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Achieving partial nitrification by inhibiting the activity of *Nitrospira*-like bacteria under high-DO conditions in an intermittent aeration reactor

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ABSTRACT

It is generally accepted that a low dissolved oxygen (DO) concentration is more beneficial for achieving partial nitrification than high-DO. In this study, partial nitrification was not established under low-DO conditions in an intermittent aeration reactor for treating domestic wastewater. During the operational period of low-DO conditions (DO: 0.3 ± 0.14 mg/L), stable complete nitrification was observed. The abundance of *Nitrospira*-like bacteria, which were the major nitrite-oxidizing bacteria, increased from 1.03×10^6 to 2.64×10^6 cells/mL. At the end of the low-DO period, the batch tests showed that high-DO concentration (1.5, 2.0 mg/L) could inhibit nitrite oxidation, and enhance ammonia oxidation. After switching to the high-DO period (1.8 ± 0.32 mg/L), partial nitrification was gradually achieved. *Nitrospira* decreased from 2.64×10^6 to 8.85×10^5 cells/mL. It was found that suddenly switching to a high-DO condition could inhibit the activity and abundance of *Nitrospira*-like bacteria, resulting in partial nitrification.

Introduction

Partial nitrification, which avoids the oxidation of nitrite to nitrate, is a critical step in some biological nutrient removal processes (*e.g.* SHARON-ANAMMOX, CANON, OLAND) (Van Hulle et al., 2010; Regmi et al., 2014). Compared to traditional nitrification–denitrification nitrogen removal processes, processes with partial nitrification are less expensive since they consume less oxygen and less carbon-source (Ahn, 2006). The establishment of partial nitrification is always the key to the implementation of these processes, but it is difficult to achieve. At present, several effective methods have been developed to achieve partial nitrification, which are mainly based on high temperature (Ge et al., 2015), high free ammonia concentrations (FA) (Zhang et al., 2014), high free nitrous acid (FNA) (Ge et al., 2015), aeration duration control (Ma et al., 2016), inhibitors and low dissolved oxygen (DO) concentration (Peng and Zhu, 2006).

Among these factors, low-DO concentration has been widely reported to successfully achieve partial nitrification in wastewater treatment systems (Peng and Zhu, 2006; Ma et al., 2009, 2011). It is commonly recognized that the activity of ammonium-oxidizing bacteria (AOB) is higher than that of nitrite-oxidizing bacteria (NOB) under low-DO conditions. This is because the oxygen half-saturation constant (K_o) of AOB is lower (Hanaki et al., 1990; Manser et al., 2005; Stenstrom, 1990), resulting in NOB being gradually selected out due to the stronger inhibition by low-DO conditions. However, Liu and Wang (2013) recently found their reactors performed complete nitrification during long-term operation

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under low-DO conditions. Similarly, Park and Nogura (2004) suggested that complete and stable nitrification could be maintained even at a low-DO level (0.12 mg/L). Moreover, Regmi et al. (2014) found that the K_o of AOB (1.16 mg-O_2/L) was significantly higher than that of NOB (0.16 mg-O_2/L) in their reactor. These contradictory findings suggest that there is a high probability of not achieving partial nitrification in some low-DO reactors, which can adversely affect processes based on partial nitrification. However, there has been limited understanding of how to start-up or recover partial nitrification in a low-DO reactor performing complete nitrification.

In our study, an intriguing phenomenon was observed in an intermittent aeration nitrifying reactor treating organic-removed domestic wastewater. Complete nitrification was maintained under the low-DO concentration, but partial nitrification was started up under the high-DO concentration. In order to determine the mechanism of the start-up of partial nitrification, the nitrogen removal performance of the sequencing batch reactor (SBR) was monitored, and the activity and community of nitrifying bacteria were detected by batch experiments and biological methods.

1. Materials and methods

1.1. Experimental bioreactor set-up and operating conditions

An SBR with a working volume of 10 L was used which included two operational stages: low-DO stage (DO: 0.3 ± 0.14 mg/L) and high-DO stage (DO: 1.8 ± 0.32 mg/L). The SBR was operated at a fixed temperature of 25°C using an electric heater. The oxygen required for the nitrification process was supplied by an air pump through a flow meter. A mechanical stirrer was used to ensure a completely mixed status during the nitrification process. The pH, DO and temperature in the reactor were monitored using an on-line multi-parameter sensor (WTW 3420, Germany). The pH was not controlled in the reactor. The operational stage in each cycle included four periods, namely 10 min feeding period, variable-length intermittent aeration (10 min aeration/10 min anoxic) period, 30 min settling period, and 15 min discharging of 5 L supernatant period. The terminal point of nitrification was judged by the real-time control of DO concentration. The endpoint values were 0.7 mg/L in the low-DO stage and 3 mg/L in the high-DO stage. The SRT was maintained at 30 days by withdrawing the activated sludge regularly. The mixed liquor suspended solids (MLSS) was maintained at 1500 ± 134 mg/L, and the effluent suspended solids (SS) was 24 ± 8 mg/L.

The influent to the nitrifying SBR was domestic wastewater from which the organics had been removed in an activated sludge bioreactor by direct aeration for a short time (35 min). The characteristics of the influent were as follows: COD: 40–55 mg/L, NH_4^+-N: 50.2–80.4 mg/L, NO_2^--N: 0.1–5.4 mg/L, NO_3^--N 0.04–1.24 mg/L, and pH 7.2–7.8. Activated sludge taken from a pilot-scale SBR (volume: 7000 L) was used as the inoculum.

1.2. Batch experiments

Batch experiments were carried out to investigate the effect of DO fluctuation on the nitrifying performance of the activated sludge acclimated to the stable DO concentration. The experiments were conducted in a lab-scale SBR with a working volume of 2.5 L at the end of the low-DO stage and the high-DO stage, separately. The sludge was taken from the operating SBR (10 L) and washed three times with deionized water before being transferred to the 2.5 L SBR. The synthetic wastewater was prepared from a solution of NH_4Cl and $NaNO_2$. The initial NH_4^+-N and NO_2^--N concentrations were 40 mg/L and 20 mg/L, respectively. Different DO concentrations (0.3, 0.5, 1.0, 1.5 and 2.0 mg/L) were applied in the batch experiment. A variable frequency air pump was used to adjust air flow automatically according to the DO probes, in order to ensure a stable DO concentration. The initial MLSS in each 2.5 L SBR was kept at 2000 mg/L. The temperature was maintained at 24°C and the pH was kept at 7.2 by adding $NaHCO_3$ solution. The batch experiments were operated in triplicate for each DO concentration.

1.3. Analytical methods

NH_4^+-N, NO_2^--N and NO_3^--N concentrations were measured using a Quickchem® 8500 system (Hach Company, Lachat Instruments, USA), while the chemical oxygen demand (COD), SS and MLSS, MLVSS were analyzed using standard methods (American Public Health Association, 1998).

1.4. DNA extraction and quantitative PCR assay

Total DNA was extracted from 0.1 g of lyophilized sludge using a Fast DNA Spin Kit for Soil (QBIOgen Inc., Carlsbad, CA, USA), following the manufacturer's instructions. The DNA samples were stored at −20°C. The concentration of DNA was measured using a Nanodrop ND-1000 UV–Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

Quantitative PCR was performed with a Stratagene Mx3000P QPCR System (Agilent Technologies, USA), using the fluorescent dye SYBR-Green approach (TAKARA, Dalian, China). Three coupled primers (Table 1) were used to amplify the AOB and NOB (*Nitrobacter*, *Nitrospira*), namely amoA-1f/amoA-2f, FGPS872f/FGPS1269r and NSR1113f/NSR1264r (Park et al., 2010; Degrange and Bardin, 1995; Geets et al., 2007). Each PCR amplification mixture (20 μL) consisted of 10 μL SYBR® Premix Ex Taq™ (Takara, Dalian, China), 0.4 μL ROX Reference Dye50, 1 μL bovine serum albumin (25 mg/mL), 0.2 μL of each primer (10 μmol/L) and a 2 μL template of DNA (1–10 ng). The PCR amplification program performed denaturation for 3 min at 96°C followed by 40 cycles of

Table 1 – List of primers.

Target	Primer name	Sequence (5′-3′)	Temp. (°C)
AOB	amoA-1f/amoA-2r	ggggtttctactggtggt/ccccctckgsaaagccttcttc	53
Nitrobacter	FGPS872f/FGPS1269r	ctaaaactcaaaggaattga/ttttttgagatttgctag	56
Nitrospira	NSR1113f/NSR1264r	cctgctttcagttgctaccg/gtttgcagcgctttgtaccg	53
	EUB338f/Ntspa0685M	actcctacgggaggcagc/cgggaattccgcgctc	53

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