



# Anammox granules formation and performance in a submerged anaerobic membrane bioreactor



Ziyin Li, Xiaochen Xu\*, Bing Shao, ShuShen Zhang, FengLin Yang\*

Key Laboratory of Industrial Ecology and Environmental Engineering, Ministry of Education, School of Environmental Science and Technology, Dalian University of Technology, Dalian 116024, PR China

## HIGHLIGHTS

- Anammox granules were achieved by selecting a proper shear stress in the SAnMBR.
- The granules reached an influent TN of 4.1 g/L with TN removal efficiency of 88%.
- The formed Anammox granules reached a doubling time of around 6.9 days.
- $\text{NO}_2^-$ -N degradation kinetics of Anammox biomass was obtained.
- Membrane operation circle prolonged with granule size growing from 287 to 896  $\mu\text{m}$ .

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## ABSTRACT

Anaerobic ammonium oxidation (Anammox) granules with the maximum specific Anammox activity (SAA) of 2.14 kg N/kgVSS d, the short doubling time of 6.9 days and the maximum endurable  $\text{NO}_2^-$ -N concentration of 130 mg/L were successfully formed in a continuously fed and completely mixing submerged anaerobic membrane bioreactor (SAnMBR). With specific input power ranging from 0.08 to 0.02 kW/m<sup>3</sup>, mean particle size (volume-weighted mean) of the Anammox flocs did not change a lot. With a specific input power of 0.003 kW/m<sup>3</sup>, the mean particle size increased rapidly from 180  $\mu\text{m}$  of the flocs to 863  $\mu\text{m}$  of the formed granules in 90 days. With maximum influent total nitrogen (TN) up to 4.1 g/L, a stable nitrogen removal efficiency of 88% was accomplished by the formed granules. The nitrite degradation kinetics was also investigated and it showed that the formed Anammox granules reached its maximum activity at 30 mg  $\text{NO}_2^-$ -N/L but was strongly inhibited at 150 mg  $\text{NO}_2^-$ -N/L. With granule size increasing from 287 to 896  $\mu\text{m}$ , the membrane operation circle prolonged by 2–2.3 times due to the relief of membrane fouling. The cultivated Anammox granules with high activity and shock resistance capacity, fast growth rate and relieved membrane fouling shows the appealing potential of the Anammox-SAnMBR in treating high-strength nitrogen wastewaters.

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## 1. Introduction

High total nitrogen (TN) removal, energy-saving and small footprint are notable features of the anaerobic ammonium oxidation (Anammox) compared to the conventional nitrification/denitrification system. In an Anammox process, ammonium is directly oxidized to nitrogen gas under anoxic conditions with nitrite as the electron acceptor [1]. Hitherto, researchers had employed various of bioreactors to achieve high-rate of nitrogen removal using Anammox bacteria, such as gas-lift reactor, up-flow anaerobic

sludge bed (UASB) reactor and up-flow fixed-bed biofilm column reactors with nonwoven fabric sheet, etc. [2–5]. However, a continuous loss of small Anammox biomass in effluent could result in lower observed biomass growth rate [6]. Moreover, the severe sludge washout due to granule floatation could lead to instability or even system collapses, especially at high loading. Therefore, it is essential to keep efficient retention of biomass for the start-up and operation of Anammox process.

Membrane bioreactor (MBR) was an effective facility for cultivating slow-growing Anammox bacteria. It could keep all the bacteria within the reactor rather than be washed away with the effluent. Researchers had explored the feasibility of combining Anammox with submerged anaerobic MBR (SAnMBR) [7–9]. However, membrane fouling is the bottleneck of the industrial

\* Corresponding authors. Tel.: +86 411 8470 6172 (X. Xu). Tel.: +86 411 8470 6328 (F. Yang).

E-mail addresses: [xxcep@dlut.edu.cn](mailto:xxcep@dlut.edu.cn) (X. Xu), [yangfl@dlut.edu.cn](mailto:yangfl@dlut.edu.cn) (F. Yang).

application of MBR in wastewater treatment. Lim and Bai [10] reported that pore blocking attributed to the main type of membrane fouling. Moreover, particle size and size distribution play an important role in pore blocking, and small particles cause much severer fouling than the larger ones. Therefore, large particles or sludge granulation could relief membrane fouling in SAnMBR. Some previous studies reported that Anammox bacteria could grow in aggregates or granules in MBRs [7,9]. Arrojo et al. [11] reported that the Anammox granular system could be successfully carried out at a certain specific input power. However, the information on how to select a suitable specific input power to impel the Anammox flocs form granules in a continuously fed and completely mixed MBR was still limited.

Anammox bacteria have high activity but a slow-growing rate with a doubling time of weeks [6–8]. Microbial growth and utilization of environmental contaminants as substrates have been studied by many researchers [12,13]. If suitable environmental and nutritional conditions were provided, the slow-growing Anammox bacteria might exhibit excellent substrate utilization performance, as well as benefiting biomass growth. Nitrite was the electron acceptor, but also a potential inhibitor for Anammox bacteria. Strous et al. [14] and some other authors [15,16] reported a  $\text{NO}_2^-$ -N concentrations higher than 100 mg/L completely inhibited the Anammox process. While Lotti et al. [17] reported a higher inhibition level (a half maximal inhibitory concentration of 400 mg  $\text{NO}_2^-$ -N/L). As a result, investigating the  $\text{NO}_2^-$ -N biodegradation kinetics of the Anammox granules is meaningful, especially for Anammox cultivation and reactor operation.

The objectives of this study were therefore (i) to impel the formation of Anammox granules; (ii) to evaluate the nitrogen removal performance and  $\text{NO}_2^-$ -N biodegradation kinetics; and (iii) to investigate the membrane fouling status in a SAnMBR during long-term operation.

## 2. Materials and methods

### 2.1. Reactor setup and operational conditions

The completely mixed SAnMBR (Fig. 1a) consisted of a glass cylindrical column (effective volume of 4 L) with a mechanical stirring. The membrane module (hollow fiber PVDF membrane) (Fig. 1b) was placed within the reactor with a mean pore size of 0.4  $\mu\text{m}$  and total membrane surface area of 0.024  $\text{m}^2$ . The

mechanical stirrer (Fig. 1c) used was a Rushton turbine (6 blades), which had an impeller diameter of 0.088 m with a blade width of 0.03 m and an impeller height of 0.015 m. The pH was computer-controlled at  $7.5 \pm 0.05$  by dosing 1% (v/v)  $\text{H}_2\text{SO}_4$  or 1% (w/v) NaOH. The temperature was kept at  $35 \pm 0.5$  °C by a heating plate sit under the reactor. The reactor was completely covered with black cloth to avoid light. The scheme of the Anammox-SAnMBR was shown in Fig. 1d.

The HRT was kept at one day. During stage I (day 1–60), the stirring speed was 150, 120 and 100 round per minute (rpm) in 20 days for each. The stirring speed was 50 rpm during stage II (day 61–180). The nitrogen concentration was increased from 260 to 4100 mg/L. During stage I, the nitrogen loading rate (NLR) was relatively low and the sludge was seldom withdrawn (except for sludge analysis) with approximately infinite sludge retention time (SRT). From day 91, as NLR increased to high values, the sludge was withdrawn regularly by removing the reactor-suspension once (during 10 min) per day, with SRT ranging from 16 to 10 days. The operation conditions were shown in Table 1.

### 2.2. Inoculum

Anammox sludge inoculated in this experiment has been cultivated in an UASB reactor (68 L; with stirrer; 30 °C; pH, not control but in the range of 7.5–8.2; 4500 mg VSS/L; NLR, 0.6 kg TN/ $\text{m}^3$  d; NRR, 0.51 kg TN/ $\text{m}^3$  d) for 1 year. The seed sludge contained about 89% Anammox bacteria *Candidatus B. anammoxidans*. In the preliminary study, when the mixed liquor volatile suspended solid (MLVSS) of the inoculum reached about 3500 mg/L, the hollow fiber membrane got fouled seriously in less than one day (data not shown). As a result, in the present study the concentration of the seed sludge was set at 2124 mg VSS/L. The mean particle size (volume-weighted mean) of the seed sludge was 183  $\mu\text{m}$  (Supplementary, Fig. S1). Before start-up, the reactor was sparged by  $\text{N}_2$  gas for 0.5 h to maintain anoxic condition.

### 2.3. Synthetic wastewater

$(\text{NH}_4)_2\text{SO}_4$  and  $\text{NaNO}_2$  were used as nitrogen sources in the synthetic wastewater. The synthetic wastewater also contained  $\text{KH}_2\text{PO}_4$ ,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , EDTA, and trace elements.  $\text{KHCO}_3$  was used as buffering agent and potential inorganic carbon source for the

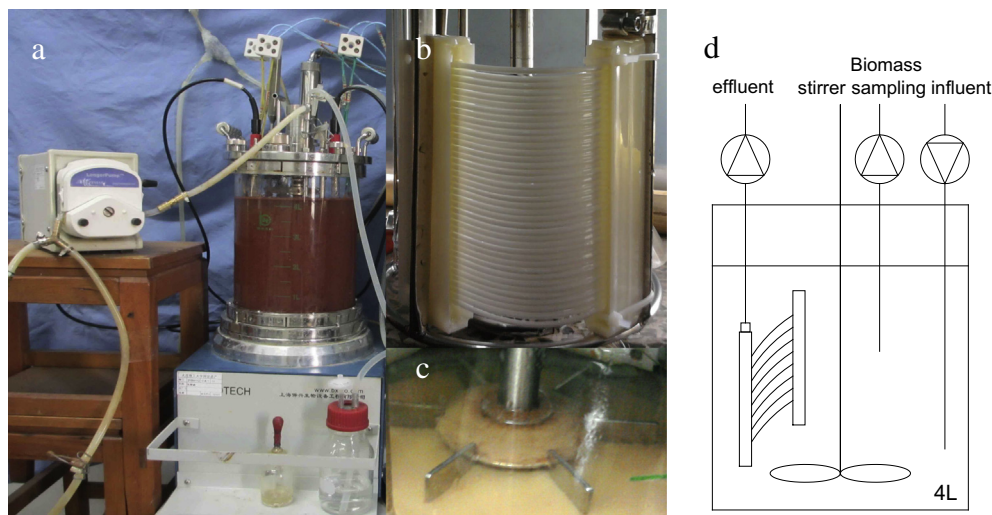


Fig. 1. Photograph of the Anammox-SAnMBR (a), the membrane module (b) and the Rushton turbine (c) and scheme of the Anammox-SAnMBR showing the position of the membrane and influent, effluent and biomass sampling lines (d).

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