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## Tertiary nitrogen removal using simultaneous partial nitrification, anammox and denitrification (SNAD) process in packed bed reactor

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## ABSTRACT

The study aimed to polish wastewater effluent from an activated treatment system for nitrogen removal by a packed bed bioreactor (PBR). The nitrogen removal via simultaneous partial nitrification, anammox and denitrification (SNAD) process was investigated with different hydraulic retention times (HRTs). The results show that both effluent ammonia- and nitrate-nitrogen concentrations approached 0 mg/L at the HRT as low as 3 h. The total nitrogen removal efficiency decreased from an average of 84% at both 18- and 24-h HRTs to 38% at 3-h HRT. This low nitrogen removal efficiency was due to the presence of incomplete degradation of organic nitrogen at shorter HRTs. The analysis using quantitative real-time polymerase chain reaction resolves that the ratio of ammonia-oxidizing bacteria (AOB) to the domain Eubacteria changed limited in the range between 2.8 and 4.0% during the continuous operation. Meanwhile, the nitrite-oxidizing bacteria (NOB) increased from 0.0 to 2.9% when the HRT was gradually reduced to 3 h. The anammox bacteria accounting for 30.5% of microbial consortia dominated the AOB and the NOB at the 3-h HRT. In sum, inorganic form of nitrogen in a typical wastewater plant effluent could be efficiently polished via SNAD process in the PBR.

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## 1. Introduction

The traditional nitrification–denitrification process is the most widespread biotechnology for ammonia-nitrogen removal in wastewater treatment plants (Pynaert et al., 2004). However, this biological process has been challenged by anaerobic ammonia oxidation (Anammox) process due to the energy concern. In anammox reaction, ammonia and nitrite act as electron donor and acceptor, respectively, to yield nitrogen gas and nitrate (Strous et al., 1997). The aeration to fully oxidize ammonia to nitrate can be saved. Additionally, the anammox reaction over traditional biological nitrogen removal process is much less nitrous oxide emission. The release of nitrous oxide (greenhouse gas) accounted for 0.6% of nitrogen loading in the anammox tank (Sri and Joseph,

2012), and therefore the greenhouse effect indirectly caused by the anammox reaction is insignificant.

The anammox reaction cannot be performed by anammox bacteria if nitrite is not present in the wastewater. Nitrite is not commonly found in the surface water because it is unstable and easily oxidized to nitrate with oxygen present. For this reason, portion of ammonia in the wastewater should be oxidized to nitrite to be served as electron acceptor. This transformation can be achieved by using the technology of either completely autotrophic nitrogen removal over nitrite (CANON) or single reactor high activity ammonia removal over nitrite –anaerobic ammonium oxidation (SHARON-ANAMMOX). In the CANON process, ammonia-oxidizing bacteria coexist with the anammox bacteria to perform partial nitrification and anammox reaction for nitrogen removal in one tank (Vazquez-Padin et al., 2009). The partial nitrification only generates nitrite but the pathway of nitrate production is restricted by limited oxygen supply (Sliekers et al., 2003). Similar to the CANON technology, the SHARON-ANAMMOX is the process also incorporating the partial nitrification with anammox, but functioning in two separated reactors (Schmidt et al., 2003).

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Nitrate is the end product of anammox process. Based on stoichiometry, removing 1 mol of ammonia will produce 0.26 mol of nitrate (Strous et al., 1998; Sri and Joseph, 2012). This nitrate needs to be removed before discharged to the water body. Accordingly, anammox integrating with other nitrogen-based removal technologies, named simultaneous partial nitrification, anammox and denitrification (SNAD), was developed to remove ammonium and nitrate from wastewater in one tank (Chen et al., 2009; Wang et al., 2010). In the SNAD process, ammonia is partially oxidized to form nitrite, and then anammox and denitrification together remove ammonia and nitrate, respectively. Because denitrification occurs in the SNAD process, portion of COD in wastewater can be removed (Lan et al., 2011). Previous studies have conducted on the nitrogen removal via the SNAD process and their operating parameters are listed in Table 1. It is of interest to note that the ratios of the COD to ammonia-nitrogen in these studies were found to be in the range from 0.2 to 0.9. This low COD to ammonia-nitrogen ratio was the pivotal parameter to ensure the stable SNAD process (Daverey et al., 2013a). In addition, long sludge retention times (SRTs) were also needed to facilitate the biodiversities of the microbial populations in the SNAD system (Chen et al., 2009; Liang et al., 2014).

The anammox process has been successfully applied to treat the synthetic wastewaters with high ammonia concentration (>200 mg N/L) at mesophilic temperature (Fux et al., 2002; Lan et al., 2011; Wang et al., 2012). However, the treatment efficacy is critical for the low nitrogen-strength wastewater because of the low growth yield (0.14 g VSS/g NH<sub>4</sub>-N) and the low specific growth rate (0.065/d) of the anammox bacteria (Strous et al., 1998). This may limit the applications of anammox process on treating diverse wastewaters, for example, the municipal wastewater with the nitrogen concentration ranging from 20 to 85 mg N/L (Metcalf and Eddy, 1991). Employing a high-rate bioreactor (Singh and Prerna, 2009; Khan et al., 2011; Bello et al., 2017) to maintain long (SRTs) is the most practical solution to overcome this obstacle. High-rate bioreactors with various configurations have been developed in the past few decades. Packed bed bioreactor (PBR) is a promising option with long SRT potential that have not been evaluated on anammox-based nitrogen removal process to date. PBR containing media is capable of supporting the growth of microbes for a long period under low shear conditions due to the immobilization of microbes within macroporous matrices. The media packing in the reactor also increase the contacting probability between nitrogen-

removing bacteria and targeted nitrogen compounds for greater nitrogen removal efficiency. It is reasonable to believe that PBR could treat the wastewaters with low nitrogen concentrations. In this study, the performance of nitrogen removal at ambient temperature in the PBR was investigated with different hydraulic retention times (HRTs). Activated sludge system effluent containing ammonia and nitrate-nitrogen was fed into the PBR operating under the SNAD environment. The microbial analysis by real-time quantitative real time polymerase chain reaction (qPCR) was carried out to explore the variations of microbial populations, and the specific anammox activity (SAA) was examined to evaluate the potential of transforming ammonia-nitrogen to nitrogen gas by the anammox bacteria.

## 2. Materials and methods

### 2.1. Seed and substrate preparations

The seed sludge containing the anammox bacteria was collected from the local landfill leachate wastewater treatment plant at the City of New Taipei, Taiwan. The levels of total solids (TS) and volatile solids (VS) of the collected sludge were 31.5 and 23.4 g/L, respectively.

The effluent of a final clarifier at the local wastewater treatment plant in the City of Taoyuan, Taiwan was used as the feed of the PBR. This wastewater treatment plant treated the wastewaters 66% from sewage, 29% from hospital, and 5% from industrial park. Table 2 summarized the water qualities of the activated sludge system effluent. This effluent quality in average contained the chemical oxygen demand (COD) of 36 mg/L, the biochemical oxygen demand (BOD) of 6 mg/L, the total suspended solid (TSS) of 8 mg/L, the

**Table 2**  
Wastewater characteristics of the PBR feed.

Parameters	Concentration (mg/L) <sup>a</sup>
TKN	35 ± 9
NH <sub>4</sub> <sup>+</sup> -N	20 ± 7
NO <sub>2</sub> <sup>-</sup> -N	1 ± 1
NO <sub>3</sub> <sup>-</sup> -N	1 ± 1
COD	36 ± 16
BOD	6 ± 3
TSS	8 ± 7
VSS	7 ± 6

<sup>a</sup> Average ± standard deviation (measurements > 5).

**Table 1**  
Summary of prior studies of biological nitrogen removal via the SNAD process.

Reactor	Temperature (°C)	Influent concentration (mg/L)	Wastewaters	TNLR <sup>d</sup> (g N/L-d)	HRT (h)	pH	DO concentration (mg/L)	References
NRBC <sup>a</sup>	35	100–150 (COD) 200 (NH <sub>4</sub> <sup>+</sup> -N) 554 (COD) 634 (NH <sub>4</sub> <sup>+</sup> -N) 3 (NO <sub>3</sub> <sup>-</sup> -N)	Synthetic wastewater Leachate	1.6	3	8 –8.2	0.4–0.6	Chen et al. (2009)
Full-Scale landfill-leachate reactor	30–33	100 (COD) 200 (NH <sub>4</sub> <sup>+</sup> -N) 17 (NO <sub>3</sub> <sup>-</sup> -N)		0.51	30.2	7.9	0.3	Wang et al. (2010)
SBR <sup>b</sup>	35	387 (COD) 662 (TKN) 519 (NH <sub>4</sub> <sup>+</sup> -N) 40 (COD) 207 (NH <sub>4</sub> <sup>+</sup> -N) 3 (NO <sub>2</sub> <sup>-</sup> -N) 6 (NO <sub>3</sub> <sup>-</sup> -N)	Synthetic wastewater	0.07–0.22	72 –216	7–8	0.5–1	Lan et al. (2011)
SBR	15–30		ADLSW <sup>c</sup>	0.05–0.18	60 –120	7–8	<0.5	Daverey et al. (2013a)
Up-Flow Biofilter	25		Synthetic wastewater	8.64	0.6	–	–	Liang et al. (2014)

<sup>a</sup> Non-woven rotating biological contactor.

<sup>b</sup> Sequencing batch reactor.

<sup>c</sup> Anaerobic digester liquor of swine wastewater.

<sup>d</sup> TNLR = Total nitrogen loading rate.

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