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# Startup and operating characteristics of an external air-lift reflux partial nitritation-ANAMMOX integrative reactor

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## HIGHLIGHTS

- AOB and ANAMMOX bacteria were partitioned culture in a single reactor.
- A reflux system was formed used the exhaust from the aeration as power.
- Reflux system alleviated parameter fluctuations and inhibited the activity of NOB.
- The environment was appropriate for the growth of the corresponding microbes.
- We realize a high nitrogen removal rate by using PN-ANAMMOX process.

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## ABSTRACT

The differences in the physiological characteristics between AOB and ANAMMOX bacteria lead to suboptimal performance when used in a single reactor. In this study, aerobic and anaerobic zones with different survival environments were constructed in a single reactor to realize partitioned culture of AOB and ANAMMOX bacteria. An external air-lift reflux system was formed which used the exhaust from the aeration zone as power to return the effluent to the aeration zone. The reflux system effectively alleviated the large pH fluctuations and promoted $NO_2^-$-N to rapidly use by ANAMMOX bacteria, effectively inhibiting the activity of NOB. After 95 d of running, the nitrogen removal rate increased from the initial 0.21 kg/($m^3$·d) to 3.1 kg/($m^3$·d). FISH analyses further demonstrated that AOB and ANAMMOX bacteria acquired efficient enrichment in the corresponding zone. Thus, this type of integrative reactor may create the environments needed for the partial nitritation-ANAMMOX processing.



## 1. Introduction

ANaerobic AMMonium OXidation (ANAMMOX) is a microbial process that has recently been utilized for biological nitrogen removal in water treatment processes. ANAMMOX bacteria use $NO_2^-$ as the electron acceptor to oxidize $NH_4^+$ to $N_2$. Many small and middle pilot-scale ANAMMOX reactors have been started successfully (Terada et al., 2011) and have deepened the understanding of the bioconversion mechanisms, pathways, conditions, and influencing factors of the ANAMMOX process (Muhammad and Satoshi, 2015). ANAMMOX nitrogen removal technology has begun to transfer from small-scale laboratory studies to engineering applications (Kallistova et al., 2016; Lackner et al., 2014).

The ANAMMOX reaction requires $NO_2^-$ to serve as the electron acceptor, yet in typical industrial wastewater nitrogen exists in the form of $NH_4^+$. Hence, a pretreatment of partial nitritation (PN) is needed. PN refers to the use of ammonia oxidizing bacteria (AOB) to convert part of the $NH_4^+$ in wastewater into $NO_2^-$ under aerobic conditions (Huang et al., 2016b). To date, researchers have succeeded in combining tandem PN and ANAMMOX reactors by adjusting control parameters (Wang et al., 2016; Durán et al., 2014) and have realized PN-ANAMMOX processing in a single reactor (Shi et al., 2016; Isaka et al., 2013) for treatment of high-strength ammonium wastewater. These techniques have successfully applied to treatment of landfill leachate (Wu et al., 2015), monosodium glutamate wastewater (Shen et al., 2012), chemical wastewater, and other industrial wastewater (Daverey et al., 2013). Compared to the conventional nitrification-denitrification process for treating high-ammonia low-carbon wastewater, the PN-ANAMMOX process possesses the great advantages of low oxygen consumption, and no need for organic matter.

However, AOB and ANAMMOX bacteria differ in physiological characteristics and survival environments. These result in certain

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conflicts when applying the combined process treatment of ammonia-containing wastewater. For example, ANAMMOX reactions increase pH while nitritation reactions reduce pH. Thus tandem processes require constant adjustment using a large amount of agents which complicates the control of process conditions (Li et al., 2014a). In addition, single nitritation reactors often lose stability due to the strong adaptability of nitrite oxidizing bacteria (NOB) (Joss et al., 2011; Sliekers et al., 2005), and thereby producing a large amount of $NO_3^-$. Furthermore, AOB need enough dissolved oxygen (DO) to oxidize $NH_4^+$ into $NO_2^-$, yet DO inhibits ANAMMOX bacteria and restricts the various functional bacteria in transforming nitrogen (Isaka et al., 2013). These certain conflicts lead to each of the biotransformation efficiency exert insufficiently. Researchers have developed granular sludge as well as biofilm to culture ANAMMOX bacteria on the interior and AOB on the exterior. Previous studies have restricted DO concentration to reduce its interference with anaerobic bacteria. However, restricting DO will limit the activity of AOB and also partially limit ANAMMOX bacteria obtain sufficient $NO_2^-$ when using a single reactor (Egli et al., 2001). Compare to nitrogen conversion rate in separate reactor of PN and ANAMMOX, the combined process is very low. In order to maximize the nitrogen removal rate (NRR) of all microbes in PN-ANAMMOX and the nitrogen removal efficiency of the overall reactor, techniques need to be developed to mitigate these conflicts and create the environments needed for the growth of various functional microbes.

To resolve the above problems, a new integrated device was used in this study. Two different environments in a single reactor were created to realize partitioned culture of AOB and ANAMMOX bacteria. Meanwhile, an airlift device utilizes the exhaust from the aeration of the nitritation process as power to return the effluent from the settling zone to the nitritation zone. This allows acid-base neutralization between the two reaction zones to alleviate the fluctuation in pH. In this study, objective was to explore the feasibility of this integrative device for nitrogen removal using autotrophic organisms in ammonia-containing wastewater.

## 2. Material and methods

### 2.1. Reactor and control parameters

The integrative device for nitritation-ANAMMOX treatment of ammonia-containing wastewater is shown in Fig. 1. The reactor is made of plexiglass with an effective volume of 3.62 L. The effective volume is comprised of a 2.8 L aerobic zone, a 0.43 L anaerobic zone, and a 0.39 L settling zone. An airway inserted in the plenum above the aerobic zone and attached the other end to the water pipe in the airlift chamber which is in turn connected to the water inlet of the settling zone. The return pipe outside the airlift chamber is connected to the bottom of the aerobic zone. The exhaust from the aeration in the aerobic zone is collected in the plenum. The water vapor in the water pipe then ascends through the airway to the airlift chamber, ultimately returning to the aerobic zone through the return pipe outside the airlift chamber.

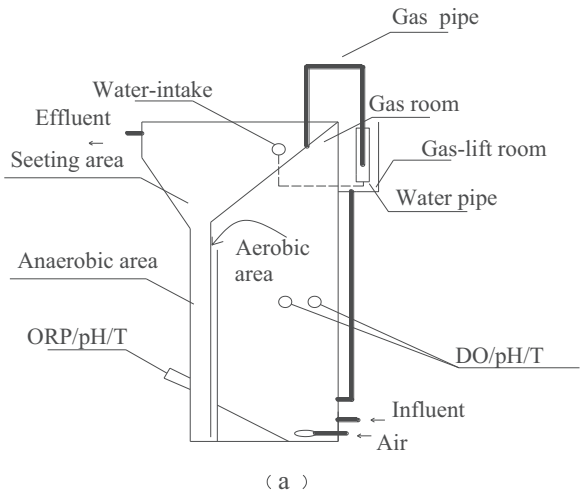


**Fig. 1.** Schematic of the partial nitritation/ANAMMOX integrative reactor.

Experiments were operated under a condition of continuous water input controlled by a peristaltic pump. The air levels in the aerobic zone were controlled by a gas meter. The temperature was kept at 32 ± 2 °C via keeping the greenhouse temperature. The oxidation reduction potential (ORP), DO, pH, and temperature of the aerobic zone and the anaerobic zone were all monitored in real-time using online monitoring equipment (Chemitc, Italy).

### 2.2. Sludge inoculation

Mature nitritation biofilm was inoculated in the aerobic zone of the integrative reactor. The carrier was cylindrical polyethylene plastic (Li et al., 2014c). The inoculated nitritation biofilm originated from a nitritation reactor after 150 d of acclimatization. The nitrite production rate (NPR) of this nitritation reactor reached 1.64 kg/($m^3$·d) (Li et al., 2014c). The volume of the nitritation film inoculated into the aerobic zone was approximately 1 L. The ANAMMOX sludge inoculated in the anaerobic zone was taken from a long-running ANAMMOX reactor in the laboratory. The NRR of the ANAMMOX reactor stabilized at approximately 28.3 kg/($m^3$·d) (Li et al., 2014d). The mixed liquor volatile suspended solids/mixed liquor suspended solids ratio of the granular sludge was 0.87 and the inoculums weighed 20 g (wet weight).

### 2.3. Wastewater composition

Artificially prepared wastewater was used. There is no organic matter in wastewater. The wastewater was composed of $NH_4Cl$ (according to experimental concentrations), 500 mg/L $NaHCO_3$, 27 mg/L $KH_2PO_4$, 136 mg/L $CaCl_2{\cdot}2H_2O$, 20 mg/L $MgSO_4{\cdot}7H_2O$, 1 mL/L Trace element I, and 1.25 mL/L trace element II. Trace element solution I contained (mg/L) EDTA 5000, $FeSO_4$ 5000; Trace element solution II contained (mg/L) EDTA 5000, $ZnSO_4{\cdot}7H_2O$ 430, $CoCl_2{\cdot}6H_2O$ 240, $MnCl_2{\cdot}4H_2O$ 990, $CuSO_4{\cdot}5H_2O$ 250, $NaMoO_4{\cdot}2H_2O$ 220, $NiCl_2{\cdot}6H_2O$ 190, $NaSeO_4{\cdot}10H_2O$ 210, $H_3BO_4$ 14. The pH of the influent was kept at 8.0 ± 0.1 and was adjusted using 1 mol/L HCl.

### 2.4. Fluorescence in situ hybridisation (FISH)

According to the FISH methodology used by Isaka et al. (2007), the aerobic zone biofilm and the anaerobic zone sludge were fixed. First, the collected biological sample was placed in freshly made 4% paraformaldehyde solution and was kept at 4 °C overnight in a refrigerator. Next, the sample was rinsed using phosphate buffered saline (PBS) and then ethanol + PBS (w/w 1:1) solution of the same volume was added. Ethanol of different concentrations (20%, 40%, 60%, 80%, and 100%) was then used for serial dehydration of the sample. Finally the prepared sample was stored at −20 °C.

EUB338, EUB338-II, EUB338-III, NSO190 and AMX368 probes were used during the hybridization. Whole-bacterial probes (EUB338, EUB 338-II, and EUB338-III) and an AOB probe (NSO190) were used to label the biofilm in the aerobic zone; whole-bacterial probes (EUB338, EUB 338-II, and EUB338-III) and an ANAMMOX bacteria probe (AMX368) were used to label the granular sludge in the anaerobic zone. For whole-bacterial probes,