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Optimization of C/N and carbon types on the denitrification biofilter for advanced wastewater treatment

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ABSTRACT

In this study, the optimization of carbon-nitrogen ratio (C/N) was investigated with methanol, sodium acetate and glucose as the external carbon source in two denitrification biofilter, which used the secondary effluent treated by triple-channel oxidation ditch in Handan. The Reactor 1 was filled with ceramsite and the Reactor 2 was filled with polyethylene polyhedral hollow ring. It showed that Reactor 1 and Reactor 2 were in stable operation by the method of artificial inoculation after 25 and 30 d, respectively. Results showed that nitrate could be effectively reduced over a wide range of C/Ns. In the case of methanol, sodium acetate and glucose as the additional carbon source, the release of N_2O was observed in the 1# and 2# columns, and when the C/N was increased to the optimum one it showed a sudden decline. And the N_2O emission factor was the smallest when methanol was used as the carbon source, and the largest when sodium acetate was used as the carbon source. And an optimum C/N of carbon source in this study was obtained. Moreover, the effluent TN of the two filters were under 15 mg/L, meeting the Grade 1A permissible discharge standard of China [18]. Through comprehensive comparison, sodium acetate was more suitable for external carbon source.

Keywords: Carbon source; C/N; Denitrification; Ceramsite; Polyethylene polyhedral hollow ring

1. Introduction

The water resources shortage has become an exacerbating issue around the world with the accelerated industrialization, urban growth and changed climatic conditions [1]. Among various measures of addressing this challenging problem, wastewater reclamation has received tremendous attention [2]. The reclaimed water from municipal wastewater treatment plant (WWTP) has been used in many ways [3]. For example, the utilization of reclaimed water in Beijing had reached 0.65 and 0.68 billion m^3 in 2009 and 2011, accounting for about 18% and 20% of its total annual water consumption, respectively [4]. In California, the reclaimed water had reached 8.64×10^8 m^3/d in 2000. In Japan, the reclaimed water had reached 0.63×10^8 m^3/d in the middle of 1900s [5]. In general, the quality of reclaimed water is affected by the available treatment processes in which many pollutants could not be thoroughly removed. In particular, WWTP effluent may still contain a relatively high concentration of inorganic nitrogen, such as nitrate and nitrite. However, some great problems would be caused by excessive nitrogen compounds which were discharged into water bodies, such as eutrophication and algal blooms [6]. So nitrogen removal is of great concern in wastewater treatment. In converting nitrate in water to nitrogen gas, denitrification is a crucial process, which is carried out by heterotrophic bacteria communities. As a result, the effective denitrification of WWTP secondary effluent is important fundamentally.

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Scholars paid more and more attention to denitrification process due to its advantages, such as smaller reactor sizes, easy operation, less demanding solids separation requirements, high nitrogen removal efficiency and so on [7,8]. The denitrification process is shown in Fig. 1, with the organic matters as an electron donor, denitrifying bacteria (chemical heterotrophic facultative hypoxic microbes) in the hypoxic conditions convert the nitrate nitrogen into nitrogen. Typical denitrification processes include the following four steps: $NO^{3-} \rightarrow NO^{2-} \rightarrow NO \rightarrow N_2O \rightarrow N_2$. The denitrification process is a process involving several enzymes (nitrate reductase [NaR], nitrite reductase [NiR], nitric oxide reductase [NOR] and nitrous oxide reductase [N_2OR] and a variety of intermediates and accompanied by electron transport and energy generation of complex biochemical reactions. Some suitable parameters of the denitrification operation were obtained in a study by Li et al. [9], where the test water was the secondary effluent of sewage treatment plant in Gaobeidian. It reported that when C/N = 4.42, the removal rate of nitrate can reach to 80% [9].

However, organic carbon resources were regarded as the electron acceptors in the progress of denitrification. But most WWTP in China were generally faced with the problem of the low efficiency of nitrogen. It was leaded to the low C/N ratio. So the carbon sources had become the important factor in the denitrification progress [10]. In order to improve the efficiency of denitrification, the carbon sources were usually added to the filter, such as methanol, sodium acetate, glucose, etc. At present, the effect of carbon source on the denitrification has been part of the researches at home and abroad. But there are few researches on the optimum C/N in the denitrification filter.

In this study, ceramsite and polyethylene polyhedral hollow ring were selected as the denitrification filter packing. After waiting for the stable operation, with methanol, sodium acetate and glucose as carbon source, the denitrifying effect and the optimum C/N of the two filters were discussed.

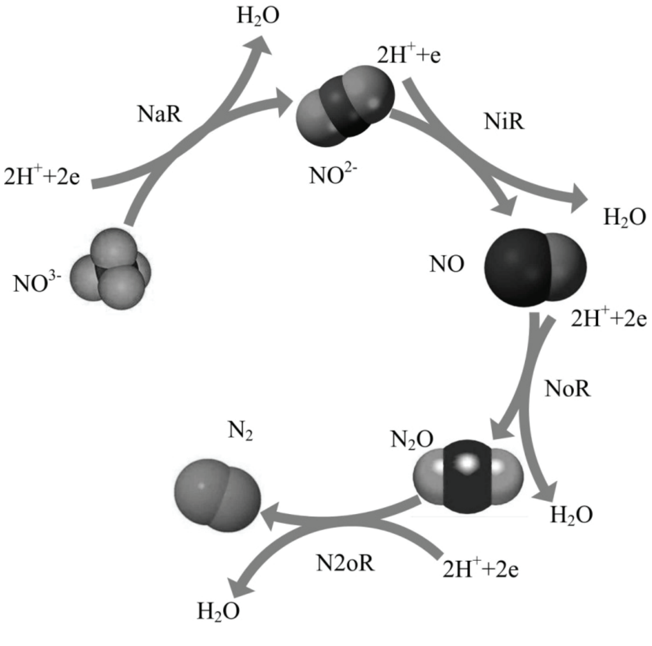

Fig. 1. Denitrification process.

2. Materials and methods

2.1. Experimental setup and operational conditions

Fig. 2 shows the schematic diagram of the experiment and Table 1 shows its parameters. In this study, Reactor 1 and Reactor 2 were operated in the same conditions. The reactors were made of plexiglass cylindrical, the diameter and height were 150 mm and 2,300 mm. The packing height was 1,000 mm, and the height of supporting layer was 300 mm. The height of the surface of the water was 300 mm. Besides, four sampling holes every 200 mm were set along the reactors. The Reactor 1 was filled with ceramsite and the Reactor 2 was filled with polyethylene polyhedral hollow ring. The diameters of ceramsite spherical and polyethylene polyhedral hollow ring were 3–4 mm and 20 mm, respectively. The pictures of ceramsite and polyethylene polyhedral hollow ring are shown in Fig. 3. Wastewater was pumped from the storage tank into the filters through inlet by peristaltic pumps, and flowed through the biofilm on the medium, and then flowed out from the bottom of the reactor. The air compressor was used to supply the air which flowed into the reactors.

2.2. Wastewater characteristics and the seeding sludge

The influent water in this study was the secondary effluent of WWTP in Handan, which applied the process of triple-channel oxidation ditch. The characteristics of raw wastewater are shown in Table 1 during the experiment. The seeding sludge was taken from the middle ditch of triple-channel oxidation ditch in Handan WWTP.

Table 2 shows that the TN of secondary effluent from WWTP was more than 15 mg/L, falling short of the level of A standard. But the ammonia nitrogen was below 5 mg/L, meeting the level of A standard. And the TN of the secondary effluent was mainly composed of nitrate. To reduce the concentration of TN, meeting the level of A standard, the denitrifying biological filter adopted to treat the secondary effluent. However, the secondary effluent COD_{Cr} was lower and was dominated by refractory organic compounds, which were not easy to be used by microorganism. So carbon source was needed to add to the denitrifying filters.

2.3. Plan of the experiment

Table 3 shows the operating conditions of the reactors. The experiment could be divided into two stages. And the first stage which completed the start-up of filters was divided into two periods. First, the WWTP secondary effluent and seeding sludge were mixed together according to the desired ratio of 3:1. Then the sludge–water mixture was added into the reactors by peristaltic pumps. When the water level reached to 300 mm on the packing medium, the peristaltic pump stopped and the air compressor was started with an air feed of 20 L/h. The hydraulic retention time was 24 h and the drainage ratio was 1/2. Such circulation was conducted for 10 d. Second, the two reactors were discharged and the air compressor was stopped. Besides, methanol was added into the influent to adjust the C/N ratio. The filtration rate of the two reactors was controlled at 1 m/h under the same operation conditions. During the start-up progress, the methanol additive quantity was determined by Eq. (1) which was proposed by IWA ASM1

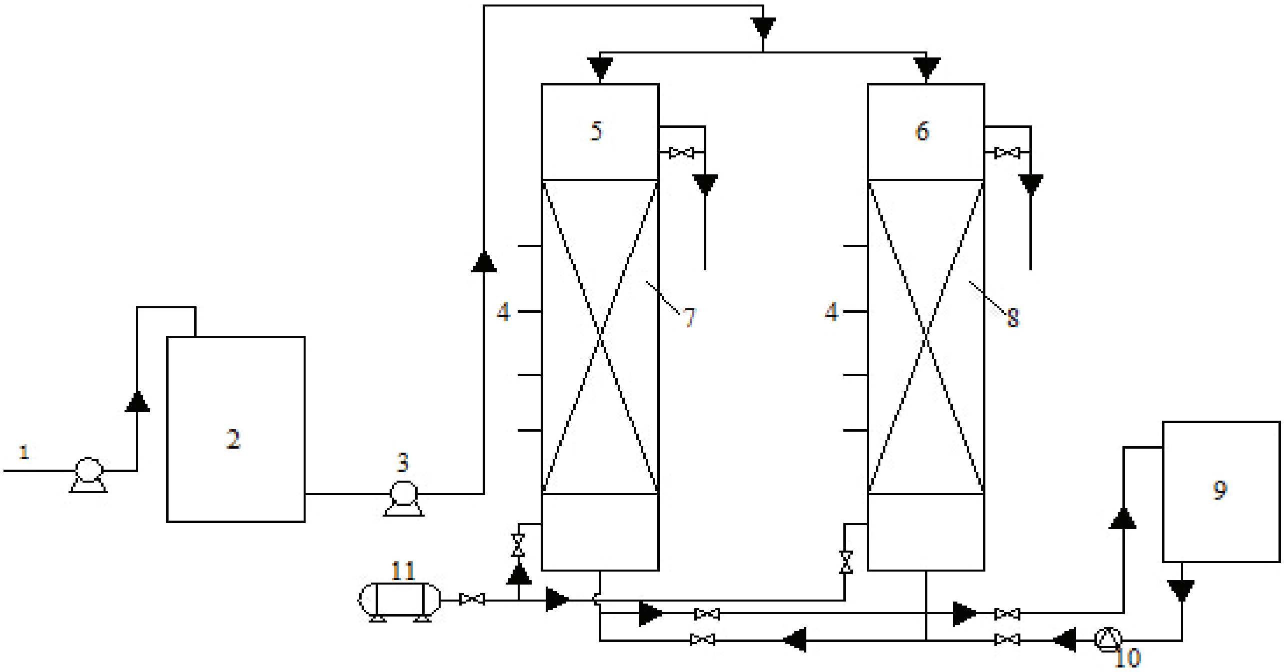

Fig. 2. Experimental schematic diagram. (1) Secondary effluent of WWTP, (2) influent tank, (3) peristaltic pump, (4) sampling holes, (5) Reactor 1, (6) Reactor 2, (7) ceramsite, (8) polyethylene polyhedral hollow ring, (9) effluent tank, (10) backwashing pump, (11) air compressor.

Table 1
Design and parameters for the reactors setup

Frame	Size (mm)
Filter column	
Diameter	150
Height	2,300
Packing height	1,000
Supporting layer height	300
Height of the surface of the water	300
Sampling holes (distance)	200

model and the batch test in the beaker, where Y_H stands for sludge yield. Then the additive quantity of methanol was confirmed to 40 mg/L (the density of methanol was 0.792), and it can keep the COD_{Cr} of influent water around 75 mg/L.

$$COD_{Cr}/NO_3^--N = 2.86/(1 - Y_H) \tag{1}$$

In the second stage, the two filters were fed with methanol, sodium acetate and glucose, respectively. Under the condition of hypoxia, with organic matter as electron acceptor, nitrate was reduced to nitrogen by denitrifying bacteria. This progress was known as traditional biological denitrification reaction. Based on the theory of biological denitrification, each 1 g nitrate needed 2.86 g carbon source [11]. But this demand value was far more than that in the actual production. It was generally believed that when C/N < 5, the effect of denitrification was poor [12]. The optimum C/N was 7.1 according to a study by Harremoes and Sinkjaer [13]. It was reported that when C/N reached 10, the nitrogen removal efficiency was more than 90% [14]. Besides, the nature of carbon source affected not only the denitrification effect but also the dosing [15]. So in this stage, the amount of carbon source and C/N were increased step by step. The operating conditions of two reactors are shown in Table 3.

The maximum nitrate, nitrite and nitrous oxide reduction rates were calculated as the difference between the NO_x production and the NO_x measured slope through linear regression. Eq. (2) shows an example of this calculation for the case of N_2O.

$$N_2O \text{ reduction rate} = N_2O \text{ production rate} - \text{measured } N_2O \text{ slope} \tag{2}$$

N_2O production rate was considered to be equal to the nitrite reduction rate based on the assumption that NO did not accumulate. In the studies when NO has been monitored, no accumulation has been detected. Also, NO is a potent cytotoxin and its accumulation causes the death of bacteria. Therefore, NO reduction reaction will always be prioritized. The specific reduction rates were calculated dividing the reduction rates by the mixed liquor volatile suspended solids concentration.

2.4. Analytical methods

The samples were taken every day in this study. Every water quality index in the experiment was carried out in

Fig. 3. Pictures of ceramsite and polyethylene polyhedral hollow ring.

Table 2
Characteristics of raw wastewater

	Range	Average
Temperature (°C)	10–20	16
COD_{Cr} (mg/L)	20–30	26
TN (mg/L)	15.17–21.09	18.18
NH_4^+–N (mg/L)	1.94–2.42	2.18
NO_3–N (mg/L)	12.68–17.58	15.13
NO_2–N (mg/L)	0.55–0.79	0.67
pH	7.4–8.0	7.7

accordance with the national standard method. The nitrate (NO_3^-N), nitrite (NO_2^-N), total nitrogen (TN) and COD_{Cr} were measured according to standard methods [16]. The pH was measured by the PHS-3C pH meter (Sartorius AG, Germany). Dissolved oxygen (DO) was measured by a DO sensor (KY-YSI55/12FT, USA).

N_2O was measured using a 6820 gas chromatograph (Agilent Technologies, USA). Gas chromatography use µ-electron capture detector (ECD) detector, the column for the GDX-104. N_2O measurement of the chromatographic conditions is as follows: inlet temperature is 120°C, column temperature is 50°C and ECD detector temperature is controlled at 300°C. Injection volume is 1 mL, with high purity N_2 as the carrier gas, flow controlled in 18 mL/min.

3. Results and discussions

3.1. Removal effects and discussions during the start-up

3.1.1. Removal efficiency of COD_{Cr}

The COD_{Cr} removal efficiency of Reactor 1 and Reactor 2 during the starting stage is shown in Fig. 4. The COD_{Cr} average concentration of the secondary effluent was 26 mg/L during the test. In the first period, the carbon source was not added to the two filters. It showed that the removal efficiency of COD_{Cr} was under 10% in the first 10 d. There were two reasons. The one was that the refractory organic compounds were the primary in the secondary effluent, and they were not easy to be used and degraded by the microorganism. The other was that the packing could only rely on physical intercept action to remove organic matters. It showed that the effluent COD_{Cr} concentration was slightly increased and COD_{Cr} removal efficiency was under 5% in Reactor 2 at the early days of the start-up. Because the interspaces of polyethylene polyhedral hollow ring were bigger than the ceramsite, leading to the lower intercept action, causing the seed sludge with the effluent out. All of these led the effluent COD_{Cr} increased. In the second period, the carbon source was added to, and the aeration was stopped at Day 11. However, the COD_{Cr} removal efficiencies were still lower in the two filters. The COD_{Cr} removal efficiencies were only 15%, 10% in Reactor 1 and Reactor 2, respectively. The reason was that when the methanol was used as carbon source, denitrifying bacteria needed a period of time to adapt [17]. After adding the carbon source (5 d), the COD_{Cr} removal efficiencies of the two filters increased rapidly. And COD_{Cr} removal efficiency of Reactor 1 grew up to 50% at Day 25, while the same removal efficiency was achieved for Reactor 2 at Day 30. After this phase, the two reactors could maintain stable operation. And the effluent COD_{Cr} of two filters was under 50 mg/L, meeting the Grade 1A permissible discharge standard of China [18].

3.1.2. Removal efficiency of nitrate

The nitrate removal efficiency of Reactor 1 and Reactor 2 during the starting stage is shown in Fig. 5. The nitrate average concentration of the secondary effluent was 15.13 mg/L during the test. In the first period, without adding carbon source, the nitrate removal efficiency was zero in the two denitrification filters. In this period, the environment of the filters was suitable for the growth of nitrifying bacteria, which could turn the rest ammonia nitrogen into nitrate or

Table 3
Operating conditions of reactors

Stage	Period	Operation mode	Operation hours	Influent rate	Carbon source	C/N
(Set-up stage)	a	Batch operation				
	b	Continuous operation	19d	1m~3.5/h	Methanol	5
2 (stable stage)	a	Continuous operation	7d	3.5 m/h	Methanol	0.8
			7d	3.5 m/h	Methanol	1.4
			7d	3.5 m/h	Methanol	2.1
			7d	3.5 m/h	Methanol	3.4
			7d	3.5 m/h	Methanol	4.5
			7d	3.5 m/h	Methanol	5.5
			7d	3.5 m/h	Methanol	6.4
			7d	3.5 m/h	Methanol	7.4
			7d	3.5 m/h	Methanol	8.4
			7d	3.5 m/h	Methanol	9.4
	b	Continuous operation	7d	3.5 m/h	Sodium acetate	0.5
			7d	3.5 m/h	Sodium acetate	1.5
			7d	3.5 m/h	Sodium acetate	2.4
			7d	3.5 m/h	Sodium acetate	3.5
			7d	3.5 m/h	Sodium acetate	4.6
			7d	3.5 m/h	Sodium acetate	5.2
			7d	3.5 m/h	Sodium acetate	6.1
			7d	3.5 m/h	Sodium acetate	7.1
			7d	3.5 m/h	Sodium acetate	8.4
	c	Continuous operation	7d	3.5 m/h	Glucose	0.9
			7d	3.5 m/h	Glucose	1.7
			7d	3.5 m/h	Glucose	3.4
			7d	3.5 m/h	Glucose	4.6
			7d	3.5 m/h	Glucose	5.7
			7d	3.5 m/h	Glucose	6.8
			7d	3.5 m/h	Glucose	7.7
			7d	3.5 m/h	Glucose	8.7
			7d	3.5 m/h	Glucose	9.8
			7d	3.5 m/h	Glucose	10.4
			7d	3.5 m/h	Glucose	11.6
			7d	3.5 m/h	Glucose	12.3
			7d	3.5 m/h	Glucose	14.1
			7d	3.5 m/h	Glucose	16.2

turn nitrite into nitrate. So the effluent nitrate concentration of two filters was slightly increased. However, after adding carbon source, the aerobic environment of filters was destroyed, then the degradation of nitrifying bacteria was restrained. Finally, the denitrifying bacteria became the advantage bacterium, and the denitrification increased. The nitrate removal efficiency of Reactor 1 and 2 reached to 11%, 9% at Day 15, and reached to 60%, 49% at Day 25, and reached to 60%, 61% at Day 30, respectively. Moreover, the effluent TN concentration of these filters was under 15 mg/L and the average TN removal rate could reach to 50%, meeting the first level A criteria after stable running. Above all, the start-up stage was completed.

3.2. Effects of denitrification after adding carbon source

In accordance with the relevant research, the more adequate of carbon source, the denitrifying was conducted the better [19]. In this study, with methanol, sodium acetate and glucose as carbon source, the denitrification and the optimum C/N were discussed.

3.2.1. Nitrate removal effect after adding carbon source

The effects of nitrate removal are shown with adding carbon source after the stable operation in Fig. 6. The average nitrate concentration in the influent was 15.13 mg/L.

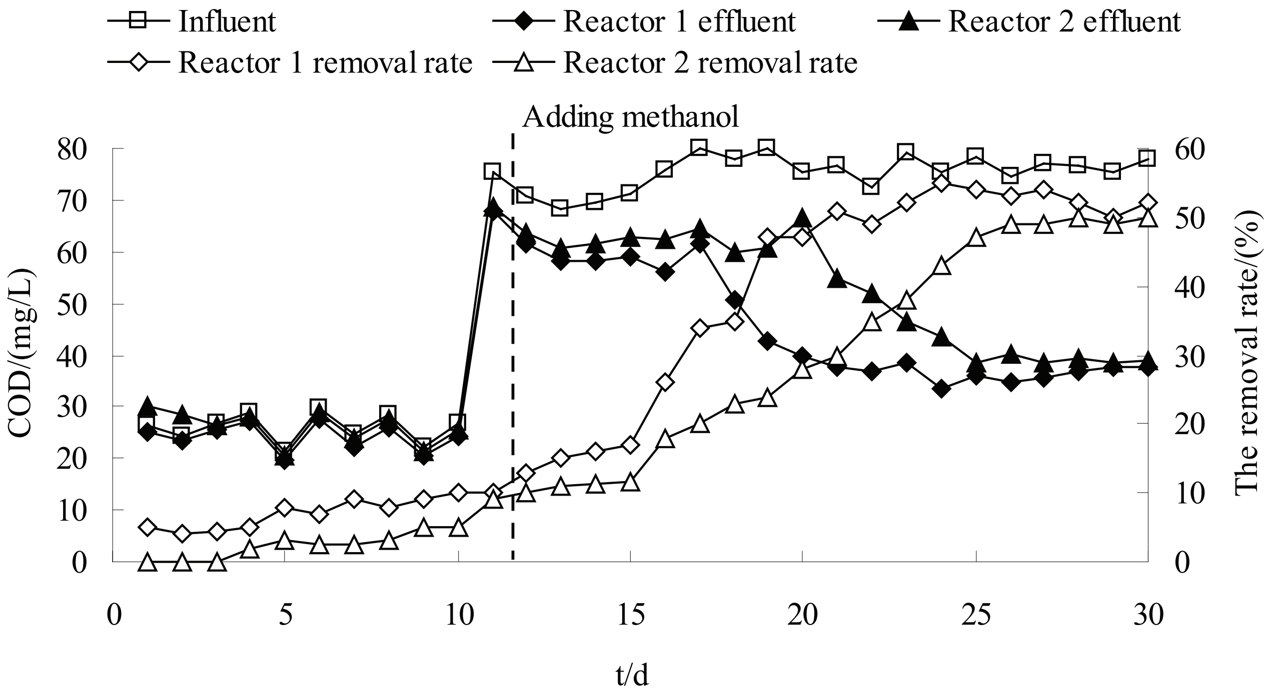

Fig. 4. COD_{Cr} removals during the start-up period.

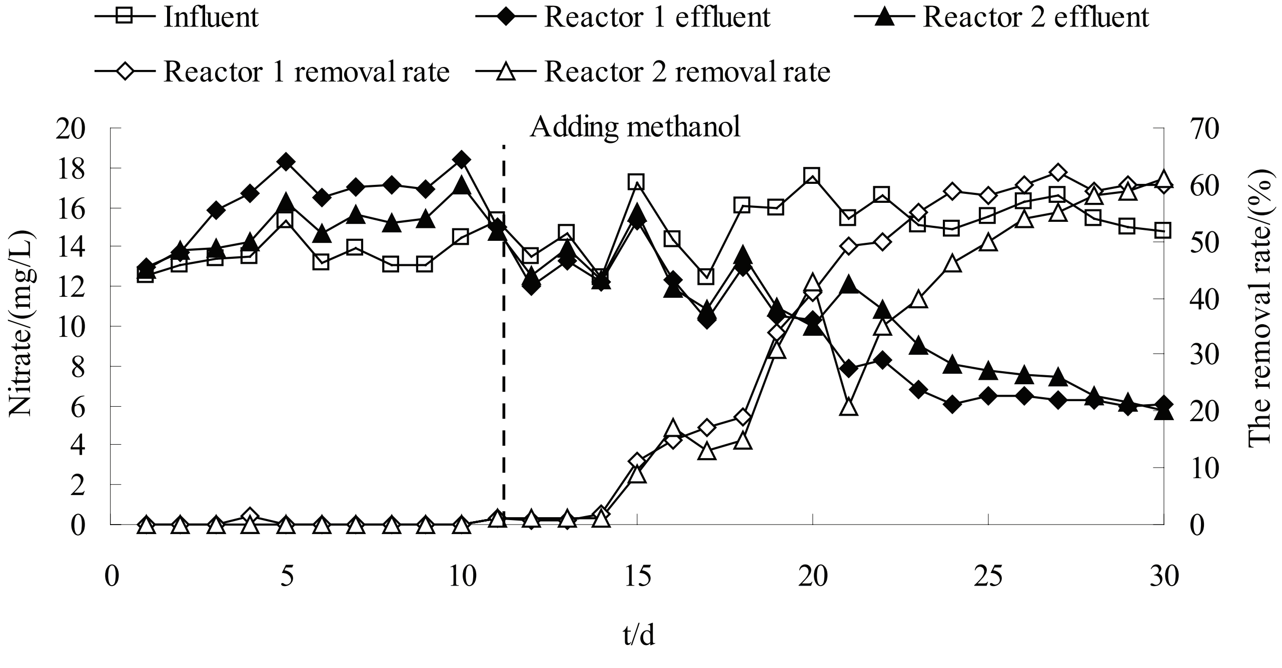

Fig. 5. Nitrate removals during the start-up period.

With methanol as carbon source, nitrate removal rate in the effluent of Reactor 1 increased from 3.3% to 96.2% when C/N increased from 0.8 to 4.5. And the removing effect was stable up 95% when C/N increased from 4.5 to 9.4. Simultaneously, nitrate removal effect in Reactor 2 increased from 1.3% to 92.3% and exceeded 90% when C/N increased from 0.8 to 5.5 and from 5.5 to 9.4, respectively.

With sodium acetate as carbon source, the nitrate removal rate went up gradually when C/N increased from 0.5 to 8.4. When C/N increased from 0.5 to 5.2, the nitrate removal rate increased from 10.2% to 97.22% and then the removal rate was stable up 95% and the effluent nitrate concentration was under 0.5 mg/L in Reactor 1. In Reactor 2, when C/N went up to 6.7, the nitrate removal rate was stable around 90% and the effluent concentration was under 2 mg/L.

With glucose as carbon source, C/N increased from 0.9 to 16.2. Nitrate removal rate in the effluent of Reactor 1 increased from 2.6% to 96.31% when C/N increased from 0.9

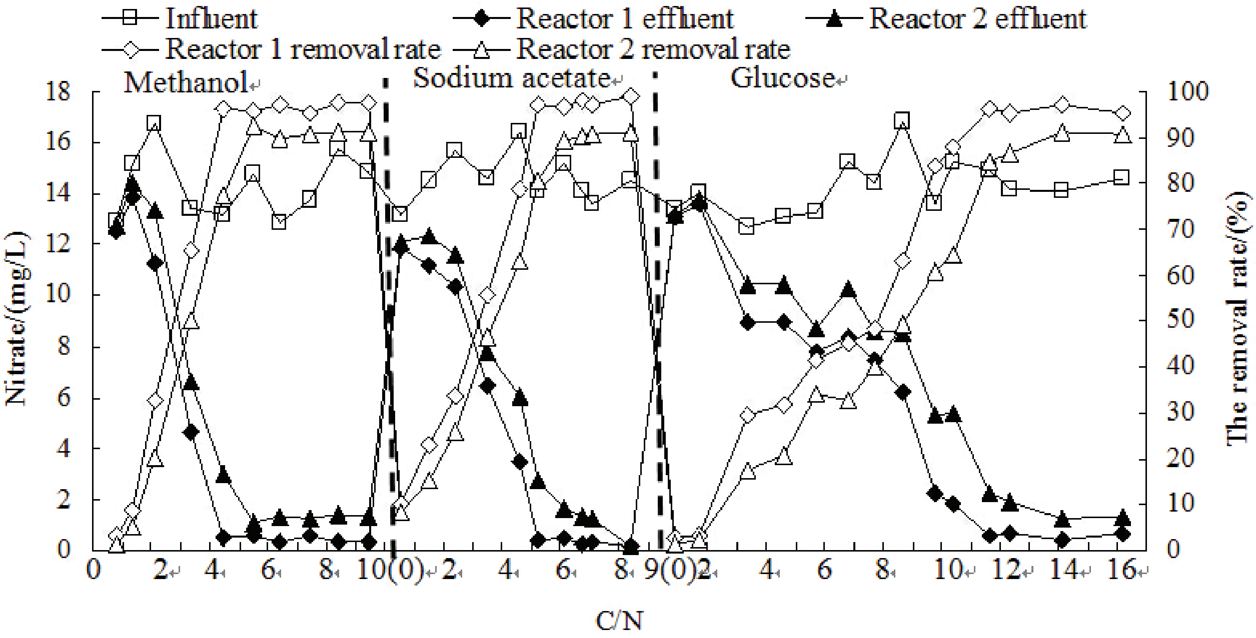

Fig. 6. Effects of nitrate removal under the different carbon source.

to 11.6. And the removing effect was stable up 95% when C/N increased from 11.6 to 16.2. The nitrate removal rate in Reactor 2 increased from 1.5% to 91.2% when C/N increased from 0.9 to 14.1. And the nitrate removal rate was stable around 90% to the end.

Using the three kinds of extra carbon source, the nitrate removal rate of the two denitrification filters rose with the increase of C/N in the early period. When C/N was low, the carbon source which was needed by the microbe was insufficient and the denitrification was not fully performed. But when C/N increased, the above mentioned problem was settled. However, the amount of the carbon source was certain that was needed by two denitrification filters. As a result, nitrate removal rate had no obvious rise while C/N continued to increase.

3.2.2. Nitrite removal effect after adding carbon source

Fig. 7 shows the nitrite removal effects after adding carbon source. The results show that nitrite accumulation appeared in both Reactor 1 and Reactor 2, and the accumulation amount was increased first then dropped with the increase of C/N. Using methanol as carbon source, when C/N = 3.4, the nitrite concentration of effluent reached to 3.56 mg/L which was the highest in Reactor 1. Then when C/N = 4.5, the effluent nitrite concentration dropped rapidly, which was lower than the influent. In Reactor 2, the nitrite accumulation amount reached to 4.01 mg/L when C/N = 4.5 and reduced next. With sodium acetate as carbon source, the nitrite accumulation amount in two filters first increased and then decreased with increasing C/N. When glucose was used as carbon source, the nitrite accumulation in two filters disappeared more slowly and the effluent nitrite concentration was higher than the influent.

There were two steps in the traditional biological denitrification reaction. The first was that nitrate was reduced and the second was that nitrite was reduced. Moreover, the reduction of nitrate was more likely to happen than the reduction of nitrite [20]. In the early stage of this study, the first step of denitrification could be only conducted due to the low C/N. So nitrite concentration went up. With the increase of C/N gradually, the amount of carbon source that was used by denitrifying bacteria increased. And the reduction of nitrate and nitrite increased as well. However, the reduction rate of nitrate was far higher than the reduction rate of nitrite. Then after a certain time, nitrite concentration accumulated maximum. However, when C/N continued to increase, the nitrite reduction dominated and the nitrite concentration of effluent dropped.

3.2.3. Ammonia nitrogen effect after adding carbon source

Fig. 8 shows the ammonia nitrogen removal effects after adding carbon source. It showed that two denitrification filters had almost no removal efficiency of ammonia nitrogen. The reason was that the DO of two filters was below 0.5 mg/L in the experiment. The nitrifying bacteria could not grow and reproduce in the hypoxia environment. In conclusion, ammonia nitrogen could be only removed through the assimilation of denitrifying bacteria and the subtractive quantity was fairly small.

3.2.4. TN removal effect after adding carbon source

Fig. 9 shows the TN removal effects under the different kinds of carbon source. The results show that TN removal efficiency was significant in these two filters. With methanol as carbon source, TN removal efficiency of Reactor 1 reached to 73.02% when C/N = 4.5 and that of Reactor 2 went up to 71.93% when C/N = 5.5. And TN removal effect of two filters was in a stable state after that. With sodium acetate as carbon source, TN removal efficiency of Reactor 1 reached to 75.31% when C/N = 5.2 and that of Reactor 2 went up to

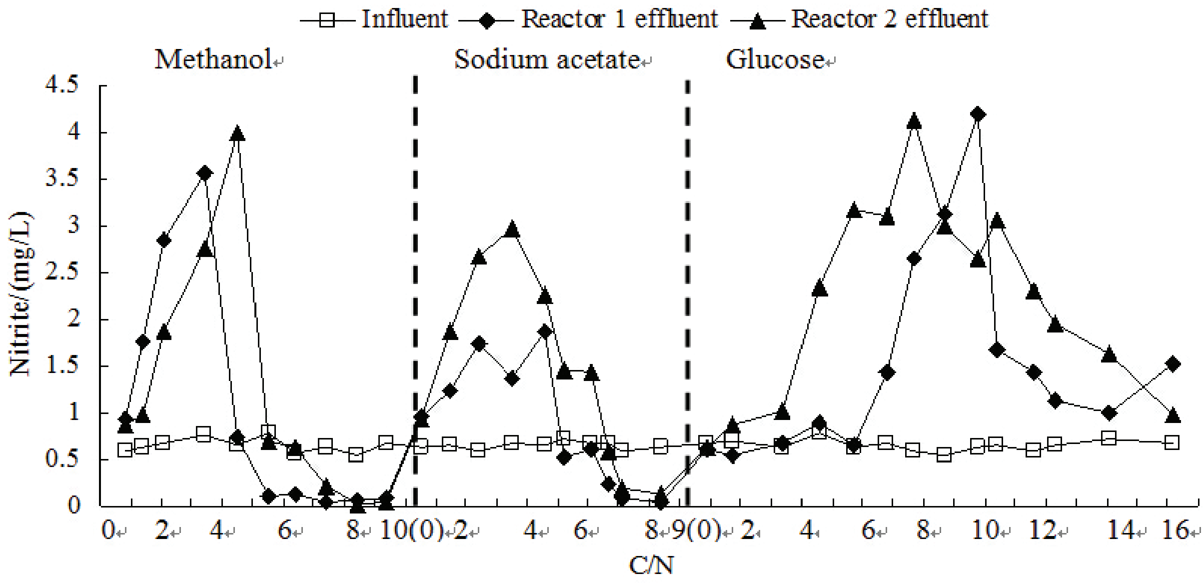

Fig. 7. Effects of nitrite removals under the different carbon source.

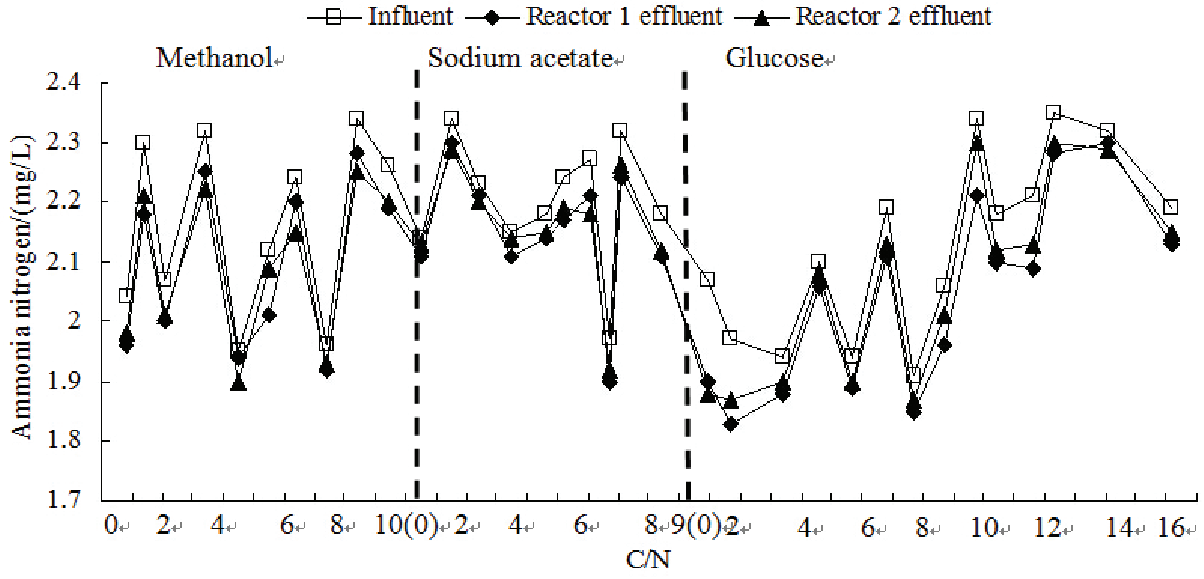

Fig. 8. Effects of ammonia nitrogen removals under different carbon source.

70.83% when C/N = 6.7. And after that, these two filters were in stable running. With glucose as carbon source, TN removal efficiency of Reactor 1 reached to 70% when C/N = 11.6 and that of Reactor 2 went up to 60% when C/N = 14.1.

The reason was that methanol and sodium acetate which were small molecule organic matter had a strong commonality. And methanol and sodium acetate could be used as nutrient substrates by denitrification reaction. However, the biodegradation pathway of glucose was slightly complex due to the bigger molecular weight. And glucose was converted to pyruvate first and then turned into ethanol when used [21]. Only in this way could be glucose used by denitrifying bacteria. So the denitrifying effect when glucose was as carbon source was inferior to the former two kinds.

3.2.5. Effects of carbon and C/N ratio on N_2O release factors

The biological nitrogen removal process is an important anthropogenic source of N_2O. Different C/N and carbon source can effect the N_2O release coefficient of the sewage treatment process obviously. The effect of the carbon source on the N_2O release factor of the 1# and 2# filters is shown in Fig. 10. When methanol is used as the carbon source, the 1# filter column is at C/N = 3.4, NO_2-N concentration reached the highest, and the release factor of N_2O increased to the highest, until C/N increased to above 7, N_2O release factor decreased rapidly to about 1.7; when sodium acetate was used as carbon source, the 1# filter column is at C/N = 3.4, the mass concentration of NO_2-N reached the highest, and the release factor

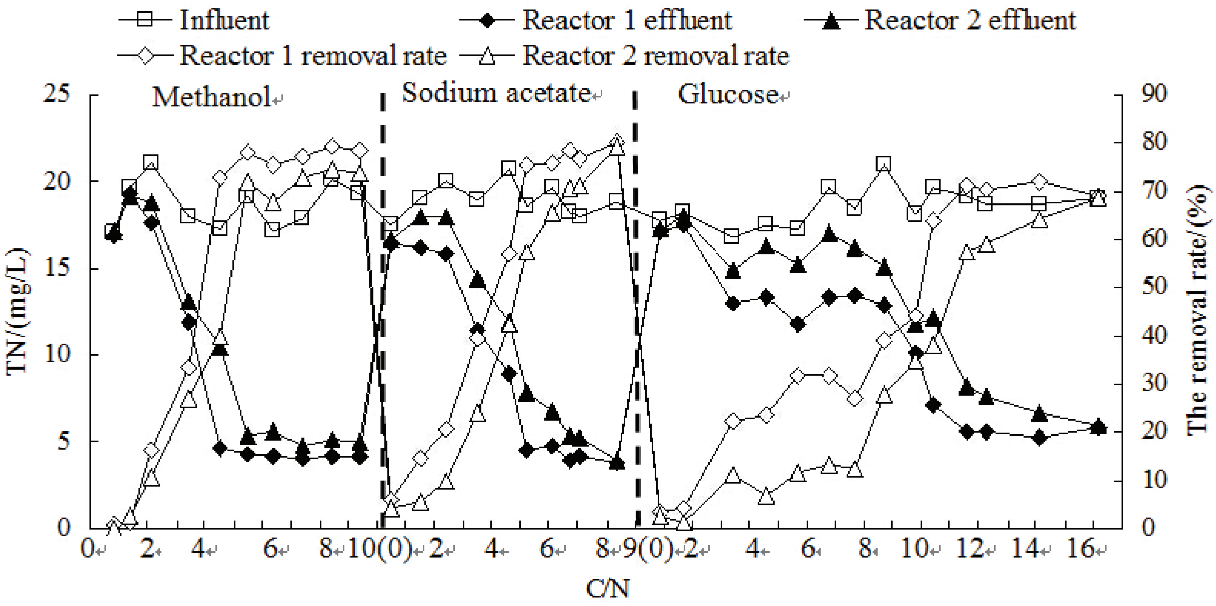

Fig. 9. Effects of TN removals under the different carbon source.

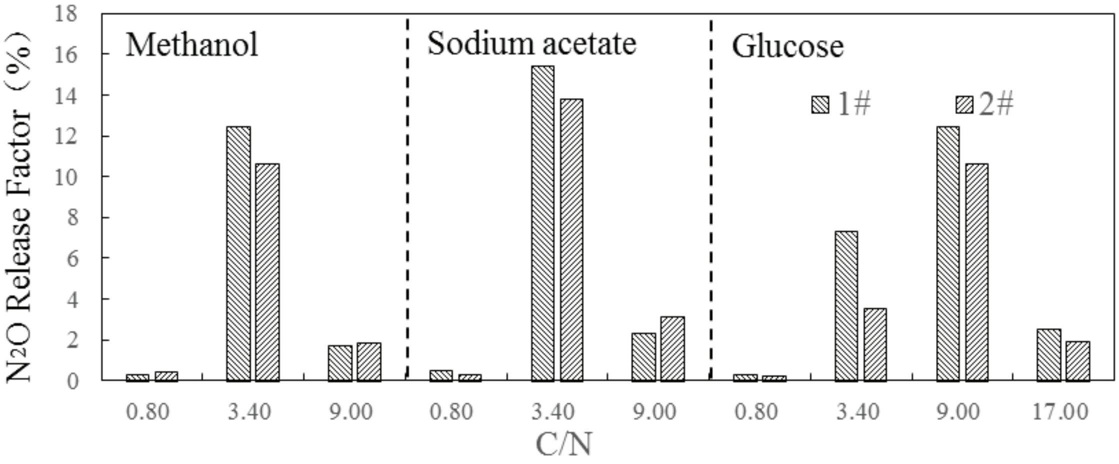

Fig. 10. Effects of carbon and C/N ratio on N_2O release factors.

of N_2O also increased to the highest, and the C/N increased to above 7, and the N_2O release factor decreased rapidly to about 2.3; when the glucose was used as the carbon source, the mass concentration of NO_2-N was the highest at C/N = 3.4 in the 1# filter column, and the release factor of N_2O increased to the highest level until C/N increased to 11 above, the N_2O release factor dropped rapidly to about 2.5. Visible under the condition of various carbon and nitrogen ratio, both the filter column appeared a phenomenon of the transgression of N_2O, and the N_2O release factor increased with the increase of C/N, and then decreased to the optimum C/N, which has difference with effect of carbon source and competition for electrons on nitrous oxide reduction in a mixed denitrifying microbial community, which was related to the effect of carbon source. The difference is mainly due to the amount of denitrification and the accumulation of NO_2-N (Fig. 7). It is generally believed that when the carbon source is insufficient, the ability of N_2OR to compete electrons is weak and reach denitrification by using intracellular carbon sources, leading to the accumulation of N_2O during the denitrification process (the effect of carbon and nitrogen ratio on N_2O production during short-range denitrification). Kishida et al. [22] studied the denitrification process for livestock and poultry wastewater. It was found that when C/N was 2.6, the release of N_2O was more than 10 times when C/N was 4.5 (effect of C/N ratio on nitrous oxide emission from swine wastewater treatment process). At the same time, the carbon source species also affect the release of N_2O, and a similar result was observed in a study by Anna et al. [23]. They have concluded that the effect is carbon source and competition for electrons on nitrous oxide reduction in a mixed denitrifying microbial community. Different carbon sources are different in efficiency. At the same time, different carbon sources tend to enrich and culture different populations of microorganisms. Some of these microorganisms do not have N_2O reductase, or their ability to metabolize N_2O is weak, which is more likely to lead to accumulation of N_2O [24,25].

3.2.6. Comprehensive comparison

The results show that when C/N > 4.5 with methanol as carbon source, C/N > 5.2 with sodium acetate as carbon source and C/N > 11.6 with adding glucose, the nitrate removal efficiency was more than 95%, the effluent nitrate concentration was under 1 mg/L and TN removal efficiency surpassed 70% in Reactor 1. When C/N > 5.5 with methanol as carbon source, C/N > 6.7 with sodium acetate as carbon source and C/N > 14.1 with adding glucose, the nitrate removal efficiency exceeded 90%, the effluent nitrate concentration was below 2 mg/L and TN removal efficiency surpassed 60% in Reactor 2. In addition, with glucose as carbon source, nitrite accumulation was more easily caused. With methanol, sodium acetate and glucose as carbon source, under the optimum C/N, the effluent TN concentration of two filters was below 15 mg/L, meeting the Grade 1A permissible discharge standard of China [18].

Through analysis and comparison, the effect of denitrification was the best with methanol as carbon source. In other words, methanol was first, sodium acetate was second, and glucose was third. However, methanol was a kind of inflammable, explosive, volatile poisonous liquid. If people exposed to the environment which methanol exist in long term, health would be threatened. Therefore, methanol was not used as extra carbon source and sodium acetate was usually applied in the actual production.

Table 4 shows the comparison of the results in previous study and this study. Either in the sequencing batch reactor (SBR) activated sludge process system or in the biological filtration system, COD and nitrate removal rates are high with adequate carbon sources and denitrification is better. The types of carbon sources and C/N all have an impact on the release of N_2O and the accumulation of nitrite in the denitrification process. In this study, the accumulation of nitrite tends to increase at first and then decrease with the increase of C/N and finally stabilize in the lower range, which is consistent with the results of Liu et al. [27], Cao et al. [30] and Du et al. [31], that is, when the carbon source is abundant, the accumulation of nitrite is small. The trend of N_2O release is consistent with that of nitrite accumulation, as evidenced by the Kishida et al. [22] study. In addition, depending on the size and nature of the molecule, the type of carbon source added also has an effect on the denitrification effect, which is why the optimum C/N occurs under different carbon sources in this study. Most of the results show that C/N is the key factor affecting the denitrification effect, however, Tang et al. [28] claimed that N_2OR might have been more strongly inhibited by high salt stress than NaR and NiR.

Table 4
Comparison of the results on previous study and this study

Investigator	Technique	Carbon source	C/N	COD removal rate (%)	NO_3-N removal rate (%)	NO_2-N cumulant (mg/L)	TN removal rate (%)
Song [26]	Biofilm	Sodium acetate	4	90	>90	0.56	86.44
Liu et al. [27]	Biofilm	Sodium acetate (start-up), acetic acid (unstable stage), methanol (stable stage)	5.3 4.5 3.8	50–82	>90	<1	–
Tang et al. [28]	Biofilm	Sodium acetate	4	72–83	65.34	–	66.33
Zhao et al. [29]	SBR	Methanol	4	–	>95	<0.15	–
Cao et al. [30]	SBR	Methanol	4	>84	>99	2.85	–
			<3.2	>84	9.3–99	–	–
Du et al. [31]	SBR	Sodium acetate	4	>56.6	>97	<0.1	–
This study	Biofilm	Methanol, sodium acetate, glucose (Reactor 1)	4.5 5.2 11.6	>52.6 (Fig. 4)	>95	0.73 0.52	>70
		Methanol, sodium acetate, glucose (Reactor 2)	5.5 6.7 14.1	>50.2 (Fig. 4)	>90	0.7 1.44	>60

4. Conclusions

The main findings of this study were as follows:

- The two denitrifying biological filters adopted artificial inoculation to start-up in this study. The progress of start-up was divided into two steps. After the first step, the removal rate of COD_{Cr} was below 10% and the nitrate removal efficiency was zero in Reactor 1 and Reactor 2. In the second step, denitrification reaction was conducted successfully. The removal efficiency of COD_{Cr} reached to 50% and the nitrate removal efficiency went up to 60% in Reactor 1 at Day 25. Reactor 2 achieved the same effect at Day 30. Moreover, these two filters were in stable running after that.
- After the stable operation, with methanol, sodium acetate and glucose as extra carbon source, the denitrifying effect was discussed. Results showed that when C/N > 4.5 with methanol as carbon source, C/N > 5.2 with sodium acetate as carbon source and C/N > 11.6 with adding glucose, the nitrate removal efficiency was more than 95% in Reactor 1. In Reactor 2, when C/N > 5.5 with methanol as carbon source, C/N > 6.7 with sodium acetate as

carbon source and C/N > 14.1 with adding glucose, the nitrate removal efficiency was more than 90%. Moreover, the effluent TN concentration of two filters was below 15 mg/L, meeting the Grade 1A permissible discharge standard of China [18].

- When methanol, sodium acetate and glucose were used as extra carbon source, nitrite accumulation appeared in both two filters. Besides, with glucose as carbon source, the accumulation of nitrite disappeared slowly, and the effluent nitrite concentration was higher than influent.
- In the case of methanol, sodium acetate and glucose as the additional carbon source, the release of N_2O was observed in the 1# and 2# columns, and when the carbon–nitrogen ratio was increased to the optimum one it showed a sudden decline. And the N_2O emission factor was the smallest when the methanol was used as the carbon source, and the largest when the sodium acetate was used as the carbon source.
- Through analysis and comparison, the utilization rate of extra carbon source in ceramsite filter was higher than that in polyethylene polyhedral hollow ring filter. So ceramsite was more suitable for the packing of denitrification filter. When methanol, sodium acetate and glucose were used as extra carbon source, the effect of denitrification with methanol was the best. From practical considerations, methanol was not suitable as extra carbon source and sodium acetate was more suitable in the actual production.

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