



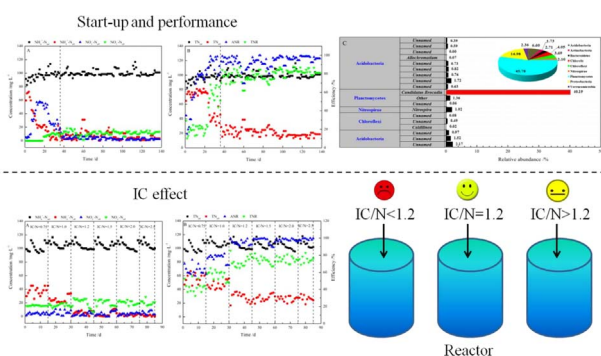
Short Communication

Start-up of the completely autotrophic nitrogen removal over nitrite process with a submerged aerated biological filter and the effect of inorganic carbon on nitrogen removal and microbial activity

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GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:

Completely autotrophic nitrogen removal over nitrite  
Submerged aerated biological filter  
Inorganic carbon  
Nitrogen removal  
Microbial activity

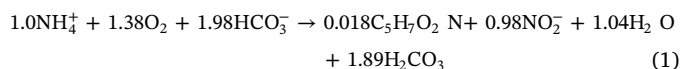
ABSTRACT

Good start-up and performance are essential for the completely autotrophic nitrogen removal over nitrite (CANON) process, and inorganic carbon (IC) is also important for this process. In this study, a lab-scale submerged aerated biological filter (SABF) was adopted for the CANON process. A 16S rRNA gene high-throughput sequencing analysis showed that the phyla Proteobacteria and Planctomycetes were the dominant microorganisms and that the genus *Candidatus Brocadia* functioned as the nitrogen remover. The effect of IC on the nitrogen removal was analyzed. The results showed that the optimum concentration ratio of IC to nitrogen (IC/N) was 1.2, which produced the highest average ammonium nitrogen removal rate (ANR) and total nitrogen removal rate (TNR) values of 95.5% and 80.3%, respectively. The average AOB and AnAOB activities were 2.45 mg·L<sup>-1</sup>·h<sup>-1</sup> and 3.57 mg·L<sup>-1</sup>·h<sup>-1</sup>, respectively. This research could promote the nitrogen removal ability of the CANON process with a SABF in the future.

1. Introduction

The completely autotrophic nitrogen removal over nitrite (CANON) process in a single stage is a microbial process that has recently been studied and utilized for biological nitrogen removal during wastewater treatment (Kartal et al., 2010). This process consists of two steps, as

described in Eqs. (1) and (2):



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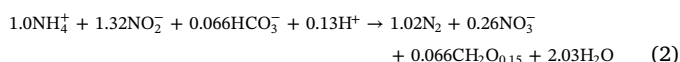
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<https://doi.org/10.1016/j.biortech.2018.01.107>

Received 29 November 2017; Received in revised form 18 January 2018; Accepted 22 January 2018

Available online 31 January 2018

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Currently, various types of reactors, such as membrane bioreactors (MBRs), sequencing batch reactor (SBR), and continuous stirred tank reactors (CSTRs), have been studied for the start-up of the CANON process (Zhang et al., 2013; Wang et al., 2017; Qian et al., 2017). However, the slow growth rate of anaerobic ammonium oxidation bacteria (AnAOB) frequently occurs because of its doubling time of 10–12 days (Van der Star et al., 2007), thereby leading to a long start-up period of the CANON process. A submerged aerated biological filter (SABF) is effective and efficient at ammonium nitrogen removal because of the growth of the attached biofilm (Rahimi et al., 2011). This reactor can help stably maintain microorganisms and has a long retention time (El-Shafai and Zahid, 2013), which is optimal for the growth of AnAOB. However, the SABF has not been investigated for use in assisting the start-up of the CANON process until now.

In addition, many factors affect the operation of the CANON process, such as the pH, DO, temperature,  $\text{N}_2\text{H}_4$  and organic carbon source, which have already been defined and optimized (Yao et al., 2013; Zhang et al., 2015). The inorganic carbon (IC) source is also a crucial parameter in the performance of the CANON process for the following reasons: (1) IC participates in the reactions of partial nitrification and anammox as a nutrient component of microorganisms. (2) IC serves as the bicarbonate alkalinity for buffering in the reaction system. In general, different types of wastewater with different  $\text{NH}_4^+\text{-N}$  concentrations will have different IC quantity requirements (Liao et al., 2008). Therefore, the concentration ratio of IC to nitrogen (IC/N) can be used to estimate the IC effect on the CANON process (Ma et al., 2015). Different optimum IC/N ratios occur for different reactors, indicating the importance of determining the effect of IC on the CANON process with a SABF, which has not been reported until now.

This paper revealed the start-up of the CANON process with a SABF under appropriate conditions. The microbial community was detected by a 16S rRNA gene high-throughput sequencing analysis. The influence of IC on the CANON process with the SABF was identified from the perspective of bacterial activity and process efficiency, thus revealing the optimum concentration ratio of IC/N for this system. This study will play an important role in the stable operation of the CANON process with the SABF.

## 2. Materials and methods

### 2.1. Reactor operation

As shown in Fig. 1, a 3 L lab-scale SABF was initiated the CANON process in biofilm mode for approximately 37 days, and it was operated for 101 days. The reaction temperature was  $30.5 \pm 1^\circ\text{C}$ , DO was  $0.1\text{--}0.3\text{ mg}\cdot\text{L}^{-1}$ , HRT was 24 h and pH was 7.8–8.3.

After preliminary operations, the effect of IC on the nitrogen removal and microbial activity of the CANON process with a SABF was studied. This reactor was operated with  $\text{HCO}_3^-\text{-C}$  concentrations of  $75\text{ mg}\cdot\text{L}^{-1}$ ,  $100\text{ mg}\cdot\text{L}^{-1}$ ,  $120\text{ mg}\cdot\text{L}^{-1}$ ,  $150\text{ mg}\cdot\text{L}^{-1}$ , and  $250\text{ mg}\cdot\text{L}^{-1}$ , which provide IC/N ratios of 0.75, 1.0, 1.2, 1.5, 2.0, and 2.5, respectively.

### 2.2. Inoculated sludge and synthetic wastewater

The inoculated sludge was obtained from secondary clarifier of the municipal sewage treatment plant of Nansha (Guangzhou, China). It was aerated for 3 days, and the following parameter values were obtained: pH: 7.0–7.2, mixed liquor suspended solids (MLSS):  $14,000\text{--}14,500\text{ mg}\cdot\text{L}^{-1}$ , and mixed liquor volatile suspended solids (MLVSS):  $8000\text{--}8500\text{ mg}\cdot\text{L}^{-1}$ .

The synthetic wastewater sample consisted of  $0.33\text{--}0.46\text{ g}\cdot\text{L}^{-1}$   $\text{NH}_4\text{Cl}$ ,  $0.03\text{ g}\cdot\text{L}^{-1}$   $\text{KH}_2\text{PO}_4$ ,  $0.01\text{ g}\cdot\text{L}^{-1}$   $\text{MgSO}_4$ ,  $0.02\text{ g}\cdot\text{L}^{-1}$   $\text{CaCl}_2$ ,

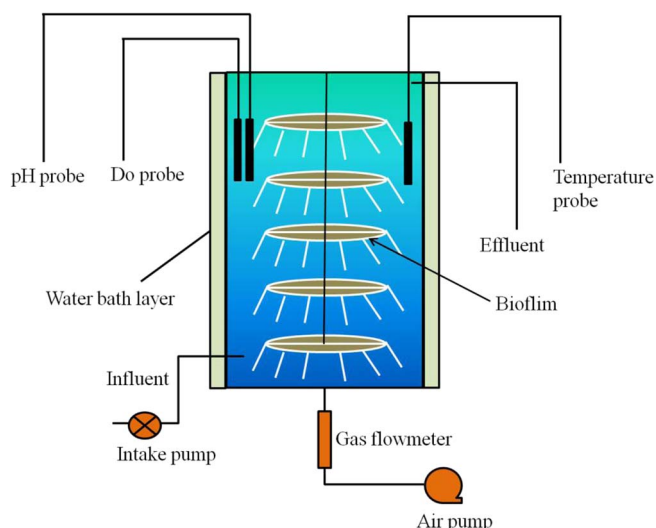


Fig. 1. Schematic diagram of the SABF system.

Table 1  
Key water quality of the influent.

Item	Unit	Value
$\text{NH}_4^+\text{-N}$	$\text{mg}\cdot\text{L}^{-1}$	82.5–116.5
$\text{NO}_2^-\text{-N}$	$\text{mg}\cdot\text{L}^{-1}$	0.0
$\text{NO}_3^-\text{-N}$	$\text{mg}\cdot\text{L}^{-1}$	0.0
COD	$\text{mg}\cdot\text{L}^{-1}$	0–16
pH	—	7.8–8.3
IC/N (Start-up period)	—	1.3–1.7

$1.00\text{ g}\cdot\text{L}^{-1}$   $\text{NaHCO}_3$  and  $0.35\text{ mL}\cdot\text{L}^{-1}$  trace element solution. The trace element solution contained  $3.52\text{ g}\cdot\text{L}^{-1}$   $\text{FeCl}_3\cdot 6\text{H}_2\text{O}$ ,  $0.36\text{ g}\cdot\text{L}^{-1}$   $\text{MnCl}_2\cdot 4\text{H}_2\text{O}$ ,  $0.08\text{ g}\cdot\text{L}^{-1}$   $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ ,  $0.30\text{ g}\cdot\text{L}^{-1}$   $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$ , and  $0.38\text{ g}\cdot\text{L}^{-1}$   $\text{CoCl}_2\cdot 6\text{H}_2\text{O}$ . Table 1 shows the qualities of the influent in this experiment.

### 2.3. 16S rRNA gene high-throughput sequencing analysis of microorganisms

The sludge samples used in this experiment were harvested on days 0, 37 and 130. The microbial community of microorganisms was analyzed using 16S rRNA gene high-throughput sequencing technology.

All sequences were compared with the reference microorganisms available in Genbank and submitted to GenBank database with the Accession numbers of MG800858–MG805313.

### 2.4. Analytical methods

The influent and effluent samples were filtered through qualitative filter papers before the analysis. The concentrations of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  and COD were measured using the procedures described in the APHA Standard Methods (APHA, 1998).

The theoretical activities of AOB (AOR), NOB (NOR) and AnAOB (NRR) were estimated based on the nitrogen balance and theoretical stoichiometry according to Eqs. (3)–(5) (Varas et al., 2015).

$$\text{AOR}(\text{mg}\cdot\text{L}^{-1}\cdot\text{h}^{-1}) = \frac{\Delta\text{NH}_4^+\text{-N} - \frac{\Delta\text{TN}}{2.04}}{24} \quad (3)$$

$$\text{NOR}(\text{mg}\cdot\text{L}^{-1}\cdot\text{h}^{-1}) = \frac{\Delta\text{NO}_3^-\text{-N} - \frac{\Delta\text{TN}}{2.04} \times 0.26}{24} \quad (4)$$

$$\text{NRR}(\text{mg}\cdot\text{L}^{-1}\cdot\text{h}^{-1}) = \frac{\Delta\text{TN}}{24} \quad (5)$$

The integrity of nucleic acids in the sludge was detected using a

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