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Perspective

Analysis of key microbial community during the start-up of anaerobic ammonium oxidation process with paddy soil as inoculated sludge

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ABSTRACT

A sequencing batch reactor (SBR)-anaerobic ammonium oxidation (anammox) system was started up with the paddy soil as inoculated sludge. The key microbial community structure in the system along with the enrichment time was investigated by using molecular biology methods (*e.g.*, high-throughput 16S rRNA gene sequencing and quantitative PCR). Meanwhile, the influent and effluent water quality was continuously monitored during the whole start-up stage. The results showed that the microbial diversity decreased as the operation time initially and increased afterwards, and the microbial niches in the system were redistributed. The anammox bacterial community structure in the SBR-anammox system shifted during the enrichment, the most dominant anammox bacteria were *Candidatus Jettenia*. The maximum biomass of anammox bacteria achieved 1.68 × 10^9 copies/g dry sludge during the enrichment period, and the highest removal rate of TN achieved around 75%.

Introduction

Nitrogen removal has taken more and more attention in the field of wastewater treatment. The conventional biological nitrogen removal process has many disadvantages such as high energy and material consumptions. However, the discovery of anaerobic oxidation of ammonium (anammox) bacteria overcame these issues (Thamdrup and Dalsgaard, 2002; Dalsgaard et al., 2003; Kuypers et al., 2003). The anammox phenomenon was first observed in the denitrifying fluidized-bed reactor (Mulder et al., 1995), and anammox bacteria can oxidize ammonium to nitrogen gas (N_2) under anoxic conditions using nitrite as the electron acceptor. The autotrophic and anaerobic properties of microorganisms save a great deal of energy and organic carbon source consumption. The anammox bacteria are affiliated with phylum Planctomycetes (Jetten et al., 2010), and five genera of anammox bacteria have been reported, "*Candidatus Brocadia*", "*Candidatus Kuenenia*", "*Candidatus Scalindua*", "*Candidatus anammoxoglobus*" and "*Candidatus Jettenia*" (Schmid et al., 2005, 2007; Kartal et al., 2007, 2008).

In recent years, autotrophic denitrification processes based on anammox have been successfully applied in high-strength ammonia wastewater treatments (Jetten et al., 1997; van Dongen et al., 2001; López et al., 2008). It is very important for the start-up of the anammox process to choose suitable seed sludge for enrichment of anammox bacteria. Generally, the seed sludge

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derives from anaerobic sludge, nitrifying sludge or mixed sludge (Zhang et al., 2004; Zheng et al., 2004; Chen et al., 2011).

Anammox bacteria has been found in many ecosystems such as wastewater plant (Bae et al., 2010), river (Zhang et al., 2007; Wang et al., 2012), lake (Schubert et al., 2006), constructed wetland (Zhu et al., 2011a, 2011b), marine (Dalsgaard et al., 2003), paddy soil, and other natural habitats with extremely cold condition and poor nutrition (Jiang et al., 2015). It shows not only that these bacteria are widely distributed in the natural environment, but also that the anammox process has great contribution to the global nitrogen cycle flux. Previous researches indicated that anammox reaction contributed 50–70% to N_2 production in marine oxygen and 4–37% to N_2 production in paddy soil (Dalsgaard et al., 2003; Kuypers et al., 2003, 2005; Arrigo, 2005; Lam et al., 2007; Zhu et al., 2011a, 2011b).

Rice paddy fields make a great contribution to the nitrogen cycle in terrestrial ecosystems. The long-term fertilization of paddy soil has a high concentration of nitrogen and the soil surface is flooded seasonally, which provides a suitable growth environment for anammox bacteria. The previous report has shown that the abundance of anammox bacteria in the 40–50 cm depth of paddy soil reaches to 1.2×10^7 copies/g dry soil (Zhu et al., 2011a, 2011b). Present researches have focused on the distribution of anammox bacteria in paddy fields. However, few studies have involved the start-up of anammox process with the sludge in paddy soil and discussed the evolution of bacteria during this process.

Hence, the present study aimed at investigating the dynamic changes of key microbial community structure during the start-up of anammox process with paddy soil as seed sludge, and finally successfully starts up an anammox process for nitrogen removal from wastewater.

1. Materials and methods

1.1. Seed sludge

A long-term fertilization paddy field soil as the sampling site in Songjiang District of Shanghai in China was selected. The depth of the collected samples was 0 to 80 cm, and the samples were placed in a sterile plastic bag and brought back to the lab under low temperature conditions. One part of paddy soil samples was inoculated in the sequencing batch reactor (SBR) to start up the anammox process, and another part was stored at −80°C for the subsequent analysis.

1.2. The SBR-anammox system

The paddy soil was inoculated in an 8-L SBR reactor (effective volume, 6 L) for anammox system in this study (Fig. 1). The reactor was equipped with on-line detection devices for detecting some important parameters, such as temperature, pH, dissolved oxygen and oxidation reduction potential. The formulated wastewater was introduced from the bottom of the reactor *via* a peristaltic pump (BT100-2J, Longer, China). The stirring rate was about 65 r/min. The temperature was controlled at 35°C using a temperature controlling belt. The anammox process in the SBR was operated with 1-day cycle. Each cycle was composed of four phases: nitrogen aeration (20 min), continuous feeding period (20 min), anaerobic reaction period (20 hr), settling (3 hr) and withdrawal (20 min). The nitrogen aeration in the beginning of each cycle was to remove oxygen from the SBR-anammox reactor system. The pH in the influent was kept between 7.5 and 8.3. The entire reaction device is wrapped with aluminum foil to achieve the dark effect. The initial sludge concentration was 6000 mg/L.

1.3. Synthetic wastewater

The influent concentrations of NH_4Cl and $NaNO_2$ were gradually increased during the start-up of the anammox system, which the concentration of NH_4Cl increased from 15 to 60 mg/L, and the concentration of $NaNO_2$ gradually increased from 15 to 78 mg/L. The composition of the mineral medium was (g/L): $NaHCO_3$: 1.85; KH_2PO_4: 0.00625; $FeSO_4 \cdot 7H_2O$: 0.018; $EDTA \cdot 2H_2O$: 0.0125; $MgSO_4 \cdot 7H_2O$: 0.2; $CaCl_2 \cdot 2H_2O$: 0.3; and 1 mL/L of trace element solution. The trace element solution contained (g/L): $MnCl_2 \cdot 4H_2O$, 0.99; EDTA, 15; H_3BO_4, 0.014; $ZnSO_4 \cdot 7H_2O$, 0.43; $CoCl_2 \cdot 6H_2O$, 0.24; $NaMoO_4 \cdot 2H_2O$, 0.22; $CuSO_4 \cdot 5H_2O$, 0.25; $NiCl_2 \cdot 6H_2O$, 0.19; $NaSeO_4 \cdot 10H_2O$, 0.21; and $NaWO_4 \cdot 2H_2O$, 0.05 (Van de Graaf et al., 1996).

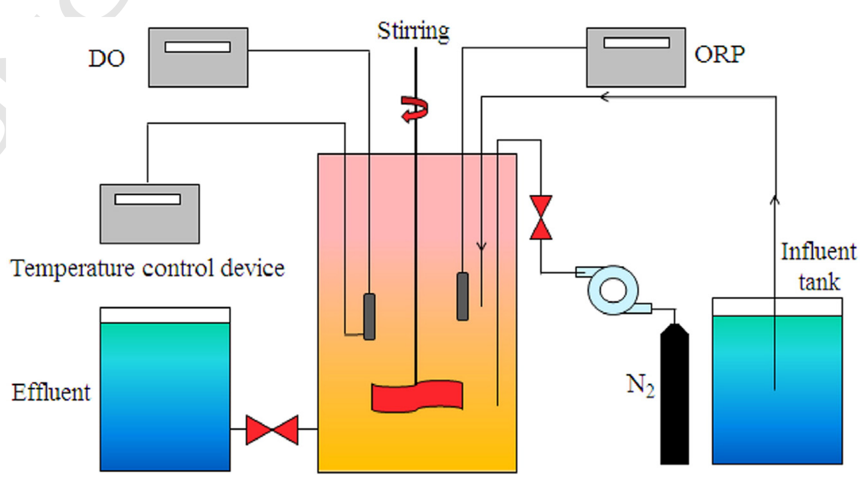

Fig. 1 – Schematic diagram of the reactor configuration. DO: dissolved oxygen; ORP: oxidation-reduction potential.

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