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Response of greenhouse gas emissions and microbial community dynamics to temperature variation during partial nitrification

Wenlin Jia[a], Yunfan Chen[a], Jian Zhang[b], Cong Li[c], Qian Wang[a], Guangchao Li[a], Weihua Yang[a,*]

[a] School of Chemistry and Materials Science, Jiangsu Normal University, Xuzhou 221116, China
[b] Shandong Provincial Key Laboratory of Water Pollution Control and Resource Reuse, School of Environmental Science and Engineering, Shandong University, Jinan 250100, China
[c] School of Environment and Planning, Liaocheng University, Liaocheng 252000, China

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ABSTRACT

This study investigated the greenhouse gas emission characteristics and microbial community dynamics with the variation of temperature during partial nitrification. Low temperature weakened nitrite accumulation, and partial nitrification would shift to complete nitrification easily at 15 °C. Based on CO_2 equivalents (CO_2-eq), partial nitrification process released 2.7 g of greenhouse gases per gMLSS per cycle, and N_2O accounted for more than 98% of the total CO_2-eq emission. The total CO_2-eq emission amount at 35 °C was 45.6% and 153.4% higher than that at 25 °C and 15 °C, respectively. During partial nitrification, the microbial community diversity greatly declined compared with seed sludge. However, the diversity was enhanced at low temperature. The abundance of *Betaproteobacteria* at class level increased greatly during partial nitrification. *Proteobacteria* abundance declined while *Nitrospira* raised at low temperature. The *nosZ* community abundance was not affected by temperature, although N_2O emission was varied with the operating temperature.

1. Introduction

High-strength ammonium wastewater, such as petrochemical and pharmaceutical effluent, fertilizer waste, swine wastewater and leachates, is required to be treated seriously to avoid the potential high ammonium pollution. However, this kind of wastewater is hard to be treated by conventional biological nitrogen removal (BNR) process due to the inhibition effect of high ammonium concentration on bacterial activity. Recently many advanced processes are developed to treat high-strength ammonium or low C/N ratio wastewater, such as CANON (completely autotrophic nitrogen removal over nitrite), SHARON (single reactor high activity ammonia removal over nitrite) and nitritation-anammox, which are technically feasible and economically favorable. In these advanced processes, nitrite accumulation efficiency is crucial for the nitrogen removal performance.

Partial nitrification (PN) can achieve high nitrite accumulation efficiency by controlling the oxidation of nitrite to nitrate during nitrification process (Zhang et al., 2016). Compared with the complete nitrification, PN can save up to 25% of the oxygen consumption and also reduce the carbon source requirement in the following denitrification process (Wei et al., 2014). In order to achieve and maintain partial nitrification, the critical procedure is to enrich ammonia oxidizing bacteria (AOB) and suppress nitrite oxidizing bacteria (NOB) in the reactors. This is usually implemented by optimizing the operating parameters of the reactor based on the differences between the two kinds of bacteria, such as oxygen affinity, specific growth rate and antitoxic capacities (Peng and Zhu, 2006). Thus partial nitrification efficiency can be affected by many operating conditions, including dissolved oxygen (DO) concentration, operating temperature, pH value, sludge retention time (SRT), and free ammonia concentration (Sui et al., 2016; Tao et al., 2012; Zhu et al., 2008). Nitrite accumulation efficiency would decline and partial nitrification could convert to complete nitrification easily if the operating condition is unfit.

Among all those factors, the temperature was a common but important parameter because it has distinct effects on the competition between AOB and NOB. Significant efforts have been made to investigate the impact of temperature on partial nitrification (Bougard et al., 2006; Reino et al., 2016; Reino et al., 2017). It was reported that raising temperature can not only promote the growth of AOB but also expand the differences of specific growth rates between AOB and NOB, which favored AOB out-compete NOB (Guo et al., 2010). In general, the optimal temperature is recommended to be above 25 °C for PN process (Zhu et al., 2008), and for SHARON process high temperature (35 °C) is a critical control parameter (Vazquez-Padin et al., 2011). For most

* Corresponding author.
E-mail address: yangwh@jsnu.edu.cn (W. Yang).

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common municipal wastewater treatment plants in China, the variation of seasonal temperature will affect the performance of partial nitrification. Moreover, most of the previous research focused on the change of nitrogen transformation and AOB/NOB abundance at different temperatures (Wu et al., 2012; Zheng et al., 2017), and there were lacking in the study on microbial community diversities and dynamics with the change of temperature during partial nitrification process.

Besides, the biological nutrients removal process is considered as an important anthropogenic source of greenhouse gases (GHGs), such as nitrous oxide (N_2O), methane (CH_4), and carbon dioxide (CO_2) (Kong et al., 2016). The GHGs are produced as a by-product or intermediate product during the biological waste treatment. The variation of temperature will alter the activities of microorganisms and diversity of the microbial community, which would change the characteristics of GHGs emission. Previous studies have investigated the emissions of N_2O, CH_4, and CO_2 from full-scale wastewater treatment processes, such as simultaneous nitrification and denitrification (SND) and A^2O process (Kong et al., 2016; Ren et al., 2015). However, there is no much knowledge on the total GHGs emission characteristics and proportion of each GHG during partial nitrification at different temperatures. Moreover, a detailed analysis of the microbial community succession is of great importance for better understanding of the variation of greenhouse gases emission characteristics at different temperatures.

The objectives of this study are: (1) to investigate the variation of nitrogen transformation and GHGs emission characteristics during the shift of biological processes at different temperatures, and (2) to further inquire into the mechanisms by detecting the dynamics of microbial community structure. To attain the purposes, partial nitrification was first achieved in an SBR and then it ran for three stages from 35 °C to 15 °C. The results would provide further information on the development and application of partial nitrification.

2. Materials and methods

2.1. Experiment setup

The experiments were carried out in one column-type SBR with an effective volume of 6 L. The reactor was seeded with activated sludge taken from the oxic tank of a local municipal wastewater treatment plant, and the mixed liquor suspended solid (MLSS) was maintained at approximately 3000 mg/L. A storage tank (25 L) was used to prepare and supply the influent for the reactor and an electric agitator with a rectangular paddle was used to keep the sludge suspended.

The reactor was operated with a successive cycle of 6 h. Each cycle consisted of 60 min for anoxic process, 210 min for oxic process, 60 min for settling, 10 min for decanting, and 20 min for idling. In the first two minutes of the anoxic period, three liters of wastewater was pumped into the reactor. The SRT was controlled at approximately 8 days by disposing excess sludge at the end of oxic period. After settling, 3 L of supernatant was discharged to obtain a hydraulic retention time of 12 h.

Synthetic ammonium-rich wastewater was used as influent of the reactor. The composition contained CH_3COONa (as COD), NH_4Cl (as nitrogen), $NaHCO_3$ (as pH buffer), K_2HPO_4 and KH_2PO_4 (as phosphorus). Trace elements were also added to maintain the metabolism of microorganisms (Sui et al., 2016). The complete influent contained 400 mg/L COD, 120 mg/L NH_4-N and 5 mg/L total phosphorus (TP).

2.2. Experimental schedule

The SBR was first started up at 35 °C to achieve partial nitrification, by controlling the DO concentration during the oxic period at a low level (< 0.8 mg/L). The nitrite accumulation efficiency was monitored every three days and the reactor obtained high and stable nitrite accumulation (> 80%) after running for about 2 months. Then the nitrogen transformation and GHGs emission characteristics, as well as the dynamics of microbial community, were studied at different temperatures. The experiments included three one-month stages with same operation condition except for temperature. The reactor was firstly operated at 35 °C (stage I), and then at 25 °C (stage II) and 15 °C (stage III), successively.

At each one-month stage, the influent and effluent samples were collected every two days to analyze the ammonium removal and nitrite/nitrate accumulation. At the last week of each stage, the nitrogen transformation and greenhouse emission during one-complete running cycle were evaluated by collect the effluent and gas samples at a certain interval. Moreover, the activated sludge of each stage was sampled on the last day of each stage to detect the change of microbial community structure.

2.3. Microbial analysis

All the collected activated sludge samples were first centrifuged at 4500 rpm for 15 min, and then the cell pellets were washed twice with phosphate buffered saline solution (pH 7.4). Total genomic DNA was extracted using the DNA extraction kit (MoBio Laboratories, USA) and the concentration was measured using an ultra-micro spectrophotometer (Nanodrop ND2000, USA).

Quantitative detections of the *amoA*, *nxrA* and *nosZ* genes, were carried out by qPCR using Roche LightCycler®480 Real-time PCR System (Roche, USA) on SYBR Green I method. The qPCR reactions were performed in triplicate in 20 μL containing 10 μL SYBR Green Mix, 0.4 μL each forward and reverse primers (10 μM), 8.2 μL RNAase-free water and 1 μL standard plasmid or DNA template. The primers used for the above genes were amoA-1F/amoA-2R (Hussain et al., 2011), F1norA/R1norA (Poly et al., 2008), and *nosZ*-F/*nosZ*-1622R (Hu et al., 2011), respectively. The qPCR standard curves were established from serial dilutions of the plasmid-carrying target genes ranging from 10^8 to 10^2 gene copies per microliter. Melting curves were tested to ensure the results. The final data were generated using Abs Quant/2nd Derivative Max by Roche LC-480 software installed.

The V3–V4 hypervariable regions of the 16S rRNA gene were amplified by PCR using the primer set 341F/805R and then the PCR products were verified by electrophoresis. The DNA amplicon on the gel was excised and purified with an AMPure XP kit (Beckman Coulter, CA). Then the sequencing of 16S rRNA gene amplicons and the subsequent data analysis were performed using a MiSeq DNA sequencer (Illumina, CA) by a biotechnology company (Sangon Biotech., Shanghai, China). Operational taxonomic units (OTUs) were set at 97% identity as the threshold. Shannon and Simpson indices, which represented the diversity of the microbial community, were calculated using the software mothur. The taxonomic classification of the sequences in each sample was conducted individually using the Ribosomal Database Project (RDP) Classifier.

2.4. Physico-chemical analysis

The analysis of COD, NH_4^+-N, NO_3^--N, NO_2^--N, and MLSS was conducted in accordance with the standard methods (APHA-AWWA-WEF, 2012). pH values and DO concentrations were measured using a pH meter (SG2, Mettler Toledo, Switzerland) and DO meter (HQ30d53LDO™, HACH, USA), respectively.

The emitted gas was collected into gas sampling bags at intervals of 15 min during the typical operating cycles at the last week of each stage. Then the concentrations of N_2O, CO_2, and CH_4 were analyzed using a gas chromatography (Shimadzu GC-2010, Japan). Then the emission rate and quantity of greenhouse gas were calculated as the method described by Kong et al. (2013). The gas concentration in the atmosphere was considered as background value and was deducted firstly before the emission rate and quantity were calculated.

Specific oxygen uptake rate (SOUR) of the sludge was measured at