Bioresource Technology 175 (2015) 189–194

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Stability and nitrite-oxidizing bacteria community structure in different high-rate CANON reactors

Yuhai Liang [a], Dong Li [a,*], Xiaojing Zhang [b], Huiping Zeng [a], Zhuo Yang [a], Shaoming Cui [a], Jie Zhang [a,c]

[a] Key Laboratory of Water Quality Science and Water Environment Recovery Engineering, Beijing University of Technology, Beijing 100124, China
[b] Henan Collaborative Innovation Center of Environmental Pollution Control and Ecological Restoration, Zhengzhou University of Light Industry, Zhengzhou 450001, China
[c] State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, China

HIGHLIGHTS

- The NOB community of four CANON reactors with high DO were studied.
- High FA concentration suppressed bioactivity of both Nitrobacter and Nitrospira.
- The introduction of organic material resulted in biodiversity decreasing of NOB.
- Strategy for suppressing NOB in CANON system with high DO was proposed.

ARTICLE INFO

Article history:
Received 1 September 2014
Received in revised form 10 October 2014
Accepted 17 October 2014
Available online 23 October 2014

Keywords:
CANON
SNAD
DGGE
Nitrobacter
Nitrospira

ABSTRACT

In completely autotrophic nitrogen removal over nitrite (CANON) process, the bioactivity of nitrite-oxidizing bacteria (NOB) should be effectively inhibited. In this study, the stability of four high-rate CANON reactors and the effect of free ammonia (FA) and organic material on NOB community structure were investigated using DGGE. Results suggested that with the increasing of FA, the ratio of total nitrogen removal to nitrate production went up gradually, while the biodiversity of Nitrobacter-like NOB and Nitrospira-like NOB both decreased. When the CANON reactor was transformed to simultaneous partial nitrification, anammox and denitrification (SNAD) reactor by introducing organic material, the denitrifiers and aerobic heterotrophic bacteria would compete nitrite or oxygen with NOB, which then led to the biodiversity decreasing of both Nitrobacter-like NOB and Nitrospira-like NOB. The distribution of Nitrobacter-like NOB and Nitrospira-like NOB were evaluated, and finally effective strategies for suppressing NOB in CANON reactors were proposed.

1. Introduction

Completely autotrophic nitrogen removal over nitrite (CANON) process has been one of the most innovative developments for treating sewage with low C/N ratio over the last decade. This process which combines partial nitrification with anammox in one reactor has shown to be a cost-effective process. It could save 63% oxygen and 100% organic carbon consumption when compared with conventional nitrification–denitrification process (Third et al., 2001). In CANON process, partial ammonia is oxidized to nitrite by aerobic ammonia-oxidizing bacteria (AerAOB), then the remaining ammonia with the produced nitrite are converted to dinitrogen gas and some nitrate by anaerobic ammonia-oxidizing bacteria (AnAOB) (Sliekers et al., 2002). The CANON process relies on the harmonious and balanced interaction between the AerAOB and the AnAOB (Third et al., 2001). However, the process performance is seriously hampered by the accumulation of nitrite oxidizing bacteria (NOB) (Pellicer-Nacher et al., 2014). Nitrate built up was one of the main operational difficulties in the full-scale installations. It has been reported in 50% of the questioned plants and throughout all technologies, such as SBR, granular and biofilm systems (Lackner et al., 2014).

Several strategies have been used to suppress NOB growth such as operation at high temperatures and sludge retention time (SRT) (Dongen et al., 2001; Van Hulle et al., 2010), high free ammonia (FA) concentration (Anthonisen et al., 1976; Van Hulle et al., 2010), low dissolved oxygen (DO) concentration (Sarah Philips et al., 2012; Van Hulle et al., 2010). In the past, CANON process was usually used to treat wastewater at low DO concentration. The reason was low DO could suppress NOB activity, and had no impact on AnAOB (Third et al., 2001). However, low DO concentra-

* Corresponding author. Tel.:+86 01067392099 4.
E-mail addresses: lidong2006@bjut.edu.cn, bjut_lidong@163.com (D. Li).

http://dx.doi.org/10.1016/j.biortech.2014.10.080
0960-8524/

tions would lead the low nitrogen removal rate (NRR). Sliekers et al. (2003) suggested that the limiting reaction step in CANON process was the ammonia oxidation by AerAOB, which was significantly affected by the oxygen supply. Several high-rate CANON systems also adopted high DO as recently reported (Furukawa et al., 2006; Qiao et al., 2012), which led to the excess nitrate production by NOB. SRT was an effective parameter under high temperature, but it could only be used for treating sewage with low loading or heat energy, since raising the temperature would be a large energy consumption. In addition, SRT could not be adjusted in biofilm systems, which was the usual reactor figuration for CANON process. Thus, new factors for inhibiting NOB should be proposed. Since some previous studies reported that organic material performed well inhibitory effect on NOB (Van Hulle et al., 2010), FA and organic material would be selective choices for inhibiting NOB in CANON reactors with high DO and room temperature.

For suppressing the NOB growth, study about the microbial community is essential. In sewage treatment system, two main species named Nitrobacter-like NOB and Nitrospira-like NOB existed (Siripong and Rittmann, 2007). They showed different affinity to substrates, Nitrobacter-like NOB preferred to survive in the system with high nitrite and high DO, while Nitrospira-like NOB showed better activity in condition of low nitrite and DO (Cebron and Garnier, 2005; Knapp and Graham, 2007). Thus, Nitrospira-like NOB was the dominant NOB specie in wastewater treatment system with low DO. However, in the present high-rate CANON systems, nitrite concentration was hoped to be very low and DO was controlled to be high for excellent NRR. Few information about the microbial community of NOB could be achieved, in such system with low nitrite and high DO, which was essential for proposing the suppressing strategy.

In this study, the nitrate production and microbial community of NOB were investigated in four reactors, which were all operated with high DO. PCR-DGGE was used to analyze the biodiversity of Nitrobacter-like NOB and Nitrospira-like NOB. The main goal of this study was to propose effective strategies for suppressing NOB.

2. Methods

2.1. Description of reactors

Four biofilters (R1, R2-I, R3 and R4) with identical set-up were adopted to in this study, which were fed with influent ammonia of 100, 200, 400 and 800 mg L^{-1}, respectively. The figuration of the reactors were depicted in the previous study (Liang et al., 2014a). The volume of four reactors were 5.3, 5.3, 5.3 and 64 L. The carrier used in this study was volcanic rock, with the porosity and particle sizes of 80% and 4.0–6.0 mm. In addition, glucose was adopted to R2 on day 201, with an influent C/N ratio of 0.2, then the reactor in this phase was named as R2-II (Liang et al., 2014b). The temperature in the four biofilters were kept at 25 ± 2 °C, and the DO concentration were all maintained at a high level (around 5 mg L^{-1}). The synthetic wastewater used in this study contained $(NH_4)_2SO_4$ and $NaHCO_3$ as main substrates, together with 0.136 g L^{-1} of KH_2PO_4 and $CaCl_2$, as well as trace element solution (1 mL L^{-1}) (van de Graaf et al., 1996).

2.2. Analytical methods

The influent and effluent water samples have collected in storage tanks and the outlet of biofilters respectively. The concentrations of nitrogen compounds in influent and effluent were daily measured according to Standard Methods (APHA, 1995). The pH and DO were measured with the online instruments (pH296/Oxi296, WTW, Germany). COD was detected by 5B-1 digestion instrument (Lianhuakeji, China).

2.3. Sampling and DNA extraction

Biofilm samples were obtained from R1, R2-I, R3 and R4 on day 198 for DGGE analysis, to investigate the ammonia effect on NOB. While one another sample was obtained from R2-II on day 262, to study the organic material effect on NOB. Some biofilm were collected and stored in 50 mL sterile plastic test tubes at −20 °C in the steady state of the reactors. Finally about 1 g wet biofilm was collected into 10 mL sterile plastic test tube for DNA extraction each biofilm sample. DNA was extracted using a bacterial genomic mini extraction kit (Sangon, China) and was detected by 0.8% (w/V) agarose gel electrophoresis.

2.4. PCR-DGGE analysis

The qualified DNA was used as template for PCR. To amplify 16S rDNA fragments of Nitrobacter-like NOB for DGGE, primers F1nxrA (with GC-clamp)/R2nxrA were used to amplify nxrA fragments (Attard et al., 2010). Primers 27F/705R (with GC-clamp) were used for the PCR of Nitrospira-like NOB (Freitag et al., 2005). Thermocycling was performed in PCR Thermal Cycler Dice (TaKaRa, Japan), the PCR products were detected by 1.5% (w/V) agarose gel electrophoresis to confirm the product size and then purified with the gel midi purification kit (Sangon, China). The primers and PCR conditions were summarized in Table 1.

The purified PCR production was used for DGGE analysis in an 8% polyacrylamide gel with 30–60% linear gradient of denaturant. DGGE electrophoresis was conducted on Dcode Universal Mutation Detection System (Bio-Rad) at 60 °C, 120 V for 7 h. After electrophoresis, the gel was stained using the silver-staining method and taken photos. The Shannon-Wiener diversity index and Simpson's index of diversity were calculated from Eqs. (1) and (2) (Zhang et al., 2013).

$$H = -\sum_{i=1}^{n} P_i \ln P_i \quad (1)$$

$$D = 1 - \sum_{i=1}^{n} (P_i)^2 \quad (2)$$

Pi was the proportion of individual band intensity relative to the sum of all band intensities.

2.5. Cloning and sequencing

Prominent DGGE bands in the stained gel were excised and dissolved in 100 μL 1× TE buffer, and stored at 4 °C overnight. 1 μL TE solution as template was reamplified with the primer pairs without GC-clamp or universal primer pair F338/R518 using the same methods as described previously, followed by purification with the purification kit (Sangon, China) and cloning in pMD19-T plasmid vector system (TaKaRa, Japan). Plasmid DNA was extracted from the cloned bacteria solutions (Escherichia coli DH5a, TaKaRa, Japan) and sequenced by Sangon Company. All sequences obtained were submitted to GenBank database by nucleotide blast tool of the National Center for Biotechnology Information (NCBI).

The GenBank accession numbers in this study were KJ023577–KJ023582 related to Nitrobacter-like NOB and KJ023563–KJ023566 related to Nitrospira-like NOB.