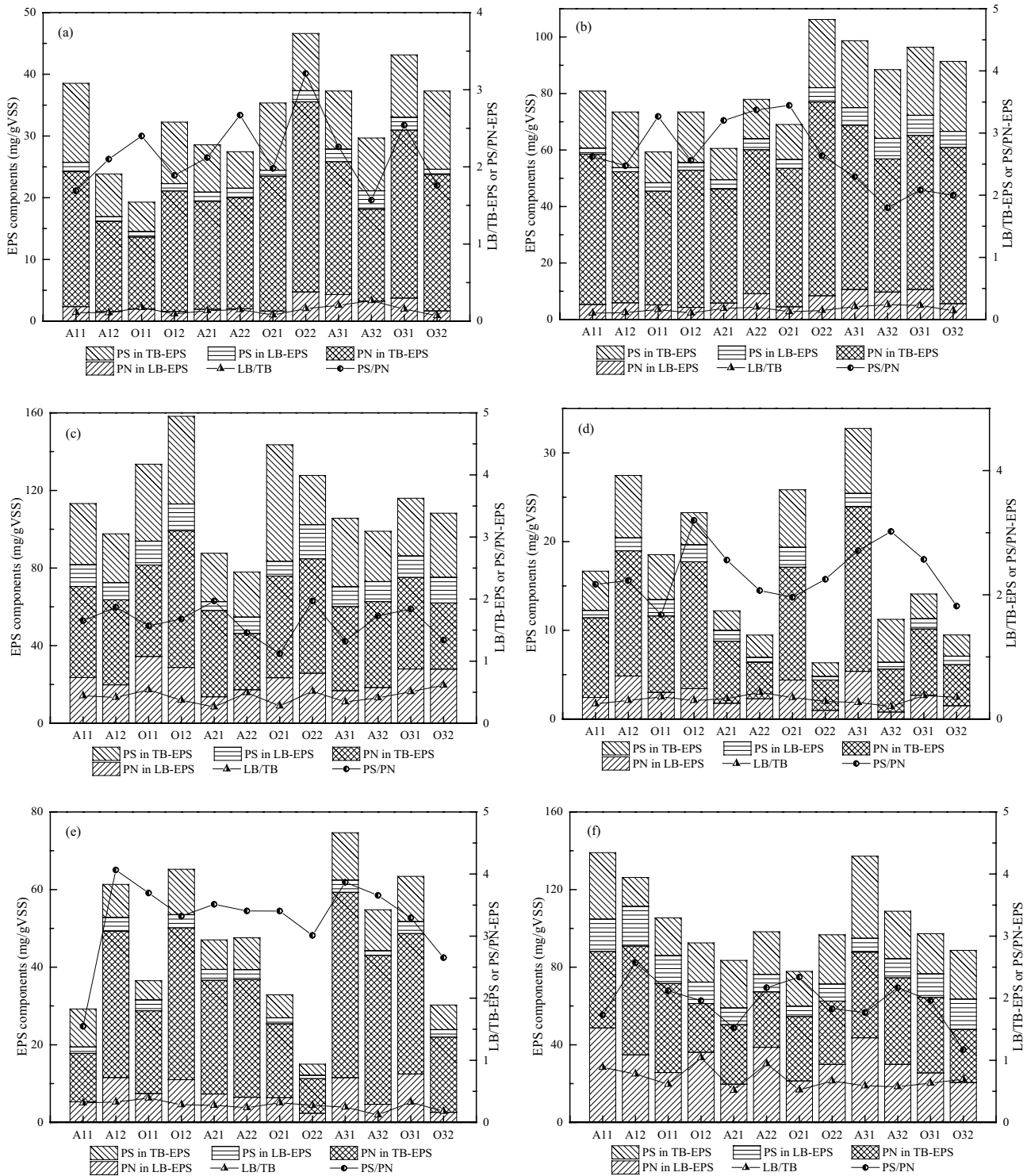


Fig. 4. Content of EPS component in biofilm and activated sludge under various working conditions along the reactor.

0.43 and 1.63, 0.71 and 1.94, respectively. However, the total amount of EPS from Run 1 to Run 3 was gradually increasing, which indicates that LB-EPS and PS increase significantly during this process. The increase in LB-EPS will be detrimental to the sedimentation of activated sludge, as LB-EPS is negative for sludge settling [11]. This

can explain the change in the sedimentation performance of the activated sludge at Run 1~Run 3 discussed earlier. The loose flock structure not only affected the treatment effect of the sewage, but also increased the burden of the secondary sedimentation tank. Combined with the results of nitrogen and EPS changes, it can be seen that the PN/

PS decline will have a certain degree of influence on the nitrification reaction. The denitrification mechanism of proteins can be used as an electrophilic-nucleophilic interaction between proteins and nitrogen atoms in ammonia nitrogen. Since proteins are generally positively charged, proteins appear as electrophiles, whereas nitrogen atoms in ammonia nitrogen contain lone electrons [24]. When HRT decreased to 6 h, the more PS covering the PN surface result from decrease PN/PS, thereby reducing the possibility of electrophilic-nucleophilic action, which is detrimental to the nitrification reaction.

3.4. Effect of HRT on DHA

Dehydrogenase is a kind of intracellular enzyme which related to oxidative phosphorylation of organics [8]. DHA is frequently measured due to it not only reveals microbial degrading ability of pollutant but also reflects the degradation of organics and the operation effect of treatment system [13]. Therefore, in order to evaluate the effect of HRT on activated sludge, DHA of activated sludge at Run 1~Run 3 was studied. Fig. 5 shows that the DHA of activated sludge increase with the decrease of HRT. This phenomenon may result from the increase in organic matter due to the increase of influent. Combined with the previous sludge concentration analysis, the organic load increased greatly because the influent volume increased while the sludge concentration decreased. The trend of DHA rising with the increase of organic load is obviously. The same rule can also be found in the changes in DHA at all levels of Run 1~Run 3. The increased organic loading did not increase the organic concentration of the effluent, mainly due to the increased in DHA and the uniformity of the load in the multi-level A/O reactor.

3.5. Analysis of denitrification in multi-level A/O coupled biofilm reactor

To better understand the nitrogen removal during the coupling process, the change of COD, $\text{NH}_4^+\text{-N}$ and TN along the reactor and the removal of each chamber under different

working conditions were detected during the stabilization period. The results are shown in Fig. 6. It can be seen from Fig. 6a that the main removal zones of organic matter in all working conditions are anoxic zones, in which the removal of organic matter in Run 1~Run 3 anoxic zones was 32.53, 48.09 and 66.64 g d⁻¹, accounting for 74.00%, 85.83% and 94.93% of the total organic matter removal. The more organic matter is used for denitrification and anaerobic phosphorus release, the higher the effective utilization of the carbon source. In

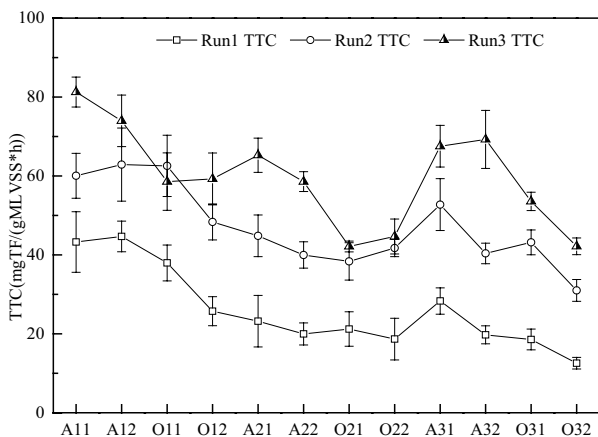


Fig. 5. DHA under various working conditions along the reactor.

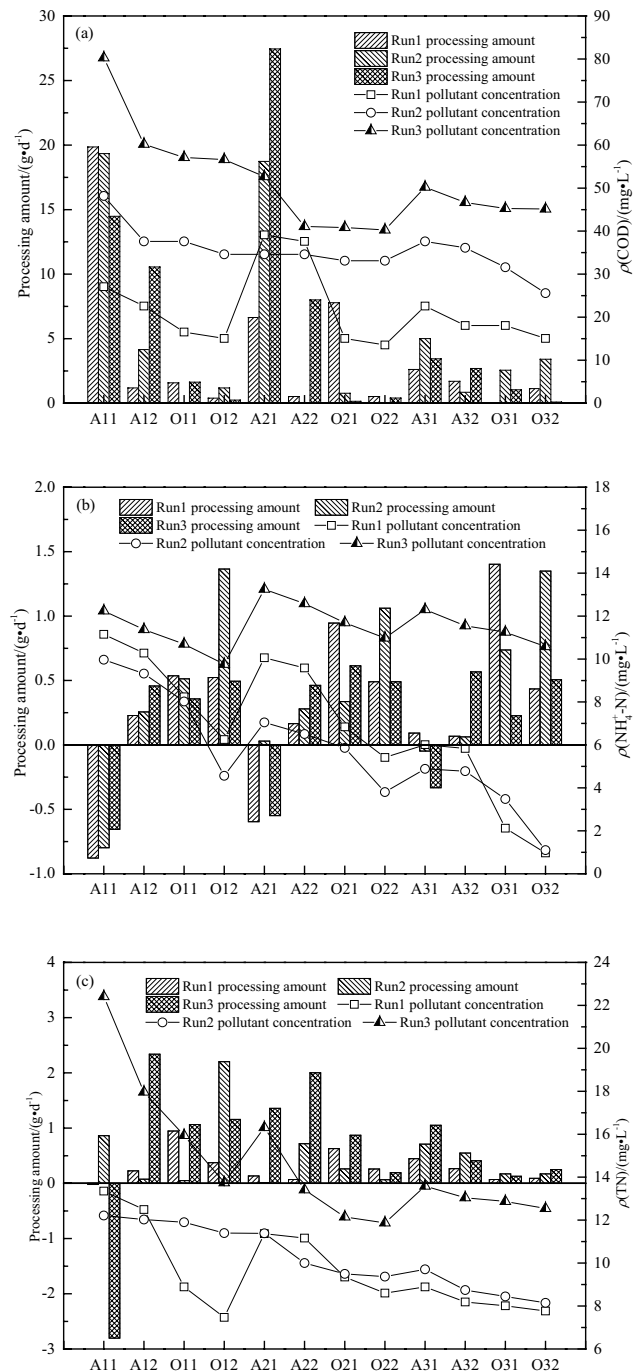


Fig. 6. Changes in COD, $\text{NH}_4^+\text{-N}$ and TN along the process and removal of each chamber under different working conditions.

terms of proportion, Run 3 seems to have an advantage in carbon source allocation. However, due to the large water inflow, Run 3 enters the aerobic zone with higher organic concentrations than Run 1 and Run 2. At the same time, less organic matter enters the aerobic zone and promotes the growth and reproduction of nitrifying bacteria [1]. Therefore, the carbon source advantage of Run 3 did not exist.

Unlike organic matter, the removal of ammonia nitrogen occurs mainly in the aerobic zones. The removal of $\text{NH}_4^+\text{-N}$ in Run 1~Run 3 was 3.41, 5.14 and 2.64 g d^{-1} . In Run 3, the total amount of influent ammonia nitrogen increased, while the amount of removal decreased relative to Run 2, indicating that ammonia nitrogen was accumulated in Run 3. It is also found in Fig. 6b that $\text{NH}_4^+\text{-N}$ was released in the first anoxic zone of the three working conditions. It is due to the ammoniation of organic nitrogen. The concentration of $\text{NH}_4^+\text{-N}$ in each level of Run 1~Run 3 was gradually decreased while Run 3 had a smaller decrease of $\text{NH}_4^+\text{-N}$ concentration in each aerobic zone. The reason was that the reduction of HRT causes the increase of influent load, and the insufficient residence time of the aerobic zone makes the nitrification reaction difficult to complete. After that, the inhibition caused by the accumulation of $\text{NH}_4^+\text{-N}$ and the decrease of the total amount of biomass in the reactor under the action of hydraulic force ultimately lead to difficulty in improving the $\text{NH}_4^+\text{-N}$ treatment effect of Run 3. It can be seen from Fig. 6c that the concentration of TN in the chamber A₁₁ of Run 3 was much higher than that in Run 1 and Run 2. The main reason was $\text{NH}_4^+\text{-N}$ carried by the sludge reflux in Run 3. Moreover, when calculated the amount of pollutants in the chambers, the pollutants carried by the sludge reflux were not included and it only participate in the flow calculation. The total amount of TN removed in the anoxic zones of Run 1~Run 3 was 1.12, 2.91 and 4.35 g d^{-1} , and that in aerobic zones was 2.37, 2.92 and 3.66 g d^{-1} . As the HRT increased, the proportion of TN removal in the aerobic zone increased. The reason was the MLSS also increased when the HRT increased. Under the same aeration conditions, longer HRTs were more likely to form the environment required for denitrification in aerobic zones. Usually, the TN that removal in aerobic zones is defined as SND. Although it can improve the nitrogen removal efficiency of the system, an excessively high proportion of SND means that the denitrification capacity of the anoxic zones was not fully utilized. Whether in the anoxic zones or the aerobic zones, the removal of TN was the highest in Run 3. However, due to the influent load, low temperature, short nitrification time, etc., the $\text{NH}_4^+\text{-N}$ in the system was accumulated, that make the HRT used by Run 3 not accepted by actual project.

4. Conclusions

This study used the three-level A/O coupled biofilm process to treat simulated low-temperature and low C/N municipal wastewater. The operation studies of three operating conditions of HRT of 12, 8 and 6 h were carried out under the same conditions. The results are as follows.

- HRT had a significant effect on the denitrification of multi-level A/O biofilm reactor at the temperature of $10^\circ\text{C} \pm 1^\circ\text{C}$. When the HRT was 12 h, the denitrification

effect was unstable due to insufficient supply of denitrifying carbon source; while HRT was 6 h, the nitrification reaction would be seriously affected.

- When the HRT were 12 and 8 h, the sludge showed slight viscous expansion, while at HRT of 6 h, the sludge viscous expansion was severe. The EPS in biofilm and active sludge increased with the decreased of HRT, especially the contents of polysaccharides. The excess polysaccharide affected the progress of the nitrification reaction. The increase of DHA with the organic load guaranteed the removal of organic matter by the reactor.
- Through the analysis along the path, it was found that the denitrification in the anoxic zone and the SND in the aerobic zone were crucial for the denitrification of the coupling process. When the HRT was 8 h, the functions of these two aspects were utilized reasonably, so that the nitrogen removal effect was better.

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