



Performance and microbial community of completely autotrophic nitrogen removal over nitrite (CANON) process in two membrane bioreactors (MBR) fed with different substrate levels

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HIGHLIGHTS

- Two identical MBR–CANON reactors were started up by different strategies.
- The bioactivity of functional bacteria was determined in two reactors.
- The genetic diversity of three kinds of functional microbes was examined.
- The distribution and quantification of functional microbes was analyzed.
- A strategy for rapid start-up and high-rate nitrogen removal was proposed.

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ABSTRACT

To study the influence of substrate on completely autotrophic nitrogen removal over nitrite (CANON) process, two membrane bioreactors (MBR) with identical setup but fed with different substrate levels (R1 with low ammonia, R2 with high ammonia), were adopted in this study. The nitrogen removal performance, bioactivity, biodiversity and distribution of the functional microorganisms in two reactors were investigated. Both the aerobic ammonia-oxidizing bacteria (AerAOB) and anaerobic ammonia-oxidizing bacteria (AnAOB) in R2 showed higher bioactivity than those in R1, while nitrite-oxidizing bacteria (NOB) showed the contrary result. *Nitrosomonas* and *Candidatus Kuenenia stuttgartiensis* were detected as predominant functional microbes in the two reactors while *Nitrobacter* only existed in R1. High influent ammonia possibly led to the higher biodiversity of AerAOB and the more densely packed distribution. Meanwhile, this study has demonstrated the feasibility of increasing ammonia for rapid start-up, and decreasing HRT for high-rate nitrogen removal in CANON process.

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1. Introduction

In conventional nitrification–denitrification nitrogen removal processes, large amount of organic carbon was needed in denitrification stage. However, most wastewaters do not contain sufficient biodegradable carbon, making them less suitable for nitrogen removal via the traditional process. Moreover, with the development of anaerobic treatment process, most organic compounds in wastewater are converted to biogas, which is feasible with the present state of the art (Kartal et al., 2010). This conversion leads to the lack of organic carbon available for denitrification and hence a large excess of nitrogen in effluent. A new innovative nitrogen removal process—completely autotrophic nitrogen removal over

nitrite (CANON) process has been developed in recent years, which could remove nitrogen without organic carbon consumption and also less oxygen consumption (Sliekers et al., 2003). Thus, this process not only offers a good alternative for nitrogen removal from wastewater with low carbon, but also eliminates the competition for carbon between nitrogen removal and biogas production, as well as decreases the power consumption.

CANON process has been successfully used to treat wastewater with high temperature or high ammonia content (Cho et al., 2011; van der Star et al., 2007), such as sludge digestion and landfill leachate. Several systems have been operated with low ammonia concentration at low temperature (De Clippeleir et al., 2011; Hendrickx et al., 2012), all of which showed worse performance than that of the systems treating high ammonia wastewater. In addition, some previous reports suggested that the systems with low ammonia encountered some challenges, such as long start-up period (Jeanningros et al., 2010), low nitrogen removal rate

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(NRR) (Liu et al., 2012) and instability. Thus, influent ammonia concentration played a significant effect on the start-up and nitrogen removal performance of CANON reactors. However, little research was done about the influence of influent ammonia on CANON, which are still not conducive to get detailed operational information of the system aiming at establishing effective nitrogen removal from wastewater with different levels of ammonia.

In CANON process, ammonia is first oxidized to nitrite by aerobic ammonia-oxidizing bacteria (AerAOB), after which the nitrite and remained ammonia are converted to nitrogen gas by anaerobic ammonia-oxidizing bacteria (AnAOB) (Nielsen et al., 2005). Thus, a co-operative relationship between AerAOB and AnAOB should be realized. Moreover, the effective inhibition on nitrite-oxidizing bacteria (NOB) is essential, which would result in an almost complete conversion of ammonia to dinitrogen gas alone with small amounts of nitrate (Third et al., 2001). Consequently, acquiring information about the functional bacteria in CANON system could be valuable for a better reactor performance. Previous phylogenetic studies indicated β -proteobacteria related AerAOB and *Planctomycetales*-like AnAOB were predominant bacteria in CANON system, and the system had a selective advantage of *Nitrobacter* over *Nitrospira*-like NOB (Ahn and Choi, 2006; Liu et al., 2008). However, the different NH_4^+ -N concentration may affect the microbial distribution and ecological features in nitrogen removal systems (Third et al., 2005; van de Vossenberg et al., 2008). Thus, the information related with microbial community and population in CANON reactors fed with different levels of influent ammonia is essential for a better understanding about the reactor. And more information of the microorganisms would contribute to a faster start-up and a better monitoring of CANON reactor, which, may further lead to the application of CANON process in treating sewage with different levels of ammonia. However, the comparison about the bioactivity, biodiversity and distribution of the functional microorganisms in CANON reactors fed with different levels of ammonia were poorly understood.

In this study, membrane bioreactor (MBR) was adopted for CANON process due to its efficient capacity of biomass retention. Comparisons of the nitrogen removal performance, bioactivity, biodiversity and distribution of functional microorganisms were studied using FISH, PCR-DGGE and gene clone libraries in two identical MBR reactors, which were fed with different ammonia levels. The main goal of this study was to measure the influence of influent ammonia level on the reactor performance and the microbial characters, which would contribute to a faster start-up and a higher efficiency of CANON reactor treating wastewater with different levels of nitrogen.

2. Methods

2.1. Experimental setup

Two MBR (named R1 and R2) with identical setup (Fig. 1) were adopted for CANON process, both of which were installed with a hollow fiber membrane module (material: PVDF; pore size: 0.1 μm ; effective area: 0.2 m^2 ; water permeability: 36 L h^{-1}), respectively. While the synthetic wastewater was fed into the reactor, the effluent was continuously pumped out from the reactor via the membrane filtration using a peristaltic pump. The entire reactor was placed in the water bath to ensure a constant reaction temperature (25 $^{\circ}\text{C}$). For constant membrane flux, the contaminated membrane module was backwashed when transmembrane pressure rose up to -80 kPa, or cleaned by tap water before soaked in 8‰ sodium hypochlorite solution for 24 h. The frequency of backwashing and cleaning was about 25 and 90 d, respectively.

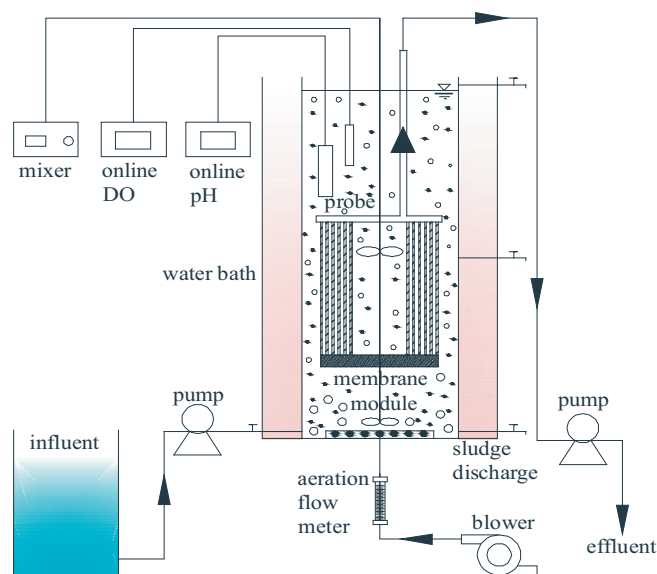


Fig. 1. Setup of the MBR used in this study.

Conventional activated sludge from a municipal sewage plant was seeded to the two reactors simultaneously. The mixed liquor suspended solid (MLSS) and mixed liquor volatile suspended solid (MLVSS) of the seed sludge were 12.9 and 10.6 g L^{-1} , respectively. The synthetic wastewater used in this study contained $(\text{NH}_4)_2\text{SO}_4$ and NaHCO_3 as main substrates, together with (in g L^{-1}) KH_2PO_4 (0.136), CaCl_2 (0.136), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.3) and trace element solution (1 mL L^{-1}) (deGraaf et al., 1996). The alkalinity concentration was varied with the influent NH_4^+ with a ratio of 10 constantly. Both the two reactors were started-up by increasing ammonia loading rate (ALR) under oxygen-limited condition. Specifically in R1, influent ammonia was kept at 80 mg L^{-1} with the HRT decreasing from 8 to 1.9 h for CANON. Simultaneously in R2, influent ammonia was increased from 70 to 200 mg L^{-1} without change of HRT. The temperature and SRT in R1 and R2 were both maintained at 25 ± 0.5 $^{\circ}\text{C}$, 100 d, respectively. Other operational conditions and the corresponding performance of the two reactors are summarized in Table 1. Sludge was obtained from the two reactors on day 178, for bioactivity, DGGE and FISH analysis.

2.2. Analytical methods

According to the Standard Methods (APHA, 1998), MLSS and MLVSS were weekly measured; concentrations of NH_4^+ , NO_2^- were daily measured using colorimetric methods, while NO_3^- was daily analyzed using ultraviolet spectrophotometric method. The temperature, DO and pH were detected using online instruments (WTW, Germany). Alkalinity was measured by potentiometric titration (ZDJ-2D).

2.3. Batch experiments

Batch experiments were carried out to determine the CANON rate, anaerobic ammonia oxidation (Anammox) rate and nitrification rate of the two reactors. Two same beakers with an effective volume of 1 L were used as two mini reactors, which were both installed with membrane module to achieve the similar setup of MBR. A peristaltic pump was used to continuously draw out water through the membrane, and then back flowed into each beaker. In each batch experiment, 1 L of mixed liquor was obtained from the two reactors, respectively, washed thrice with distilled water to minimize NO_2^- and NO_3^- present in the sludge. The influent and

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