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# Mixed nitrifying bacteria culture under different temperature dropping strategies: Nitrification performance, activity, and community

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## HIGHLIGHTS

- Nitrifiers acclimated at 20 °C for 6 days showed higher nitrification activity.
- Nitrifiers acclimated at 20 °C for 6 days showed higher RLU and least SOD fluctuation.
- *Nitrosospira* and *Nitrospira* were more preferable at low temperature conditions.
- The nitrification rate at 10 °C was mainly limited by the nitrite oxidation activity.
- Acclimation at 20 °C for 6 days improved the recoverability of nitrifiers at 10 °C.

## ARTICLE INFO

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## ABSTRACT

In this study, the nitrification performance, metabolic activity, antioxidant enzyme activity as well as bacterial community of mixed nitrifying bacteria culture under different temperature dropping strategies [(#1) growth temperature kept at 20 °C; (#2) sharp1 decreased from 20 °C to 10 °C; (#3) growth at 20 °C for 6 days followed by sharp decrease to 10 °C; and (#4) gradual decreased from 20 °C to 10 °C] were evaluated. It was shown that acclimation at 20 °C for 6 days allowed to maintain better nitrification activity at 10 °C. The nitrite oxidation capacity of nitrifiers was significantly correlated with the relative light unit (RLU) ($p < .05$) and the fluctuation of superoxide dismutase (SOD) enzyme activity ($p < .01$). With serial #3 showed the highest RLU levels and the least SOD enzyme fluctuation as compared to serials #2 and #4. Throughout the experimental period, *Nitrosospira* and *Nitrosomonas* as well as *Nitrospira* were identified as the predominant ammonia-oxidizing bacteria (AOB) and nitrate-oxidizing bacteria (NOB). The dynamic change of AOB/NOB ratios and nitrification activity in serials #2-#4 demonstrated that AOB recovered better than NOB with long-term 10 °C exposure, and the nitrification performance was mainly limited by the nitrite oxidation capacity of NOB. Applying 6 days acclimation at 20 °C was beneficial for the mixed nitrifying bacteria culture to cope with low temperature (10 °C) stress, possibly due to the maintenance of metabolic activity, antioxidant enzyme activity stability as well as appropriate AOB/NOB ratio.



## 1. Introduction

Nitrification, the microbial mediated oxidation of ammonia ($NH_4^+$-N) to nitrate ($NO_3^-$-N), plays a central role in the nitrogen cycle of aquatic environments. One of the main factors affecting the stability and efficiency of nitrification process is the decrease of temperature in cold seasons (Obaja et al., 2003; Ducey et al., 2010;

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Hoang et al., 2014). In the recent years, a few researches have been focusing on the response of nitrification performance to different temperature dropping strategies. For example, Head and Oleszkiewicz (2004) conducted an experiment on the inoculation of nitrifying biomass produced at a rapid drop of temperature from 20 °C to 10 °C to determine the impact of sharp decrease in temperature on nitrification rates. It was observed that the average nitrification rate was decreased by 58% at 10 °C. Hwang and Oleszkiewicz (2007) documented that the sharp decrease of temperature from 20 °C to 10 °C led to a 20% larger decrease of specific nitrification rate than that of gradual temperature decrease. The nitrification performance in two laboratory scale moving bed biofilm reactors (MBBRs) operated at 20 °C for six weeks and then dropped to 1 °C and operated at 1 °C for four months were investigated. The average ammonia removal rate after long-term exposure to 1 °C was determined to be 18 ± 5.1% as compared to 20 °C (Hoang et al., 2014).

It is known that low temperature mainly influences the microbial metabolic rate (Hulsen et al., 2016), which leads to the deterioration of nitrification as a result (Delatolla, 2012; Hoang et al., 2014). And this problematic is actual especially for wastewater treatment. Adenosine 5'-triphosphate (ATP), as the major energy currency existed in all of the living organisms (Tsuyama et al., 2013), is always used as a biomarker to express the activity of microorganism (Zhang and Angelidaki, 2011; Gibert et al., 2013; Vang et al., 2014; Kaarela et al., 2015). In a previous study, it was observed that the ATP levels of activated sludge was increased with the increase of temperature. At 25 °C, the ATP level was 1.5 times higher than that at 15 °C (Tobin et al., 1978). Generally, oxidative stress due to chemicals, salts or low temperature stress exerts adverse effects on microorganisms (Doughari et al., 2012; Garcia-Rios et al., 2016; Sobon et al., 2016). To alleviate the damage of microbial cells, antioxidant enzymes induced in microbial cells play a response to oxidative stress (Zhou, 2006; Huang et al., 2014; Wang et al., 2016). A recent study from our group showed that the superoxide dismutase (SOD) activity of activated sludge was closely related to the $NH_4^+$-N removal rate when temperature gradually decreased (2 °C/d) from 25 °C to 10 °C (He, 2010).

Low temperature is one of the key factors that shapes microbial community in biological nitrogen removal systems (Urakawa et al., 2008; Zhou et al., 2016). For instance, the impact of low temperatures on the bacterial community from two fixed bed systems were examined by Karkman et al. (2011). Results showed that genera *Nitrosomonas* and *Nitrospira* were the dominant nitrifiers at 10 °C, while nitrifiers closely related to the genera *Nitrosospira* and *Candidatus Nitrotoga* dominated at 5 °C. It was also observed that bacterial species richness was higher at 10 °C. Otherwise, low temperatures also exert an influence on the amount of nitrifying bacteria (Siripong and Rittmann, 2007). By using MBBRs for polluted raw water treatment, Zhang et al. (2014) observed that the percentage of ammonia oxidizing bacteria (AOB) (*β-Proteobacteria*) was 32.9% when the temperature was below 5 °C, while it reached to 35.1% and 41.5% at 14 °C and 28 °C, respectively. Similarly, the percentage of NOB (*Nitrobacter* spp.) was 21.1%, 21.9% and 25.2% when the temperature was below 5 °C, 14 °C, and 28 °C, respectively.

According to the cited researches, we can conclude that low temperature impacts the metabolic activity, antioxidant enzymes activity and microbial community of nitrifying bacteria. Otherwise, different temperature dropping strategies also lead to different nitrification performance. However, there is a lack of comprehensive understanding of which temperature-reduction strategy is more conducive to the preservation of nitrification activity at low temperature and how these indexes respond to different temperature cooling strategies. The aim of this study was to examine the nitrification rates, metabolic activity (ATP), antioxidant enzyme activity (SOD) as well as bacterial community dynamics of nitrifying bacteria with different temperature-reduction strategies, including: sudden decrease of temperature from 20 °C to 10 °C; acclimation at 20 °C for 6 days with an afterwards rapid drop to 10 °C; and gradual temperature decrease from 20 °C to 10 °C. The response difference of nitrifiers under different temperature-reduction strategies was analyzed. A strategy for potential use on nitrification at low temperature was proposed.

## 2. Materials and methods

### 2.1. Ammonia medium

Ammonia medium was composed of $(NH_4)_2SO_4$ 0.5 g/L ($NH_4^+$-N concentration 136.4 mg/L), $NaHCO_3$ 1 g/L, $Na_2HPO_4$ 0.1 g/L, $KH_2PO_4$ 0.1 g/L, $MgSO_4 \cdot 7H_2O$ 0.1 g/L, and trace element 5 mL/L.

Trace elements (Chen et al., 2014): EDTA 0.5 g/L, $ZnSO_4 \cdot 7H_2O$ 0.22 g/L, $CaCl_2$ 0.055 g/L, $MnCl_2 \cdot 4H_2O$ 0.051 g/L, $FeSO_4 \cdot 7H_2O$ 0.049 g/L, $(NH_4)_2Mo_7O_{24} \cdot 4H_2O$ 0.011 g/L, $CuSO_4 \cdot 5H_2O$ 0.0157 g/L, $CoCl_2 \cdot 6H_2O$ 0.016 g/L, pH 6.0 (KOH).

### 2.2. Nitrification performance under different temperature dropping strategies

Activated sludge from the aerobic tank of an anaerobic-anoxic-oxic process in Chengdu city (China) was inoculated into the ammonia medium, and cultured at 20 °C for 3 days in shake flasks to obtain the mixed nitrifying bacteria culture. After that, the nitrification activity of mixed nitrifying bacteria culture was investigated with different low-temperature stress regimes. Serial 1#, mixed nitrifying culture in maintained at 20 °C for 25 days was set as control; serial 2#, mixed nitrifying culture was suffer from a sudden temperature decrease from 20 °C to 10 °C and maintained at 10 °C for 25 days; serial 3#, mixed nitrifying culture was firstly acclimated at 20 °C for 6 days, and then placed to 10 °C for another 19 days; serial 4#, mixed nitrifying culture was firstly acclimated with a gradually temperature decrease (1.4 °C/d) for 6 days, and then maintained at 10 °C for another 19 days. The mixed nitrifying bacteria culture in serials #1-#4 were grown in batch mode with a processing cycle of 12 h, with the nitrogen loading rate ranged from 0.058 kg N/($m^3$ d) to 0.082 kg N/($m^3$ d). The initial pH of the ammonia medium was adjusted to 8.0 ± 0.2, the dissolved oxygen (DO) concentration was higher than 3 mg/L. The mixed liquor suspended solid was around 5000 mg/L.

The removal rate of ammonia ($\mu_{NH_4^+-N}$, mg/(L h)) and the accumulation rates of nitrite ($\mu_{NO_2^--N}$, mg/(L h)) and nitrate ($\mu_{NO_3^--N}$, (L h)) in the first 3 h were calculated by the following formulas:

$$\mu_{NH_4^+-N} = \frac{3hC_{NH_4^+-N} - 0hC_{NH_4^+-N}}{3} \quad (1)$$

$$\mu_{NO_2^--N} = \frac{3hC_{NO_2^--N} - 0hC_{NO_2^--N}}{3} \quad (2)$$

$$\mu_{NO_3^--N} = \frac{3hC_{NO_3^--N} - 0hC_{NO_3^--N}}{3} \quad (3)$$

where 0 h, 3 h $C^+_{NH4\text{-}N}$ are the $NH_4^+$-N concentrations at 0 h and 3 h in serials #1-#4, mg/L; 0 h, 3 h $C^-_{NO2\text{-}N}$ are the $NO_2^-$-N concentrations at 0 h and 3 h, mg/L; 0 h, 3 h $C^-_{NO3\text{-}N}$ are the $NO_3^-$-N concentrations at 0 h and 3 h, mg/L.

In this study, the ammonia ($NH_4^+$-N) oxidation capacity of AOB