Short communication

Effect of pH on nitrous oxide production and emissions from a partial nitritation reactor under oxygen-limited conditions

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ABSTRACT

The effect of pH on nitrous oxide (N_2O) emissions from a laboratory-scale partial nitritation sequencing batch reactor under oxygen-limited conditions was investigated from both macro- and microscopic viewpoints. During the aeration period of a single cycle, N_2O emissions decreased when the initial pH increased from 7.5 to 8.5. By application of microelectrodes, N_2O production was observed inside entire sludge aggregates, and it increased with decreasing pH from 8.5 to 7.0. At pH 8.0 and 8.5, N_2O was mainly produced in the outer layer (<1000 μm) of sludge aggregates, where nitritation mainly occurred. At pH 7.0 and 7.5, N_2O production was mainly observed in the inner layer (>1000 μm), where the dissolved oxygen was almost depleted, revealing that the dominant pathway here was denitrification. Under oxygen-limited conditions, a decrease in pH led to increased N_2O emissions from denitrification pathway.

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1. Introduction

Nitrous oxide (N_2O) is often emitted from biological nitrogen removal (BNR) processes [1–4], which is problematic as N_2O is a powerful greenhouse gas with a much stronger greenhouse effect than carbon dioxide (about 300-fold) [1]. It is generally accepted that AOB are the major contributors to N_2O emissions in BNR processes [5,6]; therefore, N_2O emissions in partial nitritation (PN) reactors are of growing concern.

N_2O emissions are affected by process parameters (e.g., dissolved oxygen (DO) concentration, pH and substrate concentrations) in BNR systems [7–10]. Low DO concentrations, high NO_2^- concentrations and variation in influent NH_4^+ concentrations have been identified as promoting N_2O formation [6,7,11]. The relationship between pH and N_2O emission in BNR process has also been reported [12,13]. Pan et al. [12] found that N_2O accumulated at a low pH value during denitrification by methanol utilizing denitrifiers. Law et al. [13] obtained the maximum N_2O emission rate at pH 8.0 in a PN system, and found that N_2O emission correlated with the ammonium oxidation rate (AOR). In previous studies, the N_2O emission rate and dynamic characteristics were well studied [7,11–13], whereas the N_2O production at micro-scale were seldom characterized.

Theoretically, N_2O is first produced inside the microbial aggregates and then is emitted to the atmosphere. Therefore, measurement of N_2O production and transformation inside sludge aggregates might reveal the most plausible pathways of N_2O production. Microelectrodes are one of the most suitable tools for microenvironmental measurements [14,15]. Satoh et al. [15] studied nitrification activities changes within biofilms under different operating conditions. Rathnayake et al. [16] observed that N_2O was mainly produced in the outer layer of a granule where AOB showed high activities, and reflected that AOB might be responsible for N_2O production. However, variation in N_2O production pathways with operating conditions has not previously been reported.

In this study, a laboratory-scale SBR fed with a synthetic inorganic wastewater was operated for PN under oxygen-limited conditions. The N_2O emissions were first investigated from a macroscopic viewpoint. Then, microelectrodes were employed to quantify the microenvironment and N transformation inside the microbial aggregates from a microscopic viewpoint. The microbial activity obtained was analyzed for correlation with the N_2O production, aiming to explore how pH affects N_2O production and emissions.

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2. Materials and methods

2.1. Experimental setup

A laboratory-scale SBR with a working volume of 4 L was used for PN. One cycle consisted of a 5 min filling period, a 320 min aerating period, a 30 min settling period, and a 5 min drawing period. The drawn volume was 2.0 L, making the exchange volume 50%. Bulk liquid (200 mL) was removed each day providing a sludge retention time of 20 days. The mixed liquor suspended solid (MLSS) was around 3000 mg L^{-1} during the period of this study.

During the aeration period, a mass flow controller was used to keep a constant air flow rate (0.32 L min^{-1}), and the average DO concentration in the bulk liquid was 0.33 ± 0.06 mg L^{-1} 5 min after aeration. The reactor temperature was maintained at 27 ± 1 °C using a water jacket.

2.2. Biomass and synthetic wastewater

In previous study, PN was successfully initiated and steadily operated by inoculating with conventional sludge, and characteristics of N_2O emissions were investigated [17]. The same biomass was used for further exploration of the effect of pH on N_2O emissions, as described below.

The synthetic wastewater contained NH_4Cl (N source), $NaHCO_3$ (C source and buffer), and trace elements. The concentrations of NH_4^+ and $NaHCO_3$ were 600 mg N L^{-1} and 5400 mg L^{-1}, respectively. Trace element solutions were added as described by Ju et al. [17]. HCl (0.5 mol L^{-1}) and NaOH (0.5 mol L^{-1}) were used to adjust to different initial pH values of 7.5, 8.0, and 8.5.

2.3. Chemical analysis

NH_4^+–N, volatile suspended solids, and total suspended solids were determined according to the standard methods of APHA [18]. Nitratenitrogen (NO_3^-–N) and NO_2^-–N were determined using ion chromatography (761 Compact IC, Metrohm, Herisau, Switzerland). The pH and the DO concentration were directly monitored using a pH meter (Unisense, Aarhus, Denmark) and a DO meter (HQ25d, Colorado, USA), respectively.

2.4. DNA extraction, PCR-DGGE, cloning and sequencing

The mixed liquor was collected from the SBR and the total DNA was extracted using a bacterial genomic mini extraction kit (Sangon, China). Universal primers F357 (with GC clamp) and R518 were used for the PCR amplification [19]. The DGGE analysis, cloning and sequencing was determined according to Lv et al. [20].

2.5. N_2O measurements

The N_2O concentration in the off-gas was measured by a gas chromatograph (PE600, PerkinElmer, USA) with an electron capture detector and a Porapak Q column (GDX-101, Gansu, China) using 30 mL min^{-1} high-purity N_2 as the carrier gas [17].

2.6. Microsensor measurements

NH_4^+, NO_2^-, pH, DO, and N_2O microelectrodes were used for microenvironmental measurements. The first four microelectrodes were manufactured and their performance was previously shown to be stable [21]. They were constructed and calibrated before measurements according to the methods of de Beer et al. [14]. The N_2O microelectrode was purchased from Unisense, Denmark. A three-point calibration (pure water, 50% N_2O solution, and saturated N_2O solution) was carried out before each measurement.
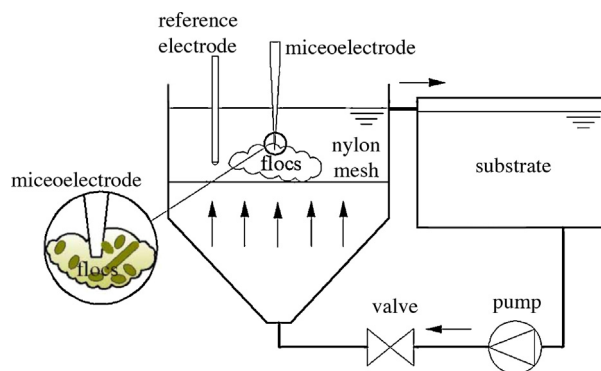

Fig. 1. The schematic diagram of the chamber for microprofile measurements.

Sludge flocs were sampled from the PN reactor and placed very gently just above the nylon net in a flowing chamber (Fig. 1) [22]. The microelectrode penetrated through the sludge aggregates under settling conditions. To obtain steady-state profiles, the sludge aggregates were left for 30 min before profile measurements started, and each microelectrode measurement was made at least three times. As the flocs had a symmetrical structure, the microelectrode only had to penetrate to half the depth of the flocs to reflect the entire substrate distribution.

The medium used in the chamber for microprofile measurements consisted of NH_4Cl (4.2 mg N L^{-1}), $NaNO_2$ (4.2 mg N L^{-1}), and $NaHCO_3$ (37.8 mg L^{-1}), and the different pH values were controlled by adding HCl (0.5 mol L^{-1}) and NaOH (0.5 mol L^{-1}). To provide the oxygen-limited condition, the DO concentration of this medium was maintained below 1 mg L^{-1} through purging with N_2. Because the pH of the PN reactor varied in the range 6.8–8.5, four different pH values (7.0, 7.5, 8.0, and 8.5) were selected for microprofile measurements.

2.7. Net volumetric rate calculations

The net volumetric rates were calculated according to mass transport equation [23]:

$$\partial C(z, t)/\partial t = D_s \cdot \partial^2 C(z, t)/\partial z^2 - Q(z) + P(z) \qquad (1)$$

where $C(z,t)$ is substrate concentration (mmol m^{-3}) at time t and depth z, respectively. Q and P are consumption and production rate (mmol m^{-3} s^{-1}), respectively. D_s is the effective diffusion coefficient (m^2 s^{-1}); D_s values of 1.38×10^{-9}, 1.25×10^{-9}, and 2.10×10^{-9} m^2 s^{-1} were used for the calculations of NH_4^+, NO_2^-, and N_2O, respectively, at 25 °C [3,16].

When steady state was achieved, the left expression is zero. Eq. (1) can be reduced to:

$$D_s \cdot \partial^2 C(z)/\partial z^2 = Q(z) - P(z) \qquad (2)$$

Defining $R(z) = Q(z) - P(z)$ is the net volumetric rate (mmol m^{-3} s^{-1}). Using Euler's formula for numeric integration, the following equation can be obtained as:

$$\partial C/\partial z_{n+1} = \partial C/\partial z_n + h \times R_n/D_s \qquad (3)$$

where h is the step size (100 μm). According to the concentration gradient, the following equation can be obtained as:

$$C_{n+1} = C_n + h \times \partial C/\partial z_n \qquad (4)$$

Substituting $\partial C/\partial z_n$ with Eq. (3), it can be calculated as:

$$R_{n-1} = D_s[(C_{n+1} - C_n)/h - \partial C/\partial z_{n-1}]/h. \qquad (5)$$