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Start-up and bacterial communities of single-stage nitrogen removal using anammox and partial nitritation (SNAP) for treatment of high strength ammonia wastewater

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HIGHLIGHTS

- The start-up the SNAP process was achieved from dewatered surplus activated sludge.
- High rate TN removal around 0.54 kg N m^{-3} d^{-1} was achieved in a single reactor.
- 16S rDNA amplicon pyrosequencing showed presence of AOB and AnAOB.
- Quantitative analysis of dominant bacteria groups arrangement in reactor.

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ABSTRACT

In this study, a lab-scale sequencing batch biofilm reactor (SBBR) was used to start up the single-stage nitrogen removal system using anammox and partial nitritation (SNAP) process seeding from surplus activated sludge. The volumetric nitrogen loading rate (vNLR) was firstly 0.075 kg N m^{-3} d^{-1} and then gradually increased to 0.60 kg N m^{-3} d^{-1}. A maximal total nitrogen (TN) removal rate of 0.54 kg N m^{-3} d^{-1} was achieved by the SNAP process after 132 days operation with NH_4^+-N and TN removal efficiency of 99.4% and 90.5%, respectively. This reactor may have applications for the SNAP process treating high strength ammonia wastewater. And dewatered surplus activated sludge was recommended as the seed sludge for engineering applications. The dominant bacterial strains were *Xanthomonas campestris*, *Nitrosomonas europaea* and *Ignavibacterium album*, corresponding to the percentage of 24%, 22% and 20%, respectively, based on the 16S rDNA amplicon pyrosequencing of the SNAP sludge.

1. Introduction

Ammonium pollution, which can cause eutrophication and be toxic to aquatic species, is becoming a serious environmental problem (Liu and Diamond, 2005). In a traditional ammonium wastewater treatment process, ammonium (NH_4^+-N) is converted to nitrogen gas via a two-step process starting with nitrification, which is the aerobic oxidation of NH_4^+-N to nitrite (NO_2^--N) to nitrate (NO_3^--N), followed by heterotrophic denitrification under anaerobic condition. While extra organic carbon is required to achieve complete denitrification (Kartal et al., 2010), which not only makes full-scale denitrification quite expensive but also causes secondary pollution, limiting its application in low C/N wastewater treatment. Thus, the anaerobic ammonium oxidation (ANAMMOX) had been recognized as a promising process to treat wastewater devoid of organic carbon (Pynaert et al., 2003; Strous et al., 1999a, 2006). Generally, major nitrogen compound in wastewater was ammonium, which must be nitrified partially to nitrite, but not to nitrate in the anammox process. Then the remaining ammonium together with the produced nitrite was converted to dinitrogen gas. This partial nitritation and anammox process can be performed in two-stage reactors such as the SHARON–ANAMMOX process (Okabe et al., 2011; van Dongen et al., 2001) or in a single-stage reactor such as CANON (completely autotrophic nitrogen removal over nitrite) process (Cho et al., 2011; Third et al., 2001) or SNAP (single-stage nitrogen removal using anammox and partial nitritation) process (Helmer et al., 2000; Lieu et al., 2005). In case that the entire nitrogen could be removed in one single reactor without organic carbon, it would greatly reduce

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investment costs and energy consumption compared with the traditional nitrification–denitrification process even if with the SHARON–ANAMMOX process.

One of the limitations about CANON process was the long system construction period, especially in the absence of inoculum source anammox bacteria. The nitrite concentration during the system startup period had a negative effect on the growth of anammox strains. Too low concentration of nitrite would lead to substrate limitation and low growth rate of anammox bacteria. While concentration of nitrite at levels above 50–150 mg N L^{-1} could be toxic to anammox organisms, and stops the process completely (Strous et al., 1999b).

Usually, there were two ways to start up the CANON process. Firstly, in the first phase, the anammox process was started up by training bacteria with NH_4^+/NO_2^- mole ratio of 1:1 in influent or strictly inoculating anammox bacteria from an anammox reactor (Qiao et al., 2012; Sliekers et al., 2002; Yamamoto et al., 2011). In the second phase, inoculated with aerobic nitrifying sludge after the anammox process was achieved (Chen et al., 2009). Then the partial nitritation and anammox was achieved in one unit process by limited aeration. Secondly, a steady partial nitritation was achieved by the strategy of limited aeration or inoculating enriched AOB (ammonia oxidizing bacteria) (Sri Shalini and Joseph, 2012). Pre-cultured nitrifying sludge in the reactor can protect anammox bacteria from O_2 inhibition. After the NH_4^+/NO_2^- mole ratio of 1:1 in effluent was obtained steadily, settled biomass from an anammox enrichment reactor was added into the reactor to complete the system construction of single-stage autotrophic nitrogen removal (Liu et al., 2008; Van der Star et al., 2007).

In this study, the combined anammox and partial nitritation reaction process was developed in a single-stage sequencing batch biofilm reactor, which was originally started as SNAP process. The anammox and partial nitritation were established simultaneously by controlling the aeration rate carefully using low activity dewatered surplus activated sludge as seed sludge. The process performance was examined. Additionally, the microbial community structure and bacterial community composition in the SNAP process was analyzed by the denaturant gel gradient electrophoresis (DGGE) and 16S rDNA amplicon pyrosequencing.

2. Methods

2.1. Reactor and operational strategy

The SBBR (sequencing batch biofilm reactor) was a plexiglas cylinder, the height of which was 500 mm and internal diameter 200 mm, with height to diameter ratio being 2.5. Semi-soft fibre fill was used as the biomass carrier and the packing rate was 50% (V/V). The reactor had a working volume of 10 L feeding with artificial wastewater. During the experiment period, the reactor was placed in a thermostatic chamber, in order to maintain the temperature constant at 30 ± 1 °C.

To start up the SNAP process, low activity dewatered surplus activated sludge was used as inoculated sludge. The SBBR was operated sequentially in 8 h-cycle, with intermittent aeration (aeration 4 h/aeration stop 4 h). Discharging and feeding were carried out during the last 10 min of each 3 cycles (24 h) and the water filling ratio was 0.25. The aeration was controlled using air pumps to regulate the DO concentration of the reactor. Initially, the SBBR was run at limited aeration stage, with the concentration of DO strictly controlled in the range of 0.8–1.2 mg L^{-1} by adjusting the air flow rate. At the aeration stop stage, the concentration of DO was at 0.1–0.2 mg L^{-1}.

The composition of the synthetic inorganic media was as follows (/L), NH_4HCO_3: 1700–13,540 mg; KH_2PO_4: 25 mg; EDTA: 25 mg; $FeSO_4$: 6.25 mg; $MgSO_4{\cdot}7H_2O$: 200 mg; $CaCl_2$: 300 mg; trace nutrient solution: 1.25 mL and the right amount of $KHCO_3$ to regulate pH to 8.0. The trace nutrient solution contained (g/L), $ZnSO_4{\cdot}7H_2O$: 0.43; $CoCl_2{\cdot}6H_2O$: 0.24; $MnCl_2{\cdot}4H_2O$: 0.99; $CuSO_4{\cdot}5H_2O$: 0.25; $Na_2MoO_4{\cdot}2H_2O$: 0.22; $NiCl_2{\cdot}6H_2O$: 0.19; H_3BO_4: 0.014; $Na_2WO_4{\cdot}2H_2O$: 0.05.

The feeding solution concentration and air flow rate increased gradually when the effluent ammonia concentration was lower than 20 mg/L and the TN removal rate was higher than 90%. The strategy of limited aeration was adopted to inhibit (nitrite oxidizing bacteria) NOB activity and prompt (anaerobic ammonium oxidation bacteria) AnAOB proliferation, as well as achieving a simultaneous growth of autotrophic AOB and AnAOB simultaneous growth.

2.2. Analysis methods

Liquid samples were taken every day. The concentrations of NH_4^+-N, NO_2^--N, NO_3^--N, TN and MLSS were measured according to standard methods for the examination of water and wastewater (APHA, 2005). The system was equipped with suitable submerged probes, such as dissolved oxygen (DO) (Hach, HQ30d, USA), pH (Hach, sension2, USA) and oxidation reduction potential (ORP) (Hach, sension2, USA).

2.3. Microbial examination

2.3.1. DNA extraction and PCR-DGGE

The sludge sample of the SNAP process was collected on day 132. For all samples in this study, DNA of sludge samples were extracted with a bead beater and three freeze–thaw cycles in boiling water and liquid nitrogen (Miller et al., 1999). Finally, detect the extraction effect by 0.8% agarose gel electrophoresis.

Before amplicon pyrosequencing, the DNA of each sample was amplified with a set of primers targeting the hypervariable V3 region of the 16S rDNA gene. The samples were subjected to PCR using universal primers targeting all bacteria: F357GC (5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG-3′) and 518 (5′-ATT ACC GCG GCT GCT GG-3′) (Muyzer et al., 1993). The PCR condition was performed using an initial denaturation step at 94 °C for 5 min and 30 cycles of denaturing (45 s at 94 °C), annealing (45 s at 60 °C) and extension (90 s at 72 °C), and the final extension for 10 min at 72 °C. The PCR products were verified by agarose gel electrophoresis (2.0% agarose, 1× Tris–acetate–EDTA (TAE)) followed by ethidium bromide staining to confirm the product size.

PCR products were analyzed by DGGE with D-code universal mutation detection system (Bio-Rad Laboratories, USA). Twenty-five microliters of each PCR product were loaded into 8% (w/v) polyacrylamide gel (containing 37.5:1 of acrylamide to bis-acrylamide) with a linear denaturant gradient ranging from 37.5% to 55% (of urea, w/v and formamide, v/v). The electrophoresis was performed at 60 °C, initially at 200 V (10 min) and then at 80 V (900 min). After the electrophoresis, the gel was stained for 25 min with ethidium bromide and immediately photographed under UV transillumination using Bio-Rad Versa Doc.

Gel images were analyzed using Quantitation Software Version 4.6.2 (Bio-Rad Laboratories, USA). DNA bands were automatically detected and the similarities in band patterns were measure as Dice coefficients (unweighted data based on band presence or absence).

2.3.2. 16S rDNA amplicon pyrosequencing

After the DNA samples were extracted, a sample quality was tested first. Then library was constructed using those qualified DNA samples: PCR amplified with fusion primer, the short