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Nitrous oxide emission in low-oxygen simultaneous nitrification and denitrification process: Sources and mechanisms

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ABSTRACT

This study attempts to elucidate the emission sources and mechanisms of nitrous oxide (N_2O) during simultaneous nitrification and denitrification (SND) process under oxygen-limiting condition. The results indicated that N_2O emitted during low-oxygen SND process was 0.8 ± 0.1 mg N/gMLSS, accounting for 7.7% of the nitrogen input. This was much higher than the reported results from conventional nitrification and denitrification processes. Batch experiments revealed that nitrifier denitrification was attributed as the dominant source of N_2O production. This could be well explained by the change of ammonia-oxidizing bacteria (AOB) community caused by the low-oxygen condition. It was observed that during the low-oxygen SND process, AOB species capable of denitrification, i.e., *Nitrosomonas europaea* and *Nitrosomonas*-like, were enriched whilst the composition of denitrifiers was only slightly affected. N_2O emission by heterotrophic denitrification was considered to be limited by the presence of oxygen and unavailability of carbon source.

1. Introduction

Nitrous oxide (N_2O) is considered as one of the critical greenhouse gas and the dominant ozone-depleting substance emitted in the 21st century (IPCC, 2007). Thus, the control of its emission has attracted increasingly more attentions over the past decade. It is generally accepted that biological wastewater treatment processes, especially those for enhanced nutrient removal, occupy an important position among the many sources of N_2O emission (Kampschreur et al., 2009; Foley et al., 2010). Recently, simultaneous nitrification–denitrification (SND) process under low oxygen condition has emerged as a promising process, due to its high nutrient removal efficiency and low energy consumption (Holman and Wareham, 2005; Liu et al., 2010; Hocaoglu et al., 2011). However, it was reported that a significant amount of N_2O may be produced during this process (Meyer et al., 2005).

Great efforts have been made to investigate N_2O emission during low-oxygen SND process. However, previous literatures mainly focused on the emission quantity (Zeng et al., 2003; Meyer et al., 2005) and influence factors such as electron acceptor (Lemaire et al., 2006), carbon source (Zeng et al., 2003; Zhu and Chen, 2011), and metal ion (Zhu and Chen, 2011). The sources and mechanisms of N_2O emission have not been seriously explored and remained unclear.

Although N_2O can be possibly produced via certain chemical pathways (e.g. hydroxylamine oxidation), nitrifier denitrification and heterotrophic denitrification are widely acknowledged to be the two main processes responsible for N_2O emission during low-oxygen SND process (Meyer et al., 2005; Wunderlin et al., 2012). However, the individual contribution of the two important biological N_2O production processes has not been quantified. In addition, it is noteworthy that N_2O emission during low-oxygen SND process is significantly different from that in conventional nitrification and denitrification processes, taking into account the greatly intensified nitrifier denitrification and heterotrophic denitrification processes. The available results regarding N_2O emission source during traditional nitrification and denitrification process therefore may not be applicable to low-oxygen SND process.

N_2O emission during low-oxygen SND process is essentially a result of microbial metabolism. A detailed analysis of microbial community is therefore of great importance for better understanding of N_2O emission mechanisms. N_2O emission during nitrifier denitrification and heterotrophic denitrification is known to be executed and accomplished by certain bacteria species, mainly ammonia-oxidizing bacteria (AOB) and denitrifiers. However, to date, no published literature is available regarding the relationship between N_2O emission and its functional bacteria (i.e., AOB and denitrifiers) during low-oxygen SND process.

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This study presented an initial attempt to determine the dominant source and mechanisms of N_2O emission during low-oxygen SND process. To this end, the contributions of nitrifier denitrification and heterotrophic denitrification to N_2O emission were evaluated by using batch experiments. Furthermore, the community structures of AOB and denitrifiers were investigated using polymerase chain reaction (PCR)–denaturing gradient gel electrophoresis (DGGE) technique, targeting ammonia monooxygenase submit A gene (*amoA*) and nitrous oxide reductase gene (*nosZ*), respectively, to gain more detailed insights into the mechanisms of N_2O emission during low-oxygen SND process.

2. Methods

2.1. SND bioreactor setup and operation

The experiments were conducted in a SND sequencing batch reactor (SBR) which was made of a transparent, rigid plexiglas cylinder with an effective volume of 15 L. The SBR was operated at room temperature (25 ± 2 °C) with a cycle time of 6 h, consisted of 6 min feeding, 90 min anaerobic stage, 180 min aeration, 70 min settling, and 14 min decant. In each cycle, 7.5 L of wastewater was fed into the bioreactor and same amount of supernatant was withdrawn after settling, resulting in a hydraulic retention time (HRT) of 12 h. For better investigation on N_2O emission mechanisms, synthetic municipal wastewater instead of real wastewater was used in this study to eliminate the influence of water quality fluctuation. Glucose and sodium acetate was used as carbon source. NH_4Cl, KH_2PO_4 and K_2HPO_4 were added as nitrogen and phosphorus. Detailed information about SBR and the composition of synthetic municipal wastewater can be found in Jia et al. (2012). The complete influent contained 350 mg COD/L, 50 mg NH_4-N/L, and 5 mg TP/L.

An electric agitator with a rectangular paddle was used to keep the sludge suspended during anaerobic stage. During the subsequent aerobic stage, air supply was regulated by using an on/off control system to keep the dissolved oxygen (DO) level between 0.35 and 0.80 mg/L. Before settling, 0.75 L mixed liquor was wasted to keep the solids retention time (SRT) at approximately 20 days. The SBR was seeded with the sludge from a local wastewater treatment plant, and the concentration of mixed liquor suspended solids (MLSS) was maintained at approximately 3000–3300 mg/L. The pH value in the reactor was monitored in the range of 7.0–7.5 over the entire experimental period. The SBR was gastight and certain amount of off gases was collected into gas sampling bags at time intervals of 15 min to measure N_2O concentrations.

2.2. Batch experiments

The use of inhibitors can help to determine the magnitude of the various processes at the origin of nitrous oxide production (Tallec et al., 2006). Allythiourea (ATU) is a common inhibitor of the first step of nitrification (Hall, 1984), and the most efficient inhibitor of the second step of nitrification catalyzed by nitrite oxidoreductase is chlorate ($NaClO_3$) (Haider et al., 2003). Tallec (2005) demonstrated that N_2O emission by heterotrophic bacteria was not significantly affected in the presence of ATU and $NaClO_3$. Therefore, the amount of N_2O produced by heterotrophic denitrification alone and by the sum of nitrifier denitrification and heterotrophic denitrification can be respectively quantified by the batch experiment with or without the use of inhibitors.

After the stable effluent nutrients levels and high SND efficiency were achieved, which indicated that the SND SBR reached steady-state, a total of 3 L of mixed liquor and sludge was taken from the parent SBR at the end of anaerobic stage and then was divided equally into three mini SBRs with working volume of 1 L. Three batch experiments were simultaneously conducted: (a) no addition of nitrite or inhibitor, (b) with addition of nitrite, and (c) with addition of both nitrite and nitrification inhibitors (ATU and chlorate). The nitrite was added for heterotrophic denitrification with the presence of inhibitors. In addition, 1 L of mixed liquor and sludge was taken and the sludge and supernatants were separated. After that, the batch experiments were conducted under the conditions of sludge resuspended with distilled water and with nitrite addition to evaluate the eliminated the effect of ammonium, and supernatants with nitrite and inhibitors addition. The nitrite, ATU and $NaClO_3$ were added at the start of experiment to have a concentration of 5.0 mg/L, 10.0 mg/L (Haider et al., 2003), and 1.0 g/L (Tallec et al., 2006), respectively.

A mixture of N_2 and air was supplied into the mini bioreactors with the ratio adjusted so as to best simulate the DO variation and hydrodynamic environment in the parent reactor. The off-gas during the experiments was collected into gasbags to quantify the emission amount of N_2O. Each experiment was triplicated.

2.3. Physicochemical analysis

The effluent COD and nutrients concentration of the bioreactor was monitored every 5 days during the start-up period until the SND efficiency stabilized at a high level (>85%). Nitrogen transformation, carbon conversion (COD and polyhydroxyalkanoates (PHA)) and N_2O emission were then evaluated.

The analysis of COD, $NH_4^+ - N$, $NO_3^- - N$, $NO_2^- - N$, TN, TP and MLSS were conducted in accordance with the standard methods (APHA, 2001). DO was measured using a DO meter (HQ30d53LDO™, HACH, USA). N_2O concentration was determined using gas chromatography (SP-3410, China) with an electron capture detector (ECD) and a Poropak Q column. PHA was measured using the gas chromatography with a flame ionization detector (FID) and a column DB-5.

The SND efficiency was calculated according to the equation described by Zeng et al. (2003). The emission rate and quantity of N_2O-N were calculated as described by Hu et al. (2010). N_2O-N conversion rate was calculated by N_2O-N/TN input.

2.4. Microbial analysis

Once the parent reactor reached steady-state, evidenced by the achieved stable satisfactory SND efficiency, the sludge sample was collected and centrifuged for DNA extraction. As control, the seed sludge in the wastewater treatment plant was also sampled before acclimation to the experimental operating conditions. The total genomic DNA was then extracted using the PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, USA).

Partial gene fragments of *amoA* and *nosZ*, which represented AOB and denitrifiers, respectively, were amplified using primers *amoA-1F*/*amoA-2R* and *nosZ-F*/*nosZ-1622R*. The PCR was conducted as the protocol described by previous literature (Hu et al., 2011). The PCR product was used for DGGE analysis using the Bio-Rad Dcode system (Bio-Rad, USA). Electrophoresis was performed at 120 V for 7.5 h in 1× TAE buffer at a constant temperature of 60 °C.

Specific bands were excised, washed, and dissolved in sterile water. They were subsequently reamplified with appropriate primes. After being purified using the UNIQ-10 column PCR Purification Kit (Sangon Biotech., China), the PCR amplicons were used for sequencing (Sangon Biotech., Shanghai, China). The obtained sequences were compared with the other available sequences in the GenBank by BLAST search. Phylogenetic trees were then conducted using the neighbor-joining method with a bootstrap of 1000 replications by using MEGA 4. All *amoA* and *nosZ* gene sequences determined in this study have been deposited in GenBank under the accession number from JQ731680 to JQ731700. The