Performance of anaerobic denitrifying upflow anaerobic sludge bed bioreactor (AD-UASB)

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Abstract. Nitrate-containing wastewater has a severely negative impact on the ecological environment, and anaerobic denitrification process is promising biotechnology for nitrate-containing wastewater treatment. The nitrogen removal performance of anaerobic denitrifying upflow anaerobic sludge bed bioreactor (AD-UASB) and granular sludge characteristics were investigated. The results showed the maximum nitrogen removal loading rate (NRR) was reached up to 28.6 kg/(m³·d) as nitrogen loading rate (NLR) was 28.8 kg/(m³·d). At the same time, the effluent NO₃⁻-N concentration and total nitrogen removal efficiency of AD-UASB was 5.09 mg/L and 99.51%, and the effluent NO₂⁻-N concentration was as low as 10.97 mg/L without obvious accumulation. As NLR was elevated to 36 kg/(m³·d), the effluent NO₃⁻-N and NO₂⁻-N concentration were sharply increased to 199.62 mg/L and 453.13 mg/L, respectively. Thus, the denitrifying metabolic pathway was strongly suppressed which further led to the accumulation of NO₂⁻-N concentration and the collapse of AD-UASB performance. The granular sludge was broken up and washed out from the reactor, and meanwhile, the concentration of MLSS and MLVSS were declined to 55.09 g/L and 24.7 g/L, respectively. The research can provide a beneficial reference for the anaerobic denitrification biotechnology application in nitrate-containing wastewater treatment.

1. Introduction
Nitrate is a common pollutant in domestic and industrial wastewater which will result in eutrophication after being discharged into waterbody without effective treatment. Nitrate also threaten human health by easily transforming to nitrite as a carcinogen[1-2]. Coupled with the further improvement of wastewater discharge standards, the limitations of nitrogen discharge are strictly controlled[3]. Anaerobic denitrification process is regarded as a highly efficient and cost-effective nitrogen removal technology in nitrate-containing wastewater treatment field[4]. Simultaneously, compared with flocculent sludge, anaerobic denitrification granular sludge has a better nitrogen removal performance and higher nitrogen removal loading rate than that of flocculent sludge due to its high biomass concentration and excellent settling capability, which can facilitate solid-liquid separation[5]. However, the maximal potential of the nitrogen removal performance of anaerobic denitrification is unknown. Therefore, the nitrogen removal performance of anaerobic denitrification process in UASB bioreactor with granule denitrifying sludge was investigated in order to provide theoretical support for biological nitrogen treatment application.
2. Materials and methods

2.1. Experimental setup

Upflow anaerobic sludge bed (UASB) reactor was applied which was made ofplexiglass with the height of 65cm, and the working zone diameter of 8cm. The total volume of the reactor was 3.5L, which included the effective volume of 1.4L. Initially, UASB was inoculated with 1.2L anaerobic digestion granular sludge taken from IC reactor treating paper-making wastewater. Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS), and the VSS/SS ratio were about 32g/L, 18.8g/L, and 0.59, respectively. The temperature was stabled at 35ºC. At the same time, the pH was set at about 7.0.

2.2. Synthetic wastewater and reactor operation

The influent COD and NO₃⁻-N were provided as CH₃COONa and NaNO₃, respectively. In order to provide sufficient carbon source for anaerobic denitrification, the C/N ratio was fixed at 4.5. The addition of trace elements and nutrient solution was 1ml/L according to Li et al. (2014)[6]. There were two different operating procedures during the whole experiment. First, the hydraulic retention time (HRT) was fixed at 12h, and the concentration of NO₃⁻-N was stepwise increased in the range from 300mg/L to 1000mg/L in startup phase termed P0. Second, the concentration was maintained in 1000mg/L and HRT was shorten progressively in running phase until the performance of UASB was deteriorated. The running phase was divided into two periods defined respectively as P1 and P2 depend on the denitrifying performance. The detailed operation procedure of AD-UASB was listed in Table 1.

| Phase | Operation time (d) | NO₃⁻-N concentration (mg/L) | HRT (h) | NLR (KgN/(m³·d)) |
|-------|-------------------|------------------------------|---------|------------------|
| P0    | 1-10              | 300                          | 12      | 0.6              |
|       | 11-15             | 500                          | 12      | 1                |
|       | 16-20             | 800                          | 12      | 1.6              |
|       | 21-24             | 1000                         | 12      | 2                |
|       | 25-30             | 1000                         | 10      | 2.4              |
|       | 31-37             | 1000                         | 8       | 3                |
|       | 38-46             | 1000                         | 6       | 4                |
| P1    | 47-51             | 1000                         | 4       | 6                |
|       | 52-58             | 1000                         | 3       | 8                |
|       | 59-65             | 1000                         | 2       | 12               |
|       | 66-78             | 1000                         | 1       | 24               |
|       | 79-83             | 1000                         | 0.83    | 28.8             |
| P2    | 84-98             | 1000                         | 0.67    | 36               |

2.3. Analytical methods

The concentration of COD, nitrate, nitrite, pH, MLSS, and MLVSS were determined by the Standard Methods[7]. The Zeta potential of granular sludge was measured by Zeta potentiometer at different NLR.

3. Results and discussion

3.1. Performance of AD-UASB bioreactor
Figure 1. Pollutant removal efficiencies and effluent pH change of AD-UASB. (a) COD, (b) NO3--N and NO2--N, (c) NLR and NRR, and (d) pH.

As illustrated in Figure 1a, the COD removal efficiency reached up to 85% in P0 and P1 phase, and the average effluent concentration of COD was 355.24 mg/L. In P2 phase, the effluent COD concentration dramatically varied from 612.67 to 1605.17 mg/L, which was 2.6 times higher, and the COD removal efficiency decreased from 85.01% to 61.20% along with the NLR gradually increase to 36 kg/(m³·d). The COD removal efficiency reduced by 28% in P2 phase compared with that in P1 phase. Moreover, the averaged effluent NO3--N and NO2--N concentration slightly increased from 1.29 and 0.75 mg/L at P0 phase to 3.24 and 0.86 mg/L at P1 phase. Therefore, the mean NO3--N removal efficiency was about 99.52% and no NO2--N accumulation in P0 and P2 phase (Figure 1b). The NO3--N removal efficiency was rapidly reduced to 81.32% and NO2--N accumulation was apparently occurred accompanied with the NLR increase to 36 kg/(m³·d). The effluent NO2--N concentration was sharply increased from 10.98 mg/L to 453.13 mg/L at P2 phase. The NO3--N removal efficiency was reduced by 18.3%, and the effluent NO3--N and NO2--N concentration were 38.8 and 40.2 times higher compared to that in P1 phase (Figure 1b). The NRR was continuously upgraded to 28.6 kg/(m³·d) along with the NLR increase. Nevertheless, as further continuing to increase NLR to 36 kg/(m³·d), the NRR was conversely declined to 15 kg/(m³·d) mainly due to the drastic accumulation of NO2--N, in this case, the nitrogen removal performance of AD-UASB was worsen (Figure 1c). Thus, the maximal NRR of AD-UASB was 28.6 kg/(m³·d) which was higher than reported the highest NRR (19.1 kg/(m³·d))[8]. With the increasing of NLR from 28.8 to 36 kg/(m³·d), the denitrification metabolic pathways were hindered, which accelerated the deterioration of the system nitrogen removal performance, and negatively affected the efficiency of nitrogen removal, even collapsed the system by inhibiting the following metabolic pathway of NO2--N reduction[9]. The effluent pH changed between 8.76 and 10.14 at P0 and P1 phase. Compared with the P0 phase, the effluent pH was increased by 15.75% in P1 phase. Conversely, the pH value was inversely decreased to 9.88 in the P2 phase which was the effluent pH reduced by 2.56% compared with that in P1 phase (Figure 1d). The results were in line with the previous study reported by Li et al. (2014) who found that the effluent pH was significantly increased owing to the rise of denitrification process.
produced OH− causing the increase of alkalinity[10]. The reduction of per mg NO3−-N to N2 produce 3.57 mg alkalinity (calculated as CaCO3).

3.2. Denitrifying granular sludge characteristics
As shown in Figure 2, the MLSS and MLVSS increased from 63.21 mg/L and 27.19 to 67.21 and 62.9 mg/L along with the increase of NLR from 0.6 kg/(m3·d) to 28.8 kg/(m3·d), respectively. The MLVSS was dramatically increased by 1.31 times at NLR of 28.8 kg/(m3·d) compared with that at the NLR of 0.6 kg/(m3·d). However, the Zeta potential was increased and the biomass of granular sludge was decreased as NLR was 36 kg/(m3·d) due to the breakup of granular sludge and washout of biomass from bioreactor. Compared with the situation under the NLR of 28.8 kg/(m3·d), the MLSS concentration, MLVSS concentration, and VSS/SS ratio were reduced by 18.17%, 60.73%, and 51.93% under the NLR of 36 kg/(m3·d) (Figure 2a). Moreover, the Zeta potential was increased by 6.57% (Figure 2b). The results suggested that the structure of granular sludge was negatively changed, and the stability of system was badly affected with the increase of NLR from 28.8 kg/(m3·d) to 36 kg/(m3·d). This phenomenon was probably due to the decrease of negatively charged microbial cells and electrostatic repulsion in the system[11]. Ren et al. (2018) also found that the system removal performance decreased with the decline of MLSS concentration[12]. This change of VSS/SS ratio indicated that the proportion of volatile organic suspended solids and the portion of active content in granular sludge was declined.

![Figure 2. Variation of biomass concentration and Zeta potential under different NLRs. (a) biomass concentration, (b) Zeta potential.](image)

4. Conclusions
The AD-UASB inoculated by anaerobic digestion granular sludge can achieve a high-rate nitrogen removal loading rate reached up to 28.6 kg/(m3·d) as NLR was 28.8 kg/(m3·d). During the maximal period of NRR, the effluent NO3−-N concentration and total nitrogen removal efficiency of AD-UASB was 5.09mg/L and 99.51%, simultaneously, the effluent NO2−-N concentration was as low as 10.97mg/L without obvious accumulation. The nitrogen removal performance was subsequently worsened with further elevation of NLR responsible for the breakup and floatation of granular sludge resulting in the washout of biomass and the decline of MLVSS concentration.

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