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

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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 micro-organisms 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⁻¹·h⁻¹ and 3.57 mg·L⁻¹·h⁻¹, 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):

\[ \begin{align*}
1.0\text{NH}_4^+ + 1.38\text{O}_2 + 1.98\text{HCO}_3^- &\rightarrow 0.018\text{C}_4\text{H}_2\text{O}_2 \ N^+ + 0.98\text{NO}_3^- + 1.04\text{H}_2\text{O} + 1.89\text{H}_2\text{CO}_3 \\
\end{align*} \]

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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, N\textsubscript{2}H\textsubscript{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 nitritation 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 NH\textsubscript{4}+-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 ± 1 °C, DO was 0.1–0.3 mg L\textsuperscript{-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 HCO\textsubscript{3} inverse of the reactions of partial nitritation 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 NH\textsubscript{4}+-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.

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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): 14,000–14,500 mg L\textsuperscript{-1}, and mixed liquor volatile suspended solids (MLVSS): 8000–8500 mg L\textsuperscript{-1}.

The synthetic wastewater sample consisted of 0.33–0.46 g L\textsuperscript{-1} NH\textsubscript{4}Cl, 0.03 g L\textsuperscript{-1} KH\textsubscript{2}PO\textsubscript{4}, 0.01 g L\textsuperscript{-1} MgSO\textsubscript{4}, 0.02 g L\textsuperscript{-1} CaCl\textsubscript{2}, 1.00 g L\textsuperscript{-1} NaHCO\textsubscript{3} and 0.35 mL L\textsuperscript{-1} trace element solution. The trace element solution contained 3.52 g L\textsuperscript{-1} FeCl\textsubscript{3}·6H\textsubscript{2}O, 0.36 g L\textsuperscript{-1} MnCl\textsubscript{2}·4H\textsubscript{2}O, 0.08 g L\textsuperscript{-1} CuSO\textsubscript{4}·5H\textsubscript{2}O, 0.30 g L\textsuperscript{-1} ZnSO\textsubscript{4}·7H\textsubscript{2}O, and 0.38 g L\textsuperscript{-1} CoCl\textsubscript{2}·6H\textsubscript{2}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 NH\textsubscript{4}+-N, NO\textsubscript{2}--N, NO\textsubscript{3}--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).

\[
\text{AOR}(\text{mg L}^{-1} \cdot \text{h}^{-1}) = \frac{\Delta \text{NH}_4^+ - \text{N}}{24} \times 25 \times 10^9
\]

\[
\text{NOR}(\text{mg L}^{-1} \cdot \text{h}^{-1}) = \frac{\Delta \text{NO}_3^- - \text{N}}{24} \times 0.26
\]

\[
\text{NRR}(\text{mg L}^{-1} \cdot \text{h}^{-1}) = \frac{\Delta \text{TN}}{24}
\]

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

1.0NH\textsubscript{4}+ + 1.32NO\textsubscript{2}-- + 0.066HCO\textsubscript{3}-- + 0.13H\textsuperscript{+} → 1.02N\textsubscript{2} + 0.26NO\textsubscript{3}-- + 0.066CH\textsubscript{2}O\textsubscript{11} + 2.03H\textsubscript{2}O (2)
The concentration and mass of nucleic acid was tested using a ND-1000 Nanodrop (Thermo Fisher Scientific Co. Ltd., America) and a Qubit 2.0 fluorimeter (Life Technologies Co. Ltd., USA). The total data and Q30 ratio were checked using a MiSeq sequencing system (Illumina Co. Ltd., America).
3. Results and discussion

3.1. Nitrogen transformation of the CANON process with a SABF

The nitrogen components in the influent and effluent of the CANON process with the SABF are summarized in Fig. 2. From day 1 to day 15, the low DO values (0.1–0.3 mg·L⁻¹) promoted AOB and inhibited NOB, leading to the NO₂⁻-N eff accumulation (increasing into 58 mg·L⁻¹) and NO₃⁻-N eff inhibition (maintaining at 0 mg·L⁻¹). From day 16 to day 37, the anammox reaction occurred, which caused the ANR and TNR increasing into 93.4% and 70.1%, respectively. Significant TN losses demonstrated the achievement of the CANON process with the SABF. From day 38 to day 138, the system maintained a high biological nitrogen removal performance. The average ANR and TNR apparently were increased into 97.9% and 82.8%, respectively. The AOB and AnAOB showed good activities and the average values were maintained at 2.36 mg·L⁻¹·h⁻¹ and 3.41 mg·L⁻¹·h⁻¹, respectively. During this period, the average ratio of △NO₃⁻-N/△NH₄⁺-N was about 0.12, closer to the theoretical value of 0.13 (Sliekers et al., 2002). This result verified that the CANON process operated stably for 101 days.

In this experiment, the CANON process was rapidly started up with the SABF due to some advantageous conditions. The combined carriers of SABF system had large specific surface areas, which could provide wide spaces for the microbial enrichment. In addition, the structure of combined carriers and low DO concentration (0.1–0.3 mg·L⁻¹) gave rise to a DO concentration gradient from the inside to outside of the biofilms.
in SABF, which supplied a suitable growth environment between the AOB as aerobic bacteria and AnAOB as anaerobic bacteria. Then, the SABF with biofilm could resist the loading fluctuation and ensure the stable enrichment of microorganisms.

3.2. Microorganisms involved in the CANON process with a SABF

As shown in Fig. 3, for the seed sludge, the dominant microorganism of the CANON process with a SABF was phylum Proteobacteria with the relative abundances of 39.74%, which affiliated with AOB, NOB, and...
3.3. Effect of IC on the performance of the CANON process with a SABF

The effects of IC on the nitrogen removal and microbial activity in the CANON process with a SABF are shown in Fig. 4. The optimum IC/N ratio was controlled at 1:2, and the highest average ANR and TNR were 95.5% and 80.3%, respectively. The average NH4+ -N eff concentration was 3.9 mg L−1, indicating that the activity of AOB was good with an average value of 2.45 mg L−1·h−1. The average ratio of \( \Delta \text{NO}_3^-/\Delta \text{NH}_4^+ \) was approximately 0.12, closer to the theoretical value of 0.13, thus illustrating that the CANON process can maintain a good performance with an IC/N ratio of 1:2.

When the IC/N ratio was decreased from 1.2 to 0.75, the average ANR and TNR were sharply reduced to 64.0% and 46.7%, respectively. Previous studies have demonstrated that the AOB activity has underlying limitations at low IC concentrations, which further affected the nitritation efficiency (Guisaclas et al., 2007). The NOB was not clearly affected by the IC/N reduction. It was the same as the recent study revealing that the IC limitation has a stronger impact on AOB than on NOB (Guisasola et al., 2007). Furthermore, the AnAOB were obviously suppressed, causing the TNR reduction because of the lack of CO2 as the actual substrate for anammox (Liao et al., 2008). In addition, the IC concentration was a significant factor that influenced N2O production by AOB (Jiang et al., 2015), which further affected the anammox efficiency.

When the IC/N ratio was increased from 1.2 to 2.5, the average NH4+ -N eff concentration was reduced to less than 1.2 mg L−1, indicating that the activity of AOB was increased and its average value was maintained between 2.66 and 2.76 mg L−1·h−1. It is shown that excessive IC had no obvious effect on the nitritation reaction. In addition, the average ratio of \( \Delta \text{NO}_3^-/\Delta \text{NH}_4^+ \) was higher than 0.19, which promoted the NOB production with the activity of 0.37–0.41 mg L−1·h−1. Moreover, the AnAOB were adversely affected by the IC increment resulting in the average TNR reduction to 75.9%. Other reports have also indicated that the AnAOB activity was enhanced with an increase of the influential IC, and then, inhibited at high bicarbonate concentrations (Ali et al., 2016). These results indicated that the IC/N ratio was an important factor for the CANON process with a SABF because NOB should be suppressed while AOB and AnAOB should be enhanced.

4. Conclusions

The CANON process with a SABF was rapidly initiated within 37 days, and stably performed for 101 days. A16S rDNA macro genome sequencing analysis revealed that the phyla Proteobacteria and Planctomycetes represented the AOB and AnAOB, respectively. IC affected the AOB and AnAOB activities and further influenced the nitrogen removal capacity of the CANON process with a SABF. The experimental results showed that the optimum IC/N ratio was 1:2, which resulted in the highest average ANR and TNR values of 95.5% and 80.3%, respectively. The average activities of AOB and AnAOB were 2.45 mg L−1·h−1 and 3.57 mg L−1·h−1, respectively.

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