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N_2O emission from nitrogen removal via nitrite in oxic-anoxic granular sludge sequencing batch reactor

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ABSTRACT

Bionitrification is considered to be a potential source of nitrous oxide (N_2O) emissions, which are produced as a by-product during the nitrogen removal process. To investigate the production of N_2O during the process of nitrogen removal via nitrite, a granular sludge was studied using a lab-scale sequence batch reactor operated with real-time control. The total production of N_2O generated during the nitrification and denitrification processes were 1.724 mg/L and 0.125 mg/L, respectively, demonstrating that N_2O is produced during both processes, with the nitrification phase generating larger amount. In addition, due to the N_2O-N mass/oxidized ammonia mass ratio, it can be concluded that nitrite accumulation has a positive influence on N_2O emissions. Results obtained from PCR-DGGE analysis demonstrate that a specific *Nitrosomonas* microorganism is related to N_2O emission.

Introduction

Nitrous oxide (N_2O) is one of the most potent greenhouse gases, with an efficient absorption of infrared radiation, and an atmospheric lifetime of approximately 120 years. The global warming equimolar concentration of N_2O is 310 times that of carbon dioxide, and 23 times methane (IPCC, 2001). It has been demonstrated that N_2O is produced during the nitrogen removal processes. Consequently, wastewater treatment plants are considered to be a potential N_2O source (Okabe et al., 2011; Wunderlin et al., 2012). Therefore, it is necessary to reduce and control N_2O emissions from wastewater treatment plants.

It has been demonstrated that nitrogen removal is performed by different groups of microorganisms, including ammonium-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB) and denitrifying microbial communities. Fluorescence *in situ* hybridization (FISH) and PCR-denaturing gradient gel electrophoresis (PCR-DGGE) analysis demonstrated that AOB are the dominant nitrifying bacteria (Yang et al., 2009; Colliver and Stephenson, 2000), with *Nitrosomonas europaea* and *Alcaligenes faecalis* being the typical microorganisms associated with N_2O emission during the nitrogen removal process (Otte et al., 1996; Poth and Focht, 1985; Jiang and Bakken, 1999). Nitrifier denitrification, where nitrite is reduced to N_2O or N_2, was reported to be an important source of N_2O production (Bock et al., 1995).

Aerobic granulation process is becoming a promising technology in wastewater treatment. But previous studies focused on N_2O production usually associated with flocculent sludge, there is little research investigating N_2O production associated with granular sludge, especially in the oxic-anoxic operating environments of sequencing batch reactors (SBRs). Therefore, this study aimed to assess the N_2O production caused by nitrogen removal in relation to well-cultivated granular sludge. This was

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achieved using a real-time control system, which enabled the identification of the relationship between N_2O emission and the ability to optimize microbial communities population using a real-time control system. The findings are useful in providing a theoretical basis for practical application in the future.

1 Materials and methods

1.1 Reactor setup and operation

The SBR with the working volume of 3.2 L was operated at 31 ± 0.5°C. The side face of the reactor was twisted with heating wire, and covered with asbestos cloth to maintain the water temperature in the reactor. Probes for monitoring dissolved oxygen (DO), pH and temperature, were inserted into the reactor from the side face. The reactor was operated under real-time control conditions for 2 cycles per day. Each cycle of SBR consisted of five steps: feeding (1 min), aeration (180 min), anoxic (40 min), settling (30 min), decant (20 min).

1.2 Inoculation

The seeding sludge was a well-cultivated granular sludge with an high removal efficiency of nitrogen and chemical oxygen demand (COD) and enhanced nitrite accumulation at the end of each cycle. The mixed liquor suspended solids (MLSSs) were maintained at approximately 2800 to 3000 mg/L. Any excess granular sludge was disposed of at the end of the anoxic detrification process to maintain a solid retention time (SRT) of approximately 25 days.

1.3 Synthetic feed

A volume of 2.6 L of synthetic wastewater was fed into the SBR at the beginning of each cycle. Each liter of influent contained: 300 ± 15 mg COD; 30 ± 2 mg NH_4^+-N; 4.0 ± 0.5 mg PO_4^{3-}-P and 1.0 mL nutrient solution. One liter of nutrient solution contained: 1.5 g $FeCl_3{\cdot}6H_2O$; 0.15 g H_3BO_3; 0.03 g $CuSO_4{\cdot}5H_2O$; 0.18 g KI; 10 g EDTA; 0.12 g $MnCl_2{\cdot}4H_2O$; 0.12 g $ZnSO_4{\cdot}7H_2O$; and 0.15 g $CoCl_2{\cdot}6H_2O$. $NaHCO_3$ was added, with the pH maintained at 7.3–7.8.

1.4 Analytical methods

During the nitrogen removal process, DO and pH levels were monitored each minute and recorded by the inserted probes (WTW 340i, WTW Company, Germany) frequency. The concentrations of N-containing ions (NH_4^+-N, NO_2^--N, and NO_3^--N), and the COD and MLSS were measured according to standard methods (APHA, 2005).

The total amount of N_2O produced consists of the N_2O emitted from the system (the emission-gas N_2O) and the amount dissolved in the solution (the dissolved N_2O). The N_2O samples emitted were collected from the SBR headspace at 1 hr intervals during the aerobic-phase, and 20 min intervals during the anoxic-phase using a gas-tight, 100 μL syringe with a lock. These samples were then injected into a GC unit (Shimadzu 2010, Japan) for analysis. The GC unit was installed with an electron capture detector (ECD), and Porapak Q column (30 m × 0.53 mm × 20 μm), using high purity nitrogen as the carrier gas. The temperatures of the injector, ECD and column were 150, 300 and 70°C, respectively. All samples were analyzed in triplicate. The dissolved N_2O samples were obtained simultaneously with the emitted samples during aerobic- and anoxic-phases from the mixed liquid in the SBR reactor. The dissolved N_2O was measured according to the headspace method, described by Shiskowski (2007).

The concentrations of N_2O emissions (from both the aerobic and anoxic phase) were determined according to Kimochi et al. (1998). The N_2O emission rate was calculated according to Liu et al. (2008).

The bacterial biomass sampled for PCR analysis was collected from the reactor set to oxic/anoxic operating mode. The primers used for the nested PCR included a reverse universal primer 518, supplied by Shanghai Songon Biology Engineering Technology and Services Co. Ltd. (China) and a bacteria specific forward primer. The forward primers used were the AOB specific primers (CTO189f and CTO654r), used to amplify bacterial 16S rDNA, and the GC-338 primer underlined below (primer 338 plus a GC clamp attached at its 57 end). The nucleotide sequences of the primers were as follows: CTO189f and CTO654r; primer GC-BSF; and primer 518. The genomic DNA extraction and PCR conditions used were according to the literature (Kowalchuk et al., 1998), with a slight deviation in the annealing temperature of the touch down PCR, which was set to 55°C.

2 Results and discussion

2.1 N_2O production during nitrogen removal via nitrite

The experiment was conducted using a SBR in oxic-anoxic operating mode. In a typical cycle, the total production of N_2O reached 1.724 mg/L during oxic nitrification, with 0.125 mg/L of N_2O produced during anoxic denitrification, which is different with the results of flocculent sludge (Liu et al., 2008). They found that the N_2O production during nitrification was 1.85 mg/L, whereas during denitrification no N_2O was produced. But our results demonstrate that N_2O is produced during both the nitrification and denitrification processes, with the greatest amount generated during oxic nitrification process.

As shown in **Fig. 1a**, during the initial stage of the oxic-phase (the initial 15 min), the concentration of DO was observed to decrease rapidly and then rebound sharply.