



## Short communication

# Feasibility of enhancing the DENitrifying AMmonium OXidation (DEAMOX) process for nitrogen removal by seeding partial denitrification sludge

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## HIGHLIGHTS

- The DEAMOX process was established for removing  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  simultaneously.
- Nitrite generated from nitrate was enhanced by seeding partial denitrification sludge.
- TN removal efficiency as high as 97% was achieved in DEAMOX process.
- ANAMMOX and denitrifying bacteria could coexist harmoniously in the DEAMOX system.

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## ABSTRACT

The recently proposed DENitrifying AMmonium OXidation (DEAMOX) process combined anaerobic ammonia oxidation (ANAMMOX) with denitrification to convert nitrate to nitrite, which was a promising way for treating wastewater containing nitrate and ammonia. This study investigated the feasibility of establishing DEAMOX process by seeding partial denitrification sludge ( $\text{NO}_3^- \rightarrow \text{NO}_2^-$ ) using sodium acetate as an electron donor in a sequencing batch reactor. Results showed that the DEAMOX process was established successfully and operated stably in 114-days operation. The average effluent total nitrogen concentration was below  $5 \text{ mg L}^{-1}$  and TN removal efficiency reached up to 97% at COD/ $\text{NO}_3^-$  ratio of 3.0 under initial  $\text{NH}_4^+$  concentration of  $25 \text{ mg L}^{-1}$  and  $\text{NO}_3^-$  of  $30 \text{ mg L}^{-1}$ . It suggested that the presence of  $\text{NO}_2^-$  in the system supplied for ANAMMOX and the relatively long sludge retention time (SRT) for denitrifiers were attributed to commendable coexistence of ANAMMOX and denitrifying bacteria.

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## 1. Introduction

The anaerobic ammonia oxidation (ANAMMOX) process has been regarded as a promising way to remove nitrogen due to its high removal efficiency and minimal energy consumption in wastewater treatment (Kartal et al., 2010; Third et al., 2005). In this process, nitrite is utilized as an electron acceptor for the oxidation of ammonia. Thus, the ANAMMOX process was usually operated with a short-cut nitrification process to produce nitrite (Ma et al.,

2011; van Dongen et al., 2001). However, the stable acquisition of nitrite has been a bottleneck in applying the process to treat the low substrate wastewater (Zhang et al., 2013), since aerobically-treated wastewater is easier to be oxidized to  $\text{NO}_3^-$  than to  $\text{NO}_2^-$ . Therefore, a nitrogen removal method from a  $\text{NO}_3^-$  and  $\text{NH}_4^+$  could be implemented as a post treatment to treat the pre-existing aerobic process.

A new biological nitrogen removal process called DENitrifying AMmonium OXidation (DEAMOX) has recently been proposed for removing nitrogen from nitrate and ammonium (Kalyuzhnyi et al., 2006b; Kalyuzhnyi and Gladchenko, 2009). The process does not require nitrite to be produced separately; it combines the anammox reaction with denitrifying conditions, generating nitrite from

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nitrate in an anoxic biofilm. Since ANAMMOX is less competitive when coupled with denitrification, sulphide is generally acted as an electron donor for the generation of nitrite from nitrate. Kalyuzhnyi et al. (2006a) had reported that a stable performance with TN removal efficiencies of about 90% was achieved in the treatment of baker's yeast effluent with sulphide-driven conversion of nitrate into nitrite. However, some limitations apply, as most of the wastewaters lack of sulphide, but significant amount of organic matter. Later, the DEAMOX process was studied using volatile fatty acids (VFAs) as a replacement for the sulfide for partial denitrification, using synthetic (ammonia + nitrate) wastewater (Kalyuzhnyi et al., 2008). The results showed that VFAs were less efficient because it was difficult to prevent the nitrite from reducing to nitrogen. Nitrogen removal via complete denitrification ( $\text{NO}_3^- \rightarrow \text{N}_2$ ) accounted for a significant percentage of the TN removal, which increased the carbon consumed and reduced the efficiency.

High generation of nitrite from nitrate is the critical factor in achieving the purpose of the DEAMOX process. A high nitrite accumulation with 80% nitrate-to-nitrite transformation ratio (NTR) was achieved with the cultivated denitrification sludge and the stability of high nitrite accumulation was demonstrated in long-term operation, as described in our previous study (Cao et al., 2013). Thus, in this work, the DEAMOX system was established in a sequencing batch reactor (SBR) by seeding the above sludge enhancing nitrite generation from nitrate, using sodium acetate as the electron donor. The main purpose of this work is to evaluate the feasibility of the sodium acetate-driven DEAMOX process, thereby introducing a new way to treat wastewater from nitrate and ammonium with a more cost-efficient method.

## 2. Material and methods

### 2.1. DEAMOX sludge and synthetic wastewater

The DEAMOX sludge was taken from the ANAMMOX reactor and the denitrification reactor with a mixed liquor volatile suspended solids (MLVSS) ratio of approximately 0.8:1, of which the ANAMMOX bacteria were cultured in a SBR with the stable  $\text{NH}_4^+$  removal efficiency of 92% and  $\text{NO}_2^-$  removal efficiency of 99% for 300 d. The denitrification sludge had a high nitrate-to-nitrite transformation ratio (NTR) as described in our previous research (Cao et al., 2013).

Synthetic wastewater was used for the DEAMOX process throughout this study, being in the form of  $\text{NH}_4\text{Cl}$  and  $\text{NaNO}_3$ , respectively. The composition of the mineral medium was: 1.25 g  $\text{KHCO}_3$ , 0.025 g  $\text{KH}_2\text{PO}_4$ , 0.3 g  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.2 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.00625 g  $\text{FeSO}_4$ , 0.00625 g EDTA per liter and 1 mL  $\text{L}^{-1}$  of trace elements solution. The trace element solution contained: 1.5 g  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ , 0.03 g  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.12 g  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 0.06 g  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ , 0.12 g  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.15 g  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ , 0.18 g KI, 0.15 g  $\text{H}_3\text{BO}_3$  and 10 g ethylenediamine tetraacetic acid (EDTA) (Du et al., 2014).

### 2.2. Sequencing batch reactor (SBR) and operating conditions

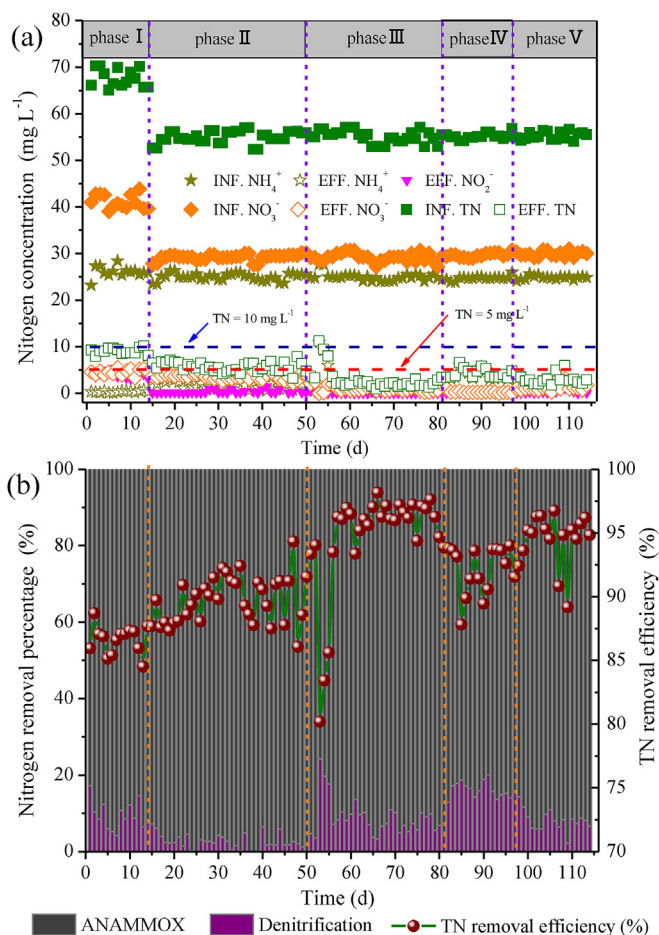
A glass-making SBR with a working volume of 2.0 L was used for this study. The reactor was operated in cycles of 8 h at room temperature (22–28 °C). Each cycle consisted of 5 min for feeding, 6 h for reaction, 50 min for settling, 5 min for withdrawing and 60 min for remaining idle. During the reaction stages, a complete mixing inside the SBR was ensured by using a magnetic stirrer at 80 rpm. At each feeding period, 1 L of synthetic wastewater was added.

The reactor operation was carried out in five phases. In phase I, the DEAMOX process was performed at a concentration of  $\text{NH}_4^+ = 25 \text{ mg L}^{-1}$  and  $\text{NO}_3^- = 40 \text{ mg L}^{-1}$  with COD/ $\text{NO}_3^-$  ratio of 2.5.

In phases II–IV, the COD/ $\text{NO}_3^-$  ratios of 2.5, 3.0 and 3.5 were investigated respectively at  $\text{NH}_4^+ = 25 \text{ mg L}^{-1}$  and  $\text{NO}_3^- = 30 \text{ mg L}^{-1}$ . And in phase V, the optimum COD/ $\text{NO}_3^-$  ratio of 3.0 was further investigated. During the experimental period, the SBR reactor ran over 114 days without sludge discharge, with an average MLVSS of  $1200 \text{ mg L}^{-1}$ .

### 2.3. Analytical methods

Influent and effluent samples were collected on a daily basis and were analyzed immediately. Water samples were analyzed according to the standard methods for the examination of water and wastewater (APHA, 1998). The parameters ammonium ( $\text{NH}_4^+$ ), nitrite ( $\text{NO}_2^-$ ), nitrate ( $\text{NO}_3^-$ ), and chemical oxygen demand (COD) were analyzed. The biomass concentration was observed as Mixed Liquor Suspended Solids (MLSS) and Mixed Liquor Volatile Suspended Solids (MLVSS). PHAs (poly- $\beta$ -hydroxyalkanoates) were determined by the sum of poly- $\beta$ -hydroxybutyrate (PHB) and poly- $\beta$ -hydroxyvalerate (PHV), and analyzed accordingly to the method reported by <http://www.sciencedirect.com/libproxy.bjut.edu.cn/science/article/pii/S0960852414009304>Zeng et al. (2003).



**Fig. 1.** Profiles of influent/effluent nitrogen compounds concentrations (a), and TN removal efficiency, percentage of ANAMMOX/Denitrification contribution on TN removal (b) in the DEAMOX system during the long-term operation.

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