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Operation stability and recovery performance in an Anammox EGSB reactor after pH shock

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ABSTRACT

An Anaerobic ammonia oxidation (Anammox) expanded granular sludge bed (EGSB) reactor was investigated with a long-term continuous operation. The nitrogen removal performance and stability of process subjected to transient pH shock (decreased from 9.0 to 7.0 and maintaining for 24 h) were evaluated. In the steady stage, the nitrogen loading rate (NLR) and nitrogen removal rate (NRR) can reach to 10 and 8.5 kg-N m^{-3} d^{-1}, respectively. However, the system had a low tolerance for transient pH shock. The deterioration of the granule sludge properties and the inhibition of specific Anammox activity (SAA) resulted in the destabilization of the EGSB after pH shock. The main factors governing the treatment performance of EGSB were the high concentration of free nitrous acid (FNA) and free ammonia (FA). However, the lower than 10% reduction of ammonia removal can be maintained when FA and FNA were lower than 15 mg l^{-1} and 15 μg l^{-1}, respectively. The process was successfully recovered by controlling the FNA and FA concentration after the low pH shock.

1. Introduction

Anaerobic ammonia oxidation (Anammox) is carried out by chemolithoautotrophic bacteria which can directly convert ammonia to gaseous N_2 using nitrite as the electron acceptor under anoxic conditions according to the following stoichiometry reaction (Eq. (1)) (Strous et al., 1998):

$$NH_4^+ + 1.32\,NO_2^- + 0.066\,HCO_3^- + 0.13\,H^+ \rightarrow 1.02\,N_2 + 0.26\,NO_3^- + 2.03\,H_2O + 0.066\,CH_2O\,0.5\,N_{0.15} \quad (1)$$

Compared with the conventional nitrification–denitrification biological processes, Anammox is considered a more economical and sustainable biological nitrogen removal process, especially for wastewaters containing a high ammonia concentration and a low COD/N ratio (He et al., 2015). This is because of a significant reduction in the aeration costs and the addition of an external carbon source as electron donor for denitrifiifation. The nitrogen removal rate (NRR) of conventional nitrogen removal biotechnologies is less than 0.5 kg-N m^{-3} d^{-1}. However, Tsushima et al. (2007) reported that the NRR of the Anammox process was 26 kg-N m^{-3} d^{-1} using a biofilm reactor under a hydraulic retention time (HRT) of 0.24 h and a TN concentration of 585 mg l^{-1}. To date, the highest recorded NRR, 76.7 kg-N m^{-3} d^{-1}, was achieved by Tang et al. (2011) in an UASB reactor under an HRT of 0.11 h and a TN concentration of 440 mg l^{-1}.

Although Anammox is a promising nitrogen removal process, its use in engineering applications would involve overcoming some challenges. One of them is the longer start-up period due to low cellular yield and the slow growth rates of Anammox bacteria (AAOB) (Strous et al., 1998). Therefore, in order to efficiently enrich AAOB in the reactor, it is very important to select an efficient strategy or reactor system which can efficiently retain the biomass. To date, different methods based on the settling ability of biomass to grow in the form of biofilm or granules and using membrane to retain biomass have been implemented with different reactors. In reactors used to enrich slow-growing microorganisms like those used in nitrification processes and anaerobic digestion, including sequencing batch reactors (SBR), membrane bioreactors (MBR), gas-lift reactors, upflow biofilters and upflow anaerobic sludge blanket (UASB) reactors, the accumulation of AAOB has been shown to be feasible (Third et al., 2005; Wang et al., 2012; Xiong et al., 2013). Among these reactors, the UASB was shown to have better stability under hydraulic shock and substrate shocks than the SBR or biofilm reactors (Jin et al., 2008). Another challenge is the low toxic

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threshold of the Anammox process: at a total nitrogen (TN) concentration of 1000 mg l^{-1} (Strous et al., 1999), the process becomes substrate-inhibited by ammonia and nitrite. However, the ammonia concentration in real wastewaters like landfill leachate often is up to several thousand milligrams. In such cases, recirculating the effluent improves the removal performance of the Anammox bioreactor, and the expanded granular sludge bed (EGSB) reactor is recommended (Chen et al., 2011). The third challenge is maintaining the stability of the Anammox bioreactor: the AAOB has been shown to have a high sensitivity to a variety of environmental factors, including dissolved oxygen (DO), temperature and organic matter etc. (Dosta et al., 2008; Jin et al., 2012; Ni et al., 2012b).

One of the most important factors in the biological process is pH. The reported pH range of the AAOB is 6.6–8.3, and the optimal pH is around 7.5–8.0 (Egli et al., 2001; Strous et al., 1999). A either too high or too low pH has the potential to adversely affect the stability of the Anammox bioreactor. It should be noted that substrate-inhibition by ammonia and nitrite has been attributed to the unionized form of ammonia (free ammonia, FA) and nitrite (free nitrous acid, FNA), and that the presence of FA and FNA in the Anammox system is pH-dependent (He et al., 2015). Therefore, pH has both a direct and indirect effect on the Anammox process due to its relationship with FA and FNA. It is feasible that the variation in the pH in Anammox process could be higher in some industrial wastewaters or due to an accident. However, little attention has been paid to the effect of transient pH shock and the accompanying inhibitions caused by FA and FNA in the Anammox process.

In this study, the pH shock on the stably stage and the recovery after inhibition was investigated in an EGSB Anammox, which had been already operated more than 3 years with a long term stable performance. This article aimed to evaluate the treatment performance by increasing the influent substrate concentration, and then investigate the stability of the Anammox bioreactor subjected to transient pH shock. Finally, the ability of the Anammox process to recover was evaluated.

2. Materials and methods

2.1. Experimental set-up and operation

As shown in Fig. 1, an EGSB reactor with a 5 L working volume was used in the experiment. The reactor had a height–diameter ratio of 8.5:1 and made with strong plexiglass. The temperature was controlled at 35 ± 1 °C with a thermostatic water jacket. The reactor was covered with a light-weight shading fabric to avoid the growth of photosynthetic microorganisms which would produce oxygen. Gas production was collected on the top and measured by a wet gas flow meter. To recirculate the effluent, it was partially pumped back to the inlet, and the reflux ratio was set to 500%. In order to avoid the detrimental effect of high pH on the treatment performance of the reactor, the pH of the reactor was adjusted by adding a 1 M H_2SO_4 solution with a peristaltic pump. The initial inoculums were a mixture of 2 l activated sludge and 3 l anaerobic digestion sludge taken from a municipal wastewater treatment plant in Sendai, Japan. The suspended solids (SS) and volatile suspended solids (VSS) of mixed sludge were 19.64 g l^{-1} and 11.64 g l^{-1}, respectively.

After stable operation for 900 days, the nitrogen loading rate (NLR) of the EGSB reactor was increased to investigate the treatment performance by changing the influent total nitrogen concentration from 500 to 1250 mg l^{-1} (phases I–III) (Table 1). After the long-term stable nitrogen removal efficiency of reactor was achieved at the NLR of 10 kg-N m^3 d^{-1}, the EGSB was subjected to transient pH shock by rapidly changing the pH of the EGSB reactor from 9.0 to nearly 7.0 and maintaining for 24 h. The effect after pH shock (phase IV) and the recovery ability (phases V–VII) were evaluated by measuring the nitrogen removal performance and granular sludge characteristics. The operating conditions applied to the Anammox reactor were summarized in Table 1.

2.2. Synthetic wastewater

Ammonia and nitrite were supplied in the form of $(NH_4)_2SO_4$ and $NaNO_2$, and according to Eq. (1), the influent ratio of NO_2^- to NH_4^+ was kept at 1.32:1. The composition of the mineral medium was as follows: 0.57 g l^{-1} of KCl, 0.688 g l^{-1} of $NaHCO_3$, 300 mg l^{-1} of $CaCl_2{\cdot}2H_2O$, 50 mg l^{-1} of KH_2PO_4, 200 mg l^{-1} of $MgSO_4{\cdot}7H_2O$. Trace element I (including $FeSO_4{\cdot}7H_2O$ 6 mg l^{-1}, EDTA 6 mg l^{-1}), and trace element II (including $ZnSO_4{\cdot}7H_2O$ 0.538 mg l^{-1}, $CoCl_2{\cdot}6H_2O$ 0.3 mg l^{-1}, $MnCl_2{\cdot}4H_2O$ 1.125 mg l^{-1}, $CuSO_4{\cdot}5H_2O$ 0.313 mg l^{-1}, H_3BO_3 0.018 mg l^{-1}, $NiCl_2{\cdot}6H_2O$ 0.238 mg l^{-1}, $Na_2SeO_4{\cdot}10H_2O$ 0.123 mg l^{-1}, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 0.275 mg l^{-1}, $Na_2WO_4{\cdot}2H_2O$ 0.664 mg l^{-1}) were based on previous studies (deGraaf et al., 1996; Schmidt et al., 2004; Trigo et al., 2006). The pH of synthetic wastewater was controlled at 7.2 ± 0.1.

2.3. Batch activity measurement

The batch activity test was carried out according to the method reported by Dapena-Mora et al. (2007). Serum flasks (120 ml) were supplied with a basal mineral medium and a trace element solution and Anammox biomass. Anammox biomass was taken from the bottom of the EGSB reactor at 23 cm and washed and suspended with a phosphate buffer (0.14 g l^{-1} of KH_2PO_4 and 0.75 g l^{-1} of K_2HPO_4) five times before it was put into the serum flasks. The initial pH value was fixed at 7.6 ± 0.1. Each flask was closed with a gas-tight coated septum capable of withstanding approximately 2 bars of pressure. The headspace and liquid phase was gasified with nitrogen gas for 15 min in order to create anaerobic conditions. The serum flasks were placed in a thermostatic shaker at 90 rpm and 35 °C. When the stable conditions were achieved, substrates of ammonia and nitrite (the ratio of NO_2^-–N to NH_4^+–N was kept at 1.32:1) were added and the pressure was equalized to atmospheric pressure. The production gas volume was measured with a time frequency depending on the biomass activity in each vial test.

The specific Anammox activity (SAA) was measured based on the nitrogen gas production rate and expressed as g-N g^{-1} VSS d^{-1}. The SAA was calculated from the maximum slope of the time course of the nitrogen gas concentration in the headspace.

2.4. Analytical procedures and methods

Influent and effluent samples were collected 2 or 3 times a week and analyzed immediately or temporarily stored at 4 °C. Before being measured, the samples were filtered through a 0.45 μm syringe filter. Ammonia, nitrite and nitrate were analyzed by ion chromatography (DIONEX, DX120). Daily records of pH and gas production were kept: the pH was determined by pH meter (TOA, HM-30V), and gas production was determined by a wet gas flow meter and the produced gas was analyzed by Gas Chromatography (Shimadzu, C-R8A). TN was calculated as the sum of nitrite, nitrate and ammonium.

The extracellular polymeric substances (EPS) were extracted based on a cation ion exchange resin (CER) method. The extraction procedure was as follows: 2 g of wet sludge taken from the reactor was transferred to an extraction beaker and 60 g g^{-1} VSS of CER and 50 mL of the buffer solution (Na_3PO_4 of 2 mmol l^{-1}, NaH_2PO_4 of 4 mmol l^{-1}, NaCl of 9 mmol l^{-1} and KCl of 1 mmol l^{-1}) were added. The suspension was stirred at 900 rpm for 1 h and then centrifuged for 15 min at 1, 2000 g and 4 °C. The supernatant harvested was filtered and stored at 4 °C for further analysis. EPS were normalized as the sum of carbohydrate and protein, which were analyzed by