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Effect of inorganic carbon on nitrogen removal and microbial communities of CANON process in a membrane bioreactor

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HIGHLIGHTS

- IC/N ratio performed significant effect on nitrogen removal of CANON.
- The shortage of IC led to the bioactivity decrease of AOB and AAOB.
- Biodiversity of AOB and AAOB both decreased with IC decreasing.
- *Nitrosomonas* sp. and *Candidatus Brocadia fulgida* could survive with IC deficit.
- Influent IC/N should be controlled at 1.5–2.0 for high-rate and stable CANON.

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ABSTRACT

In this study, a membrane bioreactor (MBR) was adopted for completely autotrophic nitrogen removal over nitrite (CANON) process. Inorganic carbon (IC) was step-wise decreased to analyze the IC influence on nitrogen removal and microbial communities, finally IC was elevated to study its recovery capability. The bioactivities of functional organisms were detected by batch experiments. Results showed that the bioactivity and biodiversity of aerobic ammonia-oxidizing bacteria (AOB) and anaerobic ammonia-oxidizing bacteria (AAOB) both decreased due to the IC shortage, while nitrite-oxidizing bacteria bioactivity showed a contrary result. When the concentration ratio of IC to nitrogen (IC/N) decreased to 1.0, the nitrogen removal sharply deteriorated, which then recovered when the ratio increased to 2.5. Denaturing gradient gel electrophoresis results showed that *Nitrosomonas* sp. of AOB and *Candidatus Brocadia fulgida* of AAOB could survive in the condition of IC deficit. The prominent IC/N ratio for high-rate and stable CANON was between 1.5–2.0.

1. Introduction

In current state, most organic material in sewage was expected be converted to biogas for use by anaerobic treatment process. As a result, the organic compounds would no longer be available for denitrification, resulting in a large excess of ammonia pollution (Kartal et al., 2010). For this problem, the completely autotrophic nitrogen removal over nitrite (CANON) process could be a feasible technology since it could remove ammonia without organic consumption. CANON has been successfully carried out in several lab-scale systems and be proved is very sensitive to environmental conditions such as temperature, pH, salinity and presence of inhibitors including nitrite, free ammonia (FA) and organic material (Gilbert et al., 2014; Kimura et al., 2011; Liu et al., 2008; Ni et al., 2012; Tang et al., 2010). Besides of these factors, inorganic carbon (IC) could also be an important factor influencing CANON process, which was still not conductively investigated. For CANON process, nitritation and Anammox reaction simultaneously occur in one single reactor, with aerobic ammonia-oxidizing bacteria (AOB) and anaerobic ammonia-oxidizing bacteria (AAOB) as functional organisms. Since AOB and AAOB both survive as autotrophic organisms and consume IC as their indispensable substrate, it is no doubt that the influent IC concentration is an important factor affecting CANON process.

Some previous studies demonstrated that the AOB bioactivity underlie limitations at low IC concentrations, which further affected the nitritation efficiency (Furukawa et al., 1993; Guisasola et al., 2007). Guisasola et al. (2007) examined IC limitation using respirometric and titrimetric techniques and proved the inhibition of AOB bioactivity at IC concentrations lower than 36 mg C L^{-1}. Similarly, Jun et al. (2000) found that suitable IC

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concentration could stimulate the bioactivity and number increasing of AOB. Another report stated that AOB suppression by FA and free nitrous acid (FNA) under IC limitation was stronger than that under non-limited IC condition, which demonstrated the strong modification of AOB dynamics under IC limited condition (Tora et al., 2010). Other reports in literature indicated that IC concentration also affected the AAOB bioactivity (Dexiang et al., 2008; Tang et al., 2009). To be specific, the AAOB bioactivity was enhanced with the increase of influent IC, and then, was inhibited as the IC was too high (Dexiang et al., 2008). Another study showed that the relationship between the influent IC concentration and AAOB bioactivity followed Michaelis–Menten kinetics, and the AAOB bioactivity could be recovered by adding a suitable amount of IC (Kimura et al., 2011). Moreover, IC limitation presented a stronger impact on AOB than on the nitrite-oxidizing bacteria (NOB), no limitation for NOB was observed while AOB was significantly affected by IC limitation (Guisasola et al., 2007). Thus, IC was definitely an important factor for CANON process since NOB should be suppressed while AOB and AAOB be enhanced.

Additionally, IC serves as the bicarbonate alkalinity for CANON process. In the nitritation stage of CANON process, acid is produced, and then alkalinity is consumed to neutralize the pH, while the Anammox stage presented the contrary phenomenon. The effectiveness of high alkalinity concentration for enriching AOB and inhibiting NOB had been stated in a previous report (Zhang et al., 2014b). While in other studies the feasibility of suitable alkalinity concentration for improving the nitritation rate and CANON efficiency had been respectively reported (Shanahan and Semmens, 2015; Wett and Rauch, 2003; Zhang et al., 2013a). These previous studies proved alkalinity concentration be crucial for CANON process, thus the IC serving as bicarbonate alkalinity could also significantly affect the CANON system. However, the effect of the IC concentration on CANON process has not yet been well investigated. More important, it was common knowledge that the IC concentration would influence the community of AOB and AAOB, since the two groups both use IC for cell growth. However, no study has else been done about the IC influence on the microbial dynamics of CANON process.

The main goal of this study was to investigate the effect of IC on the nitrogen removal and microbial communities of CANON process. Since the previous study had proved the feasibility of membrane bioreactor (MBR) for maximum retention of AAOB (Zhang et al., 2013b), an MBR was adopted for the start-up and operation of CANON process in this study. After achieved to stable operation, IC concentration was step-wise decreased, to investigate the effect on nitrogen removal performance, bioactivity and microbial communities. Finally, IC concentration was rose up again, to study the recovery capability for CANON process.

2. Methods

2.1. Experimental setup

An MBR with effective volume of 13 L was operated as CANON process, the IC influence was investigated after it reached steady state. The reactor was installed with a hollow fiber membrane module, of which the work area, filtration size and water permeability was 0.2 m^2, 0.1 μm and 36 L h^{-1}, respectively. The membrane module was backwashed when the transmembrane pressure increased to −80 kPa, or cleaned using tap water before soaking in 8‰ sodium hypochlorite solution for 24 h. Effluent was continuously pumped out via the membrane filtration, while the wastewater and air were consecutively pumped into the reactor bottom. The membrane worked continuously without relaxation during one backwash cycle, the backwashing and cleaning were performed approximately every 25 and 90 days, respectively.

The synthetic wastewater used in this study contained $(NH_4)_2SO_4$ as ammonia source and $NaHCO_3$ as IC source, together with (in g L^{-1}) KH_2PO_4 (0.136), $CaCl_2$ (0.136), $MgSO_4{\cdot}7H_2O$ (0.3) and trace element solution (1 mL L^{-1}) (van de Graaf et al., 1996). Before the experiment in present study, the MBR had been operated for more than one year as stable CANON process. The present experiment was carried out for 150 days and conducted as five phases, as shown in Table 1. The ammonia concentration was constantly 200 mg L^{-1} during the whole experiment. The IC concentration in influent was step-wise decreased from 400 to 150 mg L^{-1} in phase I to IV, with the concentration ratio of IC to nitrogen (IC/N) decreasing from 2.0 to 0.75. Finally in phase V the IC concentration was increased to 500 mg L^{-1}, to detect its capability for recovering CANON process. The hydraulic retention time, reaction temperature and DO was about 6.3 h, 25 °C and 0.15 mg L^{-1}, respectively. The mixed liquor suspended solids and mixed liquor volatile suspended solid in the reactor was around 4 and 3 g L^{-1}, respectively. Other detailed operational conditions were summarized in Table 1. Sludge samples were obtained from the reactor at the end of each phase, for denaturing gradient gel electrophoresis (DGGE) analysis.

2.2. Batch experiments for determining bioactivity

Batch experiments were carried out to determine the bioactivity of AOB, AAOB and NOB under different IC concentration, during the steady state before the continuous experiment. 5 L mixed liquor was taken out and equally filled into five same beakers with effective volume of 1 L, all of which were installed with membrane module to simulate the MBR setup. A peristaltic pump was used to continuously draw out water through the membrane, and then back flowed into each beaker. CANON, Anammox and nitrification rate was repeatedly detected through batch experiments with each IC concentration (500, 400, 300, 200, 150 mg L^{-1}). For CANON rate, the influent only contained ammonia of 200 mg L^{-1} without any other nitrogen components, DO was 0.15 mg L^{-1}; for Anammox rate, the influent contained ammonia of 86 and nitrite of 114 mg L^{-1}, without oxygen supply; for nitrification rate, the influent contained nitrite of 200 mg L^{-1} without ammonia addition, DO was 0.15 mg L^{-1}. The influent and operational conditions in each batch experiment were shown in Table 2. In all the batch experiments, the temperature was around 25 °C. During each experiment, liquid samples (5 mL each time) were collected from each reactor every 0.5 h, for detection of the nitrogen components. The reaction rates were calculated as Eqs. (1) and (2).

$$V_{\mathrm{CANON,Anammox}} = \frac{\{[\mathrm{TN}]_{start} - [\mathrm{TN}]_{end}\} \times 24}{t} \quad (1)$$

$$V_{\mathrm{Nitrification}} = \frac{\{[\mathrm{NO_2^-}]_{start} - [\mathrm{NO_2^-}]_{end}\} \times 24}{t} \quad (2)$$

2.3. Analytical methods

Concentrations of NH_4^+, NO_2^- and NO_3^- in influent and effluent were daily measured according to Standard Methods. The temperature, DO and pH were detected using online instruments (WTW, Germany). IC concentration was determined using the TOC analyzer (vario TOC cube, Elementar, Germany).

2.4. DNA extraction, PCR-DGGE and clone-sequencing

5 mL mixed liquor were collected at the end of each phase and analyzed together with seed sludge. DNA was extracted using a