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Nitrogen removal, microbial community and electron transport in an integrated nitrification and denitrification system for ammonium-rich wastewater treatment

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ABSTRACT

Partial nitrification and denitrification (PND) is a promising technology for removing nitrogen from wastewater. This study aimed to optimize the operation for a stable partial nitrification in a PND reactor with ammonia-rich influent. Metagenomics was used to analyse the functional genes and relative microbial community for nitrogen removal. Experiments with respiratory chain inhibitors were conducted to investigate the electron transport chain of denitrification. The achieved removal efficiency of ammonium nitrogen (NH_4-N) was 53.3%, and the effluent nitrite nitrogen (NO_2-N) to NH_4-N concentration ratio was 0.74. *Nitrosomonadaceae* was the dominant species of ammonia oxidizing bacteria and *Nitrospira* was the dominant nitrite oxidizing bacteria (NOB). Denitrifiers mainly belonged to *Thauera, Phycisphaerales* and *Paracoccus*. *Thauera*, *Aequorivita* and *Rhodothermus* possessing the gene *nos* were able to reduce N_2O. High NO_2-N concentrations resulted in the increasing NOB activities, which impeded the stable partial nitrification, and contributed to more N_2O emission during denitrification. A modified model of denitrifying electron transport was proposed and explained that high NO_2-N concentration improved the capacity of nitrite reductase for electron competition and caused an insufficient electron supply for N_2O reductase, which contributed to more N_2O emission.

1. Introduction

Safe and efficient nitrogen removal from ammonia-rich wastewater has received intensive attention. Partial nitrification and denitrification (PND) is a promising technology for nitrogen removal, which can reduce 25% oxygen demand and 40% carbon requirement, in comparison with the complete nitrification and denitrification process (Wei et al., 2017). The integrated system combines nitrification with denitrification within one reactor, which eliminates the need for separate tanks and consequently simplifies the design of wastewater treatment. However, stable partial nitrification and efficient nitrogen removal may be difficult to be achieved due to the different growth requirements of nitrifiers and denitrifiers, especially for ammonia-rich wastewater with low organic carbon to nitrogen ratios. Therefore, it is necessary to optimize the nitrogen removal performance in the integrated system.

Nitrite nitrogen (NO_2-N) accumulation is achieved in partial nitrification. When it happens, high concentrations of NO_2-N, and consequently high free nitrous acid (FNA) could inhibit the activity of nitrifiers (Wu et al., 2016). Ammonia oxidizing bacteria (AOB) could be inhibited by 50% at the FNA concentration of 0.42–1.72 mg/L, while nitrite oxidizing bacteria (NOB) could be inhibited by 100% at the FNA concentration of 0.026–0.22 mg/L (Laloo et al., 2018). Besides, a high NO_2-N concentration was found to result in the high emission of a greenhouse gas-nitrous oxide (N_2O) during nitrification (Castro-Barros et al., 2016; Hu et al., 2018). For sustainable development of wastewater treatment, further investigation is required to clarify the effect of NO_2-N on nitrification in the PND system.

When PND is applied, NO_2-N is the main electron acceptor rather than the nitrate nitrogen (NO_3-N) during denitrification. The FNA can also inhibit denitrifier activities. The NO_3-N reduction rate was completely inhibited at the FNA concentration of 0.2 mg/L (Zhou et al., 2011). Du et al. (2016) found that N_2O emission during denitrification via NO_2-N was higher than via NO_3-N. The N_2O conversion rate was improved with increasing NO_2-N concentrations (Sun et al., 2018). The electron acceptors for traditional denitrification include NO_3-N, NO_2-N, NO and N_2O, and denitrification requires the participation of nitrate

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reductase (NAR), nitrite reductase (NIR), NO reductase (NOR) and N_2O reductase (NOS). NAR is not included during denitrification via NO_2-N. Pan et al. (2013) illustrated that electron competition existed among denitrification reductases. The electron distribution to NAR under different organic concentrations was stable, while the electron distributions among NIR, NOR and NOS were greatly affected by organic concentrations (Pan et al., 2013). Chen et al. (2017) observed that the electron affinity of NAR was stronger than other denitrification reductases and NAR tended to have the priority to utilize electrons. Therefore, electron acceptors might affect the electron competition among denitrification reductases and the denitrification performance. It is required to further investigate the effect of electron acceptors on denitrification.

Electrons are usually originated from carbon metabolisms during denitrification. Denitrification reductases can obtain the electrons through the electron transport system. At present, there are two primary models for denitrification electron transport, i.e. the ASMN model and the ASM-ICE model (Pan et al., 2015). The ASMN model is based on a simplified hypothesis that electrons are supplied for the four denitrification steps sufficiently and independently. Based on the ASMN model, the ASM-ICE model makes some improvement and assumes that the produced electrons are firstly transferred to the intermediate electron carrier and then to the denitrification electron acceptors. If the reductases obtain the electrons from the same carrier, electron competition exists, and further investigation on denitrification electron transport would be beneficial to figure out the mechanism of electron competition.

In addition, microbial structure and functional genes are also affected by high NO_2-N concentration in the PND system. *Nitrosomonas* and *Nitrosospira* are commonly found in the nitrification process (Liu et al., 2018). *Nitrosomonas* had a better tolerance for NO_2-N than *Nitrosospira*, making them dominant at high NO_2-N concentrations (Liu et al., 2018). With increasing NO_2-N concentrations from 0 to 65 mg/L, the expression of gene *nirK* was enhanced, while similar NO_3-N concentrations had no influence on *nirK* expression (Beaumont et al., 2004). Moreover, NO_2-N concentrations led to higher mRNA concentrations of *nirK* and *nirS* and accordingly, the reduction rate of NO_2-N was accelerated (Yu and Chandran, 2010). Hence, the microbial community and nitrogen removal functional genes are worthy of investigation in the integrated PND reactor.

In this study, an integrated system for PND was conducted to treat ammonia-rich wastewater. For stable partial nitrification and efficient nitrogen removal, high-throughput sequencing and metagenomics were applied to analyse the microbial structure and nitrogen removal functional genes. In addition, the effect of NO_2-N on nitrification and denitrification performance was investigated. Finally, a modified model for denitrification electron transport was proposed to analyse the electron competition during denitrification via NO_2-N.

2. Materials and methods

2.1. Experimental setup and operation

A sequencing batch reactor (SBR) was operated at 25 ± 1 °C with an effective volume of 6 L. The operation cycle was 8 h, including 7 h reaction time (including 10 min feeding), 45 min settlement and 15 min drain. Feeding and drain were achieved using two peristaltic pumps controlled by timers. The hydraulic retention time was 16 h and the sludge retention time was 8.5 d. Seed sludge was taken from Nanshan wastewater treatment plant in Shenzhen, China.

There were six phases during the long-term operation. From Phases I to IV, the NH_4-N concentration was 100, 200, 400 and 600 mg/L, respectively. The COD to nitrogen ratio was 1:1. The reaction time included 4 h denitrification and 3 h nitrification with uncontrolled aeration. In Phase V, the reaction time included 3 h denitrification and 4 h aeration. In phase VI, the aeration rate was controlled at 1.2 m^3/h.

The SBR was fed with synthetic wastewater. Nitrogen was supplied mainly from NH_4Cl. Organic carbons of starch and peptone were supplied at the COD ratio of 1:1. Other components included 600 mg/L $NaHCO_3$, 714 mg/L $KHCO_3$, 70 mg/L Na_2HPO_4, 400 mg/L $MgSO_4$, 150 mg/L $CaCl_2$, 40 mg/L yeast extract and 0.4 mL/L trace elements.

2.2. Effect of nitrite on nitrification

Batch experiments were conducted at 25 °C to investigate the effect of NO_2-N concentrations on nitrification. A reactor with 500 mL working volume was sealed with a rubber stopper. Three ports in the stopper were used to take liquor and gas samples and purge gas, respectively. 120 mL mixed liquor was taken from the parent SBR. Supernatant was removed after centrifugation (11000 rpm) and the residual sludge was re-suspended with 500 mL synthetic wastewater. Magnetic stirrer was used for mixing. The components of synthetic wastewater were the same as Section 2.1 except for nitrogen and organic carbon. The NH_4-N concentration was 100 mg/L. The initial NO_2-N concentration was set at 0, 5, 20 and 80 mg/L to test the effect of NO_2-N on nitrification performance. 5 mL liquor and 10 mL gas samples were taken every 10 min to test the concentrations of NH_4-N, NO_2-N, NO_3-N, total organic carbon (TOC) and gaseous N_2O (dissolved N_2O was not tested due to the aeration).

2.3. Effect of electron acceptors on denitrification

The reactor was set as section 2.2. One port in the stopper was used to take the liquor, the other one was used to place a N_2O sensor. The electron acceptor was NO_3-N or NO_2-N, with the concentration of 100 mg/L. Three types of carbon source were applied, i.e. starch, peptone and a mixed carbon source (starch and peptone 1:1). The COD concentrations were 300 mg/L. Other components were the same as Section 2.1. Three-minute aeration by N_2 was conducted before experiments to remove oxygen. 5 mL mixed liquor was taken every 5 min. After sampling, the same volume of N_2 gas was injected for the pressure balance inside the reactor. Dissolved N_2O was monitored online. NH_4-N, NO_2-N, NO_3-N and TOC concentrations were tested after the experiments.

To further analyse the electron transport chain of denitrification, NO_3-N was applied as the electron acceptor, with the concentration of 100 mg/L. COD concentration was 300 mg/L with the mixed carbon source (peptone and starch 1:1). Rotenone, quinacrine dihydrochloride (QDH), antimycin A and dicyclohexylcarbodiimide (DCC) were selected as the respiratory chain inhibitors. Rotenone was added at the concentration of 0, 0.04, 0.4 and 1 mM, which could inhibit Complex I (NADH dehydrogenase) (Yu et al., 2015). QDH can inhibit Complex II (FAD dehydrogenase) and the applied concentrations were 0, 0.2, 2 and 4 mM (Yang et al., 2018). Antimycin A with concentrations of 0, 0.04, 0.2 and 0.4 mM was added to inhibit Complex III (Wang et al., 2016). DCC (0, 0.4, 0.8 and 1 mM) was added to inhibit Complex V (ATPase) (Yu et al., 2015). The experimental procedure was the same as the above denitrification experiment.

2.4. Analytical methods

NH_4-N, NO_2-N, NO_3-N and volatile suspended solids (VSS) were measured according to standard methods (APHA, 1999). During the electron transport chain experiments, ion chromatography (IC-2010, Tosoh, Japan) was used to test the NO_3-N concentration. TOC was tested by a total organic carbon analyser (TOC-L CPH, Shimadzu, Japan). Gaseous N_2O was measured by gas chromatography (Agilent 6820, Agilent Technologies, USA). Dissolved N_2O during denitrification was tested online by a N_2O micro-sensor (N_2O-100, Unisense A/S, Denmark).

The oxidation rate of NH_4-N, the reduction rate of NO_3-N and NO_2-N, the production rate of N_2O and the utilization rate of TOC were