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Study on sludge characteristics of modified A²/O activated sludge-biofilm coupling process at low temperature

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ABSTRACT

To investigate the correlation between the pollutant removal efficiency of the modified anaerobic/anoxic/oxic (A²/O) activated sludge biofilm coupling process at low temperature and sludge characteristics, experimental studies were carried out. The study used the A²/O process coupling with biofilm, and the biofilm carrier was placed in the reactor, and the test temperature was controlled at 13°C ± 1°C. Through 180 d of operation, the average removal rates of chemical oxygen demand (COD), ammonia nitrogen (NH_4^+–N), total nitrogen (TN), total phosphorus (TP) were 86.37%, 92.09%, 74.85%, and 92.76%. The modified A²/O activated sludge-biofilm coupling process could operate stably for 180 d under low temperature condition and had excellent treatment performance. The results of the study of the sludge characteristics of the system showed that the values of extracellular polymeric substances (EPS) content between compartments differed little for activated sludge and significantly for biofilm. Dehydrogenase activity values (DHA) remained similar along the course, but the activated sludge in the system had higher dehydrogenase activity. The research results of nitrification and denitrification rate of each of compartments showed that: O2 was the leading site where nitrification taken place, but the activated sludge in the O1 compartment had a more significant absorption capacity for dissolved oxygen (DO) than O2, the higher nitrification rate in the O1 compartment resulted in a higher NH_4^+–N removal rate in the O1 compartment.

Keywords: Sludge characteristics; Low temperature; Nitrification and denitrification rate; Microbial activity

1. Introduction

The activated sludge method and biofilm method are widely applies in sewage treatment. Both of them use microorganisms to remove pollutants in sewage. Due to global seasonal variations and geographic regions, sewage temperature can reduce to 8°C–15°C, even below 5°C, common in sewerage systems in temperate climates [1], most microorganisms survival are dependent on temperature, the optimum survival temperature is 20°C–35°C [2], decrease in temperature may lead to deterioration of physiological properties, decrease in microbial growth rate and microbial

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activity, altered microbial community structure and deterioration of sludge settling properties, this has a negative impact on the performance of wastewater treatment facilities [3]. Low temperature sewage significantly inhibits microbial activity, resulting in the inhibition of the metabolic action of microorganisms [4]. Some studies has shown that, when the sewage temperature at 5°C, removal rate of chemical oxygen demand (COD) by the system was 80% of that at room temperature [5]. Nitrifying bacteria are very sensitive to changes in temperature, and low temperature will greatly reduce their activity and nitrification rate, higher nitrification activity at 15°C–35°C, at temperature below 15°C, rapid decrease in nitrification rate, decreased by 50% at 12°C and 100% at 5°C. Other than that, the activity of denitrifying bacteria can also be limited by low temperature [2].

Biological nitrogen removal is usually accomplished in two steps. The first step is that under aerobic condition, oxidation of NH_4^+–N to NO_3^-–N and NO_2^-–N by autotrophic bacteria such as nitrifying bacteria (nitrification). The second step is under anoxic condition, reduction of NO_3^-–N and NO_2^-–N by heterotrophic bacteria such as denitrifying bacteria (denitrification) [6]. Heterotrophic bacteria such as denitrifying bacteria produce large amounts of high molecular weight material, which can enhance the adhesion to other bacteria, favorable to the accumulation of bacteria such as nitrifying bacteria in biofilms, nitrifying bacteria are slow-growing bacteria, usually produces a small number of EPS [7]. In the process of removal of organic matter, dehydrogenation is a crucial chemical and biochemical process to achieve the enzymatic reduction of organic pollutants from wastewater catalyzed by microorganisms, low temperature slow down protein synthesis, resulted in enzyme activity decreased [8], dehydrogenase as a critical catalyst in the process of substrate dehydrogenation, the level of activity is related to the degradation rate of organic matter and the overall treatment effect [9]. Oxygen is required for the simultaneous removal of both nitrogen and carbon, the amount of oxygen uptake by unit sludge in unit time, which can reflect the effect of metabolic intensity and a hydraulic load of microorganisms on substrate utilization, the specific oxygen consumption rate (SOUR) is used as an effective method and means to analyze microbial activity [10].

Integrated fixed film activated sludge (IFAS) has first proposed in 1994. IFAS process has been widely used in developing countries to treat some small-scale wastewater treatment such as municipal wastewater and industrial wastewater [11]. IFAS process can increase the number of various types of bacteria in the system by dosing biofilm carriers, the advantage of high numbers compensates the effect of low activity at low temperature. IFAS system can realize more distribution of nitrifying bacteria, denitrifying bacteria and other bacteria with long generation cycle and slow growth in biofilm carriers, phosphorus accumulating organisms (PAOs) and other bacteria with shorter generation cycles are more frequently distributed in suspended sludge, PAOs activity increased with the decrease in SRT, the conflict between nitrifying bacteria and phosphorus-polymerizing bacteria in terms of sludge age was resolved [12–14]. Researchers have studied the IFAS process extensively, studies tend to focus on the effect of different parameter condition on the IFAS process and its deformation process, this includes more energy-efficient operating methods, more efficient removal efficiency, etc. [15]. However, the relationship between the pollutant removal efficiency of IFAS systems and sludge characteristics had been less study, and minimal study had been conducted to reveal the role of sludge characterization indicators such as EPS, DHA, and SOUR in IFAS systems for pollutant removal in continuous flow systems.

A^2/O process was the most widely used process for treating municipal wastewater. This experiment was based on the conventional A^2/O process, dosing biofilm carriers, formed a suspended sludge coupling biofilm hybrid system, a standard IFAS-A^2/O process was developed. This experiment's design would guide the operation of the A^2/O activated sludge biofilm coupling process under low temperature condition. Detection of activated sludge and biofilm in different compartments of wastewater plants using nitrification rate, denitrification rate, DHA, SOUR indicators, understanding the activity of nitrifying bacteria, denitrifying bacteria, and heterotrophic bacteria, combined with the removal of pollutants from each of compartments, to identify the microbial activity in the different compartments of the reactor and the advantages of pollutant removal, make adjustments to the compartments in the process when appropriate, provide higher pollutant removal efficiency at low temperature for wastewater plants using the A^2/O activated sludge biofilm process as the treatment process. The primary purpose of this paper was to distinguish the sludge characteristics of other processes at low temperature and those of other modified A^2/O at room temperature. The analysis focused on the sludge characteristics of the modified A^2/O activated sludge-biofilm coupling process at low temperature. The analysis process included as fellows: (1) removal efficiency of COD, NH_4^+–N, total nitrogen (TN), and total phosphorus (TP); (2) variation pattern of COD, NH_4^+–N, TN, and TP between compartments; (3) nitrification and denitrification rates; (4) variation patterns of EPS, DHA, SOUR, and other indicators of suspended sludge and biofilm in each of compartments.

2. Materials and methods

2.1. Test device and control parameters

Fig. 1 is a diagram of the experimental device of the A^2/O coupling process. The main body of the process was made of Plexiglass panels. The size of the main body of the system was L × B × H = 60 cm × 20 cm × 40 cm, the total volume was 48 L, the practical volume was 42 L, divided into 4 compartments, which were anaerobic section A1 (Anaerobic1), hypoxic section A2 (Anoxic2), aerobic section O1, and aerobic section O2. The volume ratio was 3:3:4:4. The connection mode between the cells was water intake through the top opening and water intake through the bottom opening, the inlet water forms a "V" shaped path between the two compartments, complete contact of influent water with suspended sludge and biofilm carrier. A1 and A2 were mechanically stirred, there was a stirrer at the bottom of the tank, the aeration sand head was connected to a blower (ACO-308, HaiLi, China) to supply oxygen to the aerobic section. A rotameter was used to control. There

was a stirrer at the bottom of the tank, the aeration sand head was connected to a blower to supply oxygen to the aerobic section, and a rotameter was used to control the aeration volume (LZB-3WB, XiangJin, China). The effluent of the process flowed to the vertical flow sedimentation tank and was discharged after sedimentation. The return sludge was returned from the bottom of the sedimentation tank to the anaerobic section, and the nitrification liquid was returned from O2 to A2. Peristaltic pumps (BT300-2J, Longer Pump, China) controlled the fracture water inlet, excess sludge return, and nitrification liquid return. Reactor water inlet, excess sludge return, and nitrification liquid return were all controlled by peristaltic pumps. Biofilm filler was put into the A^2/O pool, the interior of the biofilm filler was filled with polyurethane sponge and pall rings, polyurethane sponges is a cube with side length of 3 cm, density of 16–17 kg/m^3, specific surface area of 3.8 × 10^5 m^2/m^3, bauer rings have a diameter of 25 mm, a height of 10 mm and a density of 0.95 g/cm^3, the number of polyurethane sponge blocks and bauer rings were both 5. The percentage of biofilm carriers internal filling was 30.5%. The filling ratio of the filler in each reactor compartments were 17.3%, 17.3%, 13.1%, and 13.1%. Using electric agitators (JJ-1A 160W, Zhengrong, China) in A1 and A2 to ensure that the activated sludge was in complete contact with the biofilm.

The test was carried out in a constant temperature control room, a chiller controlled the water temperature at 13°C ± 1°C, the inlet water flow was 84 L/d, hydraulic retention time (HRT) was 12 h, sludge retention time (SRT) was about 15 d, the stirring intensity was 220 rpm, dissolved oxygen concentration in A1 and A2 was controlled at 2.0–3.0 mg/L, the sludge reflux ratio was 100%, and the nitrification liquid reflux ratio was 200%. The initial dosage of inoculated sludge was held at around 4,000 mg/L.

2.2. Inoculation of sludge and test water quality

The activated sludge used in this experimental study was taken from a municipal sewage treatment plant in Changchun, China. The activated sludge was taken from the anoxic zone and aerobic zone of the sewage treatment plant and put into the reactor compartment of this research. The sludge concentration was 4,000 mg/L.

The raw water used in the experiment was prepared by using sodium acetate (CH_3COONa), starch, ammonium chloride (NH_4Cl), beef extract, peptone, and potassium dihydrogen phosphate (KH_2PO_4) to simulate urban sewage. The purity was analytically pure, the medicine was produced by Tianjin Guangfu Technology Development Co. Ltd. (China), and the specific water quality indicators are shown in Table 1.

2.3. Detection methods used in the test

The conventional water quality indicators COD, NH_4^+–N, TN, TP referred to standard methods (APHA 2007). Mixed liquor suspended solids (MLSS), mixed liquid volatile suspended solids (MLVSS) were measured by filter paperweight method, the measuring method of sludge volume index (SVI) was measured by cylinder method, the pH value was measured by a pH meter (LeiCi PHSJ-4A). DO was detected by portable dissolved oxygen meter from a DO meter (WTW Oxi3310, Germany), the NO_3^-–N and NO_2^-–N were detected by ion chromatography. The measurement method of EPS referred to previous studies [16]. The DHA was detected by using red tetrazolium (TTC) as the hydrogen acceptor [17].

Table 1
Test raw water quality

Water quality index	Concentration range	Average value
COD (mg/L)	141.50~302.50	222.00
TN (mg/L)	22.00~50.50	36.25
NH_4^+–N (mg/L)	24.47~38.39	31.43
TP (mg/L)	2.31~6.13	4.22
pH	6.98~7.53	7.13

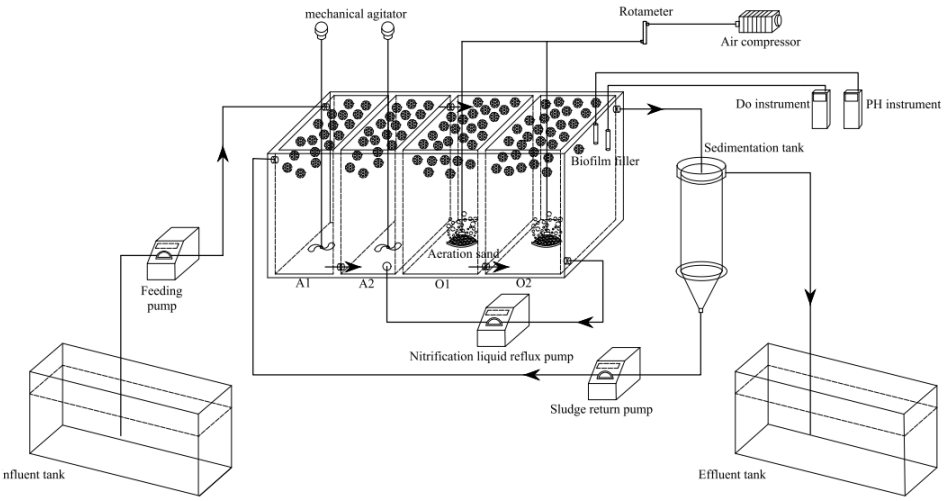

Fig. 1. A^2/O coupling process flow diagram.

The concentration of biofilm sludge on the flow-off ball packing was determined by the alkaline washing method. The carrier in the spheroid was dried and weighed, and then used the ultrasonic elution method to peel off and weight the biofilm on the packing. The difference between the two weighings were the quality of the biofilm on the test packing sample, divided by the quantity of the packing. It was the membrane biomass on a single polyurethane sponge or pall ring. Using this to calculated the total biomass of each area, divided it by the adequate volume of the site to get the biofilm sludge concentration in each of compartments [18].

Determination of aerobic nitrification rate: taken the biofilm and sludge mixture, added NH_4Cl solution, prepared the initial NH_4^+–N concentration of about 20 mg/L, the aeration device was turned on and the time was recorded. Sampling to determine NH_4^+–N, calculated NH_4^+–N reduction the amount "b", according to the relationship between the measured NH_4^+–N concentration and the sampling time, draw a regression curve, using the curve slope value "b" to indicated the speed of NH_4^+–N degradation, the mixed liquid sludge concentration MLSS, mg/L, and calculate out of the specific ammonia uptake rate (SAUR), mg/(g·MLSS/h).

$$\text{SAUR} = \frac{b}{\text{MLSS}} \tag{1}$$

Anaerobic/anoxic denitrification rate determination: taken the anaerobic and anoxic zone mixture, adding a certain amount of potassium nitrate (KNO_3) and propylene thiourea (ATU) to inhibit the activity of nitrosating bacteria, thereby inhibiting the activity of activated sludge Nitration reaction, controlled the initial concentration of NO_3^-–N in the mixed solution at 35 mg/L. Determine the concentration of NO_3^-–N in the mixed solution to obtain the curve of the change of NO_3^-–N concentration over time. Using the slope "*r*" of the curve to indicate the value of the denitrification rate. Determine the MLSS value of the mixed solution, which can be obtained by the formula-specific nitrate uptake rate (SNUR) value, mg/(g·MLSS/h).

$$\text{SNUR} = \frac{r}{\text{MLSS}} \tag{2}$$

The SOUR taking out a drifting ball in the O1 and O2 compartments by randomly, respectively, selecting a sponge filler and pall ring from the drifting ball and putting it into the SOUR bottle. Constant volume with the mixture to 250 mL, use an air pump to aerate the reaction flask for 2 min, after the DO concentration of the mixture in the flask was saturated, quickly plugged the rubber stopper and ensured that the medicine completely did not pass the dissolved oxygen meter probe, turned on the magnetic stirrer to ensure that the mixture was evenly mixed to prevent it from appearing activated sludge separation appears, the test was carried out at low temperature, and the dissolved oxygen meter was set to read a reading every 10 s automatically, and the trial was ended after the DO reading in the bottle dropped to 0.1 mg/L [19].

3. Result in analysis and discussion

3.1. Analysis of the removal effect of main pollutants

3.1.1. Removal of COD

The COD removal effect of the A²/O coupling process at low temperature is shown in Fig. 2a. After nearly 180 d of continuous operation, the average influent COD concentration of the process was 220 mg/L, the effluent concentration was below 50 mg/L, and the average COD removal rate reached 86.37%. The modified A²/O coupling process had a better removal effect on COD at low temperature condition. The carbon source was fully absorbed and utilized by the heterotrophic microorganisms in the activated sludge and biofilm filler. The result showed that the process contained many heterotrophic organisms in the early stages of operation.

3.1.2. Removal of NH_4^+–N and TN

The removal effect of NH_4^+–N and TN in the A²/O coupling process at low temperature is shown in Fig. 2b and c. The average influent concentration of NH_4^+–N in the process was 31.43 mg/L, and the effluent NH_4^+–N concentration in the early stage of operation fluctuated wildly, and the removal efficiency was low. After the process ran stably, the average NH_4^+–N effluent concentration was 0.23 mg/L, the removal rate was higher than 95.0%, and the NH_4^+–N removal effect was significant. In the first 30 d of process operation, the removal rate of NH_4^+–N fluctuated wildly. The analysis reason was that when the temperature was lower than 15°C, the aerobic compartment nitrification reaction would be significantly inhibited [20], which led to the removal efficiency of NH_4^+–N was low. After the process had been running for 30 d, all kinds of nitrogen-removing bacterial flora gradually adapted to the low temperature environment, and the biofilm cultured on the filler was steadily completed and began to play a role in removing pollutants. After the process was stabilized, a large number of nitrifying bacteria were enriched in the biofilm, which promoted the removal of NH_4^+–N at low temperature, and the removal rate remained above 80%.

The average TN concentration of the process influent was 37 mg/L, the average concentration of effluent was about 10 mg/L, and the average TN removal rate reached 74.85%. In the first 70 d of process operation, the TN removal rate fluctuated wildly. From the 70th day to the 180th day, the TN removal rate became stable, with an average removal rate of 80.77%. It could be seen that after 30 d of enrichment at low temperature, the nitrifying bacteria began to remove NH_4^+–N stably. After another 40 d, the denitrifying bacteria in the process could stably remove NO_3^-–N and NO_2^-–N. In addition to adapting to the low temperature environment, denitrifying bacteria also need to regulate and adjust to the carbon source, electron acceptors, and other resources in the process.

The removal effect of TN was better, indicating that the nitrification and denitrification reactions were carried out relatively adequately, and the nitrification and denitrification reactions could be fully completed at low temperature. It reflected that the device structure of the A²/O coupling

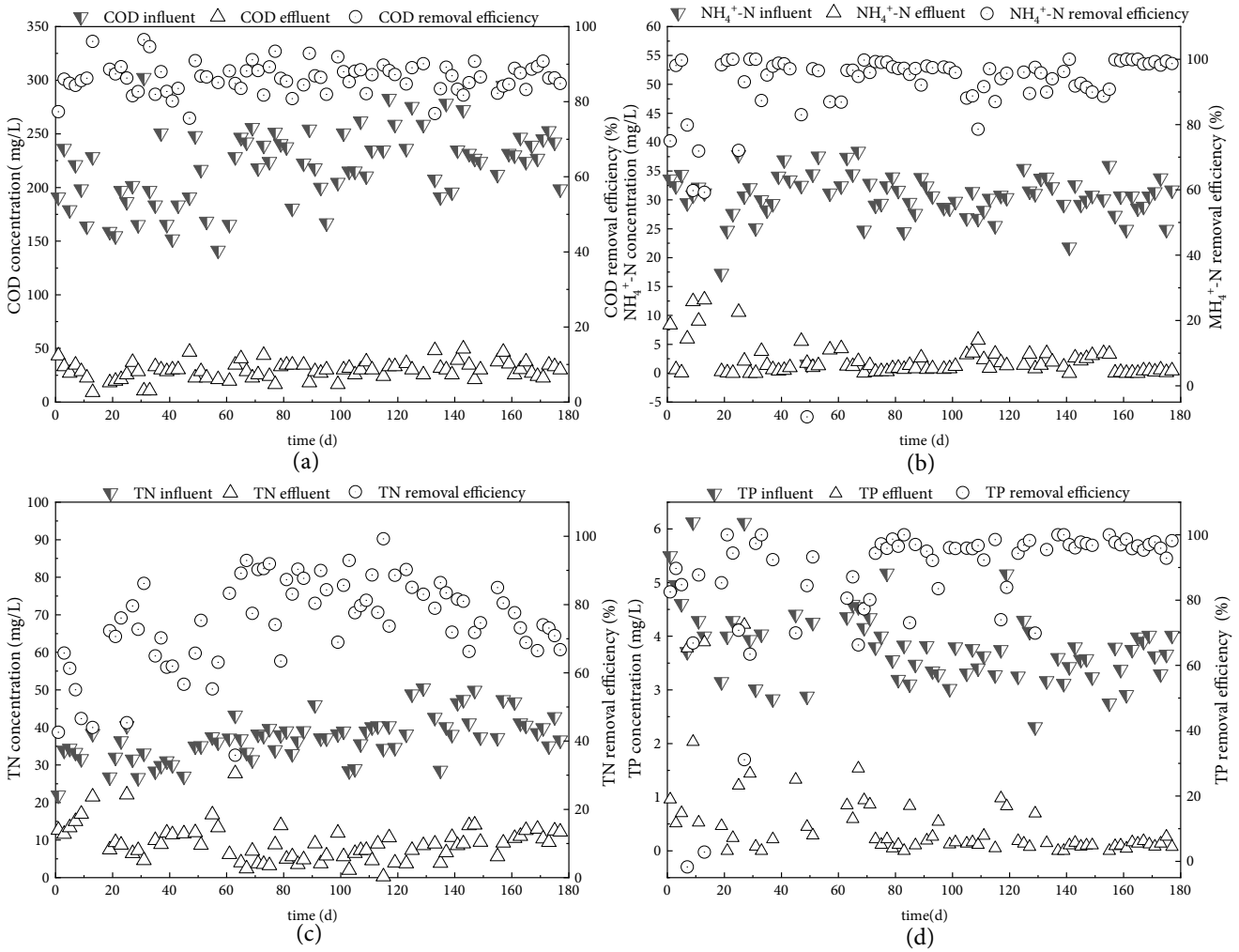

Fig. 2. The influent concentration, effluent concentration, and removal rate of COD, NH_4^+–N, TN, and TP during 180 d of low temperature operation of the modified A²/O activated sludge-biofilm coupling process. (a) COD, (b) NH_4^+–N, (c) TN, and (d) TP.

process and the biofilm had a better promotion effect on the removal of process nitrogen. The low temperature advantage nitrifying bacteria on the filler undergoes nitrification in the aerobic section, which converted NH_4^+–N into NO_3^-–N, NO_2^-–N, and then flowed back to the anoxic tank through 200% of the nitrification solution to supplement a large number of electron acceptors for the denitrification reaction process. Due to the mass transfer resistance of the biofilm on the filler, DO was unevenly distributed on the biofilm. The biofilm formed an anaerobic, anoxic, and aerobic environment from the inside to the outside [21], this created a suitable living environment for nitrifying bacteria and denitrifying bacteria, it provided the possibility of simultaneous nitrification and denitrification (SND) on the biofilm.

3.1.3. Removal of TP

Fig. 2d shows the removal of TP in the modified A²/O coupling process at low temperature. The average TP concentration of the process influent was 6.13 mg/L, and the TP effluent concentration fluctuated significantly in the 70 d before process operation. From the 70th to the 180th day of process operation, the TP effluent concentration remained stable at about 0.43 mg/L, and the removal rate was 92.76%. In the early stage of process operation, on the one hand, PAOs in the inoculated sludge need a period of time to adapt to the new environment. The nitrification reaction products such as NO_3^-–N and NO_2^-–N inhibited the anaerobic phosphorus release process [22]. Therefore, after the denitrification effect of the process became better, the NO_3^-–N and NO_2^-–N in the water were decreased, and the inhibitory effect would be reduced. The treatment effect of total phosphorus became better immediately. On the other hand, in the same process, the activated sludge and the biofilm competed with each other for carbon source, space, and DO and restricted each other's growth. After a period of operation, the competition of the two biological phases reached an equilibrium point, and the competition relationship promoted the removal of pollutants. Through the first 70 d of operation, nitrifying bacteria and denitrifying bacteria with long generation time were enriched on biofilm filler in the A²/O coupling process [23], under the premise of not affecting the efficient removal of nitrogen,

the process ability to remove phosphorus was simultaneously strengthened. By adding fillers, the contradiction between the long generation cycle time of nitrifying bacteria and the need for long sludge age and the phosphorus removal process to require a shorter SRT to discharge phosphorus rich sludge [24], the process continued to maintain stable operation for more than 100 d.

3.2. Pollutant change law in the A²/O coupling process

The pattern of pollutant removal in the modified A²/O coupling system at low temperature was selected from the most representative data through multiple tests during the system stabilization phase. The removal of pollutant law in the A²/O coupling process at low temperature condition is shown in Fig. 3. The influent COD was significantly reduced in A1. Analysis of the reason was the sludge in A1 and sludge returning from O2 urgently need to be supplemented by organic matter. After entering A1, the influent COD was quickly absorbed by the activated sludge and heterotrophic microorganisms on the biofilm, which would be used for the subsequent denitrification and anaerobic phosphorus release reaction. As shown in Fig. 3, with the increased of phosphorus concentration and no accumulation of NO_3^-–N and NO_2^-–N in A1, heterotrophic microorganisms such as phosphorus polymerizing bacteria and denitrifying bacteria consume carbon sources, conducted anaerobic phosphorus release and denitrification reaction process. Among A1 and A2, the concentration of NH_4^+–N decreased, it was probably because of that under anaerobic and anoxic condition, the anammox bacteria in the biofilm fillers process used part of NH_4^+–N and the returned NO_3^-–N and NO_2^-–N as electron acceptors to cause anaerobic ammonia oxidation [25]. Moreover, since the TP concentration of A1 anaerobic release of phosphorus increased, it began to decrease at A2, speculated that reason was that the adsorption and assimilation of microorganisms and the dilution effect of the nitrification solution back to A2. Moreover, the aggregated denitrifying phosphate accumulating organisms (DPAOs) on the biofilm filler used their stored poly-β-hydroxybutyrate (PHB) as an electron donor [26], the internal refluxing NO_3^-–N acts as an electron acceptor for phosphorus absorption, the removal of phosphorus in the aerobic phosphorus absorption process combined with PAOs was completed after the aerobic stage [21].

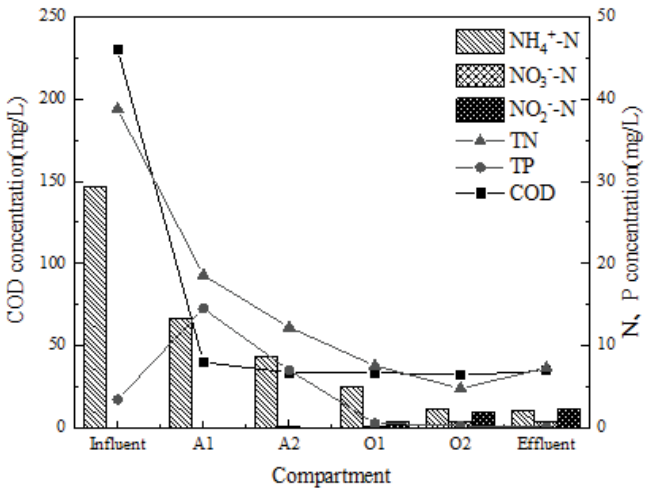

Fig. 3. Improved pollutant change rule of the A²/O coupling process.

From A1 to O2 in the process, the trend of TN and NH_4^+–N removal law was consistent, TN and the sum of NH_4^+–N, NO_3^-–N, NO_2^-–N shown in the figure had a difference, the difference was gradually decreasing, it showed that the organic nitrogen was converted into NH_4^+–N through the amination reaction, and with the original NH_4^+–N in the water, the nitrification reaction occurred in O1 and O2 to produce NO_3^-–N and NO_2^-–N. The process detected low concentrations of NO_3^-–N and NO_2^-–N, and the content of NO_2^-–N in O1 and O2 was higher than NO_3^-–N. The low NO_3^-–N content could be speculated that the NO_3^-–N and NO_2^-–N produced by the nitrification reaction were used by the denitrifying bacteria on the biofilm filler to cause denitrification, simultaneous nitrification, and denitrification reactions in O1 and O2. Moreover, the denitrifying bacteria in the process only used NO_3^-–N as the electron acceptor, and a short range nitrification and denitrification reaction occurred, which led to the accumulation of NO_2^-–N. Accumulation of NO_2^-–N in the O1 and O2 compartments, it can be interpreted that at low temperature environment, activities of AOB and NOB are simultaneously affected by low temperature, activity of AOB is higher than NOB, resulted in the accumulation of NO_2^-–N caused by low temperature [27,28].

3.3. Analysis of nitrogen removal performance of coupling process at the low temperature condition

3.3.1. Analysis of denitrification rate

The denitrification rates of A1 and A2 and the nitrification rates of O1 and O2 in the modified A²/O coupling system were both tested in the stable operation stage and selected the most representative data to illustrate. The denitrification rates of A1 and A2 in the A²/O coupling process is shown in Fig. 4. The correlation R^2 values of the denitrification rate curved both exceed 97%, indicating that the NO_3^-–N concentrations and time change had a reasonable correlation. The denitrification rate curves of A1 and A2 were obviously divided into three stages. The denitrification rate values of the three stages of A1 were 14.70, 4.49, 3.03 mg/L h, the denitrification rate values of the three stages of A2 were 11.38, 4.39, 2.51 mg/L h, the slope values of the three stages in the two compartments all gradually decreased, indicating that the degradation rate of NO_3^-–N is gradually slowing down. To analyze the reason was that the first stage mainly adsorption of microorganisms. Part of the NO_3^-–N was adsorbed by microorganisms, and the rest part of NO_3^-–N was removed by denitrifying bacteria, which used easily degradable soluble organic matter in water as the carbon resource through nitrification reaction. In the second stage, the denitrification rate decreased, indicating that the adsorption of microorganisms was weakened, and it started to use there own adsorbed NO_3^-–N, carbon sources, and non-degradable organic matter in water carbon

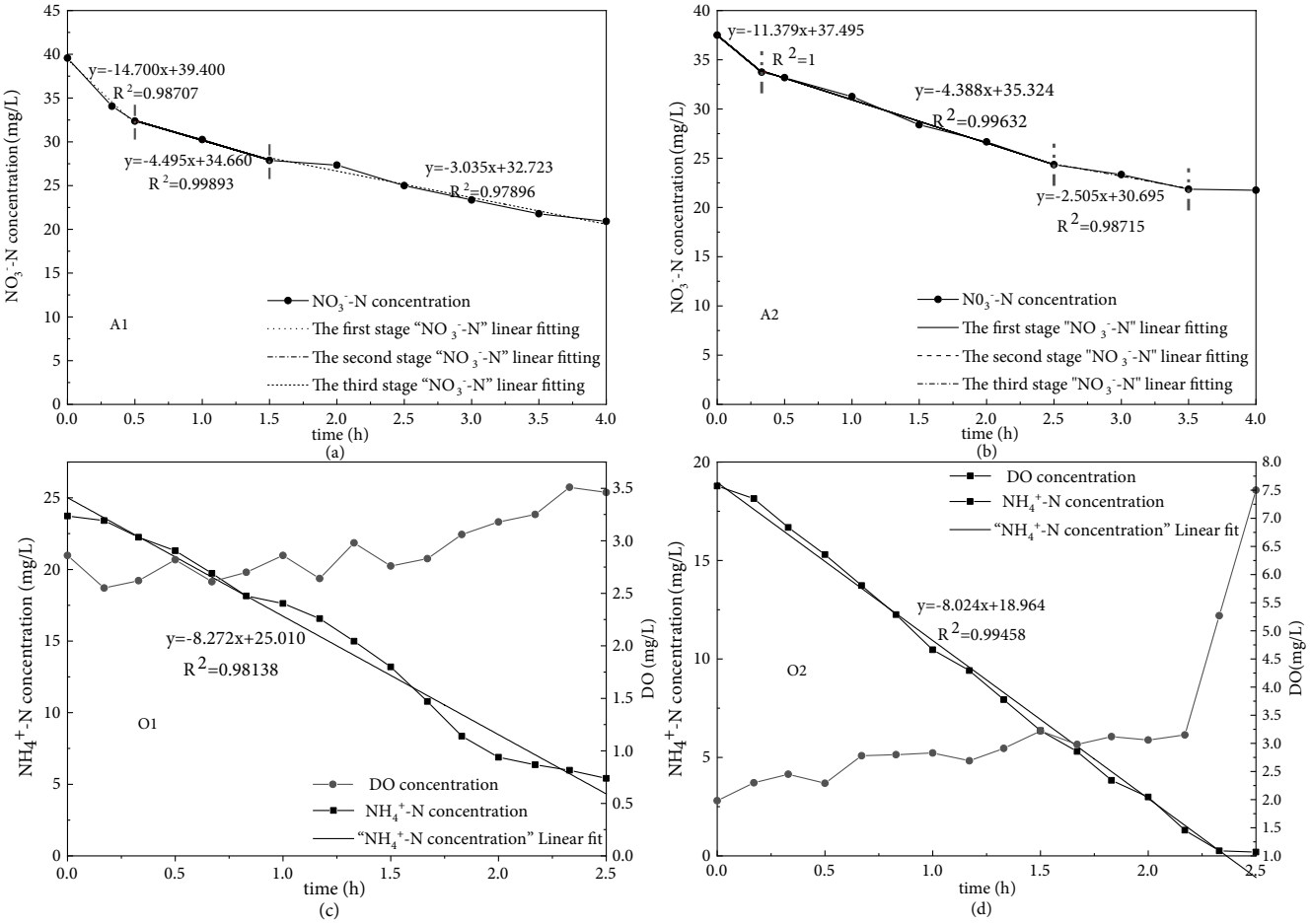

Fig. 4. Modified A²/O activated sludge-biofilm coupling process denitrification rate and nitrification rate. (a) A1 denitrification rate, (b) A2 denitrification rate, (c) O1 nitrification rate, and (d) O2 nitrification rate.

Table 2
Table of specific nitrification and specific denitrification rates of each of compartments

Nitrification/denitrification rate (mg/L h)	A1			A2			O1	O2
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3		
	14.70	4.50	3.04	11.38	4.39	2.51	8.27	8.02
Average	7.41			6.09			8.27	8.02
MLSS (g/L)	4.91			4.61			6.93	7.65
SAUR/SNUR (mg/(g MLSS/h))	1.51			1.32			1.19	1.05

sources to carry out denitrification reactions. In the third stage, the denitrification rate continued to decrease, after the second stage of the reaction process, the carbon source adsorbed by the microorganism itself was exhausted and began to use its cell material as a carbon source for endogenous denitrification reaction [29].

From the nitrification rate values of the above three stages of A1 and A2, it could be concluded that the denitrification rate values of A1 were overall higher than A2. The analysis reason was combined with the sludge concentration of the process along the way in Table 2, the activated sludge concentration and filler biomass of A1 were high, according to the theory that the more denitrifying bacteria, the denitrification rate higher [30], the number of denitrifying bacteria contained in A1 was high. In the graph of pollutant change along the course in Fig. 3, it was found that the influent COD first entered A1 and then A2, then A1 would use the carbon source in the water in preference to A2, there were more available electron donors in A1. It could be found from the figure that there was no NO_2^-–N in A1 and A2, indicating that after a hydraulic retention time treatment process, the process could complete the

treatment of NO_3^-–N, moreover, in Fig. 3, the variation range of total nitrogen in the A1 compartment was also the largest. It could be obtained by calculation in Table 2, the values of SNUR were 1.51 mg/(g·MLSS/h) for A1 and 1.32 mg/(g·MLSS/h) for A2 in the modified A²/O coupling process, indicating that the activated sludge and biofilm in A1 removed more NO_3^-–N per unit time per unit concentration.

3.3.2. Nitrification rate analysis

The nitrification rate of O1 and O2 in the aerobic section of the process is shown in Fig. 4. As the reaction proceeded, the DO concentration of O1 gradually increased, and the DO concentration of O2 increased sharply at 2.25 h. The degradation of NH_4^+–N in the process was completed, and the nitration reaction was over.

The figure shows that the R^2 values of the NH_4^+–N concentration variation curves of both aerobic sections are greater than 98%, which proved a good correlation. The nitrification rate values of O1 and O2 were 8.272 and 8.024 mg/L h. Presumably, the reason was that during the operation of the improved A²/O coupling process, the O1 influent came from the oxygen deficient environment in A2, the O2 influent came from the aerobic background in O1, high concentration of NH_4^+–N into O1, O2 continues to process the remaining NH_4^+–N in O1, the substrate concentration was relatively low, there was a fairly sufficient substrate concentration in O1, nitrifying bacteria had vigorous metabolic activities and multiply, the nitrification rate of O1 was higher. It was worth noting here that the total sludge concentration was 6,930 mg/L for O1 and 7,650 mg/L for O2. And the O1 SAUR was 1.19 mg/(g·MLSS)/h, O2 SAUR was 1.05 mg/(g·MLSS)/h, which showed that the activated sludge and biofilm in O1 remove more NH_4^+–N per unit time per unit concentration.

3.4. Sludge characteristics of the coupling process

3.4.1. Analysis of the characteristics of activated sludge and biofilm in the system

The data of activated sludge concentration and biomass of polyurethane sponge and bauer ring, EPS, DHA, and SOUR in the modified A²/O coupling system were obtained by averaging several measurements during the stable operation stage of the system to illustrate the sludge characteristics of the coupling process, the changing trend of the sludge concentration in the modified A²/O coupling process is shown in Table 3. The sludge concentration of activated sludge and biofilm filler were consistent, and both were O2 > O1 > A1 > A2. A small amount of activated sludge flowed in the direction of the water flow and finally sailed to the back O1 and O2, and O1 and O2 were the main sites where nitrification occurred in the activated sludge process and the biofilm filler process, among them, autotrophic microorganisms such as nitrifying bacteria were easier to grow and multiply and produce metabolites than the heterotrophic microorganisms in A1 and A2. The percentage of sludge concentration of biofilm packing system in the total sludge concentration of each compartment was O1, O2, A1, and A2 from the largest to the smallest; combining with the denitrification rate and nitrification rate, it could be seen that the denitrification rate of A1 was higher and the nitrification rate of O1 was higher, it showed that in the modified A²/O coupling process, the biofilm process had a significant impact on the progress of denitrification and nitrification in four compartments, and the correlation was good. Increasing the biofilm sludge ratio in each compartment to the total sludge concentration in that compartment was helpful to weaken the influence of low temperature on microbial activity. The sludge in the biofilm filler process was attached to the polyurethane sponge and the pall rings. Most of the activated sludge was attached to the polyurethane sponge, and the pall rings occupied only a tiny part. Analyzed the cause, the polyurethane filler had a high porosity, which could effectively promote the curing process of microorganisms, so that microorganisms could be efficiently and stably attached to the surface of polyurethane sponge, the function of the pall rings was to provide mass transfer channels for DO, pollutants, etc. For the microorganisms on the polyurethane, filler to fully absorb and utilized [31]. The average SVI value of the four compartments of the modified A²/O coupling process was 169.76 mL/g. The effluent quality of the process in the stable stage was not affected by the settling sludge performance. It was speculated that the biofilm filler process played a particular role in promoting the removal of pollutants.

3.4.2. Analysis of EPS

Low temperature condition were impacted on the sedimentation performance and microbial activity of activated sludge. Moreover, EPS was also greatly affected by low temperature. At low temperature, the modified A²/O coupling

Table 3
Table of the average concentration of sludge in the sludge process and biofilm process in the modified A²/O coupling process

Sludge concentration (mg/L)	Compartment				Return sludge (mg/L)
	A1	A2	O1	O2	
Activated sludge	3,845.15 ± 202.20	3,633.64 ± 193.65	3,897.29 ± 201.85	4,533.51 ± 234.65	7,010.85 ± 355.56
Polyurethane sponge	928.54 ± 51.47	844.35 ± 48.24	2,730.72 ± 138.51	2,840.13 ± 145.00	–
Ball ring	135.12 ± 6.75	133.98 ± 7.25	303.67 ± 15.95	272.04 ± 14.53	–
Total	4,908.81 ± 260.42	4,611.97 ± 249.14	6,931.68 ± 356.31	7,645.68 ± 394.18	7,010.85 ± 355.56
SVI (mL/g)	178.12	165.22	160.99	174.68	114.00

process with packed biofilm and activated sludge generated more extracellular polymers than the conventional A^2/O process under the same condition. And EPS could assist microorganisms in absorbing nutrients from the external environment when nutrients were sufficient. In Fig. 5, the EPS content in the activated sludge process in the four compartments was not much different. In the A2 and O2 compartments, the EPS content of the biofilm and activated sludge were quite different. The microorganisms in the biofilm filler in A1 secrete fewer EPS, this showed that there were fewer types and numbers of microorganisms. The content of EPS increased rapidly in A2, and EPS has the function of acting like a protective layer against changes in the external environment [32], first of all, under anaerobic environment condition, the reflux of O2 nitrification solution and NO_2^-–N were toxic substances [33], which promoted the secretion of more EPS by microorganisms. Another reason is that microorganisms are less active at low temperature, organic carbon in sewage cannot be fully utilized, resulting in the conversion of some compounds to polysaccharides, generated a large amount of EPS [34]. O1 was the place for the nitrification and denitrification reactions of the biofilm process. Microorganisms fully utilized the EPS after adsorbing nutrients, resulting in less EPS content. Higher EPS content values were detected in biofilm carriers in O2, the structure of EPS provided bacteria with a larger surface area, improving the adsorption capacity of biofilm, it is more conducive to the adsorption and removal of colloidal particles and suspended substances.

3.4.3. Analysis of DHA

The variation of DHA along the way in the modified A^2/O coupling process is shown in Fig. 6, the activated sludge dehydrogenase activity values for the four compartments A1, A2, O1, and O2 were maintained at a high level, were 43.83, 51.17, 41.13, and 43.70 mg/g·MLVSS, respectively, consistent across all four compartments. The DHA value of activated sludge was generally higher than biofilm. The reason was that activated sludge had a larger surface area to contact with organic pollutants than biofilm. Therefore, the removal of organic pollutants was mainly through the activated sludge process. Biofilm was more to assist activated sludge in removing nitrogen at low temperature, dehydrogenase obtains energy from microorganisms to degrade organic pollutants, activates unique hydrogen ions, after proper hydrogen acceptor removed hydrogen ion, the original impurities were also removed [9], it showed that the modified A^2/O coupling process had formed an apparent pollutant removal function partition phenomenon for organic pollutants and nitrogen during the 180 d operation at low temperature.

3.4.4. Analysis of SOUR

The metabolism of microorganisms and the degradation of substrates require oxygen consumption, SOUR characterize the metabolic activity of activated sludge microorganisms [35]. The oxygen consumption rate along the modified A^2/O coupling process is shown in Fig. 7. The oxygen uptake rate (OUR) values of O1 and O2 were 67.36 and 35.32 $mgO_2/L·h$, and the sludge concentration of O1 and O2 were 3,419.50 and 3,480.87 mg/L, the SOUR values of O1 and O2 were 19.73 and 9.72 $mgO_2/g·MLSS·h$. Affected by different substrate concentration values, the available flora formed in O1 and O2 were diverse. In Fig. 5, it could be seen that NH_4^+–N was reduced 3.67 mg/L from A2 to O1, O1 to O2 decreased by 2.77 mg/L, O1 had a higher substrate concentration, in O1, nitrifying bacteria preferentially used oxygen to react with NH_4^+–N, produce reaction products NO_3^-–N, NO_2^-–N, after a part of NO_3^-–N and NO_2^-–N reacted with O1 internal denitrifying bacteria, the remaining NO_3^-–N and NO_2^-–N flow to O2, the reaction products NO_3^-–N and NO_2^-–N denitrify on the O2 biofilm, the remaining NH_4^+–N in O1 used oxygen to undergo nitration reaction in O2. Therefore, the SOUR value of O1 was higher, the microbial activity in the activated sludge within O1 was higher.

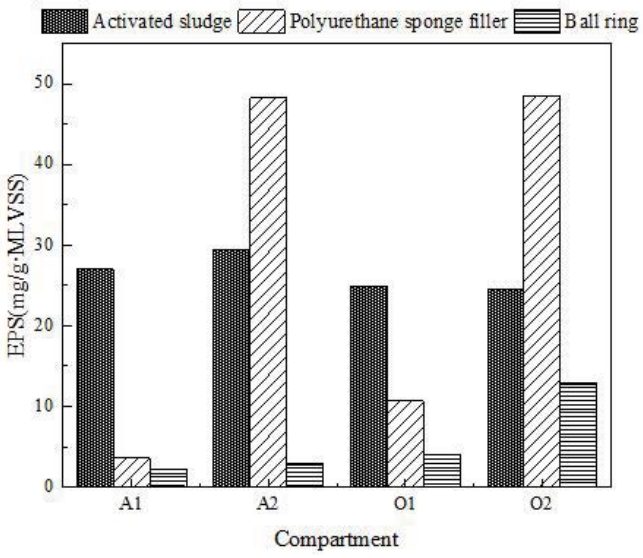

Fig. 5. EPS extracellular polymer changes along the process.

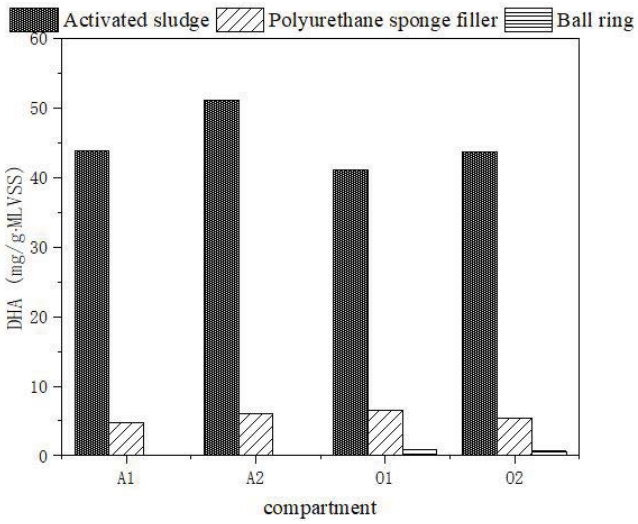

Fig. 6. Changes of DHA along the process.

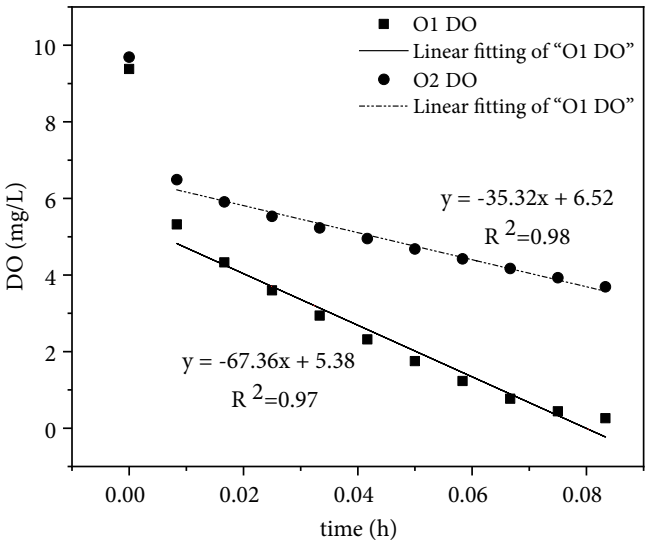

Fig. 7. Process O1, O2 activated sludge oxygen consumption rate OUR diagram.

In Fig. 4, the SAUR of O1 and O2 compartments could be found that O1 had a higher typical nitrification efficiency, in Fig. 3, it could be found the removal rate of NH_4^+–N in O1 was 55.4%, and the removal rate of NH_4^+–N in O2 was only 8%, it indicated that there was the same trend between the SOUR, SAUR, and NH_4^+–N removal rate between O1 and O2 compartments, and there was a correlation between the three.

3. Conclusion

Modified A^2/O activated sludge biofilm coupling process after 180 d of stable operation at 13°C ± 1°C low temperature, highly efficient removal of COD, N and P. Modified A^2/O activated sludge biofilm coupling process during stable operation at low temperature. High values of EPS content in biofilm carriers within A2 and O2 compartments. Dehydrogenase activity in the activated sludge of all four compartments was higher than that of the biofilm carrier. The values of SOUR, SAUR, and NH_4^+–N removal in the O1 compartment of the modified A^2/O activated sludge biofilm coupling process were higher than those in the O2 compartment, indicating that there was a correlation between pollutant removal efficiency and sludge characteristics.

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