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# Semi-nitritation process producing optimum influent for anammox process in treatment of domestic wastewater



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#### HIGHLIGHTS

• A control strategy of SN-SBR treating domestic wastewater was developed.

• The aeration time of SN-SBR was calculated using a developed equation.

• Using the control strategy, SN-SBR kept stable regardless of the influent variation.

• The inhibition of NOB in SN process was significant.

• The TN removal rate of the SN-anammox system achieved 91.7  $\pm$  0.4%.

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#### ABSTRACT

The process of anaerobic ammonium oxidation (Anammox) requires a proper ratio of NH<sup>4</sup><sub>4</sub>-N and NO<sup>2</sup><sub>2</sub>-N in the influent, which is difficult to control upon treating domestic wastewater. In this study, a control strategy of semi-nitritation (SN) process based on monitoring the pH profile and NH<sup>4</sup><sub>4</sub>-N concentration in a sequencing batch reactor (SBR) was developed. The aeration time of each cycle in SN-SBR was calculated using the established equation  $t_{SN} = t_{COD} + 0.56 a n S_0/(S_0 - S_n)$ . To verify the suitability of the control strategy, SN-SBR was operated continuously for 20 cycles, fed with real domestic wastewater with a fluctuating COD of 200–400 mg L<sup>-1</sup> and NH<sup>4</sup><sub>4</sub>-N of 65–80 mg L<sup>-1</sup>. The nitrogen removal performance of SN-anammox system using the developed control strategy was also monitored. Results showed that SN-SBR was able to generate a suitable ratio of NH<sup>4</sup><sub>4</sub>-N to NO<sup>2</sup><sub>2</sub>-N for the following anammox process, the TN removal rate of the SN-anammox system achieved 91.7  $\pm$  0.4% and the average ammonium, nitrite and nitrate concentration of effluent was only 0.50  $\pm$  0.24, 0.13  $\pm$  0.09 and 4.9  $\pm$  0.22 mg L<sup>-1</sup>, respectively. This study has potential application in the treatment of domestic wastewater using combined SN-anammox process.

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#### 1. Introduction

Nowadays, the most common method for nitrogen removal in a wastewater treatment plant is a combination of autotrophic nitrification and heterotrophic denitrification. To achieve higher nitrogen removal efficiency with lower energy consumption, many innovative biological technologies have been developed, such as partial nitrification (nitritation) and anaerobic ammonium oxidation (anammox). Compared to conventional nitrogen removal

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http://dx.doi.org/10.1016/j.chemosphere.2015.10.118 0045-6535/© 2015 Elsevier Ltd. All rights reserved. processes, nitritation saves about 25% of the original oxygen requirement and 40% carbon requirement during the denitrification process via nitrite (Turk and Mavinic, 1987; Fux et al., 2006). Additionally, nitritation can be combined with the anammox process, resulting in further reduction of required oxygen, sludge production and 100% carbon requirement for denitrification (Jetten et al., 2002). Based on these advantages, the combined seminitritation (SN)-anammox process has been confirmed as an attractive option for nitrogen removal in the past few years, particularly with regards to the treatment of high-strength wastewater (van der Star et al., 2007; Molinuevo et al., 2009; Park et al., 2010; Tang et al., 2011). In the combined SN-anammox process, an appropriate effluent property in the SN reactor was



an essential factor for the subsequent autotrophic anammox bacteria; for example, the ratio of nitrite to ammonium in the effluent should be theoretically close to 1.32:1 (Strous et al., 1998). However, when treating low-strength wastewater such as domestic wastewater, the higher fluctuation of wastewater quantity and quality makes it very challenging to acquire the appropriate effluent ratios for anammox bacteria.

Some researchers reported that a molar ratio of 1:1 for bicarbonate to ammonium in the influent could be regarded as a suitable parameter for a steady operation of a semi-nitritation reactor treating sludge reject water (Gali et al., 2007; Zhang et al., 2011). A 50% partial nitritation using a fixed bed biofilm reactor by controlling the influent  $HCO_3^-/NH_4^+$  molar ratio at 1:1 has already been achieved (Liang et al., 2011). Using a sequencing batch reactor (SBR), Ganigue also demonstrated that a suitable influent for anammox process can be produced when treating wastewater with varying levels of ammonia  $(500-3000 \text{ mg L}^{-1})$ , as long as the ratio of total inorganic carbon (TIC) to total nitrogen ammonia (TNH) is approximately 1:1 and extreme nitrogen loading rate is avoided (Ganigue et al., 2012). However, for domestic wastewater, this method is not applicable due to excess inorganic carbon and alkalinity in the influent which encourages the complete oxidization of ammonium to nitrite or nitrate. On the other hand, some researchers have pointed out that if the HCO<sub>3</sub>/NH<sup>+</sup><sub>4</sub> molar ratio in wastewater deviates from 1:1, the pH would have to be controlled in the SN process (Gali et al., 2007). However, controlling the pH in domestic wastewater is costly when the quantity of domestic wastewater is very large. Thus the availability and instability of the above combined process appear to be the primary issue to make operators reluctant to opt for this process when treating lowstrength wastewater. So far, few studies on treating domestic wastewater with the SN-anammox process (Ma et al., 2011) have been conducted. Therefore, it is desirable to explore a feasible yet efficient and cost-effective control strategy for the SN process.

Hence, the aim of this study is to (1) develop an efficient and cost-effective control strategy for the SN process in treating domestic wastewater; (2) demonstrate the effectiveness of the control strategy under fluctuating conditions; and (3) investigate the nitrogen removal performance of the SN-anammox system.

#### 2. Materials and methods

#### 2.1. Semi-nitritation bioreactor and anammox bioreactor

A 5 L plexiglass SBR was applied with a 50% exchange volume ratio (Fig. 1a). pH and DO were both continuously monitored and collected during the entire reaction phase. The temperature was maintained at 25  $\pm$  1 °C by a heater and a thermostat, and liquid mixing was performed by a mechanical stirrer. A programmable logic controller (PLC) system (Mitsubishi CPU224, Japan) was used for the reactor operation and DO concentration was controlled via PLC to maintain below 1.5 mg  $L^{-1}$  during the aeration period using ON/OFF control. The SN-SBR reactor was run 6-8 cycles per day. Each cycle of SBR consisted of 10 min of feeding, 10 min of anaerobic reaction, 90-180 min of aerobic reaction, 45 min of settling, and 15 min of decanting and idling. The mixed liquor suspended solids (MLSS) in the SN-SBR was maintained at about  $3500 \pm 350 \text{ mg L}^{-1}$ . The effective sludge retention time (SRT) was set at about 10 d by withdrawing the sludge from the reactor at the end of each aerobic cycle.

The anammox process was carried out in an upflow anaerobic sludge bed (UASB) reactor with a working volume of 1 L and diameter of 0.05 m (Fig. 1b). It was completely covered with black cloth to inhibit the growth of phototrophic organisms. The reactor was started up and operated stably for 90 d before experiments

were carried out. The operational parameters are listed in Table 1.

#### 2.2. Wastewater and inoculum of SN-SBR

Domestic wastewater used in this study was pumped from the septic tank of a residential district near the campus. The characteristics of the wastewater are described as follows: soluble COD 188–380 mg L<sup>-1</sup>, NH<sub>4</sub><sup>+</sup>-N 57.4–80.5 mg L<sup>-1</sup>, NO<sub>2</sub><sup>-</sup>-N 0.04–0.26 mg L<sup>-1</sup>, NO<sub>3</sub><sup>-</sup>-N 0.12–1.08 mg L<sup>-1</sup>, TP 5.3–6.5 mg L<sup>-1</sup>. The seeding sludge of the SN-SBR was obtained from a secondary clarifier of a pilot-scale pre-denitrification activated sludge system operated in the laboratory under limited DO conditions (0.1–0.5 mg L<sup>-1</sup>).

#### 2.3. Chemical analyses

All samples were filtered with a 0.45  $\mu$ m filter before analysis. COD, MLSS and MLVSS were measured according to APHA standard methods (APHA, 1998), while NH<sup>4</sup><sub>4</sub>-N, NO<sup>2</sup><sub>2</sub>-N, NO<sup>3</sup><sub>3</sub>-N and PO<sup>3</sup><sub>4</sub>-P were analyzed using a Lachat QuikChem 8500 flow injection analyzer (Lachat, USA). pH, DO and temperature were detected online using a pH/DO meter (WTW Multi340i, Germany).

#### 2.4. FISH

Sample fixation and hybridation steps were carried out according to the methods described by Amann (Amann et al., 1990), and fluorescent in situ hybridization (FISH) was carried out by an EUBmix (EUB338, EUB338II, EUB338III) which is specific to domain bacteria, NSO1225 and NSO190 specific for Beta-proteobacterial ammonia-oxidizing bacteria, NIT3 specific for Nitrobacter, and Ntspa662 specific for genus Nitrospira (Mobarry et al., 1996; Egli et al., 2003; Loy et al., 2003). The images of FISH samples and microscopic examinations of active sludge were captured using an fluorescence microscope (Olympus-BX52, Japan). The FISH images from 50 randomly selected fields of each sample were quantified using the Image-Pro Plus 6.0 software.

#### 3. Results and discussion

### 3.1. The performance of SN-anammox system using fixed time control

The SN-SBR was initiated with a fixed time aerobic duration control, the process of which in the start-up period is shown in Fig. 2a. On Day 1, the ammonium removal rate was only 47.6%, but the nitrite accumulation rate was 77% which was much higher than those in other studies (Blackburne et al., 2008; Zeng et al., 2010). It is suspected to relate to the seeding sludge that was obtained from a pilot-scale A/O reactor operated with limited NOB population under limited DO conditions. The aerobic duration of each cycle was fixed at 120 min in the first 16 d. On Day 12, the nitrite accumulation rate increased up to 95%. Since the specific nitrification rate increased by 1.9 times, from 0.06 (Day 1) to 0.116 gN gMLSS<sup>-1</sup> d<sup>-1</sup> (Day 15), the aerobic duration was reduced from 120 min to 90 min to maintain an effluent  $NO_2^2$ -N/NH<sup>4</sup><sub>4</sub>-N ratio of 1:1.3 after Day 16.

After 28 d of operation, the nitrite accumulation rate in the SN-SBR remained stable at over 97%. Then, the SN-SBR was combined with an anammox-UASB from Day 28 to Day 44. During the stable operation period, the aeration time for the SN-SBR remained fixed at 90 min and the average effluent ratio of  $NO_2^-$ -N/NH<sup>4</sup><sub>4</sub>-N in the reactor was 1.25. The average TN removal rate in the SN-anammox system reached 88.3 ± 3.5% and the average ammonium, nitrite and nitrate concentration in the effluent was 1.49 ± 1.51, 0.47 ± 0.97 and

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