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System for startup of partial nitritation - ANAMMOX - denitrification (PNAD) reaction

System for startup of partial nitritation -ANAMMOX - denitrification (PNAD) reaction

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Abstract. A partial nitritation- anaerobic ammonium oxidation (ANAMMOX)-denitrification (PNAD) process was activated to provide the basis and experience for engineering. A stirred up-flow reactor with surface continuous oxygen-limited aeration was used to realize partial nitritation by controlling the dissolved oxygen (DO) (0.4-0.5mg/l) of the reactor. In this study, an ASBR reactor was inoculated with ANAMMOX activated sludge with denitrification properties. After 98 days of activity recovery, the total nitrogen removal rate reached 90%. The reactor was run to the 194th day, with the total nitrogen removal efficiency reaching 95% after increasing influent nitrogen concentration on the 99th day, and the two reactors were combined in series. Up to the 212th day, the influent NH_4^+-N was 253.51mg / (l· d), and the effluent NH_4^+-N, NO_2^--N and NO_3^--N concentration were 1.67 mg / (l· d), 1.18 mg / (l· d)and 5.68mg / (l· d) respectively. In addition the activated sludge of two reactors was also identified by 16S rRNA.

1. Introduction

The ANAMMOX process is a new process in which ammonia nitrogen and nitrite nitrogen are used to produce nitrogen directly into the atmosphere in an anaerobic environment, and can be used to reduce the total nitrogen in sewage treatment and to promote nitrogen circulation [1].The process does not require an organic carbon source or aeration, so its use to treat sewage can reduce operating costs. The mainly functional bacteria of the process and anaerobic ammonia oxidation bacteria need 1.32 moles NO2--N to oxidize 1 mole NH4+-N [2-3], but there is little NO_2^--N in the actual sewage. Many researchers have conducted a series of experiments to supply NO_2^--N and perfect anaerobic ammonia oxidation such as the short-cut nitrification-anaerobic ammonia oxidation (SHARON - ANAMMOX) [4, 5], oxygen limited autotrophic nitrification-anaerobic denitrification (OLAND) [6, 7], completely autotrophic nitrogen removal process (CANON) [8, 9] and short-cut nitrification - anaerobic ammonia oxidation - denitrification (SNAD) [10, 11, 12]. Most researchers used different microorganisms including anaerobic ammonia oxidation bacteria and Ammonia Oxidizing Bacteria (AOB) in a same reactor to start or run [6, 8, 10], alleviating the need for intermediate control of pH and DO. However, when the instability of reactor operation needs to be remedied, it is not easy to analyze and solve it in detail in face of various possible causes. In order to handle properly the pH and DO required for the survival of various species, the height-diameter ratio of the reactor designed was often greater [12] or more compartments in the horizontal direction [10], so that different types of bacteria can be partitioned. In this study, the partial nitritation reactor and anaerobic ammonia oxidation-denitrification (PNAD) reaction system was run, a provision of a sewage treatment scheme.

Partial nitritation is the key control process around the expansion of ANAMMOX process, which refers to the process of oxidizing approximately 50% of ammonia nitrogen to nitrite nitrogen [13, 14]. In order to achieve NO_2^--N effluent stability, DO, pH and FA need strict control [14~18]. At present, most reactors use intermittent aeration [19, 20], which requires the relevant staff to pay close attention to the dissolved oxygen in the reactor for timely aeration. Such a reactor being actually used, intermittent aeration will bring a new burden to the sewage treatment plant. In this study, a new type of up-flow reactor with surface-level oxygen-limited continuous aeration was used. This reactor can not only effectively solve the problem caused by intermittent aeration, but also effectively prevent the occurrence of effluent water instability in short-cut nitrification reactor caused by excessive aeration.

In this study, 16S rRNA was also identified for the activated sludge domesticated in the partial nitritation reactor and anaerobic ammonia oxidation-denitrification reactor.

2. Materials and methods

Test equipment and operating conditions

2.1.1. Partial nitritation reactor. Its height was115 cm, internal diameter 9.5 cm; the effective volume was about 8 L. The influent and effluent were about 3L per day before the successful start-up. The reactor was run in a continuous flow mode. When combined, its inlet and outlet water was about 1L/d. It was at room temperature; pH was controlled at 7.5~8.2; DO was controlled at 0.1 ~ 1.7 mg / l; a stirring rod was installed in the reactor at a rotating speed of 30 r / min; sludge reflux ratio was about 80%.

2.1.2. AD reactor. The AD reactor was applied to the anaerobic sequential batch reactor (ASBR). The water intake time was about 30min; the influent pH adjustment time was about 1min; operation time was about 23h; precipitation time was 20min; filter time was about 3min.Its height was 35 cm, inner diameter 10.5 cm, and the effective volume about 3 L. A stirring rod was also installed in the reactor at a rotating speed of 30r / min. The outer layer was a water bath insulation layer, with the temperature of the circulating water bath insulation box set at 35°C. The surface of the reactor was covered with tinfoil paper to avoid the competition with photosynthetic bacteria. DO was under 0.3 mg / l; pH was controlled at 8.0 ~8.5. There was about 1L of water in and out every day, without discharging mud.

The 16S rRNA identification experimental procedure included extraction of total DNA from the microbial group, PCR amplification of the target fragment, amplification product recovery and purification, fluorescence quantification of the amplified product, preparation of the sequencing library, and high-throughput sequencing on the machine. The process was determined by the microbiological company, personalbio, in Shanghai.

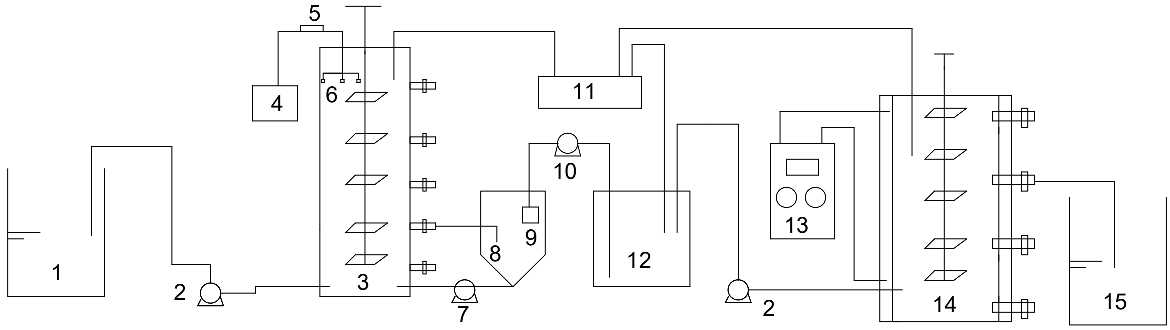

Figure 1. Schematic diagram of PNAD series combination

1: Inlet tank 2: Peristaltic pump 3: Partial nitritation reactor 4: Air pump 5: Regulating valve 6: Aerator 7: Return sludge pump 8: Discharging tube 9: Hollow fiber membrane module 10: Magnetic drive circulating pump 11: Hash water quality monitor 12: Semi-closed regulating tank 13: Thermostatic water bath 14: Anaerobic ammonia oxidation-denitrification reactor 15: Effluent tank

2.1.3. Inoculation sludge

Partial nitritation reactor: the activated sludge from an aeration tank of a municipal sewage treatment plant in Baoding was domesticated as an inoculating mud, and the SS was 4.189 g/l.

Anaerobic ammonia oxidation-denitrification reactor: the inoculated sludge was ANAMMOX activated sludge with denitrification properties, and the SS was 4.118 g/l. The sludge was taken from the aeration tank activated sludge and river sediment, domesticated and cultured for more than 1 year.

Test water

2.1.4. Partial nitritation reactor. The experimental water was artificially simulated wastewater; the initial concentration of NH_4Cl was 50 mg/l, and the added concentration was different depending on the mode of later operation of the reaction.

2.1.5. Anaerobic ammonium oxidation reactor. The experimental water was also artificially simulated; and NH_4Cl and $NaNO_2$ were the main inorganic nitrogen source provision of animus bacteria, with the concentration of the later preparation increasing proportionally. The influent COD was tested at about 200mg/l.

3. Results and discussion

In this study, a stirred up-flow reactor was used to enrich AOB, with the ammonia oxidation rate of activated sludge increasing by a high and low ammonia nitrogen method [21]. Then, the concentration of NH_4^+-N and pH of the influent water were adjusted to promote the reactor to achieve partial nitritation. The AD reactor was established synchronously. Finally, the partial nitritation reactor and the AD reactor were combined in series to realize a series process of the partial nitritation and anaerobic ammonium oxidation-denitrification (PNAD).

Establishment of partial nitritation reactor

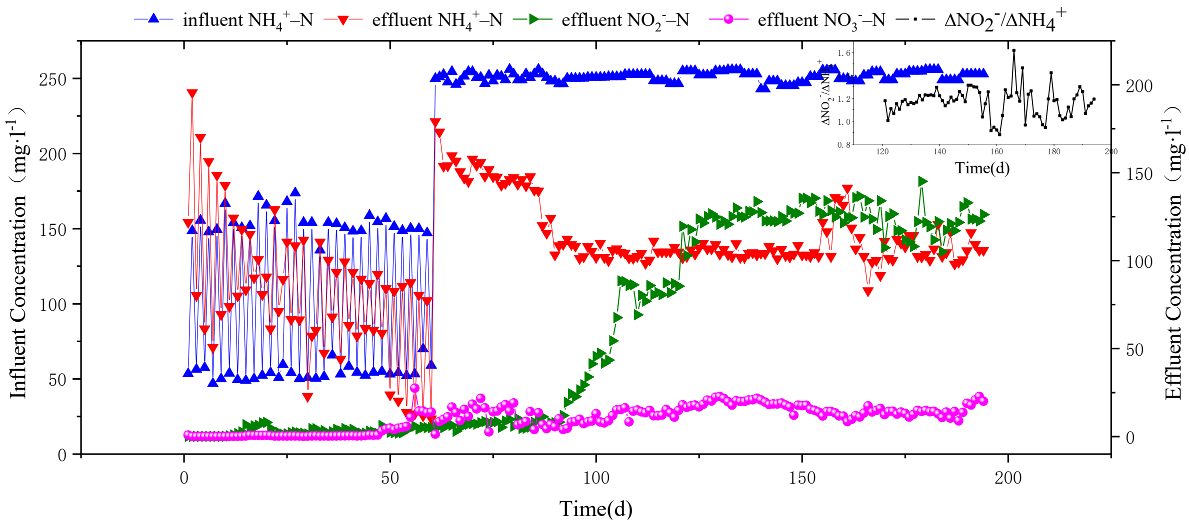

Figure 2. Establishment of partial nitritation reactor

The activated sludge was taken from the aeration tank of the municipal sewage treatment plant and was inoculated into the stirred up-flow reactor. The ammonia oxidation rate was raised by the high and low ammonia nitrogen method [21] (50mg/l and 150mg/l) at 1 to 60 days. The high and low ammonia nitrogen method, which uses different concentrations of ammonia nitrogen to the reactor, alternately, aims to stimulate and improve the activity of AOB. At 61~194d, high ammonia nitrogen (250mg/l) was used for feed, and surface oxygen-limited aeration was applied, which was intended to further increase the accumulation of nitrite. The concentration of effluent ammonia nitrogen from 1 to 50 days was higher than the influent concentration, which may be due to the fact that some microorganisms cannot adapt to this experimental condition and appear autolysis phenomenon [22]. It can be seen from Figure 2 that the ammonia nitrogen effluent continuously decreased and that the ammonia oxidation rate gradually increased from 1 to 60 days. The ammonia oxidation rate increased to 83% when the reactor was operated to the 60th day, indicating that some microorganisms had adapted and survived in the reactor during the phasing out process.

In order to provide an appropriate amount and continuous oxygen for the bacteria in the reactor and enable the reactor to efficiently produce nitrite, an upper surface oxygen-limited aeration device was implemented. Most reactors used intermittent aeration [20] and needed to stop aeration before the inflection point occurred according to the pH value [21], which inevitably imposed a burden on the operating personnel. The dissolved oxygen in the upper surface continuous oxygen-limited aeration reactor at different depths was in the range of 0.1~1.7mg/l, which was in line with the DO range of AOB [24]. Partial nitritation can be achieved by controlling dissolved oxygen of about 0.4-0.5mg/l at a depth of 15cm. The effluent nitrite concentration/ effluent ammonia nitrogen concentration in the partial nitritation reactor was in the range from 1 to 1.4 mostly, which was in line with the ratio of NO_2^--N to NH_4^+-N of ANAMMOX reaction. This mode can solve the problem from the inconvenient control of aeration during operation, and the effluent nitrite was relatively stable due to the structure and the mode of operation.

Establishment of AD reactor

The activated sludge was inoculated into an ASBR reactor to recover activity. The initial NH_4^+-N concentration was 50mg/l and the NO_2^--N concentration was 66mg/l. Up to 98d, the effluent NH_4^+-N and NO_2^--N concentration were 0.5mg/l and 1.19 mg/l, and the NO_3^--N was 9.47mg/l, an indication that the activity of anaerobic ammonia oxidation activated sludge was improved and that the ASBR reactor successfully started.

In order to provide abundant nutrients to the activated sludge of the ASBR reactor and make the reactor be connected to the partial nitritation reactor in series, the NH_4^+-N influent concentration was increased to 105 mg/l, and the NO_2^--N influent concentration was increased to 135 mg/l on the 99th day. In the face of a sudden increase in the influent load of the reactor, the activated sludge could not adapt to this change, and it was particularly obvious from the effluent NO_2^--N in Fig. 3. The effluent NO_2^--N concentration reached 114.74 mg/l at worst time, and the total nitrogen removal rate dropped to 27%. The reactor continued to run. Up to 194d, the total nitrogen removal efficiency of the reactor was increased to 96%, and the total nitrogen concentration of the effluent was 10.47 mg/l. The effluent COD was about 120mg/l. Therefore, the AD reactor could undertake the effluent NH_4^+-N and NO_2^--N from the partial nitritation reactor due to the stability of the AD reactor.

Reactor series combination

After the start-up of the partial nitritation and the AD reactor, the ammonia nitrogen and nitrite nitrogen from the partial nitritation reactor can be used to supply the anaerobic ammonium oxidation reactor. However, the pH in the effluent of the partial nitritation reactor needed to be adjusted accordingly. Because the pH of the effluent was often low (pH<8), and the effluent of the partial nitritation reactor contained aerobic bacteria. If it was directly pumped into the anaerobic ammonia oxidation reactor, it would not be conducive to the stable operation of the AD reactor and the survival and reproduction of the anammox bacteria and denitrifying bacteria.

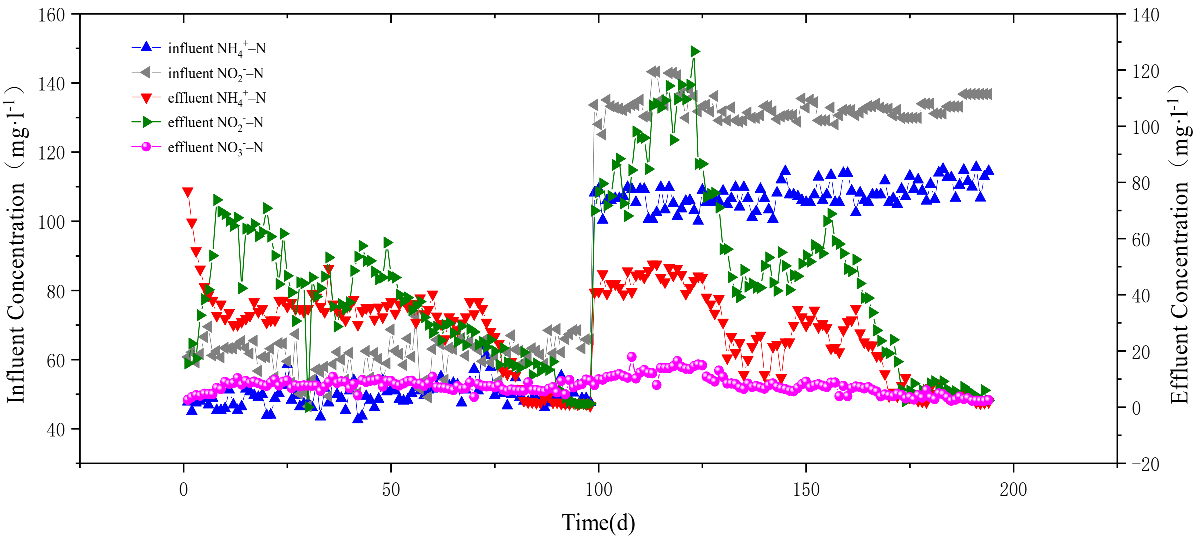

Figure 3. Establishment of anaerobic ammonia oxidation reactor

In view of the above, a hollow fiber membrane module and a semi-closed regulating tank were added between the partial nitritation reactor and the AD reactor. The circulating pump pumped the effluent water from the partial nitritation reactor into the semi-closed regulating tank through the hollow fiber membrane module; the pH and DO in the regulating tank were monitored by the water quality monitor. The pH was adjusted to 8 and the DO under 0.3 mg/l. Then the water from the regulating tank was pumped into the AD reactor with a peristaltic pump. After series connection, the water volume treated by the reactors was about 1000ml every day. As shown in Figure 4, the total nitrogen removal efficiency reached beyond 93%.

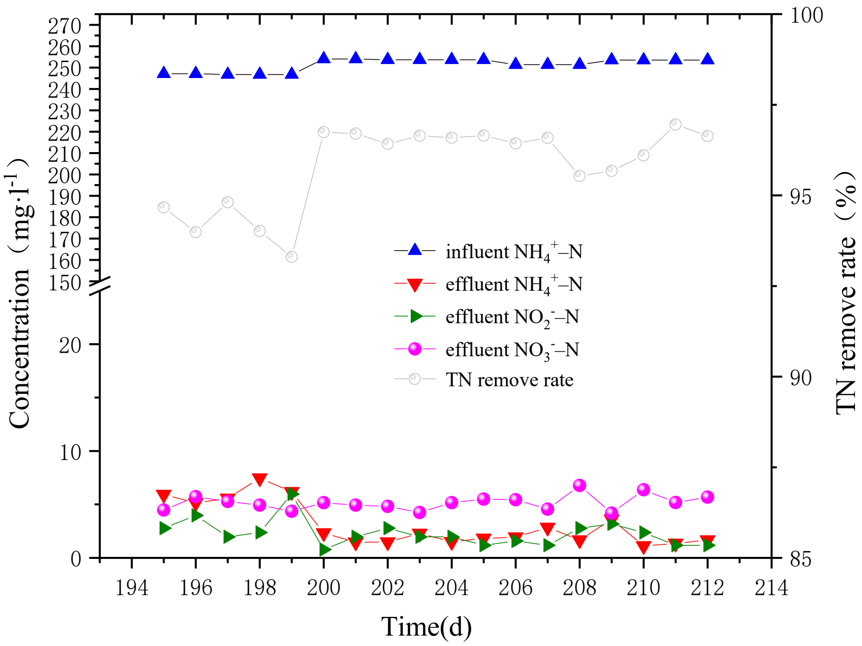

Figure 4 Experimental study of the PNAD process

16s rRNA identification

The total nitrogen removal rate was higher than the theoretical value of anaerobic ammonium oxidation process due to the activated sludge with denitrifying properties in the inoculated anaerobic ammonium oxidation activated sludge. In order to further explain the effects of denitrifying bacteria and analyze the activated sludge population structure of the two reactors, the activated sludge of the two reactors was subjected to 16S rRNA microbial identification.

The activated sludge in the partial nitritation reactor and the activated sludge in the AD reactor were passed to the microbial identification company for 16S rRNA identification. The AOB in the partial nitritation reactor was mainly of Nitrosamines, and the relative abundance was 11.86%; the anaerobic ammonium oxidizing bacteria in the AD reactor were mainly of bacterium_enrichment_culture_clone_Anammox_2 and Candidatus_Kuenenia, and the relative abundances were 2.35% and 1.74%, respectively. In the AD reactor, the denitrifying genus had Denititrasoma and Thermotonus, and their relative abundances were 2% and 0.69%, respectively.

4. Conclusion

The stirred up-flow reactor with surface continuous oxygen-limited aeration can be used to effectively accumulate nitrite, and by controlling the dissolved oxygen (0.4-0.5mg/l), the partial nitritation reactor can be activated.

The semi-closed regulating tank was connected in series, which could be more flexibly adjusted according to the partial nitritation reactor and AD reactor. Using the process, the total nitrogen removal efficiency can reach more than 93%.

The AOB of the partial nitritation reactor were mainly Nitrosomonas, the main ANAMMOX bacteria of the AD reactor were bacterium_enrichment_culture_clone_Anammox_2 and Candidatus_Kuenenia, and main denitrifying genus were Denititrasoma and Thermomonas by 16S rRNA authentication.

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