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# Inhibition factors and Kinetic model for ammonium inhibition on the anammox process of the SNAD biofilm

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#### ABSTRACT

The aim of the present work was to evaluate the anaerobic ammonium oxidation (anammox) activity of simultaneous partial nitrification, anammox and denitrification (SNAD) biofilm with different substrate concentrations and pH values. Kaldnes rings taken from the SNAD biofilm reactor were incubated in batch tests to determine the anammox activity. Haldane model was applied to investigate the ammonium inhibition on anammox process. As for nitrite inhibition, the NH $_4^+$ -N removal rate of anammox process remained 87.4% of the maximum rate with the NO $_2^-$ N concentration of 100 mg/L. Based on the results of Haldane model, no obvious difference in kinetic coefficients was observed under high or low free ammonia (FA) conditions, indicating that ammonium rather than FA was the true inhibitor for anammox process of SNAD biofilm. With the pH value of 7.0, the  $r_{\rm max}$ , Ks and  $K_{\rm I}$  of ammonium were 0.209 kg NO $_2^-$ N/kg VSS/day, 9.5 mg/L and 422 mg/L, respectively. The suitable pH ranges for anammox process were 5.0 to 9.0. These results indicate that the SNAD biofilm performs excellent tolerance to adverse conditions.

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#### Introduction

Wastewater that contains a large amount of ammonium will cause a serious eutrophication problem for the receiving water (Bagchi et al., 2012). Biological nitrification-denitrification is commonly used to remove the nitrogen from wastewater. However, these practices usually lead to the need for a large volume reactor and high operating costs. Recently, the development of simultaneous partial nitrification, anammox and denitrification (SNAD) process has been reported to remove nitrogen along with organic carbon in one single reactor (Chen et al., 2009; Daverey et al., 2015). The SNAD process is recognized as an energy saving and cost-effective biological nitrogen removal method. However, the SNAD process is mainly used to treat wastewaters with high ammonium concentrations (>500 mg N/L) and high temperatures (>30 °C) (Wang et al.,

2010; Zhang et al., 2015b). So far, there is little information about the SNAD process for treating domestic wastewater. Usually, the domestic wastewater contains low ammonium concentration with low temperature, which is not beneficial for the growth of anammox bacteria.

The anammox bacteria play a critical role in the SNAD process. Inhibition of anammox bacteria by substrates and environment conditions has been widely studied (Carvajal-Arroyo et al., 2014). Previous studies reported different nitrite inhibition concentrations for anammox process (Strous et al., 1998; Dapena-Mora et al., 2007; Lotti et al., 2012). Strous et al. (1998) observed complete inhibition of the anammox activity with  $NO_2$ -N concentration of 100 mg/L, while other studies have reported that anammox bacteria performed higher tolerance to  $NO_2$ -N with the  $IC_{50}$  values of 350 mg/L (Dapena-Mora et al., 2007) and 400 mg/L (Lotti et al., 2012). Fernández et al. (2012)

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suggested that free nitrous acid (FNA) was responsible for the nitrite inhibition of anammox process. Afterwards, other researchers confirmed that nitrite rather than FNA was the true inhibitor for anammox process using batch tests (Lotti et al., 2012; Puyol et al., 2014). As for ammonium inhibition, Strous et al. (1998) showed that the anammox activity was not inhibited with the NH<sub>4</sub>+N concentration of 980 mg/L. On the contrary, Dapena-Mora et al. (2007) reported that the IC<sub>50</sub> value of NH<sub>4</sub>-N concentration for anammox process was 770 mg/L. Additionally, several studies suggested that free ammonia (FA) seriously inhibited the anammox process (Jung et al., 2007; Fernández et al., 2012). Fernández et al. (2012) described that the anammox activity decreased by 50% with the FA concentration of 38 mg/L. However, there was no literature indicating whether FA or ammonium is the true inhibitor for anammox activity. Besides, the anammox activity could also be seriously inhibited by pH values (Strous et al., 1999; Egli et al., 2001). It is believed that the granule or biofilm performs high mass transfer resistance, which is beneficial for the anammox bacteria to tolerate higher substrate inhibition (Cho et al., 2010; Kimura et al., 2010).

Therefore, the aim of this study was to develop an efficient SNAD biofilm reactor for treating domestic wastewater. The effects of substrate concentrations and pH values on the anammox activity of SNAD biofilm were investigated. Kaldnes rings taken from the SNAD biofilm reactor were studied in batch tests. It is expected that the knowledge obtained in this study will be critical for the application of SNAD process to the treatment of domestic wastewater.

#### 1. Materials and methods

#### 1.1. SNAD biofilm reactor

Fig. 1 shows the reactor configuration scheme of the SNAD biofilm reactor. A sequencing batch reactor (SBR) with a working volume of 89.5 L was used. Dimensions of the unit were: height of 79 cm and inner diameter of 38 cm; the height to the diameter ratio was 2.07. The reactor contained 77.7 L water and the volume exchange ratio was 81%. Kaldnes rings (K3 carriers, AnoxKaldnes, Beijing) were used as biomass carriers with the apparent volume of 34 L. The carriers had a cylindrical shape (diameter of 25 mm) with a grid of 4 mm. The temperature was controlled at 30 °C by heating rods immersed in the reactor (GM1616, Jiyin, China). No pH adjustment was adopted. The air was supplied by an air diffuser with a constant rate of 500 L/hr at the bottom of the reactor. A hydraulic agitator (HJ1841, Sensen, China) was installed at the sidewall of the reactor. The reactor operated in automatic running mode with programmable logic controller (PCL-812, Advantech, USA). The dissolved oxygen (DO) and pH were detected by online instruments.

#### 1.2. Operation of the SNAD biofilm reactor

The reactor performed stable SNAD performance for more than 60 days. Each cycle contained: feeding (5 min), intermittent aerobic condition (aeration 20 min/ mixing 20 min), aeration (20 min), settling (10 min), decanting (10 min) and idling

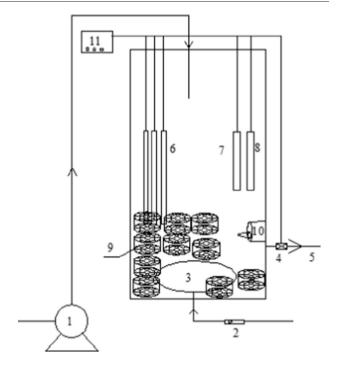


Fig. 1 – Reactor configuration scheme of biofilm reactor. 1-influent pump; 2-air flowmeter; 3-air diffuser; 4-electromagnetic valve; 5-drainage; 6-heating rod; 7-DO electrode; 8-pH electrode; 9-Kaldnes rings; 10-hydraulic stirrer; 11-programmable logic controller (PLC).

(1 min). The intermittent aerobic condition consisted of 6 aeration periods and 6 mixing periods. The average DO concentrations during aerobic and mixing periods were 5.6 and 0 mg/L, respectively. Domestic wastewater from the residential area nearby the lab was fed to the SNAD biofilm reactor. The main wastewater characteristics are as follows: pH 7.1–8.0, chemical oxygen demand (COD) 150–250 mg/L, total nitrogen (TN) 70–120 mg/L, NH $_4^+$ -N 60–90 mg/L, NO $_2^-$ -N < 1 mg/L, NO $_3^-$ -N < 3 mg/L, alkalinity (as CaCO3) 300–400 mg/L.

Appendix A Fig. S1 shows the nitrogen and COD removal performance of the SNAD biofilm reactor. The average effluent NH $_4^4$ -N, NO $_2^2$ -N and NO $_3$ -N concentrations were 3, 2 and 2 mg/L, respectively. Meanwhile, the average effluent COD concentration was 50 mg/L. The summation of NH $_4^4$ -N, NO $_2^2$ -N and NO $_3^3$ -N is calculated as the total inorganic nitrogen (TIN). The TIN removal efficiency ranged between 80% and 90% with the average TIN removal rate of 0.22 (kg TIN/m $_3^3$ /day).

#### 1.3. Batch tests

Batch tests were performed to determine the anammox activity of the SNAD biofilm. All batch tests were repeated three times in beakers (working volume of 1000 mL). In the beginning, Kaldnes rings taken from the biofilm reactor were carefully rinsed with tap water (30 °C) to remove residual substrate. Afterwards, 50 Kaldnes rings were transferred to beakers together with 1000 mL synthetic wastewater. Ammonium and nitrite were added to the synthetic wastewater in the form of NH<sub>4</sub>Cl and NaNO<sub>2</sub>, respectively. The mineral and trace element solution

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