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Interaction of anammox bacteria and inactive methanogenic granules under high nitrogen selective pressure

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ABSTRACT

Granular anammox reactors usually adopted anaerobic/aerobic granules as source sludge, in which the washout of other species and enrichment of anammox biomass were very slow because of the competition of the coexisting bacteria. In this study, inactive methanogenic granules were proved to be suitable for rapid anammox granulation under high nitrogen concentrations by investigating their interaction with anammox bacteria. The start-up nitrite concentration was significantly higher than the published toxic level for anammox bacteria and other lab-scale studies. The nitrogen loading rate increased from 141 to 480 mg/L/d in 120 days operation with a total nitrogen removal efficiency of 96.0 ± 0.6%. Anammox granules with a diameter of 1.3 ± 0.4 mm were observed over the course of three months. Molecular analysis showed that over 67% of the cells in the anammox granules were anammox bacteria after 90 days. The accommodations and proliferations of anammox bacteria in the inactive methanogenic granules might be the main reason for the high anammox purity in a short period. The important role of the extracellular polymer in the granule structure was observed via morphological observation.

1. Introduction

Granulation plays an important role in biological systems. Firstly, granulation significantly enhances the settleability of biomass, leading to effective bacterial retention in the reactor. Meanwhile, it improves physiological conditions, making them favorable for bacteria and their interactions, especially syntrophisms in the anaerobic system (McCarty and Smith, 1986). The formation of granular sludge can be considered the major reason of the successful introduction of the upflow anaerobic sludge blanket (UASB) reactor (Hulshoff Pol et al., 2004). In this work, a UASB reactor was adopted to form granules for the slowly growing bacteria.

The anammox (anaerobic ammonium oxidation) process is a novel and promising alternative to biological treatment of ammonium, in which ammonium is oxidized to nitrogen gas using nitrite as the electron acceptor autotrophically (Eq. (1)) (Mulder et al., 1995; Strous et al., 1998). The main limitation of the anammox process is the long doubling time of anammox bacteria (Strous et al., 1998). The reactor carrying out anammox must be capable of holding the biomass efficiently. Literature review shows that the UASB reactor was successfully applied to the anammox process (Imajo et al., 2004; Jung et al., 2007; Schmidt et al., 2004; Thuan et al., 2004). In previous anammox process studies, reactors containing granules were considered to be suitable for slowly growing anammox culture. With granular sludge, it is possible to maintain a large amount of active biomass in the reactor (Imajo et al., 2004). Anammox granulation usually takes an extended period of time beyond the acceptable start-up time. According to Imajo et al. (2004), red granules consisting of only anammox bacteria were observed until 180 days of operation, seeded with methanogenic granules and anammox biomass. Jung et al. (2007) indicated that only 13% of cells in the anaerobic granules were constituted by anammox bacteria after 3 months of continuous operation. Even with anammox sludge, the granulation process was time-consuming (Fernandez et al., 2008; Qiao et al., 2009). Most granular anammox processes used sequencing batch reactor (SBR) and UASB reactors seeded with anaerobic/aerobic granules (Fernandez et al., 2008; Jung et al., 2007; Liao et al., 2007; Thuan et al., 2004). Seeded with mixed activated sludge, Lopez et al. (2008) developed a granular anammox SBR. Because of the competition between the coexisting bacteria, the washout of other species and enrichment of anammox biomass were very slow. In this study, it was assumed that sludge with low bacteria activity might be better than the active sludge when starting an anammox reactor. By electron microscope examination, the inactive methanogenic cells exhibited a characteristic of hollow skeleton. For the first time, the inactive methanogenic granules were used as seed

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in this study. The interaction between anammox bacteria and the granules was studied.

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (1)$$

Since high nitrite concentrations could inhibit anammox activity (Strous et al., 1999), the anammox reactors usually started at low nitrogen concentrations of less than 70 mg N/L (Jung et al., 2007; Strous et al., 1998). At low nitrite concentrations, if nitrite removal efficiency was close to 100%, nitrite became the limiting nutrient (Lopez et al., 2008). In this study, a high nitrogen intensity start-up strategy was proposed to shorten anammox granulation time. By feeding a high nitrogen concentration during start-up, the main objective of this study was to study the interaction of anammox bacteria and inactive methanogenic granules and to evaluate the feasibility of rapid anammox granulation. The anammox granules were characterized by real-time polymerase chain reaction (PCR) and electron microscopy. The possible anammox granulation mechanisms were also discussed.

2. Methods

2.1. Reactor systems and operation

A glass vessel (height 1.10 m, diameter 0.10 m) with a gas–solid separator at the top and a 5.0 L working volume was used as the UASB reactor (Fig. 1). The sidewalls were enclosed with a water jacket to maintain a temperature of 35 °C. The UASB was fitted with a gas purge tube, a feed inflow tube, an outflow tube, a recycling tube, a gas collection port and sampling ports. Gravel of three sizes, approximately 2, 5 and 10 mm, was placed in the bottom of the reactor. Wastewater and gas were injected into the reactor below the gravel. The existence of the gravel could not only enhance the biomass retention, but also help to distribute the wastewater and gas evenly in the bottom of the reactor, which would favorite the granulation process. Peristaltic pumps (Cole-Parmer Instrument, Vernon Hills, IL) were used to control the recirculation rate and the influent feed rate. To avoid the toxic effects of the high nitrite concentrations (Strous et al., 1999), wastewater from the top of the reactor was recycled to dilute the influent at a recycling ratio of 200% on the basis of influent flow rate. Wastewater recirculation could not only create the upflow water current, which would favor the granulation process, but dilute the influent and benefit the anammox process. The pH in the reactor was adjusted to 7.8–8.0 with CO_2 gas. In order not to break the granules in the reactor, the CO_2 gas-flow was kept constant to create fine bubbles. All tubing and connectors were black butyl rubber or polyvinylchloride (PVC), which have low air permeability and light transmission.

A volume of 1.5 L of inactive methanogenic granular sludge was added into the UASB reactor. The inactive granules from a full-scale UASB reactor were stored in a sealed PVC barrel for more than two years without any feeding at room temperature in the laboratory. The mean diameter of the inoculums was 1.6 ± 0.5 mm. The suspended solids (SS) and volatile suspended solids (VSS) of the black granular sludge were 32.9 and 24.8 g/L, respectively. After the addition of inactive granules, the reactor was kept on standby for about 2 weeks in order to acclimatize the sludge and test the reactor. After 2 weeks, 50 mL anammox sludge taken from a running anaerobic reactor was added into the reactor (Ni et al., 2010), which was characterized by 3.2 g VSS/L (67% VSS/SS). According to the real-time PCR test, 97.7% of the cells in the anammox sludge were quantified to be anammox bacteria (Ni et al., 2010). The sludge source in this study constituted 0.16 g VSS anammox sludge and 37.2 g VSS inactive anaerobic granules. The UASB was then run as a continuously-fed sequence. The designated day 1 was the day when the reactor was started to run continuously. Throughout the experiment, hydraulic retention time was kept between 1.5 and 1.7 days.

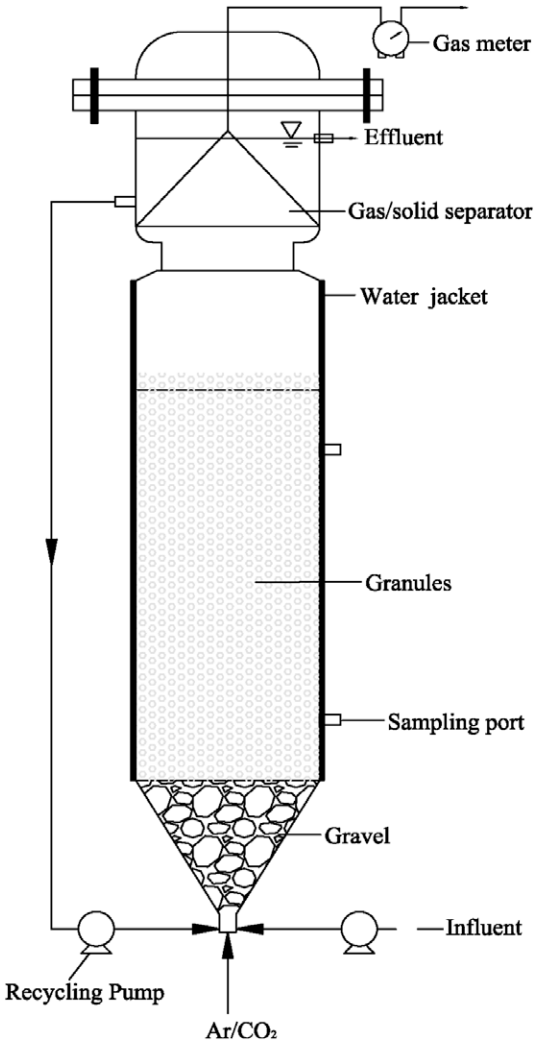

Fig. 1. Schematic diagram of the lab-scale UASB reactor.

2.2. Synthetic wastewater

Ammonium and nitrite were added to a mineral medium in the required amounts in the form of $(NH_4)_2SO_4$ and $NaNO_2$. The composition of the mineral medium was (g/L): $KHCO_3$ 0.5, KH_2PO_4 0.0272, $MgSO_4{\cdot}7H_2O$ 0.3, $CaCl_2{\cdot}2H_2O$ 0.18 and 1 mL trace elements solutions I and II (Ni et al., 2010). The synthetic wastewater was deoxygenated by flushing with argon gas before feeding to the reactor.

2.3. Analysis

After filtering through 0.45 μm syringe filter (National Scientific, Rockwood, TN, USA), nitrite and nitrate concentrations were determined by ion-chromatography (DX 500, Dionex, Sunnyvale, CA). Ammonium was measured by selective electrode according to Standard Methods (APHA, 1998). SS and VSS were determined by the weighing method after being dried at 103–105 °C and burnt to ash at 550 °C (APHA, 1998). The pH was determined via a calibrated pHS-25 acidimeter and a general purpose pH electrode (Thermo Fisher Scientific, Waltham, MA). To measure the granule