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## Journal of Environmental Management

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Research article

# An innovative U-shaped sludge bed anammox process for nitrogen removal



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ARTICLE INFO

Keywords:
Anammox
Pyrosequencing
Sludge
Mathematical model
Nitrogen removal

#### ABSTRACT

In this study, we proposed an innovative U-shaped sludge bed reactor which could be a cost effective and simplified approach for the operation of an anammox reactor. The performance for nitrogen removal and the composition of bacterial communities were investigated for about 500 days of operation. The nitrogen removal rate could be approximately 85% when the total nitrogen loading rate was about 0.54 kg N/m³/d. The 16S rRNA gene pyrosequencing analysis of the bacterial community determined that *Betaproteobacteria* (class level) of the ammonia-oxidizing bacteria (AOB) community, *Nitrospira* (genus level) of the nitrite-oxidizing bacteria (NOB) community, and *Brocadia* (genus level) of the anammox bacteria community simultaneously coexisted in the reactor sludge. These results demonstrated that simultaneous growth and coexistence of AOB, NOB, and anammox were capable within the reactor. Furthermore, a mathematical modeling system was developed to simulate the nitrification and anammox processes. The model simulation showed that the oxygen was rapidly depleted and that led to a drop in the activity of AOB and NOB, then the growth of anammox bacteria started under anaerobic conditions.

## 1. Introduction

Anaerobic ammonium oxidation (anammox) is an autotrophic bacteria-mediated process that can convert nitrite and ammonium ions into dinitrogen and water under anaerobic conditions (van de Graaf et al., 1996; Strous et al., 1998). It has become a promising biotechnology for the treatment of high-strength ammonium due to the advantages of low energy consumption and environmentally friendly operation (Ali and Okabe, 2015; Fernandez et al., 2016). Many full-scale anammox plants have been installed worldwide and the number is growing (Ali et al., 2013). However, the development of wastewater treatment processes can be troubled by the slow growth rate and complicated environmental requirements of anammox bacteria. Anammox bacteria have a slow doubling time between 10 days and 2 weeks and are extremely sensitive to the inhibitions of dissolved oxygen (DO), organic matter, and nitrite (Jin et al., 2012).

Numerous types of anammox-related bioreactors have been developed to estimate their performance for nitrogen removal. The first anammox process was accidently discovered in a denitrifying fluidized bed reactor (FBR) (Mulder et al., 1995). Thereafter, the microbial community responsible for the anammox process was enriched by running the FBR with synthetic medium (van de Graaf et al., 1996). Laboratory studies for anammox generally used a sequencing batch reactor (SBR) due to its simplicity, flexibility of parameter control, and

efficient biomass retention (Cui et al., 2016; Dapena-Mora et al., 2004; Chamchoi Nitisoravut., 2007; Vlaeminck et al., 2008). A high-rate performance with nitrogen removal rate of 74.3-76.7 kg N/m<sup>3</sup>/day could be accomplished in the upflow anaerobic sludge blanket (UASB) reactor (Tang et al., 2010, 2011). The anammox process can be combined with a partial nitritation process to provide a proper ammonium/ nitrite ratio (van Dongen et al., 2001). Furthermore, simultaneous nitritation-anammox and completely autotrophic nitrogen removal over nitrite were developed to achieve both aerobic and anaerobic ammonium oxidation in a single stage reactor (Sliekers et al., 2002; Lotti et al., 2014). Fast growth of anammox bacteria was reported in studies of membrane bioreactors (MBRs), which can significantly increase the maximum specific growth rate up to 0.33/d (Lotti et al., 2015; Zhang et al., 2017). Until now, these reactors were mainstream in reactor development that could successfully enrich anammox microorganisms. However, the inoculation of enriched anammox biomass, the complicated operating strategies, and the expensive materials (media, membrane filter, and nonwoven, etc.) had to be applied to achieve a stable and fast start-up of the anammox process. Furthermore, controlling the DO level is mandatory, because anammox activity can be irreversibly inhibited by high DO levels (> 18% of oxygen saturation) (Egli et al., 2001). For the very slow growing anammox bacteria, it cannot be cultivated using conventional microbiological techniques. The choice of reactor operating strategy is very important to apply the anammox

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process. It should be suited for long-term enrichment, cultivation and quantitative analysis (Terada et al., 2011).

In this study, we developed a novel anammox process with a U-shaped sludge bed reactor. The aim of this study was to demonstrate an innovative process for operating an anammox reactor in a simplified and cost-effective way. We estimated the performance of nitrogen removal and investigated the composition of bacterial communities using 16S rRNA gene pyrosequencing analysis. Furthermore, the bacterial growth and substrate fate in the reactor were estimated using a simulation with the plug-flow Monod model.

### 2. Materials and methods

#### 2.1. Inoculum

The reactor was inoculated with denitrifying sludge taken from a post denitrification tank of distillery wastewater treatment plant in Ansan City, South Korea. The denitrification reactor which is receiving anaerobic digester effluent is operated in aerobic/anoxic condition with a chemical oxygen demand (COD) loading rated of 1.97 kg COD/m³/d and a nitrogen loading rate of 0.68 kg N/m³/d. Different from the activated sludge in aerobic tank the denitrifying sludge experienced both aerobic notification and anoxic denitrification process by receiving a high strength ammonium wastewater (about 1386 mg N/L) which may provide the essential environment (low oxygen level) and chemicals (ammonium, nitrite, carbon source) for the presence of anammox bacteria (Strous et al., 1997; Ding et al., 2017).

#### 2.2. Reactor operation

The anammox process was carried out in an aluminum-made reactor with a working volume of 8 L. The reactor was hermetically-sealed to cut off the intrusion of light and air. As shown in Fig. 1, the reactor had a cubic column (a height of 35 cm and a width of 21 cm) structure with a U-shape bottom (depth of 5 cm). The inside of the reactor was separated by a barrier with a right-width of 8 cm and a left-width of 13 cm. The off gas valve was placed at the right side of the barrier. The height of barrier was 30 cm which has a space of 5 cm distance from the

bottom. The position of single barrier promises sufficient substrate contact with biomass and meanwhile prevents sludge loss. The sludge taken from the denitrification reactor was fed into the reactor with a mixed liquor volatile solids concentration of 9600 mg/L. The sludge skipped the adaptation step and it was immediately used for the reactor start-up. The settled sludge created a fixed biomass layer with a depth of 12 cm. The reactor was equipped with a heater to maintain the temperature at around 35 °C (Jin et al., 2012). The wastewater with a flow rate of 12 L/d was continuously flowing past the liquid and settled sludge layers at the sides separated by the barrier. Throughout the experiment, the reactor was operated with a constant hydraulic retention time (HRT) of 0.67 d. There was no intentional sludge wasting for controlling the solids retention time. We did not deoxygenize the wastewater and, hence, the presence of DO was allowed in the influent. The DO of influent water varied between 6.8 and 8.2 mg O<sub>2</sub>/L which could affect the growth of anammox bacteria during the reactor start-up (Jin et al., 2012). This operating scheme was used to reduce the influent DO from the ammonium and nitrite oxidation by the liquid suspended biomass and the upper sludge bed biomass. Therefore, anoxic conditions could be created in the bottom sludge bed and at the right side of the barrier. It was supposed that the environment inside the reactor could be separated into the aerobic mixed liquor suspended sludge phase, the anoxic mixed liquor suspended sludge phase, the anoxic sludge bed phase, and the gas phase. Furthermore, a significant anammox biomass can be maintained within the reactor due to the fixed sludge bed. This process was named U-shaped sludge bed anammox (USBA) according to the operating type.

#### 2.3. Synthetic wastewater

The anammox activity was supported by the synthetic wastewater, which mainly contained ammonium and nitrite in the form of  $(NH_4)_2SO_4$  and  $NaNO_2$ , respectively. The alkalinity, phosphorous, and minerals were supplemented by KHCO $_3$  (1.25 g/L), KH $_2PO_4$ , MgSO $_4$  (0.06 g/L), and CaCl $_2$  (0.05 g/L). The trace supplement solution (1 mL/L) contained EDTA (5 g/L), FeSO $_4$  (5 g/L), ZnSO $_4$ 7H $_2$ O (0.43 g/L), CoCl $_2$ 6H $_2$ O (0.24 g/L), MnCl $_2$ 4H $_2$ O (0.99 g/L), CuSO $_4$ 5H $_2$ O (0.25 g/L), NaMO $_4$ 2H $_2$ O (0.22 g/L), NiCl $_2$ 6H $_2$ O (0.19 g/L), NaSeO $_4$ 10H $_2$ O

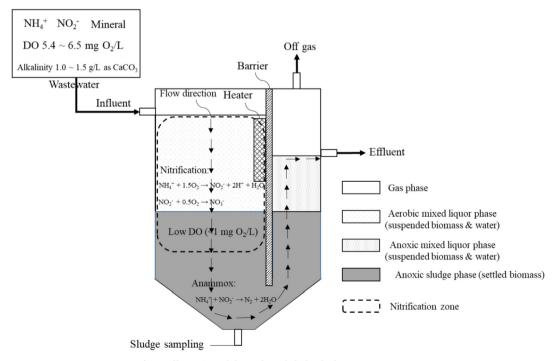


Fig. 1. Illustration of the U-shaped sludge bed anammox process.

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