Impact of reactor configuration on anammox process start-up: MBR versus SBR

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ABSTRACT

Anaerobic ammonium oxidation (anammox) is an energy saving biological nitrogen removal process which was limited to slow growth rate of anammox bacteria during start-up period. This study investigated the start-up of anammox process by a laboratory sequential batch reactor (SBR) for 218 days and subsequently modified the reactor as a membrane bioreactor (MBR) for 178 days. Modification of a SBR as MBR with installation of an external membrane module resulted in acceleration of specific anammox activity by 19 times. The acceleration of specific anammox activity with MBR was further confirmed by starting-up another MBR for a 242 day period. Molecular microbial analyses showed that *Candidatus* "Brocadia anammoxidans" and *Candidatus* "Kuenenia stuttgartiensis" were the dominant species in the inocula and biomass developed in the reactor. The start-up with MBR appeared to be more effective than SBR for the enrichment of anammox bacteria due to high sludge retention property of MBR configuration.

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1. Introduction

Excessive production of anthropogenic nitrogen causes severe eutrophication of terrestrial and aquatic systems and has already been of global concern (Galloway et al., 2008). Many countries have strictly regulated the discharge of nitrogen-rich wastewater into aquatic system. For wastewater treatment plants (WWTPs), more stringent discharge standard calls for an update of nitrogen removal technologies to replace the conventional one (e.g. full or shortcut nitrification/denitrification), which costs high investment and energy. Anammox process provides an attractive option for total nitrogen (TN) control (Kartal et al., 2010).

Anammox process was observed in a pilot denitrifying fluidized-bed reactor (Mulder et al., 1995) and has since been successfully applied to treat various types of ammonia-rich wastewater (Strous et al., 1997; Liang et al., 2009; Joss et al., 2009). Bacteria responsible for anammox are related to *Planctomycetes* (Strous et al., 1999). These organisms can couple the oxidation of ammonium with the reduction of nitrite to nitrogen gas (N_2) as the terminal product (Van de Graaf et al., 1995). Anammox bacteria grow at a slow rate (a common opinion is doubling time 8–11 days) (Strous et al., 1998; van der Star et al., 2008), and are extremely difficult to grow in vitro.

Some factors have been found to be able to influence the start-up of anammox process including hydraulic retention time (HRT), dissolved oxygen (DO), inoculum, temperature, wastewater composition and nitrogen compound concentration (Tang et al., 2010a; Tsushima et al., 2007). Recently, reactor configuration was also demonstrated to have a significant impact on cultivation of anammox bacteria (Hu et al., 2010).

A reactor configuration with high biomass retention is essential for anammox process due to their slow growth rate. Membrane bioreactor (MBR) and sequencing batch reactor (SBR) are widely-applied bioreactors for wastewater treatment (Shannon et al., 2008; Gao et al., 2010). MBR can prevent product inhibition and the outflow of suspended cells (Jagersma et al., 2009). Several studies have proved that a submerged MBR was an excellent tool for enriching slow-growing microorganisms such as methanotrophic archaea (Meulepas et al., 2009) and anammox bacteria (van der Star et al., 2008). However, the anaerobic condition of a submerged MBR could be interrupted if membrane modules are frequently replaced due to biofouling (Gao et al., 2009), which could harm anammox bacteria (Meulepas et al., 2009). An external MBR (or EMBR) could avoid such interruption. To date, studies that investigated the start-up of anammox process by using an external MBR are very limited and the comparison of SBR versus MBR in anammox start-up is also rare.

The objective of this study is to assess the start-up of anammox process with EMBR versus SBR. The performance of these reactors were compared were based on (a) nitrogen (ammonium, nitrite and nitrate) removal efficiency; (b) specific anammox activity (SAA) of the biomass enrichment in the two reactors; (c) fluorescence in situ hybridization (FISH) analysis for anammox bacteria; (d) microbial community analysis; and (e) activity variations of side populations.

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2. Methods

2.1. Reactor design

Two same sized reactors were used in this study. Reactor R1 was started as a SBR system and operated for 218 days and subsequently was modified as an EMBR for 178 days named as R2. When the reactor was operated as an SBR, no membrane module and recycle pump was installed (Fig. 1). Reactor R3 was started as an EMBR and operated for 242 days. The reactors were made of plexiglass with the identical cylindrical shape and effective volume (3.0 L). Fig. 1 shows the system design of EMBR. A mechanical stirrer kept completely mixing the sludge. The effective volume of membrane module tube was 188 mL and the flux of recycling pump was 170 mL/min. Sludge mixture stayed only 1.1 min (on average) in the module. Polyethylene (PE) hollow fiber sheets (Mitsubishi Rayon Co. Ltd., Tokyo, Japan) were used, having a mean pore size of 0.4 μm. The membrane surface is 0.03 m^2 and the maximum flux is 10 L day^{-1}. In order to continually measure the transmembrane pressure (TMP) and actual flux of the EMBR, electronic pressure and flux sensors (JYB-KO-HAG and JYB-KB-LAG, Beijing Collihigh Sensor Technology Center, China) were equipped. A PC computer was used to collect and analyze data. To prevent light penetration and the growth of phototrophic microorganisms, the reactors were entirely covered with a black shade.

2.2. Inoculation

Reactor R1 was inoculated with 500 mL of stored aerobic granular sludge from a 12 L laboratory-scale SBR treating synthetic wastewater with glucose substrate (Liu et al., 2010). The volatile suspended solid (VSS) of inoculated aerobic granules was 0.3 g L^{-1}. After 218-day operation, R1 was modified as EMBR and equipped with membrane module, recycling pump, and pressure and flux sensors as an EMBR (R2). Reactor R3 was inoculated with 3 L of activated sludge (an initial VSS content of 5 g L^{-1}) from a WWTP.

2.3. Synthetic medium

R1, R2 and R3 were fed with synthetic medium. The source of ammonium and nitrite was prepared using NH_4Cl and $NaNO_2$. The nitrogen loading of the two reactors were gradually increased by supplementing NH_4Cl and $NaNO_2$ to influent. The composition of other mineral medium was 10 mM inorganic carbon ($KHCO_3$),

0.074 mM phosphor (KH_2PO_4), 0.24 mM magnesium ($MgSO_4$·$7H_2O$), 0.034 mM calcium ($CaCl_2$·$2H_2O$), 0.040 mM iron ($FeSO_4$ + EDTA) and 1.25 mL L^{-1} of trace elements solution. The trace element solution contained (g L^{-1}) (adapted from Van de Graaf et al., 1996): H_3BO_4, 0.014; $MnCl_2$·$4H_2O$, 0.99; $CuSO_4$·$5H_2O$, 0.25; $ZnSO_4$·$7H_2O$, 0.43; $NiCl_2$·$6H_2O$, 0.19; $NaSeO_4$·$10H_2O$, 0.21; $NaMoO_4$·$2H_2O$, 0.22; and $CoCl_2$·$6H_2O$, 0.24. Inert gas (95% Ar + 5% CO_2) was purged into the influent tank during the preparation of medium to remove the oxygen (influent DO < 0.08 mg L^{-1}). The pH of influent was 7.7–8.3.

2.4. Reactor operation

The operational conditions of three reactors are summarized in Table 1. The HRT of R1, R2 and R3 were 48 h. The reactors were equipped with a temperature controller to maintain the temperature of 31–32 °C. There was no excess sludge removed from the reactors during the whole experimental trial, except for sampling for MLSS (25 mL), FISH (1.5 mL) and SAA analysis (10 mL). An R1 cycle consisted of 1 h of continuous supply of medium, 46.25 h of mixing, a settling period of 0.5 h, and 15 min to draw off liquid from above the settled sediment and biomass. Inert gas was purged to R1 several minutes before and after the filling period to drive away most oxygen left in R1. R2 and R3 were operated for 178 and 242 days, respectively, and the membrane sheets were almost bimonthly changed due to membrane fouling (Gao et al., 2009). Four membrane modules that were exactly the same were used alternately. Old membranes were replaced when the TMP exceeded 50 kPa.

2.5. Analytical methods

Ammonium, nitrite, nitrate, MLVSS were measured according to standard methods (APHA, 1998). Total organic carbon (TOC) and TN were determined with a Shimadzu TOC-VCPN-6000 analyzer. Parameters including pH and DO were tested by Handheld Multi-Parameter Instruments (pH/Oxi 340i, WTW, Germany).

2.6. Batch tests for specific activities

Batch tests were conducted for the determination of specific activities for the removal of ammonium, nitrite and nitrate to characterize the activities of different trophic groups of bacteria involved in nitrogen cycle in the biomass. The consumption rate of ammonium-nitrite was used for the calculation of the activity
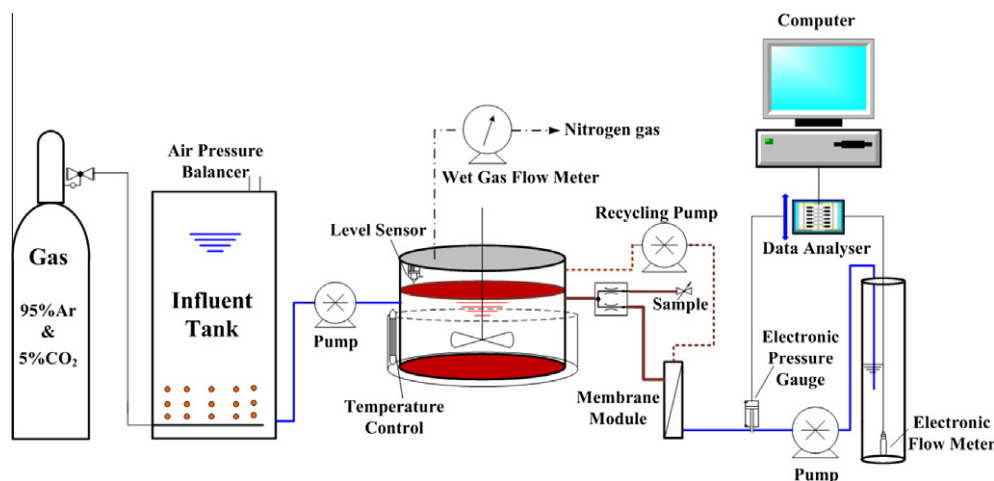

Fig. 1. Schematic diagram of the anammox system with external membrane (EMBR). The SBR system used was the same reactor but without EMBR module, recycling pump and other relevant components. The EMBR module tube had a diameter of 40 mm and height of 150 mm.