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Achieving mainstream nitrogen removal through simultaneous partial nitrification, anammox and denitrification process in an integrated fixed film activated sludge reactor

Chao Wang [a], Sitong Liu [b], Xiaochen Xu [a], Chaolei Zhang [c], Dong Wang [a], Fenglin Yang [a,*]

[a] Key Laboratory of Industrial Ecology and Environmental Engineering (MOE), School of Environment Science and Technology, Dalian University of Technology, Linggong Road 2, Dalian 116024, China

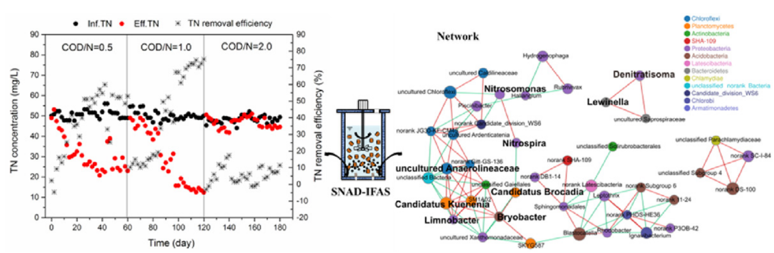
[b] Department of Environmental Engineering, Peking University, Beijing 100871, China

[c] School of Life Science and Biotechnology, Dalian University of Technology, Linggong Road 2, Dalian 116024, China

HIGHLIGHTS

- SNAD-IFAS process was feasible for mainstream nitrogen removal.
- The optimum COD/N ratio for mainstream nitrogen removal by SNAD-IFAS was 1.2 ± 0.2.
- Biodiversity of heterotrophic bacteria and NOB both increased with COD increasing.
- Anammox displayed significant co-occurrence with some heterotrophic bacteria.

GRAPHICAL ABSTRACT



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ABSTRACT

The anaerobic ammonium oxidation (anammox) is becoming a critical technology for energy neutral in mainstream wastewater treatment. However, the presence of chemical oxygen demanding in influent would result in a poor nitrogen removal efficiency during the deammonification process. In this study, the simultaneous partial nitrification, anammox and denitrification process (SNAD) for mainstream nitrogen removal was investigated in an integrated fixed film activated sludge (IFAS) reactor. SNAD-IFAS process achieved a total nitrogen (TN) removal efficiency of 72 ± 2% and an average COD removal efficiency was 88%. The optimum COD/N ratio for mainstream wastewater treatment was 1.2 ± 0.2. Illumina sequencing analysis and activity tests showed that anammox and denitrifying bacteria were the dominant nitrogen removal microorganism in the biofilm and the high COD/N ratios (≥2.0) leaded to the proliferation of heterotrophic bacteria (*Hydrogenophaga*) and nitrite-oxidizing bacteria (*Nitrospira*) in the suspended sludge. Network analysis confirmed that anammox bacteria (*Candidatus* Kuenenia) could survive in organic matter environment due to that anammox bacteria displayed significant co-occurrence through positive correlations with some heterotrophic bacteria (*Limnobacter*) which could protect anammox bacteria from hostile environments. Overall, the results of this study provided more comprehensive information regarding the community composition and assemblies in SNAD-IFAS process for mainstream nitrogen removal.

* Corresponding author.
E-mail address: yangfl@dlut.edu.cn (F. Yang).

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1. Introduction

In 2008, the Global Water Research Conference (GWRC) proposed the development goal of the mainstream wastewater treatment plants (WWTPs) in the future, that was, the wastewater treatment plants would be built into the Energy factory, the Waters factory and the Nutrient factory (Roeleveld et al., 2010; Reardon et al., 2013). Therefore, the research on energy neutral or energy positive sewage treatment technology has become a frontier issue and research hotspot in recent years. The main idea for energy neutral in mainstream wastewater treatment is to recycle organic carbon and convert it into methane, and then utilize low energy consumption technology for biological denitrification (Khiewwijit et al., 2015; Meerburg et al., 2015).

The mainstream anaerobic ammonium-oxidizing (anammox) is becoming a critical technology for energy neutral in mainstream wastewater treatment. Based on anammox technology, the high rate activated sludge-Deammonification (HRAS-Deammonification) process, anaerobic membrane bioreactor-oxygen limited autotrophic nitrification/denitrification (AnMBR-OLAND) process and chemically enhanced primary treatment-completely autotrophic nitrogen removal over nitrite (CEPT-CANON) process were proposed for mainstream wastewater treatment (Guida et al., 2007; Malovanyy et al., 2015; Lin et al., 2016). However, the main challenge for deammonification process application in mainstream wastewater treatment is suppression of nitrite-oxidizing bacteria (NOB) growth and the presence of COD in influent (Morales et al., 2015). Because the NOB could compete with ammonia-oxidizing bacteria (AOB) and anammox bacteria for substrate (oxygen and nitrite) and the presence of COD would inhibit the activity of AOB and anammox bacteria (de Clippeleir et al., 2013; Lotti et al., 2014; Zhang et al., 2015). Simultaneously the NOB could produce a part of nitrate. Due to this part of nitrate, the total nitrogen of effluent in mainstream wastewater treatment may not meet the requirement of the National Municipal Wastewater Discharge Standards of China (TN $\leq$ 15 mg L^{-1}). Although some investigations focus on the denitratation/anammox process and the simultaneous partial nitrification, anammox and denitrification (SNAD) process to treat mainstream wastewater (Wen et al., 2016; Ma et al., 2017; Liu et al., 2017), there are still some problems with these technologies, such as more oxygen consumed, higher temperature, only model predictions (the bioconversion process and microbial structure in the granule-based mainstream autotrophic nitrogen removal reactor were according to a one-dimensional biofilm model) and lower COD/N ratios requirement (COD/N ratio of 0.2–0.6, generally the COD/N ratio of 1–2 after pretreating during mainstream wastewater treatment). Therefore, the SNAD process for mainstream wastewater treatment has been remained to be further studied.

The bioreactor can be regarded as a small microbial ecosystem, like natural ecosystems, and the degradation and transformation of substrate in the reactor is carried out by a variety of microbes (Röling et al., 2007). Many factors can account for the variation in microbial community, such as COD/N ratio, substrate quality and reactor structure (Michaud et al., 2014; Peng et al., 2014; Shen et al., 2014). The COD/N ratio is a major factor influencing microbial composition and diversity in the bioreactor. It is reported that the high COD/N ratio performed inhibition on the bioactivity and biodiversity of both AOB and anammox bacteria, which then led to the decreasing of nitrogen removal in the CANON process (Zhang et al., 2015). Otherwise, the anammox activity cannot significantly be affected by the low concentration of organic matter (Ali and Okabe, 2015). However, there is limited work to explain why anammox bacteria could survive in organic matter of low concentrations. Therefore, it is important to understand the effect of different COD/N ratios on the process performance; structure of the microbial communities and bacteria correlation during SNAD process for mainstream wastewater treatment.

The objectives of this study were to investigate SNAD process for mainstream wastewater treatment in an integrated fixed film activated sludge (IFAS) reactor and the effect of COD/N ratio on process performance and microbial community structure of SNAD process. The nitrogen removal pathways in the suspended sludge and biofilm were investigated through measuring the activity of the biofilm and suspended sludge in batch tests. The microbial community structure and correlation analysis were used via Illumina MiSeq sequencing of the V3–V4 region of 16S rRNA gene in order to decipher the potential microbial interactions.

2. Materials and methods

2.1. SNAD-IFAS reactor setup and operation

A laboratory-scale IFAS reactor was used to implement SNAD process. The reactor was made of acrylic plastic with a total working volume of 8.0 L (Supporting Information (SI), Fig. S1). A 40% volume fraction of non-woven ring carriers (Wang et al., 2017a) with average density of 1.1 g cm^{-3} was employed in the SNAD-IFAS reactor. The raw material for manufacturing non-woven is polyester and the carrier-frame is made of polypropylene. A mechanical mixer installed in the reactor, and air was supplied from the bottom of the reactor, thus it can be considered as a type of homogenous reactor. A secondary clarifier was used for collecting activated sludge, and then the activated sludge returned to the system by pump. Sludge reflux ratio was set at 3.0.

The operating condition of the SNAD-IFAS reactor is displayed in Table 1. The influent flow rate (10.7 L d^{-1}) was controlled by a peristaltic pump to give a hydraulic retention time (HRT) of 0.75 d (Zekker et al., 2012; Wang et al., 2017a). The reactor was operated at an ambient temperature of 25 ± 2 °C. An air flow rate was applied in the reactor to supply oxygen for nitrifiers. The air was supplied intermittently with 1 min aerated phase and 10 min non-aerated phase each hour. The dissolved oxygen (DO) content was maintained at 0.4 mg L^{-1} (air flow rate: 2.0 L min^{-1}) during the aerated phase and at 0 mg L^{-1} during the non-aerated phase. The methodology of the aeration was adapted from Lackner and Horn (2013). The levels of dissolved oxygen (DO) and pH in the reactor were detected with a digital portable DO and pH meters (WTW, Multi 3430, Germany), respectively.

The SNAD-IFAS reactor was operated for a total of 180 days and reactor operation was divided into three stages (I, II, and III). The first stage (I) was start-up of the SNAD reactor from day 0 to day 60. Non-woven carriers were used in the reactor to form biofilm. The seed sludge was anammox sludge prepared from a laboratory-scale up-flow anaerobic sludge bed reactor (UASB, MLSS = 2.3 g L^{-1}, *Candidatus* Kuenenia stuttgartiensis, Dalian University of Technology), which had been used to enrich anammox bacteria for 2 years under ambient temperature. The partial nitrification sludge taken from a laboratory-scale continuous-flow stirred tank reactor (CSTR, MLSS = 2.7 g L^{-1}, Dalian University of Technology) for over 5 months under ambient temperature. The reactor was fed with synthetic wastewater to simulate pretreated mainstream wastewater with the COD/N ratio being approximately 0.5 according to

Table 1
Operational parameters of SNAD-IFAS reactor.

Period	Time (d)	pH	DO (mg/L)	HRT (d)	Temperature (°C)
I	0–60	7.2 ± 0.2	0.4 ± 0.1	0.75	25 ± 2
II	60–120	7.2 ± 0.2	0.4 ± 0.1	0.75	25 ± 2
III	120–180	7.2 ± 0.2	0.4 ± 0.1	0.75	25 ± 2