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High-rate nitrogen removal and microbial community of an up-flow anammox reactor with ceramics as biomass carrier

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HIGHLIGHTS

- A NLR and NRR of 1.16 g N L^{-1} d^{-1}, 1.0 g N L^{-1} d^{-1} were achieved at HRT 1.5 h.
- The DO had little influence on nitrogen removal efficiency even it was 1.0 mg L^{-1}.
- With the decrease in HRT, an enhanced for TN removal performance was achieved.
- *Candidatus Jettenia asiatica* showed high anammox activity even at 20 ± 2 °C.

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ABSTRACT

Nitrogen removal performance and responsible microbial community of anammox process at low temperatures, and long term effect of dissolved oxygen (DO) on the performance of anammox process were investigated in a biofilm reactor, which was operated at 33 ± 1 °C (159 d) and 20 ± 2 °C (162 d) with an influent DO concentration of 0.7–1.5 mg L^{-1}. Nitrogen removal recovered to 70% after 2 wk with the temperature drastically decreasing from 33 ± 1 °C to 20 ± 2 °C. At 20 ± 2 °C, the average effluent (NH_4^+–N + NO_2^-–N) concentration was 0.08 ± 0.08 mg L^{-1} at a hydraulic retention time of 1.5 h. A total nitrogen removal efficiency of the reactor of 1.0 g N L^{-1} d^{-1} was obtained for up to one month while the nitrogen loading rate was 1.16 g N L^{-1} d^{-1}. Results of T-RFLP and 16S rRNA phylogenic analysis revealed that *Candidatus Jettenia asiatica*, as confirmed to adapt to low temperature, was considered to be responsible for the stable and high nitrogen removal performance.

1. Introduction

Conventional wastewater treatment systems for nitrogen removal require a large amount of energy to create aerobic conditions for biological nitrification, and also use external organic carbon to remove nitrate by biological denitrification, therefore an economical ammonium removal process should be required. The partial nitritation–anammox system, compares to the conventional nitrification/denitrification, avoids the requirement of organic carbon source to denitrify, allows saving over 65% of the oxygen supply and produces a lower amount of sludge (Fux et al., 2002). Partial nitritation is accomplished as pre-treatment in an aerobic reactor where 50% of ammonia is oxidized into nitrite and its effluent is used as the influent for subsequence anammox process. While, the DO concentration was commonly controlled in a range of 0.4–1.3 mg L^{-1} in the partial nitritation reactor (Chuang et al., 2007; Tang et al., 2011; Zheng et al., 2012), which would lead to the inhibition of the activity of anammox bacteria caused by the oxygen existing in the effluent of the partial nitritation reactor. Moreover, it requires high cost for maintaining absolutely anaerobic condition. Therefore, the possibility and stability of cultivating anammox consortium in a reactor under DO conditions (0.4–1.3 mg L^{-1}) should be evaluated for the application of anammox process at low temperatures. While the difficulties could be overcome by using the biofilm reactor because a layer of biofilm with various bacterial species and steady bacterial community structure and function could form on the surface of the filling in the reactor (Xiao et al., 2009). Biofilms have been shown to be a simpler and more effective method of immobilizing anammox bacteria on account of their improved ability to retain biomass within the reactor systems compared to suspended sludge reactors and the large contribution of the biofilm towards total reactor activity (De Clippeleir et al., 2011; Hendrickx et al., 2012; Tao et al., 2012).

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The anammox process was successfully used to treat waste waters with high ammonia concentration (>500 mg N L^{-1}) and high temperatures (30–40 °C), such as landfill leachate, and the supernatant of digested sludge (Szatkowska et al., 2007; Van der Star et al., 2007). Due to the very low growth rate (0.003 h^{-1}) (Jetten et al., 1999) and high optimum temperature (30–40 °C) (Strous et al., 1999; Isaka et al., 2007) of anammox bacteria, the main challenge for applying anammox in municipal waste water treatment is to achieve a high rate process with good biomass retention and a low effluent nitrogen concentration (Hendrickx et al., 2012). The application of anammox processes at low temperatures (⩽20 °C) and low nitrogen concentration (<100 mg N L^{-1}) has also been a challenging problem.

The purpose of this study was to investigate the feasibility of applying the anammox process as the temperature drastically decreased from 33 ± 1 °C to 20 ± 2 °C. Meanwhile, the nitrogen removal performance was studied at 20 ± 2 °C and low nitrogen concentration by decreasing the hydraulic retention time (HRT) in the biofilm anammox reactor, of which the influent contained some DO. T-RFLP and phylogenic analysis on the 16S rRNA gene were applied to analyze the microbial community structure in this anammox reactor.

2. Materials and methods

2.1. Experimental set-up and operation strategy

An up-flow column reactor with an apparent gross volume of 3.8 L (id: 100 mm) and an effective volume of 1.5 L was used (Fig. 1), in which Shale Ceramics-grain (biocarriers) of 1–5 mm diameter were filled to strengthen biomass retention. At the bottom part of reactor, there was a supporting layer of cobblestone (diameter: 4–6 cm). The reactor was operated in the up-flow mode, whose influent was introduced at the bottom using a peristaltic pump (BT100-2J, Longer, China) and equipped with a black vinyl sheet enclosure to inhibit the growth of photosynthetic bacteria as well as the reduction of the anammox growth rate by light (Zhang et al., 2010). The temperature of the reactor was maintained at 33 ± 1 °C during initial 159 d and then at 20 ± 2 °C by using a water bath connected to the double reactor wall. For identification of microbial communities in the reactor, biomass samples were taken from the lowest sampling port of the reactor. Although biofilm samples could not be taken from the filter by using the sampling ports, it was assumed that biofilms sloughed off from the filter carriers were also present in the sample. Thus, both attached (biofilm) and non-attached (floc) microorganisms in the filter could be sufficiently represented by using the sample (Calli et al., 2006).

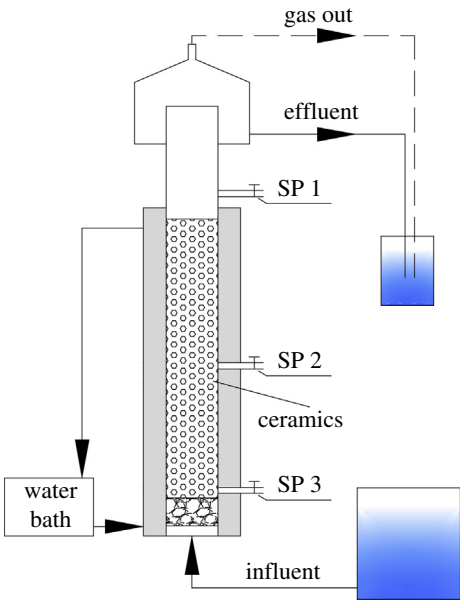

Fig. 1. Diagram of up-flow anammox column reactor in this study.

2.2. Seed sludge and feeding media

The reactor was inoculated with 1.6 L nitrifying sludge (1.7 g SS L^{-1}) from a WWTP located in Harbin, China (45°47′24″N, 126°38′37″E) which treats about 3.25×10^8 m^3 d^{-1} domestic wastewater. During the implement of the experiment, two kinds of substrates were used. Tap water containing $NaNO_3$ (~70 mg N L^{-1}) and 0.25 L L^{-1} of fresh domestic sewage were used until the nitrate removal efficiency was maintained at 90% for 18 d. The influent was then switched to synthetic wastewater (in mg L^{-1}) $(NH_4)_2SO_4$ 30–135, $NaNO_2$ 35–150, $KHCO_3$ 500, KH_2PO_4 27.2, $MgSO_4{\cdot}7H_2O$ 300, $CaCl_2$ 136, $FeSO_4{\cdot}7H_2O$ 9, EDTA 5, trace element solution (in mg L^{-1}) 1 mL L^{-1} (EDTA 15000, $CuSO_4{\cdot}5H_2O$ 250, $ZnSO_4{\cdot}7H_2O$ 430, $CoCl_2{\cdot}6H_2O$ 240, $MnCl_2{\cdot}4H_2O$ 990, $NaMoO_4{\cdot}2H_2O$ 220, $NiCl_2{\cdot}6H_2O$ 190, $NaSeO_4{\cdot}10H_2O$ 210, H_3BO_3 14) as prepared according to the previous literature (Van de Graaf et al., 1996). The latter substrate was flushed with N_2 to maintain DO concentration in the feed between 0.7–1.5 mg L^{-1} and the pH value was adjusted to 7.4–8.0 by 1 M HCl and 1 M Na_2CO_3 before providing to the reactor.

2.3. Analytical methods

The concentrations of nitrogen compounds were determined spectrophotometrically according to the Standard Methods (APHA, 1998). The pH, DO values were determined using a pH meter (inoLab Terminal 740, WTW, Germany), a DO meter (inoLab Oxi 730, WTW, Germany), respectively. Reactor temperature (Pt100) were measured and recorded.

2.4. DNA extraction and PCR amplification

DNA was extracted from the biomass samples taken at day 286 using Power Soil DNA Isolation Kit (MoBio, USA) according to the manufacturer's instructions. The DNA concentration was determined on a NanoDrop 2000 Spectrophotometer (Thermo Scientific, DE). 16S rRNA gene fragments of domain bacteria were PCR-amplified with bacterial primer set 27f (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492r (5′-GGT TAC CTT GTT ACG ACT T-3′) (Weisburg et al., 1991), using a thermo cycling program consisting of 5 min of denaturation at 95 °C and 30 cycles of 45 s at 95 °C, 45 s at 55 °C and 1 min at 72 °C, followed by a final extension step at 72 °C for 10 min. PCR reaction was performed using the C1000 Thermal Cycler (Bio-Rad, USA). The amplified products were electrophoresed in 1.5% (w/v) agarose gel and purified by EasyPure Quick Gel Extraction Kit (TransGen, China).

2.5. Cloning and sequencing of the 16S rRNA gene and phylogenetic analysis

Purified PCR products were ligated into pMD18-T vectors (TaKaRa, Japan) and transformed into competent Escherichia coli DH5α (TaKaRa, Japan), as described in the manufacturer's protocol. Nucleotide sequencing was performed with an ABI 3730XL Genetic Analyzer (Applied Biosystems, USA). All sequences (ca. 1505 bp) were compared with similar sequences of the reference organisms by BLAST search (Altschul et al., 1990) and submitted to GenBank