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Hollow fiber membrane bioreactor affects microbial community and morphology of the DAMO and Anammox co-culture system

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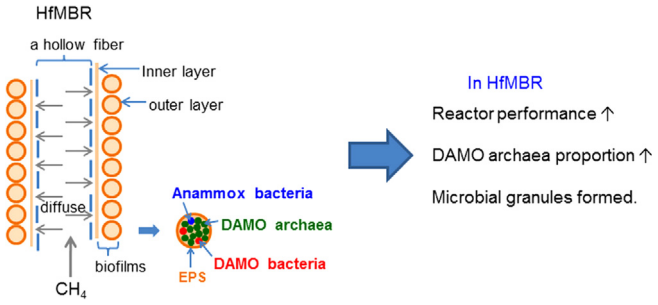
HIGHLIGHTS

- HfMBR improves nitrogen removal in the DAMO & Anammox co-culture system.
- HfMBR significantly increases DAMO archaea proportion to 74.3%.
- DAMO archaea, DAMO bacteria and Anammox bacteria form granules on fibers surface.
- Microbial spatial distribution is different with reported simulation result.

GRAPHICAL ABSTRACT



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ABSTRACT

Denitrifying anaerobic methane oxidation (DAMO) and Anammox co-culture system was investigated in hollow fiber membrane bioreactor (HfMBR) for the change of microbial community morphology and proportion. NO_3^--N and NH_4^+-N removal rates reached 85.33 and 37.95 mg/L/d on 193 d. The inoculum microorganisms were flocs and the proportion of DAMO archaea, DAMO bacteria and Anammox bacteria was 11.0, 24.2 and 0.4%, respectively, but it changed to 74.3, 11.8, 5.6% in HfMBR, respectively. Interestingly, microorganisms formed biofilms on fibers surface and the biofilms included two layers: inner layer was thin and dominated by DAMO bacteria and Anammox bacteria; while the outer layer was thick made up of granules with 100–200 μm diameter and dominated by DAMO archaea. The spatial distribution of microorganisms in HfMBR was different from simulation results in the literature. Likely, HfMBR changed the interaction between DAMO and Anammox microorganisms, and the reactor configuration was beneficial for DAMO archaea growth.

1. Introduction

Anaerobic ammonium oxidation (Anammox) is an innovative technology for ammoniacal nitrogen removal, which directly utilizes nitrite as the electron acceptor to oxidize ammonium to generate nitrogen gas (Eq. (1)). Compared with traditional nitrogen removal pathway via nitrification and denitrification, the Anammox process has many advantages, such as the faster nitrogen removal rate, no organic carbon requirement, less sludge production, etc (Kuenen, 2008; Ma et al., 2016). However, some shortages are not neglected, such as, the ratio of nitrite to ammonium should be strictly controlled and the reaction produces nitrate that requires another denitrification process to remove (Khin and Annachhatre, 2004; Shi et al., 2013).

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$$1.32NO_2^- + NH_4^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 2H_2O \quad (1)$$

The denitrifying anaerobic methane oxidation (DAMO) process is also a novel denitrifying technology (Raghoebarsing et al., 2006), which utilizes methane as the sole electron donor to reduce nitrate or nitrite (Eqs. (2) and (3)). Methane is inexpensive, widely spread, and easy to obtain through anaerobic digestion in wastewater treatment plants. The biggest challenge for the DAMO process is the long enrichment period for DAMO microbes due to the slow growth rate and low solubility of methane in the medium (Ettwig et al., 2009; Hu et al., 2009).

$$4NO_3^- + CH_4 \rightarrow 4NO_2^- + CO_2 + 2H_2O \quad (2)$$

$$8NO_2^- + 3CH_4 + 8H^+ \rightarrow 4N_2 + 3CO_2 + 10H_2O \quad (3)$$

The DAMO and Anammox co-culture system combines all advantages of each process and fills the gaps of each one. In such a system, ammonium, nitrite and nitrate are the substrates and they can be removed simultaneously under anoxic condition, and the ratio of nitrite to ammonium does not need to be strictly controlled as 1.32. DAMO archaea utilize methane to metabolize nitrate to nitrite, then nitrite is consumed by DAMO bacteria and/or removed together with ammonium by Anammox bacteria, while the byproduct nitrate can be consumed by DAMO archaea. Thus, the DAMO and Anammox co-culture system can remove nitrogen completely (Ding et al., 2014; Shi et al., 2013; Wang et al., 2016).

Methane is difficult to dissolve in water and it is a key limiting factor in the DAMO process. The activity of DAMO microorganisms directly affects the nitrogen removal rate of the DAMO and Anammox co-culture system. Hollow fiber membrane bioreactor (HfMBR) can shorten methane transfer pathway and improve methane transfer (Chen et al., 2014), in which methane is directly transferred from gas to microorganism via fibers instead of the methane dissolving process. There are only a few studies on the DAMO and Anammox co-culture system, especially the co-culture system in the HfMBR reactor (Cai et al., 2015; Shi et al., 2013). Generally, the performance of DAMO and Anammox co-culture system in HfMBR is better than other reactor configurations. Especially, Cai et al. (2015) recently reported the co-culture system reached a practically useful removal rate with 684 ± 10 mg/L/d for NO_3^--N and 268 ± 2 mg/L/d for NH_4^+-N in HfMBR. Therefore, HfMBR has become a promising reactor configuration for the DAMO and Anammox co-culture system applied in the future.

Due to synergetic and competitive relationship between DAMO archaea, DAMO bacteria and Anammox bacteria in the co-culture system, the spatial distribution of these three microbes become important for their interaction. However, the spatial distribution in the flocculation system is quite different in the literature, such as DAMO archaea and DAMO bacteria uniformly dispersed (Hu et al., 2009, 2011), DAMO bacteria aggregated together (He et al., 2015), DAMO archaea surrounded by DAMO bacteria and (or) Anammox bacteria (Ding et al., 2014; Raghoebarsing et al., 2006). At present, the morphology and spatial distribution of microbial community in HfMBR have not been comprehensively explored except the simulation results (Chen et al., 2014), in which the biofilms are stratification caused by methane and nitrogen concentration gradients: DAMO microorganisms attach to the membrane surface, DAMO archaea at the lower half and DAMO bacteria at the upper half of fibers membrane surface, moreover, Anammox bacteria mainly grow in the outer biofilm layer closed to liquid. But the simulation results also need to be certified by experimental data.

In this work, the DAMO and Anammox co-culture system was operated in HfMBR. Reactor performance, biofilm morphology, microbial community and spatial distribution of DAMO archaea, DAMO bacteria and Anammox bacteria were studied and discussed. This work will reveal the effect of HfMBR on the interaction between DAMO and Anammox microorganisms.

2. Materials and methods

2.1. Inoculum and reactor configuration

The inoculum used in this experiment was from a DAMO parent reactor (Ding et al., 2014) which the removal rates of NO_3^--N and NH_4^+-N were 7.28 mg/L/d and 0.12 mg/L/d, respectively. The two HfMBR were named R1 and R2 used in this study. The configuration of HfMBR is shown in Fig. S1. The membrane module kJ-4 × 7 MB (Kaijie, Hangzhou, China) contained a bundle of hollow fibers made of polypropylene and the filling ratio was 10% of total volume, the total surface area was 0.3 m^2. The hollow fibers were degassing membranes with 450 μm outside diameter, the wall thickness was 40–50 μm, the air permeability was larger than 8.5×10^{-2} cm^3/cm^2 S cmHg, the porosity was 40–50%, and the aperture diameter was about 0.2 μm. The total volume of HfMBR was 1340 mL, including 1260 mL of working volume, 51 mL of hollow fibers inside for gas supply, and 29 mL of the fiber material occupation.

2.2. Operating condition

The volatile suspended solid (VSS) concentration of initial sludge was 0.13 gVSS/L in R1 and R2. The mineral medium components were same to the previous work (Fu et al., 2015). The pH in the reactors was maintained 7.3–7.8 by manual injection of 1 M HCl. The temperature was maintained at 35 °C by water bath.

Both R1 and R2 (HfMBR) were operated in the same conditions, except the supplied gas. R1 was provided with a mixture of N_2 (95%) and CO_2 (5%), the gas provided to R2 was a mixture of CH_4 (95%) and CO_2 (5%). The gas in HfMBR was continually provided through the regulating valve and the pressure gauge reading was 0.1–0.5 atm. The liquid recirculating rate was 10,000 mL/min. R1 was operated at sequencing batch reactor (SBR) mode all the time. R2 included two stages, SBR stage (0–80 d) and continuous-feeding stage (80–200 d). In the SBR stage, the recirculation pump was stopped and one-third reactor liquid was exchanged with the mineral medium every month. In the continuous-feeding stage, the hydraulic retention time (HRT) was 5 days, and the concentrations of NO_3^--N and NH_4^+-N in the influent were gradually increased from 72 and 33 mg/L to 585 and 248 mg/L, respectively.

2.3. Batch experiment under different nitrogen conditions

The relationship between and among DAMO archaea, DAMO bacteria and Anammox bacteria was evaluated in the inoculum and R2 with different nitrogen sources. The concentrations of NO_3^--N, NO_2^--N and NH_4^+-N were 60 mg/L, 10 mg/L and 30 mg/L, respectively. The removal rates of NO_3^--N, NO_2^--N and NH_4^+-N were calculated with the slope of concentration and time via linear regression. Based on Eqs. (1)–(3), the activities of DAMO archaea, DAMO bacteria and Anammox bacteria in different nitrogen conditions were calculated with the following formula modified from the previous work (Fu et al., 2015): DAMO archaea r1 = rNO_3^- + 0.26 rNH_4^+; DAMO bacteria r2 = rNO_3^- − 1.06 rNH_4^+ + r NO_2^-; Anammox bacteria r3 = rNH_4^+.

2.4. Chemical analysis method

The concentrations of NO_2^--N, NO_3^--N and NH_4^+-N were analyzed with a water quality autoanalyzer (ThermoFisher, Aquakem 200,