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Characteristics of nitrogen removal and sludge reduction using a multi-redox environment coupled bioreactor

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ABSTRACT

Due to the problems of excess sludge production, low denitrification efficiency and high cost of the traditional aerobic biological treatment process, a multi-redox environment coupled bioreactor was built, which combined wastewater denitrification and *in-situ* sludge reduction. The optimal operating parameters of the reactor were as follows: multi-point influent distribution ratio of 3:4:3, dissolved oxygen (DO) content of 4.0 mg/L, Hydraulic Retention Time (HRT) of 11 h, and reflux ratio R of 1.0. Anaerobic, anoxic and aerobic micro environments were repeatedly coupled in a novel compound migration carrier to release low molecular organic carbon and significantly promote the effect of denitrification. Using domestic sewage with a low C/N ratio, the average removal rates of total nitrogen (TN), chemical oxygen demand (COD) and NH_4^+-N were 80%, 91%, and 95%, respectively, and the corresponding concentrations in the effluent decreased to 20, 30 and 5.0 mg/L, respectively. The microbial community in the reactor was diverse, and the sludge yield was only 0.10 kg MLSS/kg COD. The sludge reduction effect was remarkable and superior to other biofilm processes.

Keywords: Oxidation; Reduction; Biofilm; Nitrogen removal; Sludge reduction

1. Introduction

With the deterioration of the water environment and the improvement of emission standards for wastewater treatment plants, the one of the main goal of urban wastewater treatment plants was increasing the removal efficiency of nitrogen and phosphorus. However, a large amount of urban domestic sewage shows low COD, high nitrogen C/N features [1–3]. This type of sewage has a low organic matter content, which cannot meet carbon source requirements for the process of nitrogen removal by denitrification. As a result, the traditional nitrogen removal process is more difficult when dealing with such sewage [4,5]. In additional, the traditional aerobic biological treatment process usually produces a large excess of sludge via microbial proliferation, in the meantime, high cost, great difficulty and secondary pollution bothered sludge treatment and disposal, which have resulted in problems in the sewage treatment industry [6–8]. In view of the problems of domestic sewage with a low C/N ratio, i.e., the lack of carbon source during biological denitrification, and the larger production of excess sludge, experts at home and abroad have done much research [9]. To analyze the mechanism of sludge reduction by coupling aerobic with anaerobic conditions, Xing Xinhui and others constructed an aerobic-anaerobic repeated coupling model test system, and a good sludge reduction effect was achieved [10,11]. In this study, a multi-redox environment coupled bioreactor was established, which combines sewage denitrification with *in-situ* sludge reduction. A multi-level alternating hypoxia and aerobic environment appeared in the space of the reactor, and an anaerobic-an-

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oxic-aerobic micro environment was formed inside the carrier; in other words, a complex redox environment was formed in the reactor. The pollutant could be effectively separated, accumulated, low molecularized and anaerobic decomposed by carriers to achieve *in-situ* sludge reduction. Meanwhile, the released carbon served as a supplementary carbon source for denitrification. Therefore, the denitrification was promoted in addition to *in-situ* sludge reduction. In this paper, the domestic wastewater with low C/N ratio was taken as the research object to study the sludge reduction and nitrogen removal effect and operation characteristics of the reactor so as to determine the suitable process operating parameters.

2. Test apparatus and method

2.1. Test device

The experiment was carried out using a biofilm reactor (Fig. 1) with the size of 270 cm × 20 cm × 60 cm (L × B × H) and an effective volume of 240 L. Micro porous tubes were used for aeration. There were 9 sampling ports set at raw water tank, the bottom of each A/O phase and effluent area, respectively, and named as influent, O-1, A-1, O-2, A-2, O-3, A-3, O-4, and effluent. The volume ratio of the aeration zone to the anoxic zone was approximately 2:1, and the filler loading ratios of the aeration zone and the anoxic zone were 50 and 100%, respectively. (Fig. 1). The spherical shell was made from a polymer material,in which cubic sponge carrier filled.. The spherical surface had a net-like shape,of 8 cm in diameter, side length 1.5 cm and porosity was greater than 0.90.

The test examined operation effect under different parameters was shown in Table 1. The system was stable operation for 1 year after the biofilm formation.

2.2. Raw water quality

Raw water was the domestic sewage from living-area septic tank, which was located in the campus dormitory area, 50 m northwest of the laboratory, and the quality of raw water was as follows: the concentrations of NH_4^+-N, TN, TP, SS, BOD_5 and COD were 75~100 mg/L,110 mg/L,4.6~6.5 mg/L,90~170 mg/L,125–265 mg/L and 260– 400 mg/L, respectively. And the average concentrations of NH_4^+-N, TN, TP, SS, BOD_5 and COD were 90, 110, 5.6, 130, 214.5, 330 mg/L, respectively. Furthermore, C/N was approximately 2.5~3.0 and the BOD_5/COD was

Table 1
Operating condition

Operation condition number	Multi-point influent flow distribution ratio	HRT (h)	DO (mg/L)	Sludge reflux	Temperature (°C)
1	5:3:2	14	5.0	1.0	25
2	4:2:4	14	5.0	1.0	25
3	3.3:3.3:3.3	14	5.0	1.0	25
4	3:4:3	14	5.0	1.0	25
5	2:3:5	14	5.0	1.0	25
6	3:4:3	11	5.0	1.0	25
7	3:4:3	9	5.0	1.0	25
8	3:4:3	11	4.0	1.0	25
9	3:4:3	11	3.0	1.0	25
10	3:4:3	11	2.0	1.0	25
11	3:4:3	11	4.0	1.5	25
12	3:4:3	11	4.0	2.0	25
13	3:4:3	11	4.0	1.0	13

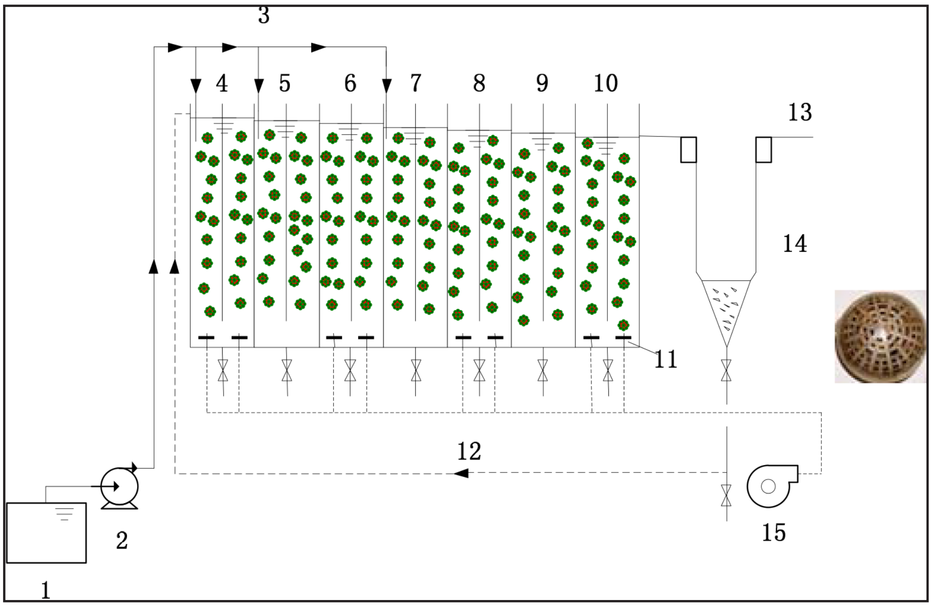

Fig. 1. Experimental device (1. Raw Water Tank; 2. The Inlet Pump; 3. Inlet Pipe; 4, 6, 8, 10. Aerobic Zone, O-1, O-2, O-3 and O-4, respectively; 5, 7, 9. Anoxic Zone, A-1, A-2 and A-3, respectively; 11. Air-blast head; 12. Sludge reflux; 13. Effluent; 14. Sedimentation Tank; 15. The Air Pump) and composite displacement porous carrier.

approximately 0.65. The reactor used return activated sludge (RAS) from a sewage plant as the inoculate sludge, and the characteristics of the sludge were MLSS 5.6 g/L and SVI_{30} 132.

2.3. Conventional physical and chemistry methods

COD_{cr}, TN, TP, NH_3-N and NH_4^+-N were determined according to standard methods (APHA, 2005). The temperature, dissolved oxygen (DO) and pH were determined by water meter (FANGJUN WNG-01), dissolved oxygen monitor (HACH LDO101) and pH meter (METTLER TOLEDO FE28), respectively.

2.4. Pretreatment and scanning electron microscope analyses

The biofilm in the aerobic zone was sampled randomly on the 120th day, when the flow distribution ratio was 3:4:3, the HRT was 11 h, the concentration of DO was 4.0 mg/L, and the temperature was 25°C, the reflux ratio R = 1.0. The samples were immediately fixed in a 2.5% glutaraldehyde-phosphate buffer for 1 h, and then were rinsed twice with phosphate buffer. After being dehydrated and processed in a graded ethanol series (30%, 50%, 70%, 90% and 100% v/v), the samples were finally air-dried, fixed on SEM stubs and vacuum-Au-coated. Electron micrograph was taken by a scanning electron microscope (SEM, HITACHI, S-4800, Japan).

3. Results and discussion

3.1. Effect of the flow distribution ratio on the nitrogen removal efficiency of the reactor

The multi-point influent was beneficial to balance the sludge load, increasing the biomass and supplying the carbon source to promote denitrification. It was very important for the operation of the reactor to rationally distribute the flow rate. The flow distribution ratios of stage O-1, A-1 and A-2 were R1 = 5:3:2, R2 = 4:2:4, R3 = 3.3:3.3:3.3, R4 = 3:4:3, and R5 = 2:3:5.

The flow distribution ratio of R1 in Figs. 2a, b shows that the distribution flow in stage O-1 was larger and the nitrification capacity was not balanced, resulting in a higher effluent ammonia nitrogen concentration. When R3 and R4 were used as the flow distribution ratio, the nitrification effect was improved remarkably, especially for R4, when the removal rate of ammonia nitrogen was 96.4%, and the concentration of NH_4^+-N in the effluent was 3.2 mg/L. Compared with R1, the nitrification effect at stage O-2 was remarkable, and less ammonia nitrogen was accumulated in front stages when the flow distribution ratio was R4. Thus, a better nitrification effect was achieved.

When R5 was used as the flow distribution ratio, distribution flows in stage A-1 and A-2 increased gradually, and the ammonia nitrogen load in stage O-1 was low, while the loads in stage A-1 and A-2 were high. Therefore, the nitrification capacity in stage O-1 was high,and it was insufficient in stage A-1 or A-2, thus affecting the nitrification reaction and resulting in more NH_4^+-N in the effluent water. The situation under flow distribution ratio R2 was similar to that of R5.

As shown in Figs. 2a,b, when R1 was used as the flow rate distribution ratio, the concentration of nitrate-nitrogen in stage A-1 or stage A-2 in the hypoxic segments was low, but it was relatively high in stage A-3,which can be explained by the phenomenon that more abundant carbon source in stage O-1 and A-1 effectively promoting denitrification reaction, while the carbon deficiency in stage A-3 became the limiting factor of denitrification reaction. Under the flow rate distribution ratio R2 or R5, stage A-2 in the hypoxic zone was assigned larger flow rate, and a higher ammonia nitrogen load occurred, while the stage A-3 could not get enough carbon source, resulting in carbon source deficiencies during the subsequent denitrification stages. As a result, the content of nitrate-nitrogen in the effluent was higher. When flow rate distribution rations were R3 and R4, the ammonia nitrogen load in each stage was relatively balanced, the carbon source was relatively sufficient, the denitrification reactions in stage A-1 and A-2 were more complete, basically there were no NO_x^-N remaining or entering in the subsequent sections, and the distribution flow rate in the end stage was not large, thus,the concentration of NO_x^--N in the effluent maintained a lower level.

The concentration of sludge in the reactor successively decreased along the reaction sections, and the corresponding nitrification capacity also decreased. When the flow rate distribution ratio was R3 or R4, the flow rate was well-distributed, and the ammonia nitrogen load was more average, thus, the nitrification capacity in each section was better utilized. It was beneficial for the removal of TN. It can be seen from Fig. 3 that the removal rate of TN reached 78.38% and the concentration of TN in the effluent was 22.7 mg/L when the flow rate distribution ratio was R4.

3.2. Effect of the hydraulic retention time on the denitrification efficiency of the reactor

The hydraulic retention time (HRT) is an important parameter to the reactor as it directly affects the volume, covered area and operating cost of the reactor. The denitrification efficiency of the reactor was examined under the HRT of 9 h, 11 h and 14 h, respectively.

When the composite outflow porous carriers were placed into the reactor, a typical biofilm process occurred, the residence time of sludge was longer than that of sewage, and sludge age in the reactor was extended. Additionally, more nitrifying bacteria, which have a long generation time, survived in the reactor; therefore, the system showed a strong nitrification capacity. As shown in Figs. 2c, d, the removal rates of ammonia nitrogen were 96.1%, 94.2% and 89.5% when the HRT were 14 h, 11 h and 9 h, respectively. A long water retention time was conducive to contact fully between the sewage and nitrifying bacteria, leading to a more thorough nitrification reaction, thus effectively promoting the removal of ammonia nitrogen.

It can be seen from Figs. 2c, d that the concentration of NO_X^--N at stage A-1 or stage A-2 was below 3 mg/L, which could be explained by the fact that denitrification reaction was sufficient at stage A-1 or stage A-2. Furthermore, the concentration at stage A-3 was nearly doubled that at stage

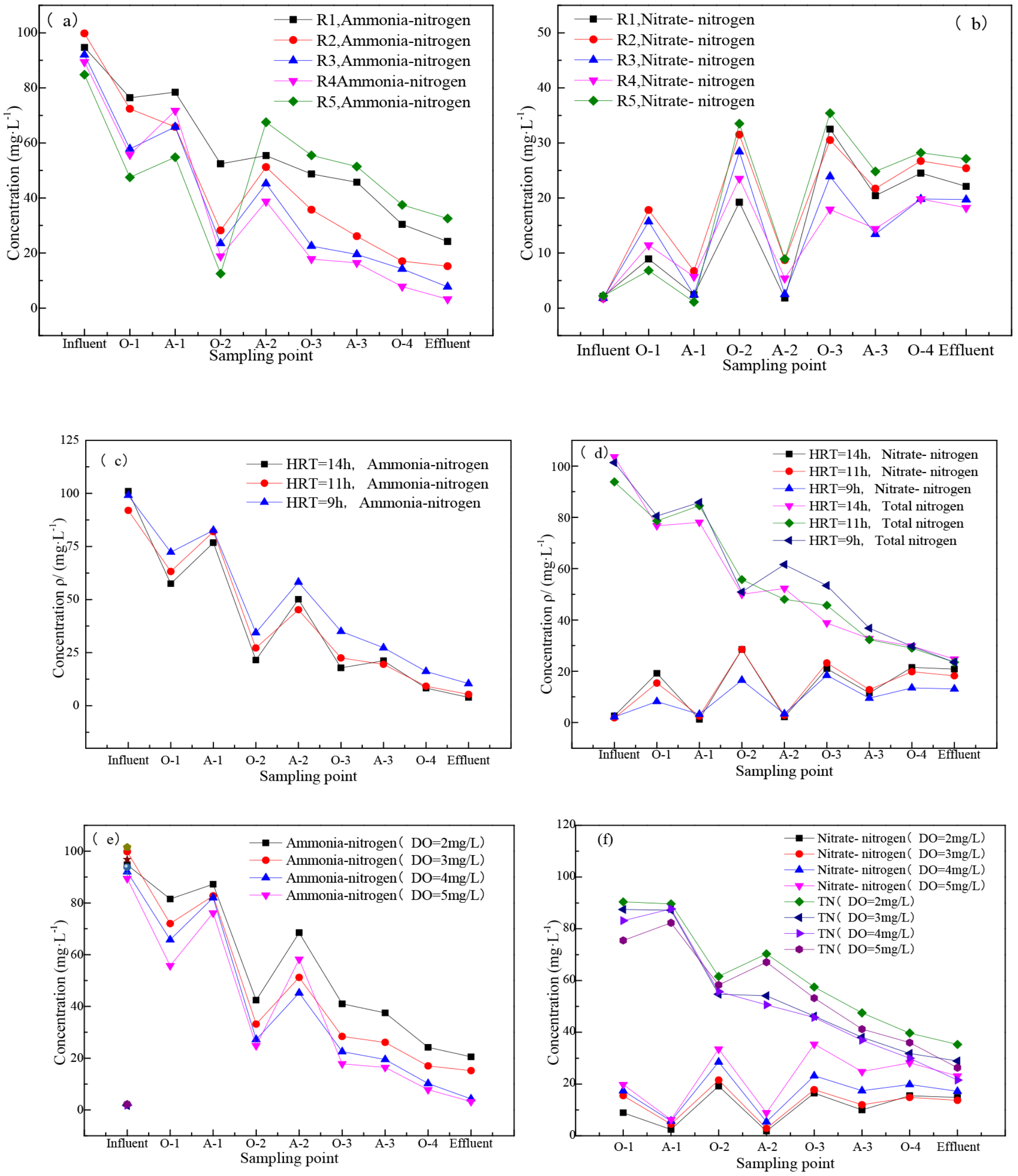

Fig. 2. Variation in the concentration of NH_4^+-N, NO_x^--N or TN at: (a,b) different influent flow rate distribution ratios, (c, d) different HRTs, (e, f) different DO levels.

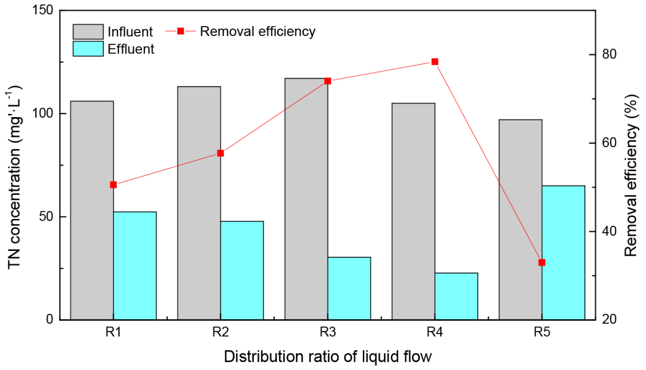

Fig. 3. Removal efficiency of TN at different flow rate distribution ratios.

A-1 or stage A-2, which was because COD was basically exhausted after three times of aerobic degradation, and the lack of carbon source affected the effect of denitrification at stage A-3.

The Hydraulic retention time is also an important parameter to the removal of TN. It can be seen from Figs. 2c, d that with the decrease of HRTs, the concentration of TN in the effluent increased, and the corresponding removal rates of TN decreased by varying degrees, they were 84.2%, 79.3%, and 64.1%, respectively. The composite outflow porous carriers could prevent nitrifying bacteria from being flowed out with water [12,13]. At small HRT, the time allowed for nitrification and denitrification was short, and the reaction was incomplete. In the mean time, the organic carbon source and NO_X^--N in sewage could not effectively enter the hypoxia microenvironment in the filler, which affected the efficiency of nitrogen removal. Considering HRTs of 14 h and 11 h, the difference in the concentrations of TN in the effluent was very small, while a longer HRT increased investment and operating costs, thus, the recommended HRT was determined to be 11 h.

3.3 Effect of dissolved oxygen on the denitrification efficiency of the reactor

The growth rate of activated sludge during biological treatment was highly influenced by the concentration of DO. In each section of the multi-stage A/O system, carbon oxidation reaction and simultaneous nitrification and denitrification reaction conducted alternatively. On the one hand, the higher concentration of DO was beneficial to remove organic pollutants,on the other hand, the denitrification reaction in the hypoxic environment would be affected when it was too high, which affected the removal effect of TN. A low concentration of DO would inhibit the nitrification reaction of ammonia nitrogen [14,15]. Therefore, it was very important for the removal of TN to determine the proper concentration of DO. The denitrification effect in the reactor was examined when the concentrations of DO were 2.0 mg/L, 3.0 mg/L, 4.0 mg/L and 5.0 mg/L, respectively.

Figs. 2e, f show the variation in the concentration of NH_4^+-N at different DO levels. With an increase in the concentration of DO, the removal rate of ammonia nitrogen were greatly changed to 78.4%, 84.8%, 95.3% and 96.4%, respectively. The concentration of DO had a great effect on the activity of nitrifying bacteria. When it was low, the corresponding activity was inhibited, and the concentration of NH_4^+-N in the effluent was high. When it became higher, the DO content basically satisfied the amount of oxygen for nitrification reaction, and it was not significant for the removal rate of ammonia nitrogen. When it was increased from 4.0 to 5.0 mg/L, the increase in the removal rate of ammonia nitrogen was only 1.1%, and the concentration of NH_4^+-N in the effluent decreased from 4.3 to 3.2 mg/L; the change in the range was not obviously.

As shown in Figs. 2e, f, with an increase in the concentration of DO, the nitrification rate increased correspondingly and most of the nitrobacteria existed in zoogloea flocs., mean while, the ability of the nitro bacteria to penetrate the biofilm was enhanced,the nitrification area was expanded, and the nitrification reaction of ammonia nitrogen was enhanced. However, to a certain extent, the high concentration of DO would affect the denitrification reaction in the hypoxia environment in the biofilm. On the other hand, the oxidation reaction of organic matter was enhanced when the concentration of DO was too high, most of the organic matter was decomposed and oxidized, the carbon source for denitrification reaction in the anoxic zone was reduced and the denitrification effect was weakened. As shown in Figs. 2e, f, in each anoxic section, the concentration of NH_x^+-N was higher under high concentration of DO than that under low concentration of DO.

As shown in Fig. 4, with an increase in the concentration of DO, the removal rates of TN were 65.8%, 72.2%, 80.2%, and 76.4%, respectively. Although a low concentration of DO was favorable for denitrification reaction in the hypoxic area, it played an important role in the nitrification rate and became a limiting factor for nitrification reaction. In additional, the low concentration of DO weakened the ability of oxygen to penetrate the biofilm, leading to the weakening of the aerobic microenvironment in the biofilm carrier, affecting the nitrification reaction. The results showed that the denitrification effect was better when the concentration of DO in aerobic zone was 4.0 mg/L (Fig. 4). The removal rate of TN was 67.8% when the concentration of DO was 5.0 mg/L, less than 71.3% when it was 4.0 mg/L,which was because with the enhanced mass transfer capacity of oxygen under high concentration of DO, the hypoxic microenvironment formed in the biofilm carrier was reduced or there were not an hypoxic microenvironment, and the denitrification reaction did not proceed adequately. In additional, as the concentration of DO increased, the degradability of organic matter was enhanced. Therefore, carbon deficiency could be another reason for performing the denitrification reaction.

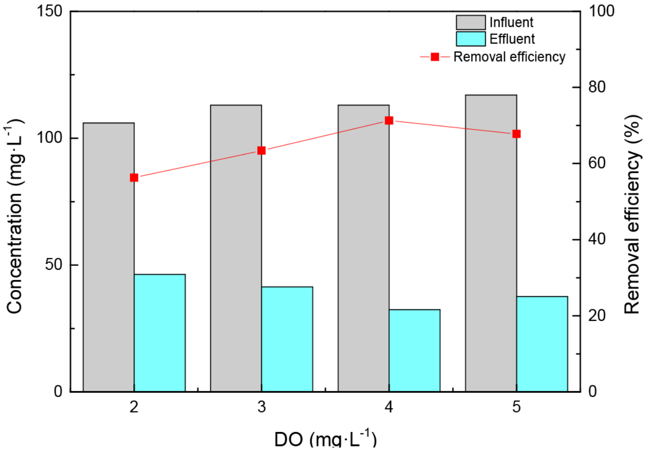

Fig. 4. Removal efficiency of TN at different DO concentrations.

3.4. Effect of temperature on the denitrification efficiency of the reactor

It can be seen from Fig. 5a that low temperatures were not conducive to the removal of NH_4^+-N and COD. The average removal rates of COD and NH_4^+-N were 84.9% and 63.8% at 13°C, and the average removal rates of COD and NH_4^+-N were 90.9% and 93.2% at 25°C. The effect of temperature on the removal of NH_4^+-N was higher than that of COD, which was because the nitrifying bacteria are auto trophic bacteria, sensitive to temperature and other environmental factors changes;temperatures between 20~30°C were appropriate for the growth of bacteria. When the temperature was low, the activity of the nitrifying bacteria was inhibited. When the temperature was below 15°C, the nitrification reaction could be greatly affected, what's more,the nitrification reaction could not proceed basically at 5°C [16].

3.5. Effect of the reflux ratio on the denitrification efficiency of the reactor

The sludge reflux ratio is an important parameter for the operation of sewage treatment plants, directly affect-

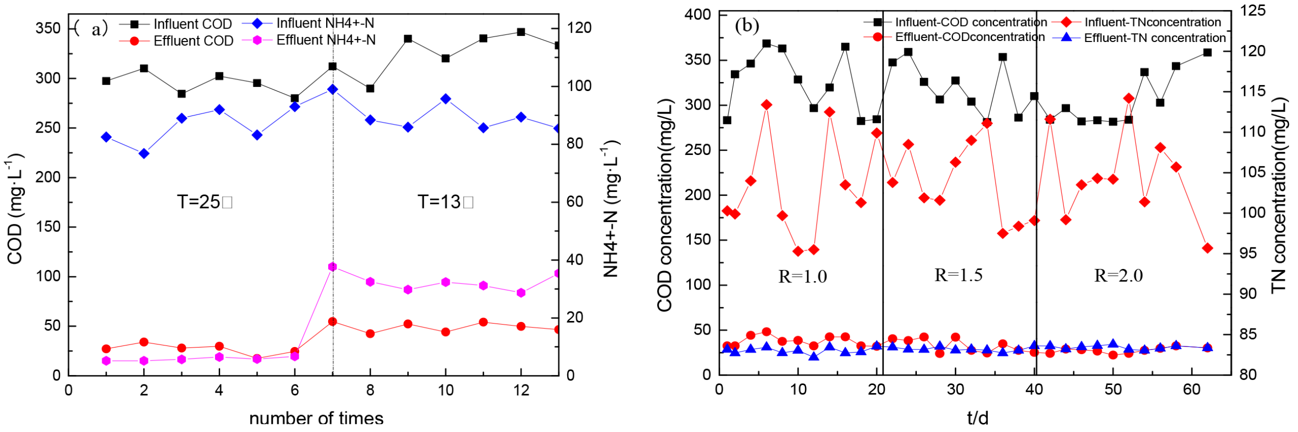

Fig. 5. Contaminants conditions: (a) Removal efficiency of COD and NH_4^+-N at different temperatures, (b) the concentrations of COD and TN at different reflux ratios.

ing the operation stability of the activated sludge. The sludge from sedimentation tank was used as reflux sludge, directly flowed back to the aerobic area at the head of the reactor.

The removal of organic matter and the denitrification efficiency at reflux ratio R = 1.0, 1.5 and 2.0 were examined. It can be seen from Fig. 5b that the removal rates of COD in the reactor were 91.4%, 92.9% and 93.5% when R = 1.0, 1.5 and 2.0, respectively. With the increase of reflux ratio, the removal rates of COD did not increase significantly, indicating that the biomass in the reactor was enough to meet the requirement for degradation of the organic matter. The removal rates of TN in the system were 81.8%, 75.5% and 70.4% when R = 1.0, 1.5 and 2.0, respectively (Fig. 5b), indicating that the denitrification effect was improved when the reflux ratio R = 1.0 (the removal rate of TN was 71.3% when there was no reflux), but with an increase in the reflux ratio, the removal rates of TN decreased. The reason for which was as followed, with the increase of reflux ratio, the biomass in the reactor increased, organic matter got a faster degradation, insufficient carbon source had become a limiting factor for the denitrification reaction.

3.6. Discussion on the changes in COD and TN under the best conditions and the mechanism

When the flow distribution ratio was 3:4:3, the HRT was 11 h, the concentration of DO was 4.0 mg/L, and the reflux ratio R was 1.0, the changes in the concentrations of TN and COD were shown in Fig. 6. The concentration of COD was lower at each stage in the reactor, and the microbial degradation was more thorough. The concentrations of COD and TN in the effluent were below 30 mg/L and approximately 20 mg/L, the corresponding removal rates were over 90% and approximately 80%, respectively. Due to the higher concentration of TN while lower concentration of COD in the influent, the removal rate of TN was difficult to further improve. Carbon source may be the main reason for this phenomenon. Fig. 6 shows that TN was removed at aerobic stages, because the unique internal structure of the filler affected the mass transfer of oxygen, resulting in a concentration gradient of DO between the interior and surface of the carrier as well as the inner and outer surface of the biofilm. On the biofilm surface of higher concentration of DO, aerobic nitrifying bacteria could become the dominant species; furthermore, oxygen could not penetrate inside the filler, so an anoxic environment could be formed. Therefore, the new compound migration carrier formed a diversity of microbial communities in which anaerobic-type, oxygen-type, aerobic-type, and suspended aerobic-type bacteria could be located from the inside out, and the carrier played the role of SND (simultaneous nitrification and denitrification). Due to the adhesion of the carrier, the suspended activated sludge and the falling biofilm was accumulated and adhered to the filler, in the meantime, they were digested and decomposed in the anaerobic microenvironment of the filler. The sludge was decomposed *in-situ* to release low molecular organic carbon, which provided a carbon source for nitrogen removal in denitrification reaction,and the promoting effect to nitrogen removal was significant.

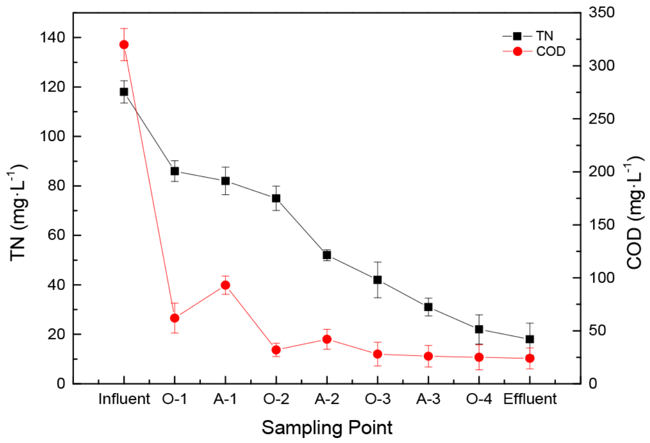

Fig. 6. Variation in TN and COD at the optimal conditions.

3.7. Performance of sludge reduction

The multi-redox environment coupled bioreactor not only has a good denitrification effect but also has a significant sludge reduction effect.. The experiment showed that the sludge yield was only 0.10 kg MLSS/ kg COD under the conditions of HRT of 11 h, flow rate of 3:4:3, the concentration of DO of 4.0 mg/L, temperature of approximately 25°C, and reflux ratio of 1.0, which was approximately 1/5~1/4 of the sludge yield obtained from the traditional activated sludge process for domestic sewage treatment (0.40~0.55 kg VSS/kg COD). There are many domestic studies on the effect of biofilm processes on sludge reduction [17,18]. Table 2 shows that the sludge reduction effect of multi-redox environment coupled bioreactor is more significant.

2.8. The mechanism of in-situ sludge reduction in multi-redox environment coupled bioreactor

2.8.1. Construction of multi-redox environment and principle of denitrification

As shown in Fig. 1, the hybrid reactor created alternative space-sequenced anoxic–oxic–anoxic–oxic zones. In the oxic compartment, the biofilm attached on the spherical porous carriers can be divided into four layers – suspended oxic layer, attached oxic layer, where nitrification occurs, the attached anoxic layer and deeper attached anaerobic layer where oxygen transportation is limited and denitrification takes place. These complicated microzones and oxic-anoxic inter phases can enhance bioactivity of the biofilm and maintain a delicate balance between nitrification and denitrification, achieving high removal efficiency of nitrogen-rich compounds via SND (simultaneous nitrification and denitrification). Besides, substrate diffusivity occurring within the biofilm microzones accompanies with alternative oxidation/reduction reactions, furthermore, the part of the substrate served as the carbon source for the denitrification (Fig. 7), producing less waste sludge and maximising sludge reduction.

Table 2
Comparison of the sludge yield in similar processes

Process	Sludge yield (kgVSS/kgCOD)
UASB + MBBR combination process	0.32
Sequencing biofilm batch reactor(SBBR)	0.17
Composite A^2/O process (plus polyurethane filler)	0.14~0.42
Casting porous plastic ball filler SBR process	0.21
Shaking-type filler-biofilm reactor	0.15

3.8.2. Microscope oberservation of the biofilm

The main causes of the good sludge reduction effect of the multi-redox environment coupled bioreactor are as follows: 1) An alternating oxic-anoxic environment formed in the space of the reactor, the appropriate microbial community and the dominant bacteria grew on each stage. The complex biological diversity created a microbial ecosystem gathering a variety of organisms, such as bacteria, vorticella, rotifers, nematodes, daphnia, etc. Under the influence of which the energy of the system and excess sludge were reduced [19]. 2) The new composite carrier filler has a variety of microbial environments. Because of its unique structure, aerobic, anoxic and anaerobic microenvironments form on the carrier from the outside to the inside. The microbial organisms on the surface of the carrier can quickly decompose soluble organic matter in the effluent during the biooxidation reaction. Due to the variety of microbial metabolism survival time, wastewater undergoes efficient *in-situ* sludge reduction.

Through observation with a 10–40× magnification electron microscope (JIANGNAN BM-1000), the activity of the sludge zoogloea was determined to be higher, the system was rich in biological resources, and there were many zooplanktons such as limb beetles, nematode, vorticella and rotifers (Fig. 8).

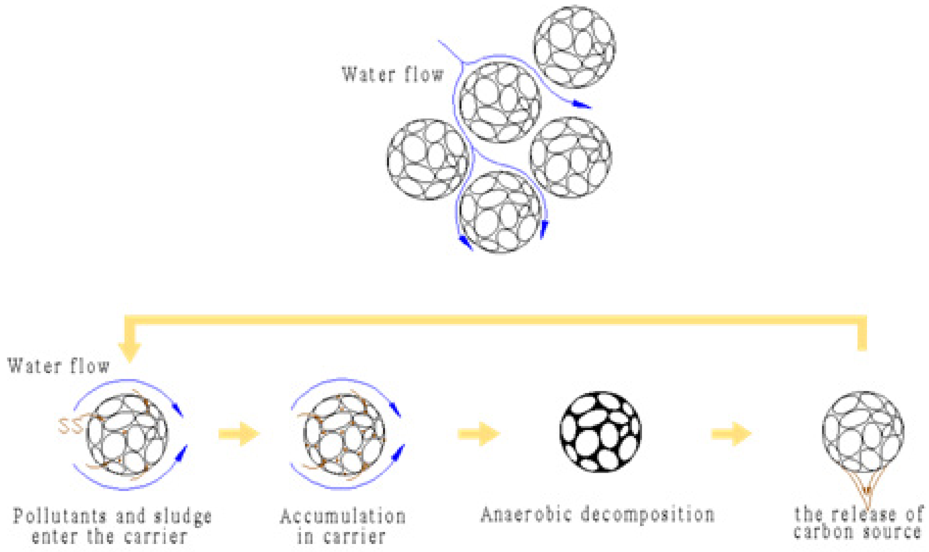

Fig. 7. Sludge decomposition occurring on the carriers' surface.

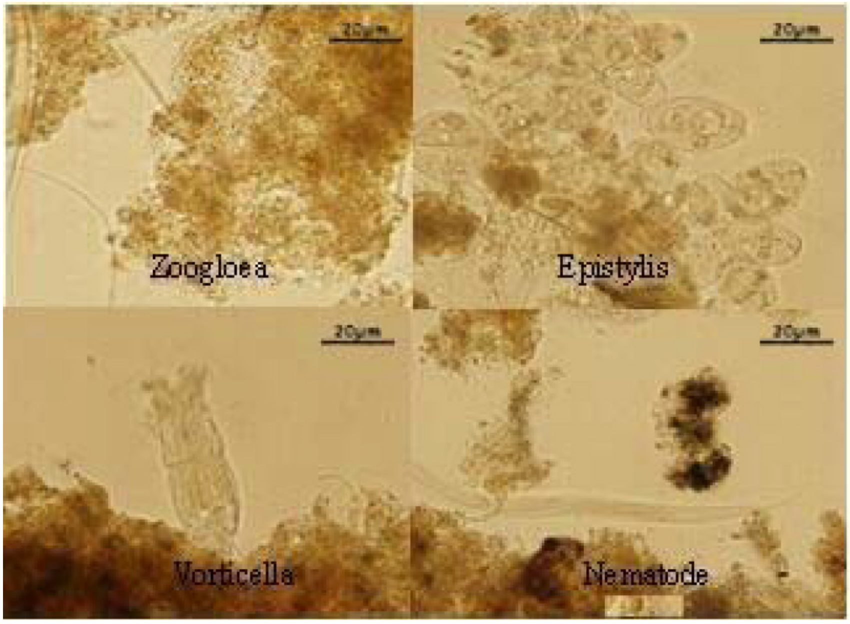

Fig. 8. Microscope images of micro-organisms on the carrier (×1000).

3.8.3. Morphological analyses of microbial ecology on biofilm

The biofilm samples were taken from the O-1, A-1, O-2, A-2, O-3, A-3 and O-4 carrier surfaces, respectively. The microbial morphology of biofilm surface in different regions was observed. The results are shown in Fig. 9. The O-1 biofilm was mainly composed of rod-shaped bacteria, as shown in Fig. 9A. The bacteria share similar physiological, biochemical and morphological characteristics. In contrast, the microbial morphology of the A-1 region was significantly looser than that of O-1, and the microbial dense structure was destroyed and a small amount of bacterial debris appeared (Fig. 9B). As the sludge passes through the continuous alternating A/O environment, microbial debris on biofilm surface gradually increased, as shown in Figs. 9C–G, the biofilm surface became loose and more porous. In section 2 of the article, the COD and ammonia concentration in the A-1 and A-2 regions were increased to some extent. Li et al. [20] also found that ammonia-nitrogen concentrations in the supernatant of hypoxia and anaerobic regions were higher than those in other areas of the reactor in an MBBR combined with continuous anaerobic aerobic reactor study. This phenomenon can be inferred that the broken microbial cells will release the intracellular soluble organics, i.e., Previous studies have found that microbes under anaerobic conditions will easily use intracellular substances released from the cell lysis phenomenon as substrate, for biodegradation and microbial growth [21,22], to achieve *in-situ* sludge reduction. Feng et al. [23] also found that the rod-shaped biofilm bacteria in the outlet area in the continuous A/O biofilm reactor seemed to shrink remarkably without compact floc morphology compared to the ones around the inlet area, (His observations was quite in agreement with Figs. 9D–G). The loose microbial morphology also contributes to the diffusion that the intracellular material released from cell lysis phenomenon in the filler flows into the water, improving the utilization efficiency of other microorganisms and promoting the recessive growth of microbes.

3. Conclusions

The multi-redox environment coupled bioreactor not only has a good nitrogen removal effect but also has a significant effect on sludge reduction. Based on these analyses, we can draw the following conclusions:

(1) The optimal operating parameters of the multi-redox environment coupled bioreactor are as follows: multi-point influent distribution ratio of 3:4:3, HRT of 11 h, the concentration of DO of 4.0 mg/L, temperature of 25°C, and reflux ratio of R = 1.0.

(2) Under the optimal operating conditions, when the concentrations of TN, NH_4^+-N and COD in the influent are 80~130 mg/L, 75~100 mg/L and 260~400 mg/L, the concentration of TN in the effluent is approximately 20 mg/L, while the concentrations of NH_4^+-N and COD are reduced to 5.0 mg/L and 30 mg/L, respectively. The average removal rates of TN, NH_4^+-N and COD are 80%, 95% and 91%, respectively. The results show a good denitrification effect.

(3) A variety of microbial environments are formed in the multi-redox environment coupled bioreactor, where there are rich microbial communities. Under the influence of which, sludge is reduced efficiently. The results demonstrate that the sludge yield in the reactor is only 0.10 kg MLSS/kg COD, which is superior to other similar biofilm processes, and it has a good sludge reduction effect.

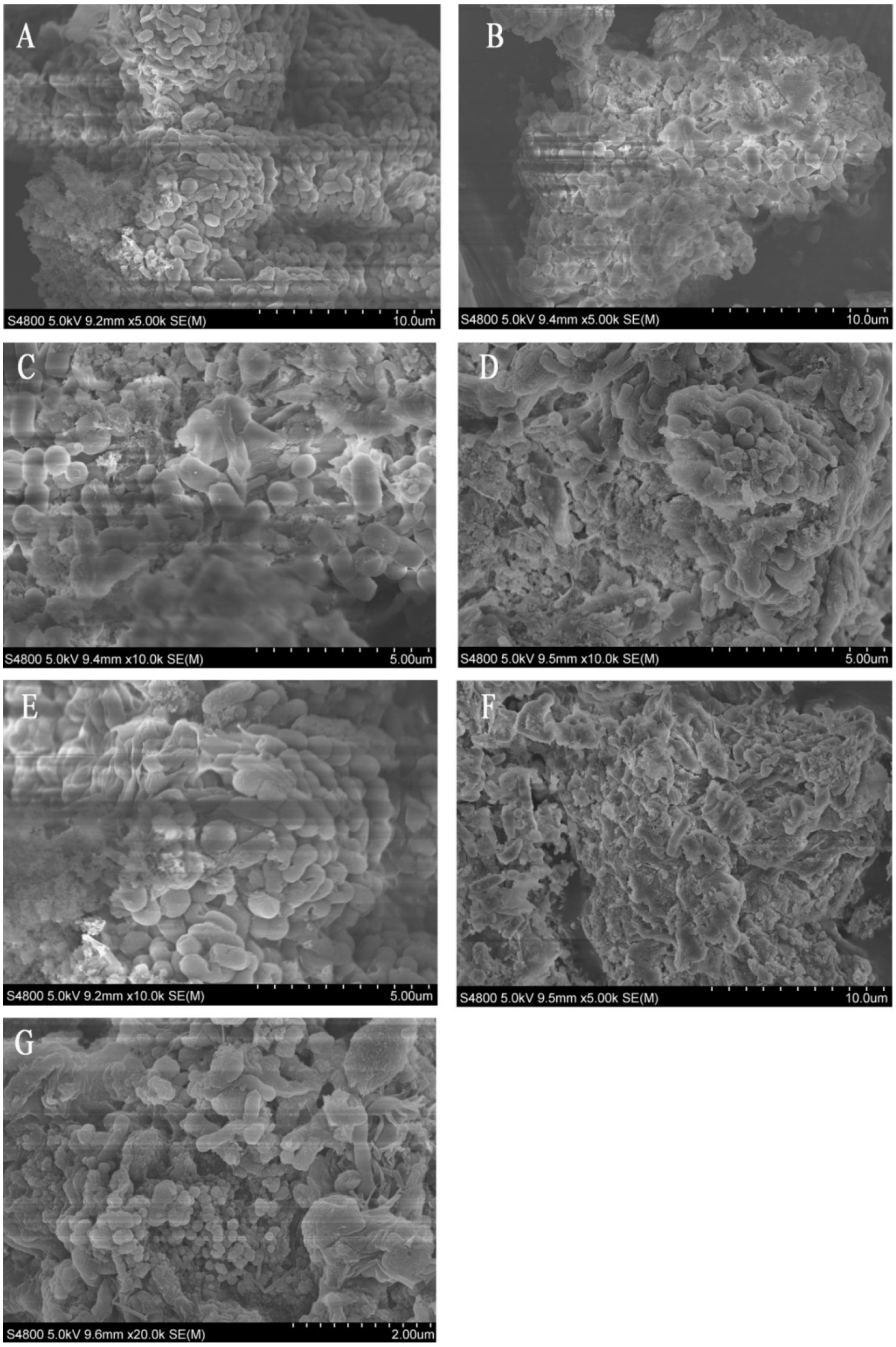

Fig. 9. SEM images of the biofilm in different regions: A:O-1; B:A-1; C:O-2; D:A-2; E:O-3; F:A-3; G:O-4

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