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Evaluating the feasibility of ratio control strategy for achieving partial nitritation in a continuous floccular sludge reactor: Experimental demonstration

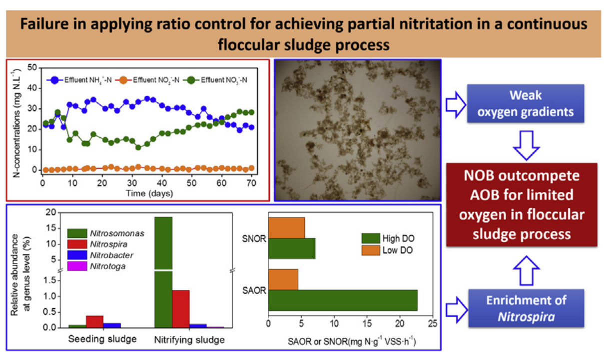
Wenru Liu, Dianhai Yang *

State Key Laboratory of Pollution Control and Resource Reuse, School of Environmental Science and Engineering, Tongji University, Shanghai, China

HIGHLIGHTS

- Applying ratio control in floccular system to achieve nitritation was investigated.
- Nitritation was not obtained by maintaining DO/TAN ratio below 0.02 mg $O_2 \cdot mg^{-1}$ N.
- The high AOB/NOB ratio of 18.75%/1.35% benefits from the excess of ammonium.
- Weak oxygen gradients and enrichment of *Nitrospira* caused the nitritation failure.

GRAPHICAL ABSTRACT



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ABSTRACT

To investigate the applicability of ratio control strategy to other systems, a continuous floccular sludge reactor was used in this study. It was found that nitrite accumulation was barely detected throughout 70 days' investigation, being the average concentration in the effluent of 0.7 ± 0.4 mg/L. Batch experiments indicated that low dissolved oxygen (DO < 0.3 mg·L^{-1}) greatly repressed the ammonium oxidizing bacteria (AOB) but only slightly inhibited the nitrite oxidizing bacteria (NOB). However, high-throughput sequencing revealed that the ratio of abundance between *Nitrospira* and *Nitrosomonas*, being the dominant NOB and AOB respectively, was considerably low (1.2%/18.7%). The weak oxygen gradients in floccular sludge and the selectively enriched K-strategist NOB *Nitrospira* under oxygen-limited conditions were both contributed to the failure of achieving partial nitritation; therefore, the rapid start-up of partial nitritation process based on proposed ratio control strategy is not feasible for continuous floccular sludge systems treating low-strength wastewater.

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1. Introduction

Nitrogen removal via nitrite (the nitrite pathway) such as nitritation/denitritation and partial nitritation/anammox could decrease the organic carbon demand and save the aeration costs for wastewater treatment (Kartal et al., 2010; Peng and Zhu, 2006; Siegrist et al., 2008). The successful implementation of the nitrite pathway relies on the reliable nitritation. To date, researchers have reported numerous approaches to the selection of ammonium oxidizing bacteria (AOB: oxidizing ammonium to nitrite) and suppression of nitrite oxidizing bacteria (NOB: oxidizing nitrite to nitrate), including maintenance of low oxygen, real-time control, high temperature plus solids retention time (SRT) manipulation, alternating anoxic and aerobic operation, combination of pH con-

* Corresponding author at: No. 1239 Siping Road, Yangpu District, Shanghai 200092, China.
E-mail address: yangdianhai@tongji.edu.cn (D. Yang).

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trol and free ammonia (FA) and free nitrous acid (FNA) inhibition, and ultrasonic treatment (Ge et al., 2015; Park et al., 2010a; Peng and Zhu, 2006; Zheng et al., 2016). However, reliable nitritation has proven difficult to rapidly achieve and chronically maintain, particularly in continuous processes treating low-strength wastewater (Ge et al., 2014; Peng et al., 2012; Xu et al., 2015). Until now, limited researches have been done on outcompeting NOB and maintaining nitritation in continuous processes from low-strength wastewater even at low temperature (Ge et al., 2014; Isanta et al., 2015; Regmi et al., 2014; Reino et al., 2016).

Recently, one promising strategy was successfully applied for achieving partial nitritation in continuous granular reactors. The strategy is to maintain the adequate ratio between dissolved oxygen (DO) and total ammonia nitrogen (TAN = N-NH_4^++ N-NH_3) concentrations in the reactor bulk liquid (DO/TAN concentration ratio control) (Bartroli et al., 2010). The strategy has the following advantages. On one hand, the effect of the DO/TAN concentration ratio on partial nitrification was fast. Previous documents indicated that partial nitritation could be achieved within one month (Bartroli et al., 2010; Isanta et al., 2015). On the other hand, the long-term stability of partial nitritation process for low-strength wastewater could be maintained even at low operational temperature (Isanta et al., 2015; Pérez et al., 2014; Reino et al., 2016). Moreover, considerably high volumetric nitrogen loading rates compared with other reported stable partial nitrification process can be obtained (Bartroli et al., 2010; Isanta et al., 2015; Reino et al., 2016), because the process achieving partial nitritation through the low DO/TAN concentration ratio control strategy without the need to be operated at aggressive SRTs. All these advantages demonstrated the efficiency of the strategy in outcompeting nitrite oxidizers. Notably, the proposed low DO/TAN concentration ratio strategy was mainly applied in the granular or biofilm systems. Because a steep oxygen gradient is typically present in the granules or biofilms and hence lead to the spatial organization of nitrifying bacteria: AOB dominate at the outer edge, while NOB distribute in a deeper layer (Matsumoto et al., 2010; Montras et al., 2008). While such a specific biomass distribution structure cause the competition for space between AOB and NOB.

However, the applicability of the low DO/TAN concentration ratio strategy in other systems with less oxygen diffusional limitation, such as continuous floccular sludge reactors remains unknown. Though Isanta et al. (2015) stated that the strategy could not be used in activated sludge systems, since there is no competition for space and lower oxygen gradients in the floccular sludge. However, their statement is still lack of experimental validation.

The objective of this research was to evaluate the feasibility of achieving partial nitritation in a continuous floccular sludge reactor based on the low DO/TAN concentration ratio strategy. The reactor was fed with synthetic influent with an average TAN concentration of 50 mg N·L^{-1} mimicking the low-strength wastewater. Accordingly, the TAN concentrations in the reactor bulk liquid will be approximately 20–25 mg N·L^{-1} when stable partial nitritation providing nitrite and ammonium for anammox is obtained. Thus, if the DO/TAN concentration ratio was maintained below 0.02, a considerably low set-point compared with previously reported values (Bartroli et al., 2010; Bernet et al., 2005; Isanta et al., 2015; Jemaat et al., 2013; Reino et al., 2016) to ensure the strong oxygen-limiting conditions for partial nitritation, the DO will be controlled lower than 0.4 mg O_2·L^{-1} in this study.

2. Materials and methods

2.1. The experiment set-up and origin of the biomass

One bench scale continuously stirred tank reactor (CSTR) (2.4 L) was set up and fed continuously with synthetic wastewater to achieve a hydraulic retention time of approximately 8 h (Fig. 1). The reactor was operated with 20 day solids retention time. The DO was maintained around 0.3 mg·L^{-1} by continuous DO measurement and air supply control. Air was provided using air diffusers connected to air pump, and mixing was achieved through steadfast overhead mechanical stirrers. Synthetic wastewater, containing 190 mg·L^{-1} NH_4Cl, 50 mg·L^{-1} K_2HPO_4, 20 mg·L^{-1} $CaCl_2·2H_2O$, 25 mg·L^{-1} $MgSO_4$ $7H_2O$, 1 mL·L^{-1} of trace element solution as described by Bellucci et al. (2011). In order to focus on the study of the nitrification process and communities, no organic matter was put into the influent. The pH in the reactor ranged from 7.0 to 7.5, controlled by a buffer containing $NaHCO_3$. Operational temperature was approximately 22 °C.

The reactor was seed with return activated sludge from Quyang WWTP (Shanghai, China), which operates an anaerobic/anoxic/aerobic (AAO) process. The process has approximately a 10-day SRT and 8 h hydraulic retention time.

2.2. Physical and chemical analyses

All water samples were filtered through a 0.45 μm filter before analyzing. Concentrations of NH_4^+-N, NO_2^--N, NO_3^--N, MLSS, MLVSS and sludge volume index (SVI) were all measured according to standard methods (APHA, 2005). DO and pH were monitored by pH/oxi1970i meter with DO and pH probes (WTW Multi1970i, Germany). The morphology of granules was measured by microscope (OLYMPUS CX41, Japan) with an attached digital camera.

2.3. Batch experiments

Batch tests were conducted to investigate the short-term effect of DO on nitrification rate. The sludge samples were taken from the operating reactor on day 70. All batch experiments were carried out in the 300 mL reactors. To each batch reactor, the MLVSS concentration was in the range of 0.53–0.68 g VSS·L^{-1}. The pH was monitored and controlled between 7.2 and 7.5 by adding $NaHCO_3$. Experimental temperature was maintained at 22 °C. For testing the first step of nitrification, the initial NH_4^+-N (NH_4Cl) concentration was approximately 25 mg/l; to evaluate the second step of nitrification, $NaNO_2$ was used instead of NH_4Cl and the initial NO_2^--N concentration was approximately 20 mg/L. During each test, concentrations of NH_4^+-N or NO_3^--N as a function of time were measured. The specific ammonium oxidation rate (SAOR) were determined through linear regression of the measured NH_4^+-N profiles. The specific nitrite oxidation rate (SNOR) were determined similarly from the measured NO_3^--N profiles. Triplicate experiments were conducted at every DO level.

2.4. DNA extraction and Polymerase Chain Reaction (PCR)

In order to analyze the microbial community structure changes in the reactor, sludge samples of Day 0 and 70 marked as 'seeding sludge' and 'nitrifying sludge' respectively were taken from the CSTR for DNA extraction using FastDNA Spin Kit for Soil (MP Biomedicals, LLC, Solon, OH). The extracted DNA was purified using ethanol precipitation. The DNA concentration was determined using Nanodrop 2000 to guarantee values of OD260/OD280 and OD260/OD230 above 1.8 and 2.0, respectively. The DNA quality was checked by running a 2 μL DNA solution on a 1% agarose gel.

2.5. Polymerase Chain Reaction (PCR) and Illumina miseq sequencing

DNA samples were amplified in triplicate by PCR using primer set F515 (5′-GTGCCAGCMGCCGCGG-3′) and R907 (5′-CCGTCAATT CMTTTRAGTTT-3′) for the V4 region of the 16S rRNA gene. The 12-nucleotide barcodes were added to the 5′ end of R907 to allow

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