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N_2O micro-profiles in biofilm from a one-stage autotrophic nitrogen removal system by microelectrode

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HIGHLIGHTS

- N_2O production from biofilm in a one-stage completely autotrophic nitrogen removal system was investigated using microelectrode technique.
- The pathways of nitrogen transformation and N_2O production was characterized by concentration micro-profiles of dissolve oxygen, nitrogen compounds, and N_2O in the biofilm.
- NH_2OH oxidation, AOB denitrification, and HD were the pathways for N_2O production from the biofilm.
- NO_2^- played a key role in N_2O production from the biofilm.

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ABSTRACT

Emission of nitrous oxide (N_2O), a greenhouse gas, is of growing concern in biological wastewater treatment. N_2O emission from biofilm in a one-stage completely autotrophic nitrogen removal system was investigated using microelectrodes in this study. It is indicated that the pathways of nitrogen transformation in biofilm mainly included partial nitrification and anaerobic ammonium oxidation (anammox), also included nitrification and heterotrophic denitrification (HD). Ammonium-oxidizing bacteria (AOB) denitrification and HD were the main pathways resulting in N_2O production in the biofilm, and hydroxylamine (NH_2OH) oxidation was a subordinate pathway. In addition, the amount of N_2O emission in test in which both NH_4^+ and NO_2^- were added (NH_4^+-N: NO_2^--N = 1:1) was about 2 times greater than that in test with NH_4^+ addition only. This result expressed that NO_2^- is an important factor affecting N_2O production in the biofilm. In conclusion, the present study provides a theoretical support for reducing N_2O production in one-stage completely autotrophic nitrogen removal system.

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1. Introduction

Methods for reducing the production of nitrous oxide (N_2O) have become a research focus, because N_2O has a more than 300-fold stronger effect on global warming than carbon dioxide (CO_2) and is contributing to ozone layer destruction (Bates et al., 2008). Researchers have found that wastewater treatment plants (WWTPs) are an important source of N_2O emission, especially those employing biological nitrogen removal processes that involve nitrification and denitrification (Kampschreur et al., 2009; Sun et al., 2015). To minimize N_2O emission, it is necessary to identify the main biological pathway of N_2O emission from WWTPs.

The one-stage completely autotrophic nitrogen removal process combines partial nitrification (PN) and anaerobic ammonium oxidation (anammox) in one reactor. It has remarkable potential for treating wastewater with high-strength ammonium and a low C/N ratio. Because this process requires less aeration and no external organic carbon addition, it is increasingly used in WWTPs (Zhang et al., 2014). However, it is reported that N_2O emission is considerable from one-stage completely autotrophic nitrogen removal process, because the process is oxygen-limited, lacks carbon sources, and involves multiple nitrogen removal pathways. 2.3% of the nitrogen load that has been found in the full-scale two-reactor nitritation-anammox process is emitted as N_2O (Kampschreur

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et al., 2008). In a one-stage granular sludge reactor using partial nitritation-anammox, 2.0% of total incoming nitrogen was found to be emitted as N_2O-N (Castro-Barros et al., 2015). In a lab-scale two-reactor PN and anammox process, the average amounts of the influential nitrogen load emitted as N_2O from the PN and anammox reactors were 4.0 ± 1.5% and 0.1 ± 0.07%, respectively (Okabe et al., 2011).

In the present study, the production of N_2O from biological nitrogen removal processes was studied according to three main pathways: hydroxylamine (NH_2OH) oxidation (Xiao et al., 2014), ammonium-oxidizing bacteria (AOB) denitrification (Sabba et al., 2015), and heterotrophic denitrification (HD) (Wang et al., 2014). N_2O is a by-product of incomplete NH_2OH oxidation during nitrification, and thus, NH_2OH oxidation plays a role in N_2O production during nitrification (Schreiber et al., 2012; Sabba et al., 2015). AOB denitrification, which uses nitrite (NO_2^-) as a terminal electron acceptor, generates N_2O as final product under oxygen-limited conditions (Ni et al., 2014). Excessive ammonia loading, accumulation of NO_2^-, and limited oxygen lead to N_2O production (Chandran et al., 2011). In addition, N_2O is an indispensable intermediate product in HD. An insufficient biodegradable C/N ratio and a higher consumption rate of NO_2^- have been shown to result in accumulation of N_2O by HD via the NO_2^- pathway (Scaglione et al., 2013; Gabarró et al., 2014). Law et al. (2012) reported that AOB denitrification is the main contributor to N_2O production rather than HD in an aerated environment, whereas Ishii et al. (2014) concluded that HD is a major pathway for N_2O production and that the contribution of HD to N_2O production was relatively greater (20–30%) than that of NH_2OH oxidation and AOB denitrification in a partial-nitritation aerobic granule reactor. However, the source of and factors affecting N_2O production are not clear in the biofilm of the one-stage completely autotrophic nitrogen removal process.

The microelectrode technique, which offers high spatial and temporal resolution, can selectively measure substrate concentrations (Hinzman et al., 2015), and it is also used to determine micro-profiles of the biofilm non-destructively, which is beneficial for analyzing nitrogen compound transformation and N_2O production from biofilm.

This study investigated N_2O production from the biofilm in the one-stage completely autotrophic nitrogen removal process via microelectrode measurement. Several batch experiments were carried out under low oxygen conditions, and the tested biofilm was sampled from a sequencing batch biofilm reactor (SBBR). The transformation pathways of nitrogen compounds in the biofilm were analyzed according to concentration micro-profiles of DO, NH_4^+, NO_2^-, and NO_3^-. In addition, potential pathways and influencing factors of N_2O production from the biofilm were deduced from the N_2O concentration micro-profiles and the transformation pathways of nitrogen compounds.

2. Materials and methods

2.1. Operation of SBBR

The one-stage completely autotrophic nitrogen removal process was carried out in this sequencing batch biofilm reactor (SBBR) with an effective volume of 15 L (Fig. S1). Polyacrylonitrile-activity carbon fiber (PAN-ACF) was used as the biofilm support material. Water-bath heating outside the reactor was used to keep the constant temperature of the SBBR. Magnetic stirrers were used to mix the wastewater. The SBBR was operated at steady-state with continuous aeration. The concentration of DO is 2.00 ± 0.20 mg L^{-1}. Inorganic synthetic wastewater was supplied to the SBBR as the nutrient source for the biofilm and consisted of NH_4HCO_3 (200 mg L^{-1} NH_4^+-N), KH_2PO_4 (20 mg L^{-1} TP), $NaHCO_3$ (330 mg L^{-1}), pH adjusted to 8.0 ± 0.2, and 2 mL L^{-1} of a trace element solution that was described previously (Chen et al., 2012). The temperature was kept at 30 ± 2 °C, and the hydraulic retention time was 24 h. One period of the SBBR contains the influent process about 5 min, reaction process about 23 h, sedimentation process about 50 min, and effluent process about 5 min.

2.2. Tests design

Three tests were carried out as described in Table 1 to investigate the nitrogen transformation pathways in the biofilm and elucidate the main pathways and factors responsible for N_2O production from the biofilm. A schematic diagram of the tests system for measuring substrate concentration in the biofilm is shown in Fig. 1. It mainly contains an oxygen and nitrogen gas mixing system, a special 500 mL measurement vessel, and magnetic stirrers, and oxygen meter (YSI), and microelectrodes. Test biofilm samples which were taken from the SBBR, washed with ultrapure water. The washed biofilm was weighted about 20 g (wet weight), and cultured in the special measurement vessel without substrates or aeration approximately 10 h to remove residual NO_2^- and NO_3^- completely before tests. The concentrations of $NaHCO_3$ and trace elements in the tests were the same as those in the SBBR, and temperature was kept at 30 ± 2 °C. The measurement vessels were flushed with O_2 and N_2 mixing gas throughout the tests to maintain the low oxygen condition, and magnetic stirring was used to mix the wastewater. Micro-profiles of DO, N_2O, NH_4^+, NO_2^-, and NO_3^- concentrations at 4 h (after substrate addition) were acquired using microelectrodes within the biofilm over the range from −200 μm to 750 μm (the biofilm surface was at the depth of 0 μm).

2.3. Chemical analyses of the SBBR

To monitor the performance of the SBBR, influent and effluent samples were collected at an interval of 2 h. NH_4^+-N, NO_2^--N, NO_3^--N, and TN concentrations were measured using standard methods (Nepa, 2002). The DO concentration and temperature (T) were measured using a portable digital dissolved oxygen meter (YSI, Professional ODO™, YSI Co., USA).

2.4. DNA analyses

Sludge samples were collected from the biofilm which was taken from different depth in the SBBR. DNA was extracted by 3S DNA centrifugal Kit for environmental samples (Shenergy Biocolor Biotech, Shanghai, China). Illumina Miseq PE300 sequencing was

Table 1
The design of tests.

Test number	NH_4^+ (mg L^{-1})	NO_2^- (mg L^{-1})	Potential main nitrogen transformation pathways	Purpose
A	100	0	PN, anammox	Analyze N_2O production pathways
B	0	100	nitrification, HD	
C	100	100	PN, anammox, nitrification, HD	

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