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Realization of microbial community stratification for single-stage nitrogen removal in a sequencing batch biofilter granular reactor

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HIGHLIGHTS

- Nitritation-anammox process successfully started up in SBBGR.
- The SNAP process performance was gorgeous with ammonium removal efficiency >90% and TN removal efficiency >80%.
- The observed specific microbial stratified distribution in SBBGR contributed to the co-existence of AOB and anammox.
- *Proteobacteria* and *Planctomycetes* were dominant functional organisms on the phylum level.

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ABSTRACT

A permanent microbial stratified nitrogen removal system coupling anammox with partial nitrification (SNAP) in a sequencing batch biofilter granular reactor (SBBGR) was successfully constructed for the treatment of ammonia-rich wastewater. With a nitrogen loading rate of 0.1 kg N $m^{-3} \cdot d^{-1}$, the maximal ammonia and total nitrogen removal efficiencies could reach up to 96.08% and 84.86% on day 108, respectively. The pH, DO profiles revealed a switch of functional species (AOB and anammox) at a typical intermittent aeration cycle. qPCR and high throughput analyses certified a stable spatial microbial stratified community structure. Although, anammox preferred strict anaerobic environment while AOB needed oxygen, a special stratified community structure contributed to conquer this obstacle. Moreover, *Bacteroidet*, *Chlorobi*, *OD1*, *Planctomycetes*, and *Proteobacteria* were the dominant species in the SBBGR. Although we have predicted the possible pathways of nitrogen transformation, further studies are needed to validate the pathways in enzymology.

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1. Introduction

Nitrogen removal from wastewater is extremely important to protect the aquatic environment because excess release of nitrogen into water could cause eutrophication and acidification problems. In traditional processes, nitrogenous compounds especially ammonia removal from waste water can be accomplished through the combination of sequential nitrification and denitrification processes. However, this process is seldom applied in cases with low values of carbon/ammonium ratios. For the treatment of these wastewaters, more external carbon is needed, and the costs become higher due to the oxygen and additional organic matter requirements. Thus, novel processes have been expected to develop to reduce these costs. Anaerobic ammonium oxidation (Anammox) process was a novel technology to remove ammonia from the waste water with nitrite as an electron acceptor devoid of organic carbon. In the early 1990s, the anammox was first discovered in a denitrifying fluidized bed reactor. Since then, novel processes and technologies based on anammox have been boomed and investigated (Meng et al., 2014). The recognized bio-chemical reaction of the anammox described as follows (Eq. (1)).

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (1)$$

Nitrite is absent from ammonia-rich wastewaters, which is the basic requirement of anammox process. To fulfill the requirements of anammox process, one more step should be included to produce the nitrite. Normally, the nitrite can be obtained from partial nitrification (Eq. (2)) (Ma et al., 2013) and partial denitrification (Cao et al., 2016) (Eq. (3), glucose as the electron acceptor). Thus, different processes based on anammox such as SHARON-ANAMMOX process (Okabe et al., 2011), CANON (completely autotrophic

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nitrogen removal over nitrite) process (Cho et al., 2011), SNAP (single-stage nitrogen removal using anammox and partial nitrification) process (Zhang et al., 2014) or heterotrophic DEAMOX (a strategy combining anammox with partial denitrification) process (Cao et al., 2016) have been developed for ammonia removal.

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O \tag{2}$$

$$CH_3COO^- + 4NO_3^- \rightarrow 2H_2O + 2CO_2 + 4NO_2^- + H^+ \tag{3}$$

In these processes, due to the slow growth rate of anammox bacteria (Strous et al., 1999), the most challenging task is to retain biomass in the reactor and the suppression of NOB growth. Up till now, several methods have been adopted to immobilize the organisms. For example, biomass carriers such as ceramics (Ren et al., 2014) and polyurethane spheres (Daverey et al., 2013) as well as fixed-biofilm (Zhang et al., 2015) have been proved to retain the organisms in the reactor efficiently. On the other hand, NOB will compete for nitrite with anammox, and decrease the total nitrogen (TN) removal efficiency. Therefore effective control strategy must be taken to regulate the amount and activity of NOB. Because NOB has lower affinity for oxygen than AOB (Guisasola et al., 2005), NOB will be washed out under limited DO condition. Previous studies showed that AOB dominated over NOB under DO concentration below 1.0 mg/L (Sinha and Annachhatre, 2007). Meanwhile, the DO concentration would influence the activity of anammox. Reversible and irreversible anammox inhibition have been observed at the DO concentration of 0.08 mg/L and 1.44 mg/L, respectively (Egli et al., 2001). Thus, DO concentration is another crucial parameter and the drastic changing in this parameter should be avoided to keep the system in balanced form. To overcome these complications, new methods such as partial nitrification-anammox granular technology have been developed (Ali et al., 2016). In the established granular model, AOB was gathered in the outer layer while anammox bacteria appeared to be the dominant species in the core space. This spatial distribution structure was corresponding to the oxygen concentration gradient in the granules. The anaerobic environment in the granule core can protect the anammox against the oxygen inhibition. And at the same time, the good settleability of nitration-anammox granular can decrease the loss of biomass to the greatest extent. Rodriguez-Sanchez et al. (2016) established a single-stage partial nitrification/anammox granular sludge bioreactor successfully to remove nitrogen at low temperature. What is more, sequencing batch biofilter granular reactor (SBBGR), as an innovative technology, combining all the advantages of a granular SBR based on a submerged bio-filter operating in a batch mode, was successfully applied in the treatment of textile waste water (Van de Graf et al., 1996). In SBBGR, the biomass is confined in the bed of the system, thus allows better sludge retention in the reactor. Moreover, the substrate and oxygen were dissolved in liquid phase, which passed through bioreaction bed to be supplied to the organisms. Due to the oxygen consumption of partial nitrification process, certain anaerobic areas were expected in SBBGR, which were suitable for the growth of anammox. Thus, aerobic and anaerobic areas formed separately in SBBGR by controlling the DO concentration, which would benefit the enrichment of anammox and AOB separately. However, to the best of our knowledge, there was no published research about the realization of SNAP process in SBBGR based on spatial microbial diversity in different zones of SBBGR.

In this study, to retain more bacteria and provide a suitable environment for coexistence of AOB and anammox bacteria, a single-stage partial nitrification-anammox process using SBBGR in an intermittent aeration model for the treatment of ammonia-rich rejected water was constructed. It is expected that the spatial microbial partition of SBBGR will contribute to the co-work of AOB and anammox, with a better nitrogen removal performance. The key objectives of this study were to fulfill the feasibility of autotrophic nitrogen removal in the SBBGR system for the treatment of ammonia-rich wastewater, to understand the effects of intermittent aeration on the activities of dominant species and what is more important, to verify the microbial spatial distribution and stratified structure of important nitrogen-cycle shareholders in SBBGR.

2. Materials and methods

2.1. SBBGR setup and operation

Fig. 1 shows the schematic diagram of the lab-scale SBBGR system. The reactor was made of plexiglas materials which have an effective volume of 5 L with an internal diameter of 0.1 m. The temperature was controlled at 31 ± 1 °C by a water bath with water recirculation through the outer chamber. The surface of the reactor was wrapped by silver papers to prevent the underlying growth of phototrophic organisms.

The reactor was divided into two halves, part A (aeration and mixture of waste water) and part B (bio-reaction bed). A constant air-flow rate was kept in the part A, while nutrients were supplied from the bottom by a peristaltic pump and distributed through a multihole diffuser. An external loop via re-circulation pump guaranteed that the homogeneous liquid in part A can pass through part B steadily. The bio-reaction bed was filled with compacted polyethylene media (680–710 m^2/m^3 of specific surface, 0.675 of porosity) which were hold by a sieve at the bottom of the reactor. Two multihole diffusers were installed on the top and bottom of the bio-reaction bed to immobilize the media. A gravel layer (3 cm thick) was lying under the bottom of the bio-reaction bed, and an inflow tube was buried in it. The system was controlled by programmable logic controller (PLC) automatically, and its

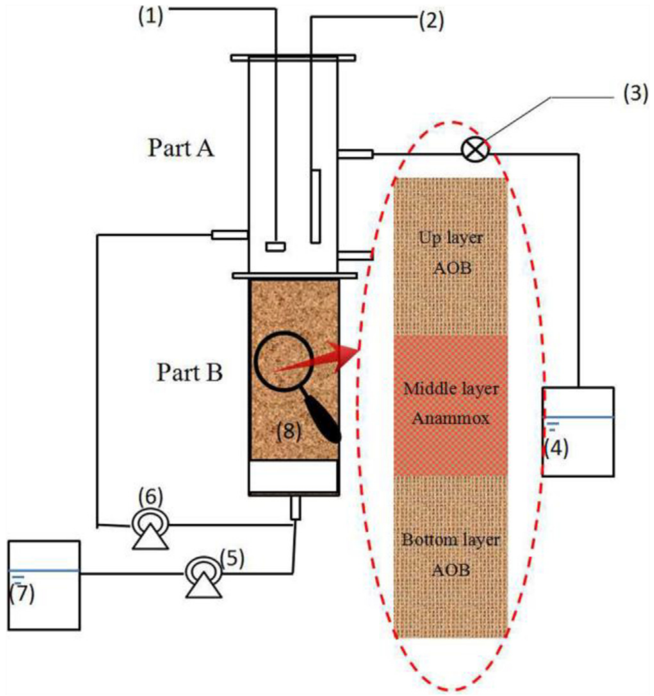

Fig. 1. Schematic diagram of SBBGR system. (1) Air compressor (2) pH, DO online minotor (3) electromagnetic valve (4) effluent tank (5) influent peristaltic pump (6) recycle pump (7) influent tank (8) polyethylene filter particles.

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