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## Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere



# Simultaneous domestic wastewater and nitrate sewage treatment by DEnitrifying AMmonium OXidation (DEAMOX) in sequencing batch reactor



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#### HIGHLIGHTS

- DEAMOX was developed to treat domestic wastewater and NO<sub>3</sub>-N sewage simultaneously.
- TN removal efficiency achieved 95.8% with influent NO<sub>3</sub>-N/NH<sup>‡</sup>-N ratio of
- Performance recovered rapidly after deterioration for mass transfer limitation.
- The dominant Thauera general possibly played key role in high NO<sub>2</sub>-N accumulation

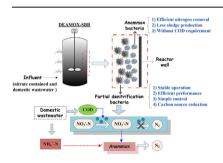
#### ARTICLE INFO

Article history:
Received 20 May 2016
Received in revised form
7 December 2016
Accepted 3 February 2017
Available online 4 February 2017

Handling Editor: Chang-Ping Yu

Keywords:
Partial denitrification
Anammox
Domestic wastewater
Nitrite accumulation
High-throughput sequencing

#### G R A P H I C A L A B S T R A C T



#### ABSTRACT

A novel DEAMOX system was developed for nitrogen removal from domestic wastewater and nitrate  $(NO_3^-N)$  sewage in sequencing batch reactor (SBR). High nitrite  $(NO_2^-N)$  was produced from  $NO_3^-N$  reduction in partial-denitrification process, which served as electron acceptor for anammox and was removed with ammonia  $(NH_4^+-N)$  in domestic wastewater simultaneously. A 500-days operation demonstrated that the efficient and stable nitrogen removal performance could be achieved by DEAMOX. The total nitrogen (TN) removal efficiency was as high as 95.8% with influent  $NH_4^+-N$  of 63.58 mg  $L^{-1}$  and  $NO_3^--N$  of 69.24 mg  $L^{-1}$ . The maximum  $NH_4^+-N$  removal efficiency reached up to 94.7%, corresponding to the  $NO_3^--N$  removal efficiency of 97.8%. The biomass of partial-denitrification and anammox bacteria was observed to be wall-growth. The deteriorated nitrogen removal performance occurred due to excess denitrifying microbial growth in the outer layer of sludge consortium, which prevented the substrate transfer for anammox inside. However, an excellent nitrogen removal could be guaranteed by scrapping the superficial denitrifying biomass at regular intervals. Furthermore, the high-throughput sequencing analysis revealed that the *Thauera* genera (26.33%) was possibly responsible for the high  $NO_2^-N$  accumulation in partial-denitrification and *Candidatus Brocadia* (1.7%) was the major anammox species.

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#### 1. Introduction

Anammox process capable of converting  $NH_4^+$ -N to nitrogen gas  $(N_2)$  using  $NO_2^-$ -N as the electron acceptor has attracted much

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attention (Mulder et al., 1995; Jetten et al., 1998) for its low demand of oxygen, no organic carbon sources required (Van Dongen et al., 2001), high nitrogen removal and low sludge yield (Jetten et al., 1998). Autotrophic anammox process has been applied for ammonia-rich wastewater (Peng et al., 2008; Joss et al., 2009), but limited studies focused on its widely application in treating low-strength sewage, such as the domestic wastewater.

The main challenge of anammox for domestic wastewater treatment is the stable source of NO<sub>2</sub>-N generation (Lackner et al., 2014). Traditional NO<sub>2</sub>-N production was via nitritation process, in which NH<sup>‡</sup><sub>4</sub>-N is oxidized to NO<sub>2</sub>-N without going further to NO<sub>3</sub>-N. Successful nitritation-anammox process is favorable for ammonium-rich wastewater due to the free ammonia (FA) inhibition on nitrite oxidation bacteria (NOB). However, nitritation is difficult to maintain stably for the low-strength wastewater (Ma et al., 2009; Peng et al., 2012), consequently the effluent contains undesirable concentration of NO<sub>3</sub>-N due to NO<sub>2</sub>-N oxidation by NOB (Fux et al., 2004; Erguder et al., 2008).

On the other hand,  $NO_2^-N$  can be produced from partial-denitrification process. According to our previous study,  $NO_3^-N$  could be reduced to  $NO_2^-N$  with the transformation ratio about 80% in the denitrification (Cao et al., 2013). Moreover, excess  $NO_3^-N$  in effluent of anammox reactor could be converted to  $NO_2^-N$  efficiently and removed with  $NH_4^+-N$  by backflow to anammox reactor for advanced nitrogen removal performance (Du et al., 2015a). This demonstrated the feasibility of providing  $NO_2^--N$  for anammox by  $NO_3^--N$  reduction, which hold great potential for treating low-strength wastewater by integrating partial-denitrification to anammox process.

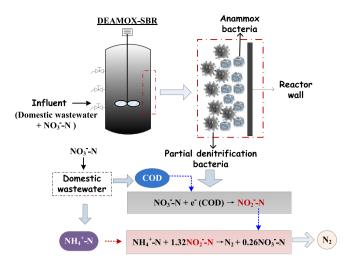
In the DEAMOX system, NO<sub>2</sub>-N is generated from heterotrophic NO<sub>3</sub>-N reduction by inoculated partial-denitrification sludge, then the NO<sub>2</sub>-N and NH<sub>4</sub>-N was removed by anammox bacteria in single reactor. In this case, NO<sub>2</sub>-N could be accumulated without complex control (Cao et al., 2013) compared with nitritation process. Therefore, it provided an efficient option for simultaneous nitrogen removal from domestic wastewater and NO<sub>3</sub>-N sewage, such as some industrial wastewater for fertilizer, metal finishing and explosive, which usually contained high concentration of NO<sub>3</sub>-N. Previous study reported that the average nitrogen removal was 81% in an anammox-denitrifying system when fed with synthetic wastewater containing NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, and acetic acid (Gao et al., 2012). Kalyuzhnyi and Gladchenko (2009) found that it was less efficient when using organic matter as electron donor for DEAMOX with NH<sub>4</sub>-N removal around 40% compared with that using sulphide. However, the efficiency and stability of NO<sub>2</sub>-N production during partial-denitrification as well as the microbial community of the system have not been well understood, especially for the treatment of real domestic wastewater.

In this study, the DEAMOX based on partial-denitrification for simultaneous  $NO_3^-N$  sewage and domestic wastewater treatment was developed for the first time. Denitrifying sludge with high  $NO_2^-N$  accumulation was inoculated to supply substrate for anammox (Fig. 1). The nitrogen removal performance and the characteristics of active sludge were investigated over a 500-days operation. The microbial community of the system was also estimated by high-throughput sequencing.

#### 2. Materials and methods

#### 2.1. The DEAMOX SBR

The DEAMOX SBR (working volume: 2.0 L, diameter: 120 mm, and height: 250 mm) was operated with 8 h cycles per day, consisting of four stages: 5 min for feeding with 1.2 L wastewater, 6 h for anaerobic reaction with a magnetic mixing (250 rpm), 60 min



**Fig. 1.** Schematic of the cooperation between partial-denitrification and anammox in the DEAMOX system and mechanism for nitrogen removal from domestic wastewater and  $NO_3^--N$  sewage.

for settling, 5 min for discharging (1.2 L supernate), and 50 min for idle. The system was operated at  $28 \pm 0.5$  °C to provide a suitable condition for anammox bacteria and keep efficient microorganism retention. The domestic wastewater and NO $_3$ -N was pumped into the SBR using a peristaltic pump, as well as sodium acetate solution was pumped separately as external carbon source, as shown in Fig. 1. The SBR was covered with a black PVC material (5 mm thickness) to prevent the penetration of light (van de Graaf et al., 1996).

#### 2.2. Seeding sludge and wastewater

The DEAMOX SBR was inoculated with the anammox and partial-denitrification sludge. The seeding anammox sludge was collected from a lab-scale upflow anaerobic sludge blanket (UASB) reactors (working volume of 3.6 L), which had been operated for 7 months with the influent NH<sub>4</sub>-N and NO<sub>2</sub>-N concentration of 300 mg  $L^{-1}$  and 400 mg  $L^{-1}$ , respectively. The mixed liquor suspended solid (MLSS) and mixed liquor volatile suspended solid (MLVSS) of seeding sludge was about 3.08 g  $L^{-1}$  and 1.79 g  $L^{-1}$ , respectively. The partial-denitrification sludge was collected from an enriched partial-denitrification system as previously reported (Cao et al., 2013). It reduced NO<sub>3</sub>-N to NO<sub>2</sub>-N with the NO<sub>3</sub>-N to NO<sub>2</sub>-N transformation ratio (NTR) of 80% when feeding with synthetic wastewater containing  $NO_3^--N$  (30 mg  $L^{-1}$ ) and sodium acetate. The MLSS and MLVSS of the seeding partial-denitrification sludge were 3.0 g  $L^{-1}$  and 1.8 g  $L^{-1}$ , respectively. The MLVSS in the DEAMOX SBR was 2.5 g  $L^{-1}$  after inoculation.

The DEAMOX reactor was fed with domestic wastewater collected from an on-campus sewer line and the wastewater characteristics were listed as follows: COD: 125.2–264.8 mg L $^{-1}$ , NH $_{+}^{+}$ -N: 47.32–79.09 mg L $^{-1}$ , NO $_{2}^{-}$ -N: 0–0.02 mg L $^{-1}$ , NO $_{3}^{-}$ -N: 0.01–0.46 mg L $^{-1}$ , and pH: 7.0–7.6.

Increasing nitrogen loadings (NL) were examined during the whole operation. At the start-up phase, domestic wastewater was diluted before feeding, then sodium nitrate was added into the wastewater to achieve an NO<sub>3</sub>-N/NH<sub>4</sub><sup>+</sup>-N ratio of 1.0–1.3, in order to simulate the mixture of NO<sub>3</sub>-N contained wastewater and domestic wastewater. The increasing NH<sub>4</sub><sup>+</sup>-N loading was achieved by adding ammonia chloride into the mixed wastewater. Since the concentration of biodegradable organic matter in domestic wastewater dropped after dilution, sodium acetate solution was pumped into

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