Impact of temperature reduction on partial nitrification in a batch biofilter

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Abstract. The impact of temperature reduction on the stability and recoverability of partial nitrification in a batch biofilter were investigated in present study. The results showed that NH$_4^+$-N removal rate, NO$_2^-$-N accumulation rate and effluent NO$_2^-$-N/NH$_4^+$-N ratio of the batch biofilter could maintain at about 60%, 95% and 1.3 respectively when the temperature was controlled at 20~25°C. Within this temperature range, the batch biofilter had good partial nitrification performance and could provide suitable and stable influent conditions for the subsequent anaerobic ammonia oxidation (ANAMMOX). When the biofilter was shocked by low temperature, its COD removal ability and partial nitrification performance could both basically restore to its original level via resuming 25°C for a period of time.

1. Introduction

Temperature is one of the most important factors affecting the survival of microorganisms. Its influence on the growth and reproduction of microorganisms is mainly manifested in the following aspects [1-3]: i). The activity of enzymes is greatly influenced by temperature. Metabolism in organisms needs to be catalyzed by enzymes, most of which belong to proteins. Temperature changes can affect the rate of enzymatic reaction, and then affect the efficiency of cell synthesis. Different types of enzymes have different adaptability to temperature. When the temperature is higher or lower than the optimum range, the enzyme activity will decline or even be completely destroyed; ii). The fluidity of cell membrane is also affected by temperature. The fluidity of cell membrane is the basis of cell completing many physiological functions. When the temperature changes, the content of lipid unsaturated fatty acids is affected, the fluidity of cell membrane changes, and then affects the absorption efficiency of nutrients and the secretion of metabolites; iii). The solubility of nutrients is affected by temperature. Nutrients can only be used by microorganisms if they enter microbial cells. The solubility of nutrients in the medium affects the degree of difficulty of entering cells. The change of temperature will affect the solubility of nutrients, and then affect the absorption capacity of microorganisms to nutrients. Therefore, the change of temperature has an important impact on the performance of the wastewater treatment system, and it is necessary to explore the suitable temperature range for the stable operation of the system [4].
In recent years, partial nitrification (PN) - anaerobic ammonia oxidation (ANAMMOX) process has been considered as the most efficient and economical biological denitrification method [5, 6]. Compared with traditional biological nitrogen removal, PN-ANAMMOX process can reduce oxygen consumption by 62.5% without any additional carbon source [7]. It can effectively solve the problems of high energy consumption and insufficient carbon source in traditional biological denitrification, and has gradually become a research hotspot in the field of wastewater biological denitrification [8-10]. To achieve PN-ANAMMOX, the premise is to achieve partial nitrification, that is, to control 50% ~ 60% of NH$_4^+$-N oxidation to NO$_2^-$-N, so that the concentration of NO$_2^-$-N and NH$_4^+$-N in effluent is close to the theoretical NO$_2^-$-N/NH$_4^+$-N ratio of ANAMMOX (1.32) [11]. Researchers have achieved partial nitrification by controlling blower frequency, pH, dissolved oxygen and free ammonia [12-15]. However, most of these studies are focused on activated sludge system, and it is rare to carry out in a batch biofilter. The impact of temperature reduction on partial nitrification in biofilter is still not clear.

In this study, the impact of temperature reduction on the stability and recoverability of partial nitrification in a batch biofilter were investigated. The research results could provide scientific reference for the stable operation of partial nitrification in batch biofilters, and also provide technical reference for other similar wastewater treatment reactors.

2. Materials and Methods

2.1. Experimental Reactor

The experimental reactor is shown in Fig. 1. The reactor simulation column was made of PVC material, with a column height of 100 cm and an inner diameter of 7.04 cm. The filter layer is 75 cm high. The filter media is composed of natural river sand, shell sand and zeolite sand with a particle size of 0.5~1.0 mm and the volume ratio is 6:3:1. In addition, a 2.5 cm thick gravel layer (5~18 mm in diameter) was laid on the top and bottom of the filter media, which served as buffer and support respectively. The biofilter operated intermittently for two cycles a day, each lasting 12 h, with 3 h of water intake, 9 h of drying and a hydraulic load of 1.0 m/d. Adjustable flow rate pump and rotor flowmeter were used to adjust water intake, relay was used to control water intake time, and constant temperature circulating water bath device was used to control the reaction temperature.

![Figure 1. Schematic diagram of experimental reactor.](image-url)
2.2. Wastewater and Inoculated Sludge

Synthetic wastewater was used in the experiment. By adding C₆H₁₂O₆, NH₄Cl, NaNO₂, KNO₃, KH₂PO₄ to tap water to regulate the concentration of COD, NH₄⁺-N, NO₂⁻-N, NO₃⁻-N, TP to 120~140, 45~50, 0~0.5, 2.5~4.2 mg/L. By adding 0.1 mol/L HCl and 0.1 mol/L NaOH to regulate the pH value of wastewater to 7.2~7.8. By adding 120~200 mg/L NaHCO₃ to supplement alkalinity. At the same time, 0.1 mL nutrient solution (KCl 2100 mg/L, NaCl 1500 mg/L, CaCl₂·2H₂O 2800 mg/L, MgSO₄·7H₂O 2000 mg/L, FeSO₄·7H₂O 150 mg/L, ZnSO₄·5H₂O 50 mg/L, MnCl₂·4H₂O 50 mg/L, CuSO₄·5H₂O 30 mg/L) was added to every 10 L synthetic wastewater.

In order to improve the biofilm hanging efficiency, the backflow sludge with a mixed liquor suspended solid concentration of 7600 mg/L from the secondary sedimentation tank of a sewage treatment plant was inoculated before the filter material was mixed into the reactor. When the hydraulic load reached 1.0 m/d and the removal of COD and NH₄⁺-N was stable, the biofilm had been successfully hung.

2.3. Experimental scheme

A partial nitrification biofilter with stable operation at 25°C was selected as the reactor. The reaction temperature was reduced from 25°C to 20°C, 15°C and 10°C in turn. Each temperature condition was operated for 10 to 20 cycles to be stable. The impact of temperature reduction on the stability of partial nitrification was investigated. Then, the temperature was adjusted back to 25°C for a period of time to investigate the recoverability of partial nitrification after temperature reduction.

2.4. Analysis Methods

The contents of COD, NH₄⁺-N, NO₂⁻-N and NO₃⁻-N were tested by Potassium Dichromate, Nessler Reagent Spectrophotometry, N-(1-naphthyl)-ethylenediamine Spectrophotometry and Ultraviolet Spectrophotometry Method respectively.

3. Results and Discussion

3.1. Impact of Temperature Reduction on COD Removal

Fig. 2 shows the impact of temperature reduction on COD removal in partial nitrification biofilter.

![COD removal during the process of temperature reduction and increase.](image)

It can be seen that the COD removal rate remained above 80% when the temperature dropped from 25°C to 20°C, indicating that proper temperature reduction has little effect on the removal of COD. When the temperature dropped to 15°C and 10°C, the COD removal rate decreased gradually, but still maintained above 70%. It can be seen that the heterotrophic microorganisms in the batch biofilter had
stronger ability to withstand low temperature shock. When the temperature rose to 25°C, the activity of heterotrophic microorganisms could be gradually restored and the ability to degrade organic pollutants could be gradually enhanced. After 6 days of operation under 25°C, the COD removal rate was restored to more than 80%.

3.2. Impact of Temperature Reduction on Nitrogen Transformation

Fig. 3 shows the impact of temperature reduction on nitrogen pollutant transformation. As seen, when the temperature dropped from 25°C to 20°C, the concentration of NH$_4^+$-N, NO$_2^-$-N and NO$_3^-$-N in effluent changed little. The removal rate of NH$_4^+$-N, the accumulation rate of NO$_2^-$-N and the ratio of effluent NO$_2^-$-N/NH$_4^+$-N were maintained at about 60%, 95% and 1.3 respectively. The partial nitrification performance of the biofilter was stable in the temperature range of 20~25°C. When the temperature dropped to 15°C, the activity of ammonia oxidizing bacteria (AOB) was inhibited, the oxidation of NH$_4^+$-N to NO$_2^-$-N was blocked, the concentration of NH$_4^+$-N in effluent increased, and the concentration of NO$_3^-$-N decreased. However, the inhibition effect of temperature on the activity of nitrite oxidizing bacteria (NOB) was light, so the concentration of NO$_3^-$-N changed little. The activity of AOB and NOB was severely inhibited when the temperature continued to drop to 10°C. The amount of NH$_4^+$-N oxidized to NO$_2^-$-N was greatly reduced. The amount of NO$_2^-$-N available for NOB transformation was also greatly reduced. The concentration of NH$_4^+$-N in effluent increased further, the concentration of NO$_3^-$-N and NO$_2^-$-N decreased, and the concentration of NO$_3^-$-N decreased more significantly. The removal rate of NH$_4^+$-N, the accumulation rate of NO$_2^-$-N and the ratio of effluent NO$_2^-$-N/NH$_4^+$-N were about 17%, 92% and 0.15 respectively. The accumulation rate of NO$_3^-$-N was still high, but the concentration of NO$_3^-$-N in effluent was only about 5 mg/L. The partial nitrification performance of the biofilter was at a relatively low level.
Guo et al. [16] studied the impact of temperature on nitrification in SBR system and found that the ammonia oxidation rate at 25°C was 15.7% higher than that at 15°C, and the NO$_2^-$-N accumulation rate at 15°C was 12.1% lower than that at 25°C. Rong et al. [17] showed that the removal rate of NH$_4^+$-N and the accumulation rate of NO$_2^-$-N were 17.3% and 6.9% respectively when the temperature was reduced from 25°C to 5°C, which were 79.2% and 88.3% lower than that at 25°C, respectively. It was also found that the nitrification performance of the biofilter at 10~15°C was much weaker than that of 20~25°C, and the impact of low temperature (10~15°C) on the activity of AOB was greater than that of NOB. For the temperature coefficient of NOB was smaller than that of AOB, AOB was more sensitive to temperature change than NOB.

When the temperature rose back to 25°C, the concentration of NH$_4^+$-N decreased, the concentration of NO$_2^-$-N increased, and the concentration of NO$_3^-$-N increased slightly. From the 11th day of operation, the removal rate of NH$_4^+$-N, the accumulation rate of NO$_2^-$-N and the ratio of effluent NO$_2^-$-N/NH$_4^+$-N basically returned to the original level. It is inferred that low temperature (10~15°C) mainly weakened the metabolic activities of AOB and NOB, and inhibited their growth and reproduction, resulting in slow or stagnant growth. This is also a way that AOB and NOB resisted low temperature stress. Although low temperature inhibited its growth and reproduction, it did not cause a large number of deaths. They could still survive for a long time. When the temperature increased, the activity of AOB and NOB increased gradually, and the partial nitrification of the biofilter recovered gradually with the temperature rising. In summary, the partial nitrification in the biofilter could achieve stable operation when the temperature was within the range of 20~25°C.

4. Conclusion
The batch biofilter could keep good partial nitrification performance when the reaction temperature was controlled at 20~25°C. Its NH$_4^+$-N removal rate, NO$_2^-$-N accumulation rate and effluent NO$_2^-$-N/NH$_4^+$-N ratio were maintained at about 60%, 95% and 1.3 respectively, which could provide suitable influent conditions for the subsequent ANAMMOX. After being shocked by low temperature, the COD removal rate, NH$_4^+$-N removal rate, NO$_2^-$-N accumulation rate and effluent NO$_2^-$-N/NH$_4^+$-N ratio of the biofilter could basically restore to its original level after resuming 25°C for a period of time.

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