

# The Effects of Different External Carbon Sources on Nitrous Oxide Emissions during Denitrification in Biological Nutrient Removal Processes

Xiang Hu<sup>1,\*</sup>, Jing Zhang<sup>2</sup> and Hongxun Hou<sup>2</sup>

<sup>1</sup>Anhui Guozhen Environmental Protection Sci. &Tech. Co., Ltd., Hefei 230088, Anhui Province, China, xiangh1215@outlook.com

<sup>2</sup>Anhui Guozhen Environmental Protection Sci. &Tech. Co., Ltd., Hefei 230088, Anhui Province, China

\*Corresponding author

**Abstract.** The aim of this study was to investigate the effects of two different external carbon sources (acetate and ethanol) on the nitrous oxide (N<sub>2</sub>O) emissions during denitrification in biological nutrient removal processes. Results showed that external carbon source significantly influenced N<sub>2</sub>O emissions during the denitrification process. When acetate served as the external carbon source, 0.49 mg N/L and 0.85 mg N/L of N<sub>2</sub>O was produced during the denitrification processes in anoxic and anaerobic/anoxic experiments, giving a ratio of N<sub>2</sub>O-N production to TN removal of 2.37% and 4.96%, respectively. Compared with acetate, the amount of N<sub>2</sub>O production is negligible when ethanol used as external carbon addition. This suggested that ethanol is a potential alternative external carbon source for acetate from the point of view of N<sub>2</sub>O emissions.

**Keywords:** Nitrous oxide (N<sub>2</sub>O); Carbon source; Denitrification.

## 1. Introduction

Nitrous oxide (N<sub>2</sub>O), an extremely potent greenhouse gas, can be emitted from wastewater treatment, significantly contributing to the greenhouse gas footprint [1]. N<sub>2</sub>O emissions from biological denitrification processes are one of the major sources of N<sub>2</sub>O emissions during the biological nutrient removal (BNR) process in wastewater treatment. In the full-scale wastewater treatment plants (WWTPs), addition of external carbon sources is usually required for satisfied biological nutrient removal (BNR) performance and low effluent nutrients levels. Different carbon sources may affect the activities of denitrifying enzymes during denitrification process, leading to a different amount of N<sub>2</sub>O production [2, 3]. A few studies have focused on the impact of carbon sources on N<sub>2</sub>O generation. Park et al. [4] reported that addition of methanol as an external carbon source led to an appreciable reduction of the N<sub>2</sub>O emission from 4.5% to 0.2% of the nitrogen load. Li et al. [3] reported that acetate was a better carbon source for the promotion of denitrification efficiency and reduction of N<sub>2</sub>O production than glucose or sucrose. Adouani et al.[5] investigated the effects of carbon sources (acetate, ethanol, a mixture composed of ethanol and acetate, and two long carbon chain compounds) on N<sub>2</sub>O emissions during biological denitrification. Their results revealed that the highest and lowest N<sub>2</sub>O emissions occurred when acetate and ethanol were used as a carbon source, respectively. The aim of this work was to investigate the effects of two different and widely used external carbon

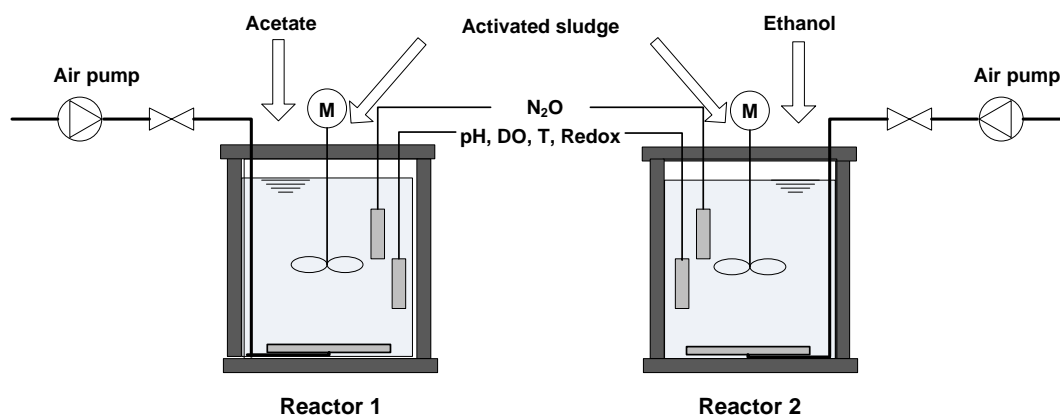


sources (acetate and ethanol) on the  $\text{N}_2\text{O}$  emissions during denitrification in biological nutrient removal processes.

## 2. Methods and Materials

### 2.1. Laboratory Apparatus

The main part of a specially designed and constructed experimental apparatus (Fig. 1) was two parallel batch reactors (max. volume of 4 L). The reactors were equipped with electrodes and probes (WTW, Germany) for a continuous monitoring of pH (SenTix 21), oxidation-reduction potential (ORP) (SenTix ORP), temperature and dissolved oxygen (DO) (CellOx 325). The nitrous oxide ( $\text{N}_2\text{O}$ ) concentrations in liquid during the denitrification processes were measured on-line using a miniaturized Clark-type sensor (Unisense, Denmark). The automated control systems for heating/cooling allowed maintenance of temperature around 20 °C. The mixed liquor in the reactors was mixed with mechanical stirrers at the velocity 180 rpm.



**Figure 1.** Experimental set-up used in the batch experiments

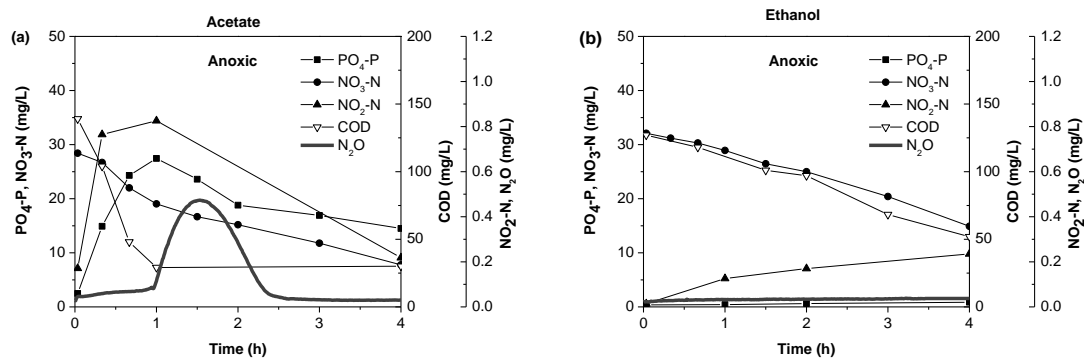
### 2.2. Batch Experiments

Two types of batch experiments, including one-phase (anoxic) experiments and two-phase (anaerobic/anoxic) experiments, were carried out in the parallel bioreactors to evaluate the effect of acetate and ethanol as different external carbon sources on the  $\text{N}_2\text{O}$  emissions during denitrification. Fresh mixed liquor from a full-scale A<sup>2</sup>/O plant (60 000 m<sup>3</sup>/d) was used in the experiments. In the one-phase experiments, a source of nitrate ( $\text{KNO}_3$ ) and the external carbon source were injected in the ratio of 6 g COD/g  $\text{NO}_3\text{-N}$  at the beginning and the test was run for 4h. In the two-phase tests, process biomass and sodium acetate were mixed and kept under anaerobic conditions for 2.5 h before injecting nitrate and the external carbon sources. Samples of the mixed liquor were frequently (every 5-60 min) withdrawn from the batch reactors, filtered under vacuum pressure on the Whatman GF/C filter and analyzed for  $\text{PO}_4\text{-P}$ ,  $\text{NO}_3\text{-N}$ ,  $\text{NO}_2\text{-N}$  and COD. The mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) concentrations were determined at the beginning of the tests.

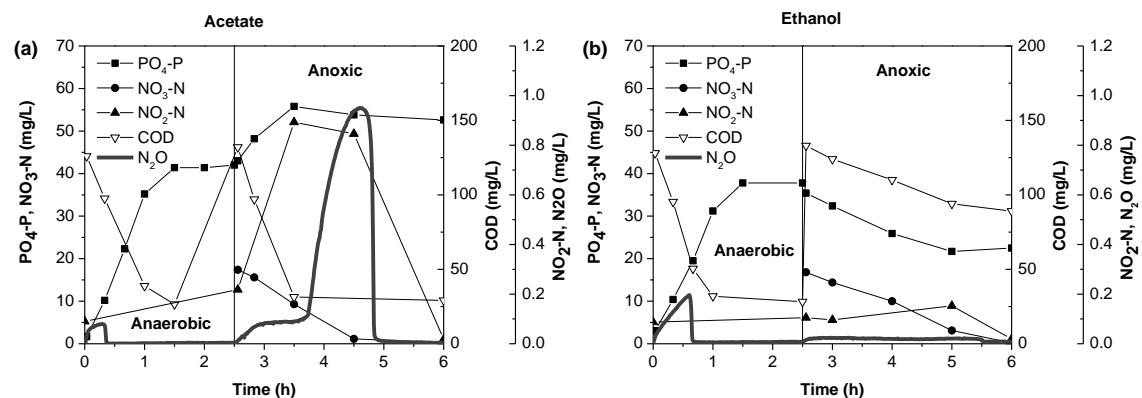
## 3. Results and Discussion

The results of different external carbon sources (acetate and ethanol) on  $\text{N}_2\text{O}$  emission during denitrification in anoxic experiments and anaerobic/anoxic experiments were shown in Fig. 2 and Fig. 3, respectively. As shown in Fig. 3a and 3b, increased  $\text{N}_2\text{O}$  emission was observed at the beginning of anaerobic phase in response to the addition of acetate (but not ethanol). Afterwards, the generation of  $\text{N}_2\text{O}$  was undetectable. This probably related to the metabolism of bacteria in reactors needing time to respond to the rapidly change in environmental conditions, resulting in substantial peak emissions of  $\text{N}_2\text{O}$  [6]. In the anoxic phase, when acetate was used as external carbon source, higher  $\text{N}_2\text{O}$  emissions

were observed in both anoxic experiments and anaerobic/anoxic experiments, with the peak  $\text{N}_2\text{O}$  concentrations achieved at 0.47 mg/L and 0.95 mg/L, respectively (Fig. 2a and 3a). Overall, approximately 0.49 mg N/L and 0.85 mg N/L of  $\text{N}_2\text{O}$  was produced during the anoxic phase, giving a ratio of  $\text{N}_2\text{O}$ -N production to TN removal of 2.37% and 4.96% respectively (Table 1). Both values are lower than the value of 7.77% obtained in an anaerobic/anoxic/oxic SBR acclimated with acetate as sole carbon source [7]. When ethanol was used as carbon source, conversely, very negligible  $\text{N}_2\text{O}$  emission was observed in either one- or two-phase experiment during the anoxic phase (Fig. 2b and 3b). Obviously, the amount of  $\text{N}_2\text{O}$  production is remarkably lower when ethanol as external carbon addition compared with acetate.



**Figure 2.** Effect of external carbon source addition on  $\text{N}_2\text{O}$  emission in anoxic experiments



**Figure 3.** Effect of external carbon source addition on  $\text{N}_2\text{O}$  emission in anaerobic/anoxic experiments

**Table 1.** Comparison of  $\text{N}_2\text{O}$  production during denitrification with different carbon sources

Experiments	Item	Acetate	Ethanol
One-phase (anoxic)	Anoxic $\text{N}_2\text{O}$ emission (mg N/L)	0.49	0.13
	TN removal (%)	72.7	53.6
	Ratio of anoxic $\text{N}_2\text{O}$ to TN removal (%)	2.37	0.78
Two-phase (anaerobic/anoxic)	Anoxic $\text{N}_2\text{O}$ emission (mg N/L)	0.85	0.06
	TN removal (%)	98.6	98.7
	Ratio of anoxic $\text{N}_2\text{O}$ to TN removal (%)	4.96	0.39

It should be noted that the dramatically increase of  $\text{N}_2\text{O}$  emissions occurred when acetate was depleted around 1 h and 3.5 h in one- and two-phase experiments, respectively (Fig. 2a, 3a). Carbon source concentration is an important factor influencing  $\text{N}_2\text{O}$  emission and limited availability of biodegradable organic carbon would increase  $\text{N}_2\text{O}$  emission during denitrification [8,9]. Schalk-Otte et

al.[10] observed that up to 32-64% was emitted as  $N_2O$  when organic carbon became limiting in a pure culture study of *Alcaligenes faecalis*. The same authors also demonstrated that  $N_2O$  accumulated as soon as bacteria started to consume internal storage compounds (PHAs), which might be a general factor related to  $N_2O$  emission [6]. A possible mechanism for  $N_2O$  emission by organisms growing on PHAs is the fact that PHAs consumption is the rate-limiting step in these organisms, which leads to competition for electrons between the denitrifying enzymes, apparently resulting in a higher NO reduction rate compared to the  $N_2O$  reduction rate and finally the  $N_2O$  generation. In the one-phase experiments in this study, the carbon source for denitrification was from the external carbon addition (acetate and ethanol), whereas both external carbon source and anaerobically synthesized PHAs were served as carbon sources in two-phase experiments. This also attributed to the higher  $N_2O$  emission in two-phase experiments than that in one-phase experiments.

High nitrite ( $NO_2-N$ ) concentration during denitrification has been reported to inhibit the activity of nitrous oxide reductase, decrease denitrification rate and be relevant to  $N_2O$  emissions during biological denitrification[11]. According to the measured  $NO_2-N$  value during denitrification in this study, it was found that the dramatically increase of  $N_2O$  emissions also occurs after a concentration peak of nitrites (0.83 and 0.89  $NO_2-N/L$  in one- and two-phase experiments, respectively) with acetate as carbon source. In the case of experiments with ethanol, however, rather low and stable nitrite concentrations (below 0.20 mg  $NO_2-N/L$ ) were observed. These observations suggest that  $N_2O$  emissions are linked to nitrite accumulation and concentration levels [5]. However, some recent studies reported that the protonated species of nitrite, free nitrous acid (FNA), rather than nitrite itself, is likely the actual inhibitor of the  $N_2O$  emissions in BNR systems [11,12]. Further research is needed to clarify this issue in the future.

#### 4. Conclusion

External carbon sources significantly influenced the  $N_2O$  emissions during the denitrification processes. When acetate served as the external carbon source, 0.49 mg N/L and 0.85 mg N/L of  $N_2O$  was produced during the denitrification processes in anoxic and anaerobic/anoxic experiments, giving a ratio of  $N_2O-N$  production to TN removal of 2.37% and 4.96%, respectively. When ethanol served as external carbon addition, the amount of  $N_2O$  production in anoxic and anaerobic/anoxic experiments is 0.13 mg N/L and 0.06 mg N/L, respectively, remarkably lower compared with acetate. Lower amount of  $N_2O$  emission during denitrification process suggested that ethanol is a potential alternative external carbon source for acetate from the point view of  $N_2O$  emissions.

#### 5. Acknowledgement

This research was financially supported by the project “CARbon BALancing for nutrient control in wastewater treatment (CARBALA)” (PIRSES-GA-2011-295176) and the National Water Pollution Control and Management Project (2015ZX07218001).

#### 6. References

- [1] J. K. Marlies, T. Hardy, K. Robbert, S.M. J. Mike, C.M. L. Mark, Nitrous oxide emission during wastewater treatment, *Water Res.* 43 (2009) 4093-4103.
- [2] F. Kargi, A. Uygur, Effect of carbon source on biological nutrient removal in a sequencing batch reactor, *Bioresour. Technol.* 89(2003) 89-93.
- [3] Q.H. Li, P. Li, P.P. Zhu, J.H. Wu, S.Z. Liang, Effects of exogenous organic carbon substrates on nitrous oxide emissions during the denitrification process of sequence batch reactors, *Environ. Eng. Sci.* 25(2008) 1221-1228.
- [4] K.Y. Park, Y. Inamori, M. Mizuochi, K.H. Ahn, Emission and control of nitrous oxide from a biological wastewater treatment system with intermittent aeration, *J. Biosci. Bioeng.* 90(2000) 247-252.
- [5] N. Adouani, T. Lendormi, L. Limousy, O. Sire, Effect of the carbon source on  $N_2O$  emissions during biological denitrification, *Resources, Conservation and Re.* 54(2010) 299-302.
- [6] M.J. Kampschreur, H. Temmink, R. Kleerebezem, M.S.M. Jetten, M.C.M. van Loosdrecht, Nitrous oxide emission during wastewater treatment, *Water Res.* 43(2009) 4093-4103.

- [7] Y.Y. Wang, J.J. Geng, G. Guo, C. Wang, S. Liu, N<sub>2</sub>O production in anaerobic/anoxic denitrifying phosphorus removal process: The effects of carbon sources shock, *Chem. Eng. J.* 172(2011) 999-1007.
- [8] Y.C. Chung, M.S. Chung, BNP test to evaluate the influence of C/N ratio on N<sub>2</sub>O production in biological denitrification, *Water Sci. Tech.* 42(2000) 23-27.
- [9] Q. Yang, X.H. Liu, C.Y. Peng, S.Y. Wang, H.W. Sun, Y.Z. Peng, N<sub>2</sub>O production during nitrogen removal via nitrite from domestic wastewater: main sources and control method, *Environ. Sci. Technol.* 43(2009) 9400-9406.
- [10] S. Schalk-Otte, R.J. Seviour, J.G. Kuenen, M.S.M. Jetten, Nitrous oxide (N<sub>2</sub>O) production by *Alcaligenes faecalis* during feast and famine regimes, *Water Res.* (2000) 34, 2080-2088.
- [11] Y. Zhou, M. Pijuan, R. Zeng, Z. Yuan, Free nitrous acid inhibition on nitrous oxide reduction by denitrifying-enhanced biological phosphorus removal sludge, *Environ. Sci. Technol.* 42 (2008) 8260-8265.
- [12] . Zhou, A. Oehme, M. Lim, V. Vadivelu, W.J. Ng, The role of nitrite and free nitrous acid (FNA) in wastewater treatment plants, *Water Res.* 45(2011) 4672-4682.