



# Reduction of nitrous oxide emissions from partial nitrification process by using innovative carbon source (mannitol)



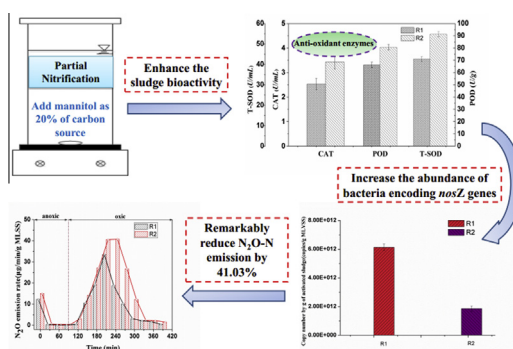
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## HIGHLIGHTS

- Mannitol could reduce N<sub>2</sub>O emission from PN process by 41.03%.
- N<sub>2</sub>O reductase inhibited by high NO<sub>2</sub><sup>-</sup> concentration could be alleviated by mannitol.
- Mannitol had a significant influence on NAR and TN removal.
- The abundance of *nosZ* genes increased in mannitol system.

## GRAPHICAL ABSTRACT



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## ABSTRACT

The purpose of this study was to evaluate the effect of mannitol as carbon source on nitrogen removal and nitrous oxide (N<sub>2</sub>O) emission during partial nitrification (PN) process. Laboratory-scale PN sequencing batch reactors (SBRs) were operated with mannitol and sodium acetate as carbon sources, respectively. Results showed that mannitol could remarkably reduce N<sub>2</sub>O-N emission by 41.03%, without influencing the removal efficiency of NH<sub>4</sub><sup>+</sup>-N. However, it has a significant influence on nitrite accumulation ratio (NAR) and TN removal, which were 19.97% and 13.59% lower than that in PN with sodium acetate, respectively. Microbial analysis showed that the introduction of mannitol could increase the abundance of bacteria encoding *nosZ* genes. In addition, anti-oxidant enzymes (T-SOD, POD and CAT) activities were significantly reduced and the dehydrogenase activity had an obvious increase in mannitol system, indicating that mannitol could alleviate the inhibition of N<sub>2</sub>O reductase (N<sub>2</sub>OR) activities caused by high NO<sub>2</sub><sup>-</sup>-N concentration.

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## 1. Introduction

With the rapid development of sustainable nitrogen elimination biotechnologies, it is of great concern that discovering an efficient strategy to reduce the operational cost of wastewater treatment. In recent years, partial nitrification (PN) process (100% NH<sub>4</sub><sup>+</sup>-N

conversion to NO<sub>2</sub><sup>-</sup>-N) is considered to be a cost-effective method based on the fact that nitrite is an intermediate compound in both nitrification and denitrification. Compared with conventional activated sludge system, PN system could reduce 25% of oxygen supply in nitrification step and 40% of carbon source requirement in subsequent denitrification step (Ge et al., 2014). Therefore, it has been successfully applied for treating various municipal and industrial wastewaters, especially when treating high strength ammonia wastewater.

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Although PN process expressed great potential in engineering applications, the significant generation of nitrous oxide ( $N_2O$ ) during this process should not be ignored (Itokawa et al., 2001). During PN process, ammonium is converted to nitrite resulting in nitrite accumulation. The accumulation of nitrite is a trigger for  $N_2O$  emission, since high  $NO_2^-$  concentration has strong inhibition on  $N_2O$  reductase ( $N_2OR$ ) activities (Kampschreur et al., 2008). Ahn et al. (2011) studied  $N_2O$  emissions in a lab-scale bioreactor operated sequentially in full-nitrification and partial-nitrification modes, suggesting a much higher degree of  $N_2O$  emitted from PN process than that of conventional full-nitrification process. As a potent greenhouse gas,  $N_2O$  has a lifetime of 120 years, and its global warming potential is about 300 times higher than that of carbon dioxide ( $CO_2$ ) (IPCC, 2007). Additionally,  $N_2O$  is regarded as the dominant ozone-depleting substance in the stratosphere during the 21st century (Ravishankara et al., 2009). Therefore, it is necessary to provide an effective method to reduce  $N_2O$  emission in PN process in order to achieve the objective of cost-effective wastewater treatment and  $N_2O$  reduction. However, there is a lack of research on the control and mitigation of  $N_2O$  emission during PN process.

$N_2OR$  catalyzes the reduction of  $N_2O$  to  $N_2$  in the final step of denitrification process, which plays a critical environmental role in preventing release into the atmosphere of  $N_2O$  (Ghosh et al., 2003). It is a dimeric protein with two copper centers (Pauleta et al., 2013). The  $N_2OR$  activities could be inhibited by some parameters including low temperature, high nitrite, oxygen,  $H_2S$ , low chemical oxygen demand (COD)/N, and solids retention time (SRT), which would result in  $N_2O$  accumulation (Itokawa et al., 2001). To date, several strategies which could increase the activity of  $N_2OR$  of denitrifiers have been reported in the literature to reduce the generation of  $N_2O$  (Zhu et al., 2013; Granger and Ward, 2003). Manconi et al. (2006) observed that the addition of copper to denitrifying sludge system could eliminate the  $N_2OR$  inhibition by  $H_2S$ . Carbon source has significant effect on denitrification enzyme activities, because it directly influences the growth of denitrifying bacteria (Ray et al., 2014). However, to date, little attention has been paid to improve  $N_2OR$  activity by using innovative carbon source.

Mannitol ( $C_6H_{14}O_6$ ) is a hyperosmolar agent and has been considered as an effective scavenger of the cytotoxic hydroxyl radical (Magovern Jr et al., 1984). Some researchers used mannitol to protect oxidation from hydroxyl radical and to control the raised intracranial pressure followed by brain injury (Wakai et al., 2013; Shen et al., 1997). Therefore, it is postulated that mannitol as carbon source might alleviate the inhibition of  $N_2OR$  activities caused by high  $NO_2^-$  concentration. However, till now, the effect of carbon source on  $N_2OR$  activity and  $N_2O$  emission during PN process has not yet been documented.

Therefore, the objective of the present study was to investigate the feasibility of  $N_2O$  emission reduction by using mannitol as carbon source during PN process. To achieve this purpose, two lab-scale PN-SBRs were acclimated under different carbon sources, i.e., sodium acetate and mannitol. Mechanisms of  $N_2O$  reduction were investigated through measurement of microbe abundance and the activity of dehydrogenases and anti-oxidant enzymes.

## 2. Materials and methods

### 2.1. Experiment set-up

Two column-type SBRs (R1 and R2) were set-up to evaluate the effect of different carbon sources (i.e., mannitol and sodium acetate) on  $N_2O$  emission during PN process. Each reactor had a working volume of 3 L with an internal diameter and working height of

12 cm and 25 cm, respectively. The schematic of the reactors has been reported in previous paper (Zhang et al., 2015). The influent wastewater was prepared in a storage tank (25 L) and was introduced into each reactor using a peristaltic pump. Low DO concentration was selected as a controlling factor to achieve PN processes (Ciudad et al., 2005). Oxygen concentration was controlled between 0.3 and 0.8 mg/L by using an air diffuser at the bottom of reactor. The SBRs were operated at room temperature ( $25 \pm 2$  °C).

A successive cycle of 8 h was operated in each reactor by alternating anoxic and oxic reaction processes. Each cycle consisted of 5 min for filling influent, 85 min for anoxic process, 300 min for aeration reaction, 20 min for settling, 10 min for decanting the effluent and 60 min for idling.

The seeding sludge was obtained from the second wastewater treatment plant of Everbright Water Ltd. in Jinan, China. Mixed liquor suspended solids (MLSS) of each reactor was maintained at approximately 3000 mg/L. SRT was controlled at approximately 20 days by disposing excess sludge at the end of aeration phase.

### 2.2. Nitrogen-rich wastewater

The two SBRs were fed with synthetic nitrogen-rich wastewater containing different carbon sources. Table 1 lists the compositions of the synthetic nitrogen-rich wastewater. All the chemicals were purchased from Tianjin Da mao chemical reagent factory (China) and of analytical reagent grade.

### 2.3. Real-time quantitative PCR

After acclimated under different carbon sources for over three months, 30.0 mL of sludge mixed liquor was sampled at the end of oxic phase of each reactor. The mixed liquor was treated with MOBIO PowerSand™ DNA kit to extract the total genomic DNA and stored at  $-20$  °C. Quantitative detection of the *nosZ* gene was achieved by quantitative PCR using Roche LC-480 (USA) according to the procedure described in our previous work (Zhang et al., 2015). The final qPCR data was generated using the Abs Quant/2nd Derivative Max provided with the Roche LC-480 system.

### 2.4. Enzyme activity

Dehydrogenases activity (DHA) is a reliable index of sludge microbial activity (Gabbita and Huang, 1984). DHA was measured with Triphenyltetrazolium chloride (TTC) method using sodium sulfide as reductant and toluene as extractant. In this study, experiments were conducted in 10-mL test tubes. To each single tube, 1 mL of 0.2%  $Na_2SO_3$ , 1 mL of 0.2% TTC, and 8 mL of activated sludge were added. Each sample was mixed and then incubated in the dark at room temperature. Incubation was stopped after 30 min. Samples were centrifuged for 5 min (4000 rpm) and decanted. 8 mL of methanol was added to the extraction of the red TF, and samples were then shaken thoroughly and centrifuged again for

**Table 1**  
Compositions of the synthetic nitrogen-rich wastewaters.

| Concentration (mg/L)    | R1   | R2   |
|-------------------------|------|------|
| $NH_4Cl$ (as $NH_4-N$ ) | 200  | 200  |
| $CH_3COONa$ (as COD)    | 160  | 200  |
| $C_6H_{14}O_6$ (as COD) | 40   | 0    |
| $K_2HPO_4$              | 112  | 112  |
| $NaHCO_3$ (buffer pH)   | 1200 | 1200 |
| $MgSO_4 \cdot 7H_2O$    | 25   | 25   |
| $FeSO_4 \cdot 7H_2O$    | 20   | 20   |
| $CaCl_2 \cdot 2H_2O$    | 30   | 30   |

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