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Effect of COD/N ratio on nitrogen removal and microbial communities of CANON process in membrane bioreactors

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HIGHLIGHTS

- COD/N in low ratio could improve nitrogen removal of CANON.
- The suppressing threshold of COD/N ratio on CANON was 1.7.
- Biodiversity of AOB and AAOB both decreased with COD increasing.
- Denitrifiers and AAOB showed different relationship under different COD/N ratio.
- Strategies for treating sewage with different COD/N ratio were proposed.

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ABSTRACT

In this study, the effect of COD/N ratio on completely autotrophic nitrogen removal over nitrite (CANON) process was investigated in five identical membrane bioreactors. The five reactors were simultaneously seeded for 1 L CANON sludge and be operated for more than two months under same conditions, with influent COD/N ratio of 0, 0.5, 1, 2 and 4, respectively. DGGE was used to analyze the microbial communities of aerobic ammonia-oxidizing bacteria (AOB) and anaerobic ammonia-oxidizing bacteria (AAOB) in five reactors. Results revealed the harmonious work of CANON and denitrification with low COD concentration, whereas too high COD concentration suppressed both AOB and AAOB. AOB and AAOB biodiversity both decreased with COD increasing, which then led to worse nitrogen removal. The suppressing threshold of COD/N ratio for CANON was 1.7. CANON was feasible for treating low COD/N sewage, while the high sewage should be converted by anaerobic biogas producing process in advance.

1. Introduction

Completely autotrophic nitrogen removal over nitrite (CANON) process has been developed in recent years as the most efficient and economical method to remove ammonia from wastewater (Sliekers et al., 2002; Third et al., 2001). With the development of anaerobic treatment process, most organic compounds in wastewater are converted to biogas without consumption of ammonia (Kartal et al., 2010), and CANON process was suggested to be an alternative process to treat the effluent with high concentration of ammonia (Liang et al., 2014). Recent researches indicated that CANON process is very sensitive to environmental conditions such as temperature, pH, salinity and presence of inhibitors including nitrite, free ammonia and organic material (OM, expressed as COD in this study) (Gilbert et al., 2014; Kimura et al., 2011; Liu et al., 2008; Ni et al., 2012; Tang et al., 2010a). Specially, one factor could not be ignored was the presence of OM since CANON process was a completely autotrophic reaction without organic carbon consumption. The functional organisms including aerobic ammonia-oxidizing bacteria (AOB) and anaerobic ammonia-oxidizing bacteria (AAOB) both utilize CO_2 as the source of carbon for ammonia oxidation.

Generally, wastewaters containing ammonia are not free from OM, and the anaerobic process for biogas production could not totally remove the organic carbon, such as anaerobic digestion process. Previous studies have reported the adverse effect of the presence of OM on the growth of both AOB and AAOB. On one hand, the presence of COD would result in the survival of heterotrophic bacteria, which would compete DO with AOB (Ji et al., 2012; Zhi and Ji, 2014). Furthermore, previous research provided evidence that the organic carbon source affects the make-up of AOB community in mixed cultures (Racz et al., 2010). Researchers are unanimous when it comes to the inhibitory effect

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of high COD concentration on AAOB. As much, AAOB are not able to compete with denitrifying bacteria (Jia et al., 2012; Ni et al., 2012). Further study revealed that both COD concentrations and COD to N ratio (COD/N) affect the performance of AAOB (Chamchoi et al., 2008; Hao and Loosdrecht, 2004; Molinuevo et al., 2009). Considering the adverse effect on both AOB and AAOB, the COD and COD/N ratio would also significantly affect the nitrogen removal performance by CANON. Only one or two studies focused on the relative low COD/N ratio influencing CANON so far. Chen (Chen et al., 2009) observed a significant reduction of the nitrogen removal efficiency (NRE) from 79% to below 52% when influent COD/N ratio was increased from 0.5 to 0.75, while Lackner and Horn (Lackner and Horn, 2013) reached 80–85% NRE with an influent COD/N ratio of 1 in a sequencing batch reactor. The available studies have not mentioned the performance of CANON process under a higher COD/N ratio, and it could not be conclusive to determine the COD/N ratio effects from the limited studies. In addition, since the reactor performance relies on the functional bacteria (Zhi et al., 2014), it is important to know the responses of microbial communities to the presence of different COD/N ratio. However, to the best of our knowledge, limited work has been carried out to explore the performance and microbial communities of CANON with different COD/N level.

The main goal of this study was to investigate the effect of influent COD/N ratio on the nitrogen removal performance and microbial communities of CANON process. Membrane bioreactor (MBR) was chosen to be the experimental equipment in this study due to its effectiveness for CANON process (Zhang et al., 2013b). Five identical MBRs with same seed sludge were simultaneously started-up, and fed with different COD/N levels. The nitrogen removal and denitrification ratio of the five MBRs were compared, and the microbial communities of each reactor were analyzed using DGGE and clone-sequencing.

2. Methods

2.1. Reactors and seed sludge

Five identical lab-scale MBRs (effective volume: 1 L, diameter: 100 mm, height: 200 mm, Supplementary material) were adopted to this study, named R0, R1, R2, R3 and R4, respectively. Each reactor was installed with a hollow fiber membrane module (material: PVDF; pore size: 0.1 μm; effective area: 0.05 m^2; water permeability: 9 L h^{-1}). For each reactor, it was entirely placed in a water bath to ensure a constant reaction temperature (25 °C). Effluent was continuously extracted from the reactor using a peristaltic pump, while the synthetic wastewater was fed into the reactor. Oxygen was supplied continuously from an aeration ring locating at the bottom of the reactor which was connected to an air blower, and the aeration was controlled by the gas flow meter. A mixer was set in each reactor to assure homogeneous condition.

A running MBR with identical setup was adopted as the seeding reactor, it showed an excellent nitrogen removal ability with the NRR of 0.6–0.7 kg m^{-3} d^{-1} before seeding. The five experimental MBRs were simultaneously inoculated with 1 L CANON sludge from the running MBR. The mixed liquor suspended solid and mixed liquor volatile suspended solid of the seed sludge were 4.1 and 3.1 g L^{-1}, respectively.

2.2. Feed and experimental setup

The effect of COD/N on CANON process was tested with synthetic wastewater. To avoid influent impact, the influent for the five experimental reactors were totally same as the seeding MBR. The main synthetic wastewater used in this study contained (in g L^{-1}): $(NH_4)_2SO_4$ (0.942), $NaHCO_3$ (3.357), KH_2PO_4 (0.136), $CaCl_2$ (0.136), $MgSO_4{\cdot}7H_2O$ (0.3) and 1 mL L^{-1} of trace element solution (van de Graaf et al., 1996). Influent ammonia for the five reactors were 200 mg L^{-1} during the whole experiment, the only difference was the COD concentration in influent. As a logical choice of organic substrate, glucose was considered as the harmless one to AOB (Ni et al., 2012). So glucose was added to the influent of R1, R2, R3 and R4, to adjust the influent COD concentration to 100, 200, 400 and 800 mg L^{-1}, with COD/N ratios of 0.5, 1, 2 and 4, respectively. For comparison, no OM was added to R0.

The five reactors were running in a continuous mode at a HRT of 6.4 h. The pH in each reactor was all around 7.8 without adjustment. It was demonstrated that AAOB had a long growth cycle and low growth rate (l = 0.0027 h^{-1}, doubling time = 10.6 d) (Strous et al., 1998; Zhang et al., 2013a), so this study were conducted for more than two months. Sludge samples were obtained from each reactor on day 61, for DGGE analysis. Since previous studies (Zhang et al., 2013a,b) showed the prominent performance when DO was 0.15 mg L^{-1}, and the seeding reactor was also operated under this value for a long time, DO was controlled as 0.15 mg L^{-1} in the five experimental MBRs, to eliminate the oxygen impact on the reactor performance. Other operational conditions of the reactors are summarized as Table 1.

2.3. Analytical methods

According to the methods mentioned by APHA, concentrations of NH_4^+-N and NO_2^--N were daily measured using visible spectrophotometer (Shanghai Yoke Instrument Co., 722S, China) with different colorimetric methods. NO_3^--N was analyzed using ultraviolet spectrophotometric (Shanghai Yoke Instrument Co., UV755B, China). The temperature, DO and pH were detected using online instruments with specific probes (WTW, Oxi1296-TriOxmatic700-7 and pH296-Sensolyt 650-7, Germany). COD was detected by digestion instrument (Lianhua Technology Co., 5B-6CV8.0, China).

Denitrification ratio was defined as the ratio of nitrogen removal by denitrification to the total nitrogen removal. The nitrogen removal by denitrification was equal to the decreased amount of NO_3^--N when compared to the amount generated by CANON reaction. Denitrification ratio was calculated as Eq. (1).

$$\text{Denitrification ratio}(\%) = \frac{\{[NH_4^+\text{-N}]_{inf} - [NH_4^+\text{-N}]_{eff}\} \times 0.11 - [NO_3^-\text{-N}]_{eff}}{[TN]_{inf} - [TN]_{eff}} \tag{1}$$

2.4. DNA extraction, PCR–DGGE, cloning and sequencing

Since DGGE was suitable for determining the specie difference and the biodiversity variation of the same kind of bacteria, it was chosen to characterize the microbial communities of AOB and AAOB under different influent COD/N ratio in this study. Some mixed liquor was respectively collected from the five reactors on day 61. DNA was extracted using a bacterial genomic mini extraction kit (Sangon, China) according to the manufacturer's manual and was detected by 0.8% (w/V) agarose gel electrophoresis.

Table 1
Operational conditions of the five experimental reactors.

Reactor	COD_{inf} (mg L^{-1})	COD_{eff} (mg L^{-1})	HRT (h)	Aeration rate (L min^{-1})	DO (mg L^{-1})	pH –
R0	0	0	6.4	0.2	0.15	7.84
R1	97.9	13.72	6.4	0.25	0.15	7.80
R2	198.2	32.56	6.4	0.3	0.15	7.78
R3	399.2	74.51	6.4	0.35	0.15	7.86
R4	800.2	252.4	6.4	0.4	0.15	7.88