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Nitrifiers activity and community characteristics under stress conditions in partial nitrification systems treating ammonium-rich wastewater

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ABSTRACT

Long-term exposure of nitrifiers to high concentrations of free ammonia (FA) and free nitrous acid (FNA) may affect nitrifiers activity and nitrous oxide (N_2O) emission. Two sequencing batch reactors (SBRs) were operated at influent ammonium nitrogen (NH_4-N) concentrations of 800 mg/L (SBR_H) and 335 mg/L (SBR_L), respectively. The NH_4-N removal rates in SBR_H and SBR_L were around 2.4 and 1.0 g/L/day with the nitritation efficiencies of 99.3% and 95.7%, respectively. In the simulated SBR cycle, the N_2O emission factors were 1.61% in SBR_H and 2.30% in SBR_L. N_2O emission was affected slightly by FA with the emission factor of 0.22%–0.65%, while N_2O emission increased with increasing FNA concentrations with the emission factor of 0.22%–0.96%. The dominant ammonia oxidizing bacteria (AOB) were *Nitrosomonas* spp. in both reactors, and their relative proportions were 38.89% in SBR_H and 13.36% in SBR_L. Within the AOB genus, a species (*i.e.*, operational taxonomic unit [OTU] 76) that was phylogenetically identical to *Nitrosomonas europaea* accounted for 99.07% and 82.04% in SBR_H and SBR_L, respectively. Additionally, OTU 215, which was related to *Nitrosomonas stercoris*, accounted for 16.77% of the AOB in SBR_L.

Introduction

Wastewaters such as landfill leachate and anaerobic digester effluent usually contain high concentrations of ammonium nitrogen (NH_4-N). When the conventional nitrification–denitrification process is applied to treat the ammonium-rich wastewater, it requires a high-energy input. Usually, the organic carbon contained cannot satisfy the requirement of denitrification. On the other hand, partial nitrification rather than full nitrification possesses advantages of 25% lower oxygen consumption, 40% lower external organic carbon requirement, and 63% lower sludge production (Bernet et al., 2001; Fux et al., 2006; Wang and Yang, 2004). Therefore, partial nitrification (nitritation) processes have been developed to treat ammonium-rich wastewater (Lv et al., 2016).

How to achieve high loading rates and how to maintain stable and high nitritation efficiencies are two key aspects for partial nitrification. Maintaining high substance gradients can improve microbial activities efficiently. Therefore, sequencing batch reactor (SBR) systems with the substrate gradients can be applied to achieve high pollutant removal. In the partial

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nitrification system operated at high loading rates, activated sludge would be exposed to high concentrations of free ammonia (FA), free nitrous acid (FNA) and salinity during the long-term operation. Generally, stress conditions such as high salinity may inhibit the activities of microorganisms due to the plasmolysis (She et al., 2016). However, Bassin et al. (2012) found that the proportion of ammonia oxidizing bacteria (AOB) in microbial communities increased with increasing the salt concentration (up to 20 g/L).

The key point for achieving the stable nitritation is to inhibit the activity of nitrite oxidizing bacteria (NOB) while maintaining the activity of AOB. FA and FNA affect nitrite accumulation because their inhibition effects on NOB are stronger than those on AOB. For example, Anthonisen et al. (1976) reported that the inhibition concentrations of FA on AOB were 10–150 mg/L, while those on NOB were 0.1–1.0 mg/L. In addition, AOB can adapt to high concentrations of FA up to 122–124 mg/L after the long-term acclimation, and yet the activity of NOB was mostly inhibited under the same condition (Liang and Liu, 2007). The activity of AOB was still maintained at a relatively high level (the specific NH_4-N oxidation rate of 0.9 g/g/day) at the FNA concentration of 4.1 mg N/L (Kinh et al., 2017). However, the activity of NOB was completely inhibited at the FNA concentration of 0.02 mg/L (Vadivelu et al., 2007). Further studies about the inhibition effects of FA and FNA on these nitrifiers should be carried out, especially for those after the long-term acclimation of microbial communities under high concentrations of FA and FNA.

High concentrations of FNA may enhance nitrous oxide (N_2O) emission. N_2O is a strong greenhouse gas, and its greenhouse effect is 300 times higher than that of CO_2 with the life cycle of 114 years (IPCC, 2007). Therefore, even low amounts of N_2O emission influence a lot to the environment. Previous studies about N_2O emission during nitritation treating ammonium-rich wastewater mainly focused on nitrifiers acclimated under relatively low loading rates. For example, Castro-Barros et al. (2016) investigated the effect of nitrite on N_2O emission in a nitrifying lab-scale reactor fed with low-strength ammonium wastewaters and indicated that the N_2O emission factor increased linearly from 0.16% to 1.5% with the step wise increases in the nitrite concentration from 0 to 150 mg/L. Besides, Law et al. (2011) investigated the effect of pH on N_2O emission for activated sludge acclimated under a low loading rate, and found that N_2O emission was influenced little by FA and FNA.

In this study, with a special focus on the nitritation under high ammonium loading rates, the responses of system performance to multiple stressors (*i.e.*, high concentrations of FA, FNA and salinity) and the effects of these stressors on the AOB and NOB activities and N_2O emission were investigated so as to evaluate the sustainable high efficiencies of nitrogen removal.

1. Materials and methods

1.1. Experimental system

Two cylindrical plexiglass SBRs (50 cm in height and 15 cm in diameter) with the working volume of 6 L were used to acclimate nitrifiers at 30 ± 1 °C. Each 4-hr SBR cycle consisted of phases of 10-min filling, 180-min aeration, 35-min settling and 15-min withdrawal/idle. The feeding volume in each SBR cycle was 3 L, and the same volume was discharged, resulting in a hydraulic retention time of 8 hr. The feed and withdrawal of reactors were realized by timer-controlled peristaltic pumps. The inoculated sludge was eluted from backwashed biofilm carriers taken from Xili Reclaimed Water Plant, Shenzhen, China.

The two SBRs were operated at different NH_4-N loading rates. The SBR with a high loading rate (SBR_H) contained five stages with the NH_4-N concentration gradually increased from 100 to, 200, 400, 600 and 800 mg/L, and correspondingly $NaHCO_3$ was increased from 1800 to 3600, 7200, 10,800 and 14,000 mg/L to adjust the alkalinity. During each stage, when the effluent NH_4-N concentration was below 15 mg/L, the influent NH_4-N and $NaHCO_3$ concentrations were increased to the next stage. The influent NH_4-N concentration was 335 mg/L for SBR with a low loading rate (SBR_L), and accordingly the concentration of $NaHCO_3$ was 6000 mg/L. Other components of the synthetic wastewater were the same: 50 mg/L Na_2HPO_4, 70 mg/L $CaCl_2$, 400 mg/L $MgSO_4$ and 0.4 mL/L of trace element solutions. The specific components of trace element solution were prepared according to Smolders et al. (1994).

The sludge retention time (SRT) was controlled at 10 days during the initial acclimation phase, and there was no sludge discharge after three SRTs (30 days) to increase the concentration of the mixed liquor suspended solids (MLSS).

1.2. Batch experiments

Batch experiments were carried out to investigate effects of FA and FNA on the AOB and NOB activities and N_2O emission for the acclimated nitrifiers. The batch reactors with the working volume of 500 mL were made from capped glass flasks, each with four ports on the cap for liquid sampling, gas sampling and aeration. During the batch experiments, the glass bottles were placed into water bath at 30°C and mixed with magnetic stirrers.

For the effects of FA on the AOB and NOB activities and N_2O emission under the dissolved oxygen (DO) concentrations of around 1.0 mg/L, 1500 mL activated sludge was taken from the parent reactors before the end of the aeration phase and then divided into 5 portions with each volume of 300 mL. After centrifugation, supernatant was discharged and the residual sludge was re-suspended with synthetic wastewater to 500 mL to achieve different initial concentrations of NH_4-N (100, 200, 400, 800 and 1600 mg/L), and then transferred into batch reactors. Ten-milliliter mixed liquor was taken from each batch reactor to measure MLSS and mixed liquor volatile suspended solids (MLVSS). Finally, batch reactors were aerated to achieve the DO concentrations of 1.0 mg/L. Each batch experiment was lasted for 1 hr, water and gas samples were taken at internals of 10 min, and simultaneously, pH and DO were measured. After experiments, nitrogen including NH_4-N, nitrite nitrogen (NO_2-N), gaseous N_2O-N and aqueous N_2O-N concentrations in both water and gas samples were analyzed.

Effects of FNA on AOB and NOB activities were also examined under the DO concentrations of around 1.0 mg/L. Experimental methods were similar to those of the experiment of FA effect. During the experiments, the initial NH_4-N concentration was fixed at 100 mg/L, while the initial NO_2-N concentrations applied were 100, 200, 400, 800 and 1600 mg/L.

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