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Impact of internal recycle ratio on nitrous oxide generation from anaerobic/anoxic/oxic biological nitrogen removal process



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ABSTRACT

To evaluate the effects of internal recycle ratio on nitrous oxide (N₂O) generation, we set up three laboratory-scale anaerobic–anoxic–oxic (A²O) wastewater treatment processes, with internal recycle ratios of 100%, 200%, and 300%, respectively. Total nitrogen (TN) removal was markedly enhanced from 45.8% to 61.9%, as the internal recycle ratio increased from 100% to 300%. N₂O generation was increased from 3.47 to 9.81×10^{-2} mg/L during the treatment process, with the anoxic section showing the largest N₂O increment from denitrification. This phenomenon is attributed to the increased amount of nitrate (NO₃⁻-N) substrate available for denitrification, due to the increased volume of internal recycle liquid, as well as the increased amount of oxygen, which could restrain the activity of nitrous oxide reductase brought to the anoxic section. Nitrous oxide reductase was more sensitive to oxygen than nitrate and nitrite reductases. Microorganism analysis indicated that the population of *nosZ* gene-containing bacteria was only slightly affected by the recycle ratio. However, the number of *nosZ* gene copies decreased as the internal recycle ratio increased from 100% to 300%; this result reveals noticeable decreases in the denitrification capacity of the system for reducing N₂O to N₂.

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1. Introduction

The contribution of greenhouse gases (GHGs) to global warming has elicited great concern from various sectors in the recent decades. Wastewater treatment plants (WWTPs) are considered artificial GHG emission resources because of nitrous oxide (N₂O) emissions during the biological nitrogen removal (BNR) process. It is arrestive that N₂O is about 300 times more greenhouse effective than carbon dioxide (CO₂), and its emission increased with a high rate of around 0.3% per year [1]. About 3.2% of all global anthropogenic N₂O emissions originate from the BNR processes of WWTPs [2]. Considering the widespread use of BNR processes, as well as the hazards of N₂O, investigation of the effect of key operating parameters and sources of N₂O emissions on the overall BNR process is an important undertaking.

N₂O can be produced from both nitrification and denitrification in BNR processes. Nitrification refers to the stepwise autotrophic oxidation of ammonia (NH₄⁺) to nitrite (NO₂⁻) by ammonia-oxidizing bacteria (AOB), and then to nitrate (NO₃⁻)

by nitrite-oxidizing bacteria (NOB). During nitrification, N₂O is produced through two pathways. The first is the aerobic hydroxylamine oxidation pathway, in which N₂O is produced from intermediates of biological hydroxylamine oxidation, probably related to significant imbalances in the metabolic activity of AOB, or by chemical decomposition of hydroxylamine and chemical oxidation with NO₂⁻ as an electron acceptor (chemo-denitrification). The second pathway is nitrifier denitrification, which involves reduction of NO₂⁻ by AOB in combination with ammonia, hydrogen, or pyruvate as electron donors, for example, at oxygen-limited conditions or elevated nitrite concentrations [3,4].

Denitrification, on the other hand, refers to the reduction of NO₃⁻ to atmospheric nitrogen (N₂) by heterotrophic denitrifiers, with NO₂⁻, nitric oxide (NO) and N₂O as obligatory intermediates [5]. N₂O, a well-known intermediate emitted in this process, is released in high quantities in environments with high dissolved oxygen (DO) and NO₂⁻ concentrations and low C/N water quality [3,6,7].

In present studies, the amounts of N₂O emitted during wastewater treatment process are reported with high range of variation. Kampschreur et al. [2] concluded that 0.0–95.0% and 0.0–14.5% of the influent nitrogen could be removed through N₂O generation during lab-scale and full-scale wastewater treatment, respectively.

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To reduce N₂O emissions, in recent years, significant efforts have been made to understand the effect of various WWTP operating parameters on N₂O production [8–10]. As a result, several parameters favoring N₂O production have been identified: low dissolved oxygen concentration [11–13], accumulation of nitrite [3], rapidly changing (dynamic) conditions [14], a low ratio of chemical oxygen demand (COD) to N-compounds during heterotrophic denitrification [15,16], sludge retention time (SRT) [17], pH [18], or toxic compounds [19].

The internal recycle ratio in BNR WWTPs performs an important function in the nitrogen removal process [20]. For example, increasing the internal recycle ratio from 100% to 400% decreases the nitrate concentration in the effluent and, hence, improves the nitrogen removal efficiency of the system [21]. The environmental conditions in the nitrification and denitrification sections of WWTP systems are also influenced by the internal recycle ratio. Therefore, our hypothesis is that a close relationship exists between internal recycle ratio and N₂O generation. At present, however, the effects of internal recycle ratio changes on N₂O emissions in BNR wastewater treatment processes have not been extensively studied.

The purpose of this study is to investigate the effect of internal recycle ratio on N₂O emission characteristics and production mechanisms in BNR process. Three parallel laboratory-scale A²O wastewater treatment processes were acclimated under different internal recycle ratios of 100%, 200%, and 300%, and the effect of changes in internal recycle ratio on N₂O generation in each unit of the A²O process was studied. Denitrifier community compositions and gene copy numbers in each unit under different internal recycle ratios were analyzed using a clone library and real-time quantitative PCR (qPCR), targeting functional genes of nosZ.

2. Materials and methods

2.1. Reactor setup and operation

Three parallel laboratory-scale A²O wastewater treatment processes were operated under different internal recycle ratios of 100%, 200%, and 300%. Except for the internal recycle rate, three parallel processes were carried out under same operating parameters, as follows. The A²O process had a total effective volume of 50 L (Fig. 1). The volumes of the anaerobic section, anoxic section, oxic section, and secondary sedimentation tank were 5, 10, 30, and 5 L, respectively. The treatment system was seeded with sludge from the aerobic tank of Luotuowan Wastewater Treatment Plant (Xinxiang, China). Influent wastewater from a residential area in Henan Normal University was loaded at a rate of 85 L/d. Water quality results showed the following contents: COD 175.6 ± 30.2 mg/L, NH₄⁺-N 38.7 ± 3.2 mg/L, NO₃⁻-N 1.2 ± 0.2 mg/L, TN 39.5 ± 3.3 mg/L, TP 7.2 ± 2.4 mg/L. The water temperature was kept constant at 25 °C by three temperature controllers in anaerobic, anoxic and oxic sections throughout the research period. The SRT of system and mixed liquor suspended solid (MLSS) concentration of the water was maintained at 15 d and approximately 3000 mg/L, by controlling the emission amount of excess sludge. The sludge recycle ratio of 100% was controlled by a peristaltic pump, which brought the sludge from secondary sedimentation tank to anaerobic section with the flow of 85 L/d (Fig. 1).

The anaerobic and anoxic sections of the experimental process were stirred by a magnetic stirrer (85-2 Sile Shanghai) at a rotating speed of 500 r/min to keep the activated sludge suspended in the water. Two dismountable sealing covers were on the top of anaerobic and anoxic section to maintain the anaerobic and anoxic conditions, respectively. The DO of the oxic section was maintained at 2 mg/L by an air pump with an adjustable air flow meter. A mechanical stirrer was installed in the secondary sedi-

mentation tank (US-52 Oteli Beijing) and operated at a rotating speed of 30 r/min. Gas and liquid samples were obtained at the same time; liquid-phase samples were used to measure water quality parameters of COD, NH₄⁺-N, NO₃⁻-N, NO₂⁻-N, TN and TP. All measurements were carried out after the A²O process had been acclimated under each internal recycle rate (100%, 200%, and 300%) for over 2 months and reached stable performance, as indicated by the stable COD, NH₄⁺-N, TN and TP concentrations observed in the effluents.

2.2. Sampling and analysis methods

2.2.1. Sampling for anaerobic section, anoxic section, and secondary sedimentation tank

A modified closed-chamber technique was used to measure fluxes in non-aerated surfaces (anaerobic section, anoxic section and secondary sedimentation tank) [22]. There were three closed chambers for each one of the non-aerated surfaces. Each tank and its constant chamber had the same diameter. There were flanges at the lower rim of the chamber and the upside of tanks. During gas sampling, closed chambers were fixed on the top of tanks, by joining the flanges. The pressure of the sampling device and joint was checked at each sampling. Four samples were collected from the headspace of the chambers through polytetrafluoroethylene tubes, into 50-mL polypropylene syringes, at 3-min intervals. The gas flux, F (mg/m² min), from the liquid surface in the static chamber was calculated using the equation:

$$F = \left(\frac{V}{A}\right) \rho \left(\frac{\Delta c}{\Delta t}\right) \quad (1)$$

where V (m³) is the volume from the liquid level to the top of chamber, A (m²) is the enclosed surface area, ρ (mg/m³) is the density of the gas at the temperature recorded in the chamber, and Δc (v/v)/ Δt (min) is the change of gas concentration in the chamber per unit time during sampling. The gas density (ρ) is calculated by the equation:

$$\rho = \frac{10^{-6}MP}{RT} \quad (2)$$

where M is the molecular weight of N₂O (44.02 g/mol), P is the atmospheric pressure (1 atm), R is the gas constant (0.082 L atm/K mol), T is the temperature (K).

2.2.2. Oxic section sampling

During gas sampling, a closed gas-tight chamber with a gas-sampling outlet was fixed to the tank. The pressure of the sampling device and joint was checked at each sampling. The entire system was gas-tight and the effluent air was collected into gas sampling bags. Three parallel samples were taken at each sampling, and the gas flux, F (mg/m² min), was calculated by

$$F = \frac{\rho c Q_a}{A} \quad (3)$$

where ρ (mg/m³) is the density of the gases at the sampling temperature, c (v/v) is the sample gas concentration, Q_a (m³/min) is the aeration rate, and A (m²) is the total surface area.

2.2.3. Dissolved gas sampling

To collect gas samples dissolved in wastewater, the headspace gas method described by Kimochi et al. [23] was used. Thirty milliliter each of water and argon gas were sealed in a 50-mL syringe; 1 mL of 20 mmol/L mercury(II) chloride was added to this mixture to prevent biological degradation. After vigorous shaking, the syringe was left at room temperature for 1 h without moving. The resulting gas phase in the syringe was collected as a gas sample.

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