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Greenhouse gas emission and microbial community dynamics during simultaneous nitrification and denitrification process

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HIGHLIGHTS

- SND reactor released 4.28 g of greenhouse gases each cycle.
- 2.91% of the incoming nitrogen load was emitted as N_2O.
- EPS contents in activated sludge increased during the SND process.
- Bacterial species richness increased during the SND process.

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ABSTRACT

This study evaluates greenhouse gas emission and the microbial community dynamics during simultaneous nitrification and denitrification (SND) process. Based on CO_2 equivalents, the SND reactor released 4.28 g of greenhouse gases each cycle. 2.91% of the incoming nitrogen load was emitted as N_2O. The CO_2 and N_2O emissions mainly occurred in the aerobic stage and CH_4 emissions were consistently near zero. Extracellular polymeric substance (EPS) contents in activated sludge increased during start-up the SND process. High-throughput sequencing showed increases in bacterial species richness, leading to changes in EPS content and composition observed using 3D-EEM fluorescence spectra. For denitrifying bacteria, the relative abundance of *Pseudomonas* significantly increased during the SND process, while *Paracoccus* decreased significantly. For phosphorus-accumulating bacteria, the relative abundance of *Rhodocyclaceae* also significantly increased. The relative abundance of other functional microbes, such as *Nitrosomonadaceae* (ammonia oxidizer), *Nitrospirales* (nitrite oxidizer) and *Planctomyces* (anammox) decreased significantly during the SND process.

1. Introduction

In recent years climate change has become a major concern for the entire international community. The increase of greenhouse gases (GHGs) has been established as the main factor that poses a major challenge for global climate. The three most important types of long-period GHGs are generally considered to be CO_2, CH_4 and N_2O, which together contribute to almost 80% of the greenhouse effect (Ren et al., 2015; Yan et al., 2014). Biological treatment, a widely used process for sewage treatment, has been recognized as an important source of GHG emissions. During the biological treatment of wastewater, the transformation of organic matter and nitrogen leads to the generation of GHGs, regardless of the technology employed (Bani Shahabadi et al., 2009; Kyung et al., 2015). The organic carbon in sewage is decomposed by microorganisms through catabolism, anabolism and mineralization, accompanied with the generation of CO_2 and CH_4 (Cakir and Stenstrom, 2005). In addition, during nitrogen and phosphorus removal, N_2O, as a by-product and intermediate product, is produced through processes of nitrification and denitrification (Kampschreur et al., 2009). Thus, direct GHG emission from the biological treatment of wastewater can offset the environmental benefits of these treatment methods (Liang et al., 2015).

Simultaneous nitrification and denitrification (SND) process under oxygen-limited conditions is a new technique for the biological removal of nitrogen from wastewater with low energy consumption and high nutrient removal efficiencies and is a process that has been widely studied. In the aerobic state, nitrification and denitrification proceed simultaneously in the same reactor to achieve improved sewage treatment performance (Jia et al., 2012, 2013). During the last two decades, previous studies have investi-

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gated the emissions of CO_2, CH_4 and N_2O from the full-scale anaerobic/anoxic/aerobic(A^2O), anoxic/anaerobic/aerobic (reversed A^2O), aerobic, anaerobic and hybrid anaerobic/aerobic processes biological treatment of wastewater, and have identified the main emission sources (Ren et al., 2015; Yan et al., 2014; Bani Shahabadi et al., 2009). Some researchers have focused on effect of phosphorus load, PHB and oxygen uptake rate N_2O emission during low-oxygen simultaneous nitrification and denitrification process (Jia et al., 2012, 2013). However, to our knowledge, no studies have examined the CO_2, CH_4 and N_2O emission during the SND process. Because of the low dissolved oxygen (DO) concentration in the SND process which could increase the GHGs emissions (Zhang et al., 2016), the characteristics of emission of GHGs may differs from those in conventional processes. Moreover, this difference is also indicated by differences in microorganism products and microbial community composition.

In this article, the characteristics of GHG emission during the biological treatment of sewage through the SND process were investigated in anaerobic–aerobic bench-scale sequencing batch reactor (SBR). In addition, changes in extracellular polymeric substances (EPSs) and the characterization of microbial community in activated sludge during the SND process were also evaluated using three dimensional excitation–emission matrix (3D-EEM) and high-throughput sequencing techniques, respectively.

2. Methods

2.1. Experimental set-up and operation

One SBR was used in this experiment. The reactor was made from polymethyl methacrylate, with an inner diameter of 14 cm and an effective volume of 6 L. Water was drawn into the reactor from its bottom using a peristaltic pump. Water discharge was regulated by a relay and an electromagnetic valve.

The operating cycles lasted 6 h. During stable operation, the procedures in one cycle were as follows: water intake for 5 min, oxygen deprivation for 90 min, aeration for 180 min, static settling for 75 min and water discharge for 10 min. The volumetric exchange ratio was 50% resulting in hydraulic retention time of 12 h. During the experiment, the temperature of water was kept at (23 ± 2 °C) (Liu et al., 2010; Pochana and Keller, 1999). A magnetic stirrer was equipped in the reactor to maintain the suspension of activated sludge throughout the whole reaction. In the last phase of the aerobic stage, an appropriate amount of suspended sludge was discharged each day to keep the concentration of sludge in the reactor at about 3000 mg/L. The sludge retention time was 20 d. An air pump was applied for blast aeration, with adhesion sand lump as the microporous aerator and a rotameter for the control of gas flow to control the DO level in the system.

2.2. Seed sludge and synthetic wastewater

The inoculated sludge with initial concentration was 3160 mg/L in mixed liquor suspended solids (MLSS) was collected from the First Water Purification Factory of Guangda Water Affairs Co., Ltd., in Jinan, China. In order to realize SND, the concentration of DO in the SBR was maintained at 0.35–0.8 mg/L by controlling the aeration intensity (Liu et al., 2010). After acclimation, sewage treatment demonstrated favorable performance and the quality of effluent water quality was stable.

Artificial wastewater was applied in the experiment and water intake was 3 L/cycle. In water intake, the chemical oxygen demand (COD) was 400 mg/L and (NH_4^+-N) was 50 mg/L. The composition of synthetic wastewater per liter was as follows: 0.26 g glucose; 0.26 g NaAC; 0.191 g NH_4Cl; 0.018 g $K_2HPO_4{\cdot}3H_2O$; 0.011 g KH_2PO_4; 0.01 g $CaCl_2$; 0.01 g $MgSO_4{\cdot}7H_2O$; 0.01 g $FeSO_4{\cdot}7H_2O$; 0.25 g $NaHCO_3$, and 1 ml trace element solution. One liter of trace element solution contained: 1.5 g $FeCl_3{\cdot}6H_2O$; 0.15 g H_3BO_3; 0.15 g $CoCl_2{\cdot}6H_2O$; 0.12 g $MnCl{\cdot}4H_2O$; 0.12 g $ZnSO_4{\cdot}7H_2O$; 0.06 g $NaMoO_4{\cdot}2H_2O$; 0.03 g KI; and 0.03 g $CuSO_4{\cdot}5H_2O$. $NaHCO_3$ was added into the feeding solution to maintain the reactor pH in the neutral range between 7.0 and 7.8.

2.3. Analytical methods

Water samples were filtered through syringe nylon membrane filters (0.45 mm pore-size) to remove biomass. COD, ammonium (NH_4^+-N), nitrite (NO_2^--N), nitrate (NO_3^--N) and MLSS concentration of sludge samples were measured regularly according to standard methods (APHA, 2005). The DO and pH values were measured using probes (HQ30d53LDO, Hatch, USA).

In the sealed SBR reactor, gas was sampled with a portable gas sampling pump into a gasbag every 15 min. Concentrations of N_2O, CH_4 and CO_2 were determined through chromatographic detection. The collected samples were analyzed within 24 h, based on recommendation (Mosier et al., 2002). N_2O was detected using a GC-ECD (Agilent 7890B, USA). The chromatographic conditions were as follows: the temperature at the injection port was 50 °C, the column temperature was 50 °C, and the temperature of the detector was 390 °C (Wu et al., 2009). Gaseous CH_4 and CO_2 were detected with GC-FID (Agilent 7890B, USA). The chromatographic conditions were as follows: the temperature of the transformation furnace was 375 °C, the temperature of the column was 40 °C, and the temperature of the detector was 200 °C. The emission rates of GHGs were calculated according to the method described by Kong et al. (2013a).

2.4. EPS extraction and analysis

The activated sludge samples in SBR were collected in triplicate on day 0, 43, and 97 after treatment initiation. First, sludge mixtures were centrifuged at 2000*g* for 20 min (Sigma3k15, Germany), after which the sludge pellets were re-suspended to the primary volume. Ultrasound was applied and then the chemical reagents formamide and NaOH were added to extract EPSs from the activated sludge. Next, the suspensions were transferred to beakers adding the cation exchange resin with a dosage of 80 g per g SS. The mixtures were stirred for 1 h at room temperature, settled 5 min to remove the cation exchange resin, and then centrifuged at 7000*g* and left to stand at room temperature for 1 h. Finally, all the supernatants were filtered with 0.45 μm acetate cellulose membranes to obtain the EPSs (Miao et al., 2014).

In the EPSs, protein content was measured according to the modified Lowry method with bovine serum albumin as the standard (Frçlund et al., 1995). Measurements of 3D-EEM fluorescence spectra were obtained using a fluorescence spectrophotometer (F-7000, Hitachi, Japan). The scanning speed was set at 1200 nm/min. The emission wavelength was set from 220 nm to 550 nm by varying in 10 nm increments, while the excitation wavelength was set from 220 nm to 420 nm at 10 nm increments. The 3D-EEM data were handled by the software package Origin 7.0 (OriginLab, USA).

2.5. Sample collection, DNA extraction, PCR amplification, sequencing and data analysis

The activated sludge samples were withdrawn and centrifuged at 6500*g* for 15 min to remove biomass (Sigma3k15, Germany). The cell pellets were washed twice by phosphate buffered saline solution (pH 7.4). Total genomic DNA was extracted from each sample referring to the manufacturer's instructions of the