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Effect of temperature on functional bacterial abundance and community structure in CANON process

Tao Liu [a], Dong Li [b,*], Jie Zhang [b], Yang Lv [c], Xie Quan [a]

[a] Key Laboratory of Industrial Ecology and Environmental Engineering (Ministry of Education), School of Environmental Science and Technology, Dalian University of Technology, Dalian 116024, China
[b] Key Laboratory of Water Quality Science and Water Environment Recovery Engineering, Beijing University of Technology, Beijing 100124, China
[c] School of Civil Engineering, Dalian University of Technology, Dalian 116024, China

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ABSTRACT

Completely autotrophic nitrogen-removal over nitrite (CANON) has been considered as a promising nitrogen-removal technology in treating high ammonia and high temperature wastewater. In this study, the exact effect of different temperatures on process performance and microbial features based on two lab-scale CANON reactors was investigated. Results showed extraordinary nitrogen removal performance with mean total nitrogen (TN) removal efficiency of 80.01% and 66.90% under high TN removal loading of 2.09 $kg\,m^{-3}\,d^{-1}$ and 1.02 $kg\,m^{-3}\,d^{-1}$ at 30 °C and ambient temperature (16–23 °C) respectively. Biodiversity analysis indicated *Nitrosomonas*-related aerobic ammonia oxidizing bacteria (AerAOB) and *Candidatus Brocadia*-like anaerobic ammonia oxidizing bacteria (AnAOB) were predominant functional microorganisms that coexisted without distinguishable niche on the volcanic carriers in both two CANON reactors. Temperature could noticeably impact functional bacterial population: the population of AerAOB and AnAOB decreased to one to two orders of magnitude whereas *Nitrospira* and *Nitrobacter* increased at ambient temperature. Based on the bacterial experimental results, some feasible strategies have been discussed to improve the bioactivity and increase the function bacterial population, aiming at enhancing nitrogen-removal capacity at ambient temperature.

1. Introduction

Despite conventional nitrification–denitrification processes have been widely applied all over the world aiming at removing N-compound pollutant from wastewater, there is still a challenge unsolved that large amount of organic carbon is definitely necessary in denitrification stage and the issue followed is in most cases there is not sufficient biodegradable carbon in the influent. Consequently, extra organic carbon has to be introduced to the wastewater to provide denitrifying bacteria with carbon and energy source. To overcome the drawbacks of conventional nitrogen removal processes, some novel technologies have been recently developed, among which completely autotrophic nitrogen-removal over nitrite (CANON) is regarded as a representative and promising technology. This process relies on the harmonious balance between aerobic and anaerobic ammonia oxidizing bacteria, in which ammonium is autotrophically oxidized to dinitrogen gas with nitrite as the electron acceptor in oxygen-limited conditions [1]. So far, most CANON processes are implemented to treat high strength wastewater with higher temperature (>30 °C) like sludge digestion and landfill leachate. The relatively high and stable temperature of the wastewater provides beneficial conditions for the slowly growing anaerobic ammonia oxidizing bacteria (AnAOB), which, is also applicable in the selection of ammonia oxidizing bacteria (AerAOB) and exclusion of nitrite oxidizing bacteria (NOB) to maintain partial nitrification process in CANON system [2–4]. However, its potential application in large scale as a post-treatment technology for nitrogen removal from effluents of the main stream at wastewater treatment plants (WWTPs) is limited by the more challenging conditions of lower temperature, less substrate and presence of residual organic carbon. In fact, several studies have already demonstrated that while temperature declines, bioactivity of AnAOB and AerAOB is suppressed significantly and partial nitrification is destroyed easily since NOB shows relatively competitive growth rate and bioactivity than AnAOB and AerAOB at the temperature below 25 °C, leading to an immediate large decrease in nitrogen removal efficiency [5–8]. In addition, another main difficulty faced for applying

* Corresponding author. Fax: +86 10 67392099.
E-mail address: lidong2006@bjut.edu.cn (D. Li).

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CANON at ambient temperature is to achieve a high rate process with longer biomass retention but shorter hydraulic retention time (HRT). Therefore, fixed/moving carriers or granule sludge that was known as ideal biomass retention material may be the optimal candidates. Given it is not cost-effective to heat the domestic sewage to around 30 °C to meet CANON's requirements, it is more reasonable to maintain the functional bacterial balance in CANON system to achieve stable nitrogen removal capacity at ambient temperature when treating domestic sewage.

Up to now, some studies have been conducted to investigate the process performance of CANON at room temperature (usually at a range of 15–25 °C) (Table 1). Based on the results, the mechanisms of reported CANON's implementation at room temperature rely on the following three aspects: (i) AnAOB may show certain adaptability to low temperature when exposed to low temperature for a long time; (ii) NOB can be inhibited by free ammonia (FA) with an appropriate concentration so as to achieve partial nitrification at low temperature since AerAOB and NOB have different tolerance to FA concentration [9]; (iii) NOB shows lower affinity to nitrite in comparison to AnAOB and can be eliminated during the nitrite competition with AnAOB [10,11]. Actually, although these studies have confirmed the feasibility of CANON process at low temperatures, the TN removal loading decreases remarkably and the system usually operates unstably comparing with that at higher temperature. In order to improve nitrogen removal capacity and further promote CANON's implementation at ambient temperature, it is vital to investigate the accurate response of the microbial features to different temperatures. However, considering the microbial features in the unstable CANON system are unconvincing and unrepresentative, it is essential and foremost to build long-term operating CANON systems with relatively high and stable nitrogen removal efficiency.

In this study, a lab-scale CANON reactor exposed to ambient temperature (16–23 °C) has been operated stably for a couple of months with relatively advanced nitrogen removal capacity. Thus, the microbial features in this reactor were expected to be convincing and representative to some extent. The other CANON reactor was operated at high temperature (30 °C). The nitrogen removal performance and functional bacterial features in these two lab-scale CANON reactors were studied to evaluate the effects of temperatures on the process stability and functional microorganisms' features. The biomass morphology on the mature CANON biofilm, functional bacterial spatial distribution, bacterial community structure and quantitative feature of different types of nitrogen removal microorganisms obtained were expected to provide us with inspirations on the improvement of nitrogen removal capacity at ambient temperature and further promote CANON's implementation in domestic sewage treatment in the long term.

2. Materials and methods

2.1. Experimental reactors and operational conditions

Two lab-scale up-flow bioreactors made of polymethyl methacrylate that filled with volcanic rock (4–6 mm in diameter and 80% in porosity) was used in the study (Fig. 1). Mature sludge from another CANON reactor that operated at high NH_4^+-N concentration (400–480 mg L^{-1}) and high temperature (30–35 °C) in the laboratory was inoculated originally to the reactors. DO was controlled at around 0.5 mg L^{-1} at both reactors. HRT of R1 and R2 was 1.0 h and1.3 h respectively. Total volume and working volume of R1 was 40.0 L and 9.0 L while these two parameters of R2 were 8.2 L and 1.8 L respectively. The reactors were fed with synthetic wastewater, containing of $NaHCO_3$ (C source and buffer), $(NH_4)_2SO_4$ (N source) and KH_2PO_4 (P source and buffer) together with a small

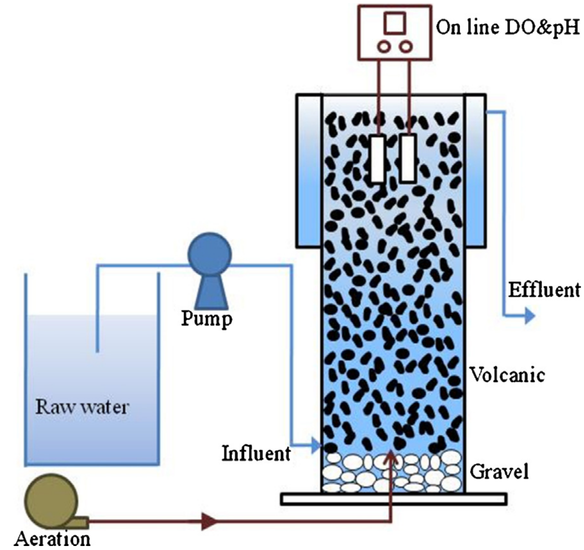

Fig. 1. Schematic diagram of CANON reactor.

amount of trace element solution. The wastewater was pumped continuously from the bottom of the reactor and output from the upper outlet (Fig. 1). Reactor 1 (R1 in abbreviation) had a double jacket to maintain constant temperature (30 °C) while Reactor 2 (R2 in abbreviation) exposed to ambient temperature (16–23 °C). In this study, the two reactors had been operated stably at an influent nitrogen concentration of 400 ± 8 mg L^{-1} for a couple of months with steady nitrogen removal capacity.

Concentrations of NH_4^+-N, NO_2^--N and NO_3^--N in influent and effluent were daily measured according to standard methods [19]. The pH value, temperature and dissolved oxygen (DO) were all detected by the online multi-function instruments (WTW inoLab-StirrOx).

2.2. Scanning electron microscope (SEM)

Some pieces of volcanic carriers were collected randomly and fixed with 2.5% (V/V) glutaraldehyde for 1 h followed by dehydration in 50%, 70%, 90% and 95% (V/V) ethanol for 10 min per each step. Afterwards, the samples was steeped in hexamethyl disilazane (HMDS) twice for 10 min, air dried, and coated with gold. Morphology characterization was conducted on Hitachi S-4300 device.

2.3. DNA extraction, PCR-DGGE, sequencing and phylogenetic analysis

Some pieces of volcanic carriers were collected from the middle site of the volume and stored at −20 °C. Biomass was removed from the carriers and genomic DNA was extracted on basis of the methods described previously [20]. The biodiversity of total bacteria, AerAOB and AnAOB was evaluated by PCR-DGGE technique. The primers targeting different types of microorganisms were used and the relevant annealing temperatures of each pair of primers were described elsewhere [20]. Thermocycling was performed in Takara PCR Thermal Cycler Dice and PCR products were detected by 1.5% (w/V) agarose gel electrophoresis to confirm the product size and purified with the TIANgel midi purification kit (Tiangen) according to the manufacturer's instructions before DGGE.

DGGE analysis was conducted at a constant voltage and temperature (120 V, 60 °C) for 7 h on a Dcode Universal Mutation Detection System (Bio-Rad). The 8% (for total bacteria and AerAOB) or 6% (for AnAOB) polyacrylamide gels with a 30–60% linear gradient of urea-formamide denaturant was prepared for DNA fragments sep-