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Performance and microbial response during the fast reactivation of Anammox system by hydrodynamic stress control

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ABSTRACT

Anaerobic ammonium oxidation (Anammox) has become a promising method for biological nitrogen removal. However, this biotechnology application is always limited due to the low growth rate and biomass yield of Anammox bacteria. This study investigated the process of fast reactivation of an Anammox consortium idled for 2 years *via* hydrodynamic stress control. The results showed that the Anammox system was efficiently and quickly reactivated by shortening of the hydraulic retention time (HRT) of the reactor from 12 to 6 hr within 68 days of operation. Moreover, at a 4-hr HRT with an influent total nitrogen loading rate of 1.2 kg N/(m^3·day), the reactor maintained high biological performance with an ammonium removal loading rate of 0.52 kg N/(m^3·day) and a nitrite removal rate of 0.59 kg N/(m^3·day). In the reactivated Anammox reaction, the stoichiometric coefficients of NH_4^+–N to NO_2^-–N and NH_4^+–N to NO_3^-–N were 1:1.04 ± 0.08 and 1:0.31 ± 0.03, respectively. The specific Anammox activity and hydrazine oxidoreductase activity, both of which represent the degree of Anammox bacteria present, increased as the hydrodynamic stress increased and were maximally (125.38 ± 3.01 mg N/(g VSS·day) and 339.42 ± 6.83 μmol/(min·g VSS), respectively) at 4-hr HRT. Microbial response analysis showed that the dominant microbial community was obviously shifted and the dominance of Anammox bacteria was enhanced during the hydrodynamic selection.

Introduction

Anaerobic ammonium oxidation (Anammox) is a novel and promising bioprocess for treating nitrogenous wastewater that involves the oxidation of ammonium with nitrite as the electron acceptor and uses CO_2 as the only carbon source without the need for aeration or additional external carbon sources (Mulder et al., 1995; Strous et al., 1998). The process is usually applied to the treatment of wastewater with a low C/N ratio, such as anaerobic digester supernatant, pig manure effluents, and landfill leachates (Abma et al., 2007; Innerebner et al., 2007; Joss et al., 2009; van der Star et al., 2007). However, the practical application of the Anammox process is always limited due to the low growth rate and biomass yield of functional bacteria. Many studies focused on enhancing biomass retention and concentration by optimizing the reactor configuration. For instance, biofilm reactors were developed for the stable immobilization of Anammox sludge using various types of biomass carriers such as a polyethylene glycol gel carrier (Kimura et al., 2011) and non-woven material (Furukawa et al., 2002). Gas lift reactors such as the up-flow anaerobic sludge bed (UASB) have also been used in the Anammox process (Li et al., 2012). In addition, Wang

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et al. (2009, 2011) used sequencing batch reactors and membrane biological reactors in Anammox operations. Reports have also shown that Anammox cultivation was accelerated using a magnetic system (Liu et al., 2008) and that the activity was enhanced by packing with a Fe electrode (Zhang et al., 2012).

The use of granulation and immobilization technologies is the most acceptable way to maintain high densities of Anammox bacteria within the reactor. Hydrodynamic stress, gas flow, and mechanical shear are significant factors of sludge granulation. In fact, more compact, stable, and dense biofilms as well as aerobic and anaerobic granules can form under relatively high hydrodynamic stress (Arrojo et al., 2006; Dapena-Mora et al., 2004; Liu and Tay, 2002; Qin et al., 2004). Furthermore, stress has significant influence on structure, mass transfer, exopolysaccharide production, and metabolic/genetic behaviors of biofilms as well as granular formation. Therefore, in an engineering sense, hydrodynamic stress can be manipulated as a control parameter to enhance microbial performance as well as the granulation process.

An Anammox mixed culture was used to accelerate the start-up of an Anammox process (Bettazzi et al., 2010; Li et al., 2012; Ni et al., 2011). Some studies investigated the effects of mixed culture storage time on Anammox system reactivation (Bettazzi et al., 2010; Yang and Jin, 2013). Longer storage of Anammox bacterial sludge partially decreased its nitrogen removal capability and created a requirement for more time for reactivation. Therefore, the current study investigated the requirements for fast reactivation of Anammox consortium idled for 2 years using hydrodynamic stress control. Scanning electron microscopy (SEM) was used to observe microorganism morphology and distribution. Furthermore, the shifts of microbial community and biochemical characterization under hydrodynamic selection pressure were revealed using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) analysis and enzymatic determination, respectively.

1. Material and methods

1.1. Inoculum

Reactors were seeded with Anammox mixed culture idled for 2 years. The original mixed culture was cultivated with nitrifying sludge in our previous reactor, and then was preserved without nutrient addition at ambient temperature. After a long idle state, the mixed culture turned black, and its specific Anammox activity (SAA) was only 0.20 mg N/(g VSS·day). The initial total suspended solid content of inoculum in the reactor was around 10 g mixed liquor suspended solids (MLSS)/L, and the ratio of mixed liquor volatile suspended solids (MLVSS) to MLSS was 0.68.

1.2. Reactor configuration and influent composition

The configuration of the UASB reactor is depicted in Appendix A Fig. S1. The cylindrical reactor made of Plexiglass had a total working volume of 11 L and an effective volume of 7.9 L, and was equipped with a thermostatic jacket to maintain a fixed temperature of 35°C. The produced gas was discharged *via* portholes in the top of the reactor. Black vinyl sheet was used to inhibit the growth of photosynthetic bacteria. To avoid oxygen disturbance, the reactor was kept strictly leak-tight during the whole operation. The mineral composition in synthetic wastewater fed to the reactor was as described in a previous study (Wang et al., 2011). The influent NH_4^+/NO_2^- molar ratio was around 1, and the pH was maintained between 7.5 and 8.0.

1.3. Anammox reactivation via hydrodynamic stress control

The reactivation strategy applied is described in Appendix A Table S1. The influent ammonium and nitrite concentrations were both 100 mg N/L during the whole operation. Based on biological performance, the hydraulic retention time (HRT) was gradually decreased from 12 to 4 hr during 108 days of operation, with up-flow velocity and total nitrogen (TN) loading rate increasing from 0.1 to 0.3 m/hr and from 0.4 to 1.2 kg TN/(m^3·day), respectively.

1.4. Chemical analysis

According to the APHA standard methods (APHA, 1998), NH_4^+–N and NO_2^-–N were determined using the phenate method (4500-NH_3 F) and colorimetric method (4500-NO_2^- B) respectively, while NO_3^-–N and TN were measured spectrophotometrically (4500-NO_3^- B and 4500-N C). The pH and oxidative redox potential (ORP) were analyzed by a digital portable pH/ORP electrode (pH-300T, SUNTEX, Kunshan City, China). MLSS and MLVSS were determined by weighing methods (2540 D and 2540 E). Every determination was done in triplicate. The removal efficiencies (E) of ammonium, nitrite or TN were calculated according to Eq. (1). Nitrogen loading rate (NLR) and nitrogen removal rate (NRR) were calculated according to Eqs. (2) and (3).

$$E = \frac{C_{in} - C_{eff}}{C_{in}} \times 100\% \quad (1)$$

$$NLR = \frac{C_{in(TN)} \times 24}{HRT \times 1000} \quad (2)$$

$$NRR = \frac{(C_{in(TN)} - C_{eff(TN)}) \times 24}{HRT \times 1000} \quad (3)$$

where, C_{in} and C_{eff} represented the influent and effluent concentrations of NH_4^+–N, NO_2^-–N, or TN, respectively.

1.5. SEM observation

The morphology and structure of sludge samples were observed by SEM (FEI Quanta 200, Hillsboro, Oregon, USA). The samples were prepared as mentioned by Baloch et al. (2008).

1.6. Assays of SAA and hydrazine-oxidizing enzyme (HZO) activity

The assay of SAA was determined according to the procedures described by Dapena-Mora et al. (2007). The SAA was calculated from the maximum N_2 gas production rate divided by the biomass concentration in the reaction system.

The HZO activity was assayed by the method mentioned in a previous study with some modifications (Shimamura et al., 2007). The sludge sample was washed, suspended and disrupted. Sodium cholate was added to the homogenate to a concentration of 1% (*wt/vol*) and sodium deoxycholate to a concentration of 0.5% (*wt/vol*), followed by mixing for 1 hr at 4°C. The mixture was further centrifuged at 16,000 ×*g* for 1.5 hr, and the supernatant