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Effect of temperature downshifts on biological nitrogen removal and community structure of a lab-scale aerobic denitrification process

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ABSTRACT

Aerobic denitrification bacteria are currently being explored as promising microorganisms with which to treat wastewater containing concentrated ammonium and organic carbon at psychrophilic temperatures. However, little is know regarding the mixed culture of these microbes under low-temperature shocks. In this study, a lab-scale aerobic denitrifying process was established in a sequencing bioreactor at 25 °C, and successfully performed in a short-term temperature downshift period that ranged from 25 °C to 5 °C. The specific aerobic denitrification rate was maximized at 15 °C [15.33 mg N/(g SS h)], and this process effectively removed chemical oxygen demand (COD) and nitrate (above 90%) even at 5 °C. The results of Illumina MiSeq 16S rRNA amplicon analysis revealed a relatively stable microbial community across the transitional periods of temperature. *Pseudomonas* was the most abuntant genus below 15 °C, and the amount of species *Pseudomonas alcaligenes* significantly increased at 5 °C with potassium nitrate as the primary nitrogen source. The communities of aerobic denitrifying bacteria in the temperature downshift period were all highly organized functionally, and they were relatively stably across the temperature gradient. These results will enhance understanding regarding the microbial ecology of aerobic denitrifiers in mixed cultures, leading to a more effective controlling of aerobic denitrification process during temperature transition states.

1. Introduction

Temperature is one of the primary factors that control microbial metabolism and community structures in many natural systems [1]. The temperature of the vast majority of the Earth's biosphere is either permanently or seasonally cold (below 5 °C). Hence, microbial growth and metabolism in low-temperature environments are currently of great interest and have been explored in multiple studies [2–5]. Reports on bacterial growth in permafrost or extreme environments with subzero temperatures have significantly enhanced our understanding of microbes on the Earth [4,6–8]. Cold-adaptive bacteria have also been introduced into the biological wastewater treatment field. For instance, the development of cold-adapted biological nitrogen removal (BNR) processes has generally been vital to wastewater treatment plants (WWTPs) in cold regions [9–11]. Therefore, environmental researchers and engineers have exerted extensive effort to improve low-temperature BNR performance [11,12]. Unlike these conventional approaches, however, the aerobic denitrification process is investigated for its capability to remove low-temperature nitrogen [13,14]. In a normal-temperature environment, aerobic denitrification has been shown to be a promising technology for the treatment of highly concentrated nitrogen discharges [15]. For example, carbon and nitrogen compounds of swine wastewater were successfully eliminated by *Alcaligenes faecalis* strain through heterotrophic nitrification–aerobic denitrification [15]. The ammonium removal rate achieved by the *A. faecalis* consortium was 5-fold–10-fold higher than those of conventional nitrification bacteria [15]. Recently, Novel aerobic denitrifiers, such as *Acinetobacter* sp., *Pseudomonas* sp., and *Aeromonas* sp. have recently been isolated from various environments [13–17]. Some of the denitrifiers can oxidize ammonia to gaseous nitrogen in aerobic condition and have exhibited a notable tolerance to low temperatures [13,14,16]. These strains could efficiently removal of ammonia and nitrate under aerobic conditions at 10 °C. In particular, strain *Aeromonas* sp. HN-02 obtained an ammonia removal rate of 0.90 mg L^{-1} h^{-1} even at 5 °C [16]. The Arrhenius temperature correction coefficient of HN-02 was lower than autotrophic nitrifiers during sudden temperature drop tests, suggesting that heterotrophic nitrifiers were more powerful to cold shocks than conventional nitrifiers [16].

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Table 1
Overview of the operation mode in different periods.[a]

Periods (days)		DO[b] (mg/L)	Temperature (°C)	Nitrogen substrate	C/N values (Inf.)	Cycle mode		
						Feeding (h)	Aeration (h)	Settling and withdrawing (h)
Enrichment of aerobic denitrifiers in SBR (a)	1–122	6.1 ± 0.3	24.8 ± 0.4	KNO_3, NH_4Cl	10.9 ± 2.1	0.5	06–11	0.5
Temperature downshift operation in SBR (b)	I (123–132)	6.1 ± 0.3	24.5 ± 0.5	KNO_3, NH_4Cl	11.8 ± 2.8	0.5	11	0.5
	II (133–146)	6.7 ± 0.8	16.6 ± 0.5	KNO_3, NH_4Cl	9.7 ± 4.1	0.5	11	0.5
	III (147–159)	7.5 ± 1.2	10.4 ± 0.5	KNO_3, NH_4Cl	12.4 ± 4.1	0.5	11	0.5
	IV (160–173)	8.8 ± 0.7	5.3 ± 1.0	KNO_3, NH_4Cl	14.9 ± 4.2	0.5	11	0.5
	V (174–188)	7.8 ± 0.6	8.5 ± 0.8	NH_4Cl	8.9 ± 2.8	0.5	11	0.5

[a] Values are given as mean ± standard deviation.
[b] DO values were measured at the end of aeration phase.

From an engineering viewpoint, aerobic denitrification is an appropriate approach to eliminate nitrogen from wastewater with high *C*/*N* values under moderate or low temperatures. Seasonal temperature drop is a primary factor that adversely influences nitrifiers activities in an actual BNR system [18,19]. Recently, a new BNR concept that involves the coupling of enriched autotrophic nitrifying and aerobic denitrifying consortia has developed [20]. This concept is applied to remove nitrogen from low-strength synthetic wastewater at 10 °C [20]. The potential advantages associated with this mixed culture include its low energy consumption and carbon source requirement given that nitrification and denitrification can be conduct in a single aerobic reactor. The optimized combination of aerobic nitrifiers and denitrifiers can be regarded as a new pattern of cost-effective method for mainstream wastewater treatment during winter. This process concept is similar to the application of partial nitrification–anammox to treat low-temperature sewage [21,22].

Although the unique properties of aerobic denitrifiers and their potential nitrogen removal efficiencies have been exploited, knowledge regarding the mixed culture of aerobic denitrifying bacteria in cold-temperature shocks remains insubstantial. Considerable knowledge must be obtained regarding the variation of microbial community structures and process functions during temperature transition for successful aerobic denitrification. Over the past decade, rapid progress has empowered the culture-independent techniques that are used to investigate microbial communities in combination with omics data processing approaches more than before [23]. These methods can be employed to exploit the relationship between the microbial community and bioreactor functions [23–25]. The process function can be predicted by effectively integrating diversity indices into a quantitative predictive framework [26]. Therefore, the detailed characterization of microbial ecology of aerobic denitrifiers in the course of temperature transition states is significant in maintaining a stable full-scale aerobic denitrifcation process [25,27]. The goals of this research were to (1) start up an aerobic denitrification using activated sludge as inoculum at room temperature followed by operation at several short-term temperature decrease periods; and (2) describe the temporal dynamics in bacterial community structures during aerobic denitrification under temperature downshifts and to either elucidate or identify the relationship between microbial community dynamics and temperature transition of this periods.

2. Materials and methods

2.1. Reactor setup and operation strategy

Aerobic denitrification was successfully established and stably conducted in a 6.5 L laboratory-scale sequencing batch reactor (SBR) (a) (Fig. S1) at 24.8 ± 0.4 °C using a thermostated jacket prior to the periodical cooling experiment. Activated sludge was collected from a secondary settling tank in a municipal WWTP (oxidation ditch process, 24° 35′N, 118° 06′E, Xiamen City, China) and used as inocula to enrich aerobic denitrifiers.

The SBR (a) operated under a 12 h consecutive cycle during the establishment of aerobic denitrification process. Settling, decanting, and feeding phases were performed during the final 1 h of each cycle. The aeration and anoxic time in one cycle was maintained at 6 h and 5 h at the beginning of the enrichment experiment. Then, the total aeration time was gradually increased from 6 h to 11 h in one cycle over time (Table 1). Dissolved oxygen (DO) concentration was regulated at 6.1 ± 0.3 mg O_2/L during the aeration period, and the exchange volume of the SBR (a) was maintained at 50%. The SBR (a) was fed with a synthetic heterotrophic medium (Table S1). That is, KNO_3 was selected as the main nitrogen source, and the NO_3^-–N concentrations of the influents were maintained at 172.8 ± 37.0 mg/L.

The effect of temperature downshift on the activity and community structure of aerobic denirification was conducted in a 3.5 L SBR [SBR (b)] (Fig. S1) for 66 d in a incubator (SHP-150, Jinghong, China) with temperature control accurate to ±0.5 °C. Mixed biomass that contained aerobic denitrifiers were collected from SBR (a), and the operation mode is shown in Table 1. SBR (b) was operated for five short-term periods, and the temperature was lowered from 25 °C to 5 °C. Given that a rapid shift in operational conditions can destabilize the biological system, experiments at cold environment were performed by lowering the temperature gradually, especially when the operational temperature was below 10 °C.

2.2. Physicochemical analysis

The concentrations of NH_4^+–N, NO_2^-–N and NO_3^-–N were monitored with a flow injection analyzer (QuickChem 8500, USA). Total nitrogen (TN) was measured with a Shimadzu analyzer (TNM-1, Shimadzu, Japan). COD was analyzed using a colorimetric method (Lian-hua, China). Temperature, pH and DO values were determined by a HACH analyzer (HQ40d18, Hach, USA). Total suspended solids (TSS) concentration of biomass was measured by a standard method [28]. The temperature coefficient was expressed as an Arrhenius equation to show the temperature dependence of aerobic denitrification rate [29].

$$r_T = r_{293} \times \theta^{T-293}$$

where r_T is the reaction rate at temperature T and θ is the temperature coefficient.