Pol. J. Environ. Stud. Vol. 29, No. 1 (2020), 901-908
DOI: *10.15244/pjoes/104656* ONLINE PUBLICATION DATE: 2019-09-10

Original Research

Characteristics of N_2O Accumulation during the Endogenous Denitrification of Nitrite

Ding Xiaoqian[1], Zhao Jianqiang[2,3]*, Hu Bo[4], Wang Sha[2], Li Xiaoling[4], Lan Lan[2]

[1]School of Architecture and Civil Engineering, Xi'an University of Science and Technology, Xi'an, Shaanxi, China
[2]School of Environmental Science and Engineering, Chang'an University, Xi'an, Shaanxi, China
[3]Key Laboratory of Subsurface Hydrology and Ecological Effects in Arid Region (Chang'an University), Ministry of Education, Xi'an, Shaanxi, China
[4]School of Civil Engineering, Chang'an University, Xi'an, Shaanxi, China

Received: 17 December 2018
Accepted: 23 February 2019

Abstract

Emissions of nitrous oxide (N_2O) occur during the biological denitrification process, but a few studies have focused on N_2O emissions during denitrification under endogenous conditions. To investigate the characteristics of N_2O emissions and accumulation during the process of endogenous denitrification, experiments were carried out to explore the action mechanisms of nitrite (NO_2^-) on N_2O production during the denitrification process, with intracellular polymers serving as the electron donor. The results indicated that the N_2O production factors increased with increasing initial NO_2^--N concentration due to the slow biological degradation of the intracellular polymers and the inhibitions of free nitrite acid (FNA) on the activity of nitrous oxide reductase (Nos). When the NO_2^--N concentration was further increased, exceeding 100 mg N/L, the N_2O production factors decreased, potentially on account of the inhibition of increased FNA on the activities of nitrite reductase (Nir) and nitric oxide reductase (Nor), resulting in declined nitrite removal efficiency and increased NO production. Studying the mechanisms of N_2O accumulation during endogenous denitrification is beneficial for controlling N_2O emissions during the biological nitrogen removal process in wastewater treatment.

Keywords: nitrous oxide, nitrite, endogenous denitrification, accumulation

Introduction

Nitrous oxide (N_2O) is an important greenhouse gas (GHG) with a global warming effect equal to 298 times that of CO_2 in a 100-year perspective [1]. N_2O in the atmosphere can also destroy the ozone layer [2, 3] and produce acid rain and other hazards [4]. A wastewater treatment plant (WWTP) is an important anthropogenic source of N_2O emissions; the range of N_2O emissions from afull-scale WWTP reported in the literature is 0~25% [5]. N_2O generation and accumulation in WWTPscome mainly from the biological nitrogen removal (BNR) process, including the processes of aerobic nitrification and anoxic denitrification [6, 7]. Low DO (dissolved oxygen) levels and high nitrite accumulation in both the nitrification stage and the denitrification stage and insufficient biodegradable substrate (carbon source) in the denitrification stage are

*e-mail: 626710287@qq.com

the main factors for N_2O accumulation and emissions in the BNR process [8].

It is well known that the traditional nitrification and denitrification processes can be achieved through nitrite instead of nitrate in order to achieve nitrogen removal. Compared with nitrate-based nitrification and denitrification technology, approximately 25% of aeration and 40% of carbon source addition can be reduced in this short-cut BNR process, and sludge production can also be reduced to an extent [9, 10]. In addition, on the condition that polyhydroxyalkanoate (PHA), poly-b-hydroxybutyrate (PHB) and other intracellular polymers are used as electron donors for denitrification, sludge production can be further reduced by approximately 20% compared with that when extracellular organics are used as electron donors [11]. Therefore, the prospect for application of the nitrite-type endogenous denitrification technology is very broad. However,as free nitrite acid (FNA) can inhibit the metabolic behavior of the denitrifying bacteria, once nitrite (NO_2^-) accumulates in large quantities during endogenous denitrification, N_2O will also be produced in this process [12]. Likewise, in the endogenous denitrification process with intracellular polymers (such as PHA, PHB and glycogen) serving as the electron donors for oxynitride reduction, more accumulation and emission of N_2O would be observed [13, 14]. Therefore, due to the large accumulation of nitrite and the use of intracellular polymers as carbon sources for denitrification, the short-cut BNR process may generate and release large amounts of N_2O [15, 16].More attention should be paid to N_2O emissions for the short-cut biological nitrogen removal with a high nitrite accumulation [17]. Most of all, it is of great significance to study the mechanisms of N_2O production in the process of endogenous denitrification of nitrite. However, much of the prior research has studied N_2O emission mechanisms of the denitrification systems with different carbon sources from the sewage serving as electron donors [17, 18]; few studies have focused on N_2O accumulation and emissions from endogenous denitrification of nitrite with intracellular polymers as electron donors.

In this paper, N_2O production during endogenous denitrification at different initial NO_2^--N concentrations was studied by experimental methods, aimed at clarifying the mechanisms of nitrite on N_2O accumulation in the process of endogenous denitrification. To explore the accumulation mechanism of N_2O in the BNR process is helpful in mitigation of GHG emissions from WWTPs.

Material and Methods

Experiment Setup

The experiment was carried out on a six-stand agitator. Six identical open beakers (with an effective volume of 500 mL) containing a mixture of activated sludge were placed on the six-strand agitator to conduct the reaction. At the beginning of the reaction, sodium nitrite ($NaNO_2$) solution was added to each beaker. The stirring rate of the six-strand agitator was approximately 100±10 r/min, and the reaction temperature was at the room temperature of 23.6-25.4ºC. The reaction device is shown in Fig. 1.

Experiment Design

The activated sludge used in the experiment was taken from an aeration tank in a continuous flow wastewater treatment reactor from our laboratory. The laboratory-scale reactor was operated in the anaerobic-anoxic/aerobic (A^2/O) mode, with an effective volume of 37.5 L. The influent of the reactor was composed of synthetic wastewater to simulate domestic sewage, and the hydraulic retention time (HRT) and the sludge retention time (SRT) of the system were 15 h and 20 d, respectively [19]. The reactor was operated for more than 6 months and the heterotrophic bacteria were enriched with acetate as the source of organic carbon. The effluent COD, ammonia nitrogen (NH_4^+-N), nitrite nitrogen (NO_2^--N) and nitrate nitrogen concentrations in the aeration tank were 10 mg/L, 1.17~1.61 mg N/L, 0 mg N/L and 0 mg N/L, respectively, and the dissolved

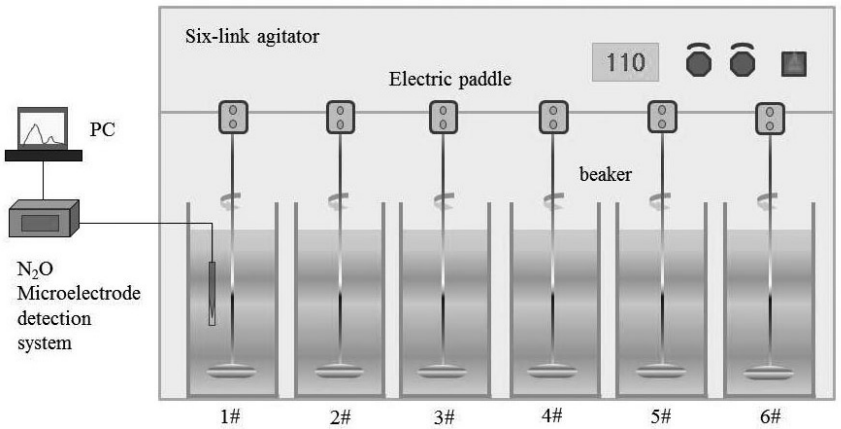

Fig. 1. Schematic diagram of the reaction devices.

N_2O in the effluent was 0 mg N/L. In the experiment, six parts of the active sludge mixture with the same volume (400 mL each) were initially added into the six beakers. The concentration of the mixed liquor suspended solids (MLSS) in the six beakers was approximately 3650 mg/L, and the concentration of the mixed liquor volatile suspended solids (MLVSS) was approximately 2865 mg/L. The initial dissolved oxygen (DO) concentration was approximately 2.2 mg O_2/L, and the initial pH of the mixture was 6.85. Then the six beakers were placed on the six-strand agitator simultaneously. Before starting the reaction, six solutions of $NaNO_2$ with different NO_2^--N concentrations were added to the beakers, making the initial NO_2^--N concentration in six beakers to be 10 mg N/L (1#), 30 mg N/L(2#), 60 mg N/L (3#), 100 mg N/L (4#), 200 mg N/L (5#) and 250 mg N/L (6#), respectively. The nitrite was continuously reduced under anoxic stirring conditions. The variation trends of N_2O production in each beaker were measured successively with a measurement interval of 30-60 min. The water quality indexes, sludge concentration, DO and pH at the beginning and end of the reaction in the six beakers were analyzed and determined.

Analysis Methods

Experimental monitoring items, which included COD, NH_4^+-N, NO_2^--N, NO_3^--N, MLSS and MLVSS, were analyzed according to standard methods [20]; DO, temperature and pH were monitored by online probes. The total nitrogen (TN) concentration was based on the sum of ammonia nitrogen, nitrite nitrogen and nitrate nitrogen. N_2O produced by the system was comprised of the dissolved N_2O in the mixture and the N_2O emitted into the air. The dissolved N_2O in the mixture was determined using the Unisense Microelectrode System (Arhus, Denmark), and the emission of N_2O was obtained by the calculation methods described in the literature [21, 22]; N_2O production was based on the sum of the amounts of the dissolved N_2O and the emitted N_2O.

Results and Discussion

Reaction of the Endogenous Denitrification Process

Biological denitrification is a process in which microorganisms reduce nitrate or nitrite to N_2 by anoxic respiration with organics as electron donors and nitrate or nitrite as electron receptors [9]. When the carbon source in the influent is insufficient, microorganisms can also consume their own cellular (intracellular polymers of PHA, PHB and glycogen, etc.) as the carbon source for denitrification, which is called endogenous denitrification [7, 17]. Therefore, endogenous denitrification by heterotrophic bacteria is a process of decreasing microbial cell materials. The microorganisms in this study came from the continuous flow A^2/O reactor. The heterotrophic bacteria stored most of the organic substrate in the influent during the anaerobic stage in the original A^2/O reactor, followed by use of the formed intracellular polymers for growth and metabolism in the subsequent anoxic and aerobic stage [25]. Since the biodegradation rate of organic matter stored in microbial cells is lower than that of extracellular organic matter [26], the heterotrophic bacteria prioritize the use of extracellular substrate(the biodegradable organics in influent) for aerobic and anoxic growth and the use intracellular polymers after the depletion of the biodegradable substrate [7]. Therefore, under certain conditions, the residual intracellular polymers were still present in microorganisms in the aerobic sludge of the A^2/O reactor.

The activated sludge containing the residual intracellular polymers was used in the experiment to investigate N_2O production during the process of endogenous denitrification at different initial NO_2^--N concentrations. Intracellular polymers stored in the microbial cells were used as the electron donor to reduce nitrite to NO, N_2O and N_2 in sequence. The experimental results are shown in Fig. 2. As the results show, because certain DO (2.2 mg O_2/L, brought from the activated sludge mixture of the aerobic tank) existed at the commencement of the reaction and a small amount of O_2 from the air was brought into and dissolved in the mixture due to the mechanical stirring, nitrite was oxidized to nitrate by autotrophic bacteria in the mixture and, accordingly, the denitrification of nitrate was observed in the experiments (Fig. 2e). As a consequence, at the end of the reaction, as DO was consumed by the growth process of autotrophic bacteria, the DO concentration in the mixture decreased to below 0.15 mg O_2/L (Fig. 2c). In addition, during nitrite denitrification, as the biological reduction reaction continuously produced alkalinity (OH^-), the pH of the mixture at the end of the reaction increased from 6.84 to a range of 6.87-7.15. The higher the initial concentration of NO_2^--N, the more OH^- was produced by the reaction, with a consequent increase in pH (Fig. 2b).

The denitrification process lasted approximately 300~330 min. At the end of the reaction, the removal rate of NO_2^--N was in the range of 12.86~74.80% and gradually decreased with the increase in the initial concentration of NO_2^--N (Fig. 2d). The highest removal efficiency of nitrite (at 100 mg N/L)was probably because more nitrite was oxidized to nitrate rather than reduced to NO, N_2O or N_2, as the TN removal efficiency continually decreased with the increasing initial concentration of NO_2^--N (Fig. 2f). This result is mainly due to the ratio of the intracellular carbon to nitrogen decreasing in experiments 1#through 6#. The insufficient electron donors resulted in the low removal efficiency of nitrite and nitrate. Meanwhile, the removal rateof NO_3^--N decreased with the increase

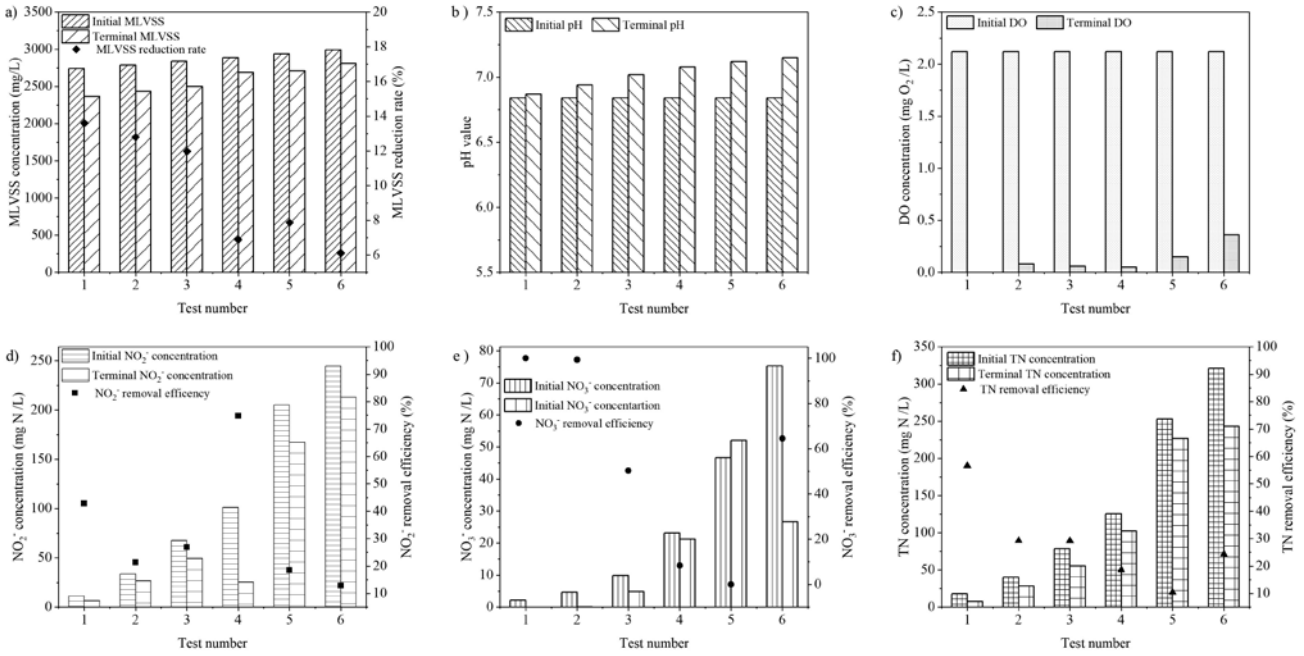

Fig. 2. Concentration and reduction rate of MLVSS a), pH b), DO c) and concentration and removal efficiency of NO_2^--N (a), NO_3^--N B), TN C) at the beginning and at the end of the endogenous denitrification process.

in the initial concentration of NO_2^--N. In cases where the initial concentration of NO_2^--N was low (1# and 2#), the initial concentration of NO_3^--N was also low, so it could be completely reduced. As the initial concentration of NO_2^--N continued to increase, reaching 100 mg N/L (4#), the initial concentration of NO_3^--N reached 23.10 mg N/L, and the NO_3^--N removal rate was only 8.32%. As the initial concentration of NO_2^--N and NO_3^--N continued to rise (5# and 6#), the removal of NO_3^--N was not observed (Fig. 2e). The TN removal process was similar to that of NO_3^--N, with a removal rate of 9.19~56.49% (Fig. 2f). NH_4^+-N was scarcely detected during the reaction. Since no extra carbon source was provided in the experiment, the heterotrophic bacteria derived the energy for nitrite reduction from the microorganisms' growth process on their intracellular polymers, so the decrease in MLVSS was observed in different reactions, as shown in Fig. 2a). Because the intracellular polymers are part of the microorganism itself, when these materials were used as electron donors for denitrification, the microorganism cell material and its own weight were reduced [25, 26]. The decreased rate of MLVSS observed in the experiment was approximately 6.12-13.61% and decreased with the increase in the initial concentration of NO_2^--N. In general, the reaction rate of the heterotrophic bacteria, including the reduction rate of nitrite and nitrate and the uptake rate of intracellular polymers, declined with the increased initial nitrite concentration. This result was mainly due to the inhibition effect of FNA on the activities of microorganisms and the decreased ratio of the intracellular carbon to nitrogen.

Mechanism of N_2O Accumulation

In the biological denitrification process, nitrate is reduced to NO_2^-, NO, N_2O and N_2, successively [27], catalyzed by four different reductases: Nar (nitrate reductase), Nir (nitrite reductase), Nor (NO reductase) and Nos (N_2O reductase), respectively [28]. As mentioned above, the final product of the traditional denitrification process is N_2, and N_2O is the intermediate product [29]. N_2O production in the endogenous denitrification of nitrite at different initial NO_2^--N concentrations was measured in this study, and the results are shown in Fig. 3. The results showed that N_2O production increased with the increase in the initial concentration of NO_2^--N. N_2O production was 0.20~24 mg N/L on the condition that the initial concentration of NO_2^--N was in the range of 10~100 mg N/L (experiments 1#~4#). However, N_2O production decreased to 17.76 mg N/L (5#) and 14.84 mg N/L (6#) when the initial concentration of NO_2^--N exceeded 100 mg N/L (experiments 5# and 6#). In other words, N_2O production decreased with the increase in the initial concentration of NO_2^--N on the condition that the initial concentration of NO_2^--N exceeded 100 mg N/L (5# and 6#) (Fig. 3b).

Studies have found that in cases where the available organics are scarce, the electrons provided for complete denitrification by heterotrophic bacteria will be insufficient, often leading to electron competition between four denitrification enzymes [4, 30]. Therefore, due to the low degradation rate of the intracellular polymer, the degradation process could not provide enough electrons. As a consequence, electron competition exists in denitrification systems with

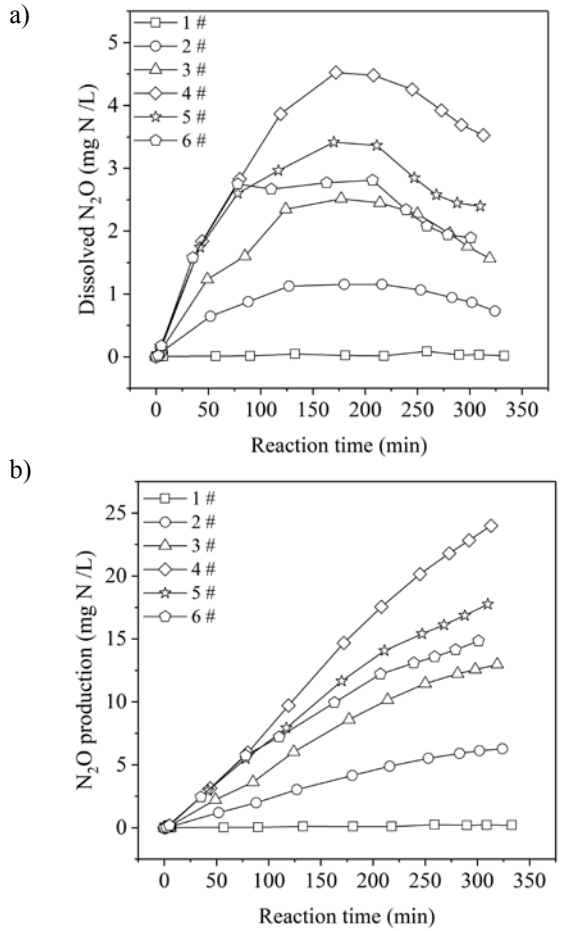

Fig. 3. Concentration of the dissolved N_2O a) and the production amount of N_2O b) of the endogenous denitrification process.

intracellular polymers serving as the electron donor [6, 7]. Under these circumstances, due to the reduction of N_2O to N_2 being the last step of denitrification, Nos had a weaker ability to compete for electrons, making it more difficult for Nos to obtain sufficient electrons to reduce N_2O to N_2 [2, 6]. In addition, it has been reported that the low level of dissolved oxygen present during denitrification stimulates N_2O accumulation, as Nos is inhibited by oxygen [8, 30]. Therefore, accumulation of N_2O was inevitable in this denitrification system with intracellular polymers serving as electron donors and the presence of dissolved oxygen in the mixture [31, 32].

In addition, adverse changes in environmental factors, such as pH, temperature and FNA, will intensify electron competition and eventually lead to the accumulation of N_2O [30]. Especially in the process of endogenous denitrification by heterotrophic bacteria, the main reason for N_2O accumulation is the stronger inhibition on Nos activity by FNA than on Nir and Nor [33]. Additionally, some studies have reported that the endogenous denitrification of nitrate did not result in N_2O accumulation, pointing out that FNA might be the inducing factor for N_2O accumulation, so nitrite played a significant role in N_2O accumulation during denitrification [34, 35]. It can be inferred that the higher the initial concentration of NO_2^--N, the higher the concentration of FNA [36], consequently resulting in a stronger inhibition of FNA on Nos activity and leading to higher N_2O production factors (the ratio of N_2O production to TN removal), which was observed in experiments 1#-4#, as shown in Fig.4. Based on calculation of the nitrogen balance, the endogenous denitrification reaction removed all of the TN as N_2O without producing NO and N_2, on condition that the initial NO_2^--N concentration was 100 mg N/L (4#). During the reaction, the strong inhibition of FNA on Nos activity resulted in all N_2O produced by NO reduction accumulating in the reactor; therefore, N_2O became the sole end product of the denitrification process. Due to the broad depression of FNA on the activities of denitrifying bacteria, the resultant increased NO_2^--N concentration led to a slowed degradation rate of intracellular polymers, thus further leading to slowed denitrification rates of NO_3^-, NO_2^-, NO and N_2O. However, the denitrification rate of N_2O was slowed more than others due to its weak electron competition ability [21], which resulted in more N_2O production in the case where initial NO_2^--N concentration gradually increased (experiments 1#-4#). This result is consistent with the research results of Wei et al. [37]. However, when the initial concentration of NO_2^--N exceeded 100 mg N/L, the observed range of suppression by FNA increased in this study, including not only Nos but also Nir and Nor. Consequently, as NO_2^--N concentration continued to rise significantly, the FNA inhibitory effect on Nor was enhanced as well, causing NO to accumulate in the reactor. As has been reported, NO is highly reactive and toxic and contributes to ozone depletion and air pollution [38]. In addition, since NO has a mutagenic effect in bacteria, it is toxic to a wide range of organisms [39]. Therefore, NO has an inhibitory effect on many nitrogen-metabolizing microorganisms,

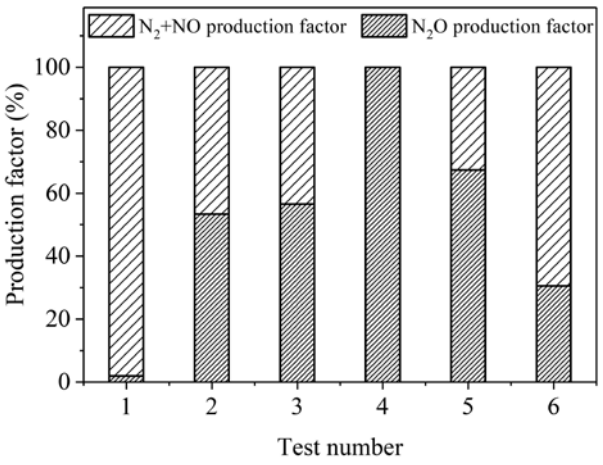

Fig. 4. The production factor of N_2O and the sum of NO and N_2 of the endogenous denitrification process.

including ammonium-oxidizing bacteria, nitrite-oxidizing bacteria, and denitrifiers [40,41]. Due to the microbial inhibition by NO [42], a large amount of accumulated NO suppressed Nir and Nor, leading to the decrease in the removal rate of nitrite (Fig. 2d) and the production factors for N_2O. Accordingly, the production factors for NO and N_2 increased in experiments 5# and 6# (Fig. 4).

Furthermore, it can be seen that the rules of behavior dictating the relationship of dissolved N_2O concentration with the initial NO_2^--N concertation in the six experimental processes was consistent with total N_2O production (Fig. 3). It should be noted that the phenomenon of dissolved N_2O concentration rising at the commencement of the reaction and then declining at the end of the reaction was observed in different experiments. This phenomenon was mainly a result of N_2O being easily soluble in water (Henry coefficient of 0.024 M/atm [6]), so N_2O generated during the reaction quickly dissolved in the mixture, but at the same time the N_2O dissolved in the mixture quickly escaped into the air due to mechanical stirring. In the first half of the reaction (0~175 min), due to the high content of intracellular polymers and the availability of sufficient electrons, the generation rate of N_2O was higher than the emission rate of N_2O, so more N_2O was dissolved in the mixture,and the concentration continued to increase. After a period of reaction, due to the consumption of intracellular polymers, electron availability decreased, leading to an N_2O production rate that was lower than the emission rate. Consequently, the concentration of dissolved N_2O decreased with time in the latter half of the reaction. This result was a unique phenomenon of N_2O accumulation in the sequential batch biological denitrification reactors [43].

Conclusions

The mechanisms of N_2O generation and accumulation were investigated in the process of endogenous denitrification of nitrite. It was found that,due to the inhibition of FNA on Nos, the yield and production factors of N_2O increased with the increase in the initial concentration of NO_2^--N. On the condition that the initial concentration of NO_2^--N reach 100 mg N/L, the final product of the denitrification process was entirely N_2O, while NO and N_2 were not observed. However, in the case of the initial NO_2^--N concentration being higher than 100 mg N/L, the high concentration of FNA inhibited the Nor activity as well, and NO accumulated in the reactor, while the production factor of N_2O decreased. Studying the relationship between N_2O accumulation and the nitrite concentration during endogenous denitrification is beneficial to mitigating GHG emissions from the BNR process in wastewater treatment.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (grant No. 51778057) and the Ph.D. program of Xi'an University of Science and Technology (grant No. 2017QDJ062).

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. IPCC. The final draft Report, dated 7 June 2013, of the Working Group I contribution to the IPCC 5th Assessment Report. In: Climate Change 2013: the Physical Science Basis. **2013**.
2. LI C., ZHANG J., LIANG S., NGO H.H., GUO W., ZHANG Y., ZOU Y. Nitrous oxide generation in denitrifying phosphorus removal process: main causes and control measures. Environ. Sci. Pollut. Res. Int., **20** (8), 5353, **2013**.
3. MONTZKA S., REIMANN S.C.L. A., O'DOHERTY S., ENGEL A., KRÜGER K., STURGES W.T. Ozone-depleting substances (ODSs) and related chemicals, in Chapter 1 in Scientific Assessment of Ozone Depletion: 2010. Geneva, Switzerland; Global Ozone Research and Monitoring Project. **2011**.
4. LU H., CHANDRAN K. Factors promoting emissions of nitrous oxide and nitric oxide from denitrifying sequencing batch reactors operated with methanol and ethanol as electron donors. Biotechnology and Bioengineering, **106** (3), 390, **2010**.
5. LAW Y., YE L., PAN Y., YUAN Z.. Nitrous oxide emissions from wastewater treatment processes. Phil. Trans. R. Soc. B. **367** (1593), 1265, **2012**.
6. KAMPSCHREUR M.J., TEMMINK H., KLEEREBEZEM R., JETTEN M.S., VAN LOOSDRECHT M.C.M. Nitrous oxide emission during wastewater treatment. Water Research, **43** (17), 4093, **2009**.
7. SCHALK-OTTE S., SEVIOUR R.J., KUENEN J.G., JETTEN M.S.M. Nitrous oxide (N_2O) production by Alcaligenes faecalis during feast and famine regimes. Water Research, **34** (7), 2080, **2000**.
8. WUNDERLIN P., MOHN J., JOSS A., EMMENEGGER L., SIEGRIST H. Mechanisms of N_2O production in biological wastewater treatment under nitrifying and denitrifying conditions. Water Research, **46** (4), 1027, **2012**.
9. DOSTA J., GALI A., BENABDALLAH EL-HADJ T., MACE S., MATA-ALVAREZ J. Operation and model description of a sequencing batch reactor treating reject water for biological nitrogen removal via nitrite. Bioresource Technology, **98** (11), 2065, **2007**.
10. ZENG R.J., LEMAIRE R., YUAN Z., KELLER J. Simultaneous nitrification, denitrification, and phosphorus removal in a lab-scale sequencing batch reactor. Biotechnology and Bioengineering, **84** (2), 170, **2003**.
11. THIRD K.A., NEWLAND M., CORD-RUWISCH R. The effect of dissolved oxygen on PHB accumulation

in activated sludge cultures. Biotechnology and Bioengineering, **82** (2), 238, **2003**.
12. LIU Y., PENG L., CHEN X., NI B.J. Mathematical Modeling of Nitrous Oxide Production during Denitrifying Phosphorus Removal Process. Environmental Science & Technology, **49** (14), 8595, **2015**.
13. ZHOU Y., LIM M., HARJONO S., NG W.J. Nitrous oxide emission by denitrifying phosphorus removal culture using polyhydroxyalkanoates as carbon source. Journal of Environmental Sciences, **24** (9), 1616,**2012**.
14. LEMAIRE R., MEYER R., TASKE A., CROCETTI G.R., KELLER J., YUAN Z. Identifying causes for N_2O accumulation in a lab-scale sequencing batch reactor performing simultaneous nitrification, denitrification and phosphorus removal. Journal of Biotechnology, **122** (1), 62, **2006**.
15. LAW Y., LANT P., YUAN Z. The confounding effect of nitrite on N_2O production by an enriched ammonia-oxidizing culture. Environmental Science & Technology, **47** (13), 7186, **2013**.
16. JIA W., ZHANG J., XIE H., YAN Y., WANG J., ZHAO Y., XU X. Effect of PHB and oxygen uptake rate on nitrous oxide emission during simultaneous nitrification denitrification process. Bioresource Technology, **113**, 232, **2012**.
17. WU G., ZHAI X., LI B., JIANG C., GUAN Y. Endogenous Nitrous Oxide Emission for Denitrifiers Acclimated with Different Organic Carbons. Procedia Environmental Sciences, **21**, 26, **2014**.
18. LI C., LIANG S., ZHANG J., NGO H.H., GUO W., ZHENG N., ZOU Y. N_2O reduction during municipal wastewater treatment using a two-sludge SBR system acclimatized with propionate. Chemical Engineering Journal, **222**, 353,**2013**.
19. DING X., ZHAO J., CHEN Y., HU B., MA B. Comparison of nitrogen and phosphorus removal performances for traditional and modified A^2/O process. Chinese Journal of Environmental Engineering, **12** (5), 1480, **2018**.
20. APHA. Standard Methods for the Examination of Water and Wastewater. American Public Health Association (APHA), Washington DC, USA. **2005**.
21. GAO K., ZHAO J., GE G., DING X., WANG S., LI X., YU Y. Effect of Ammonium Concentration on N_2O Emission During Autotrophic Nitritation Under Oxygen-Limited Conditions. Environmental Engineering Science, **34** (2), 96, **2016**.
22. ZHANG M., LAWLOR P.G., LI J., ZHAN X. Characteristics of nitrous oxide (N_2O) emissions from intermittently-aerated sequencing batch reactors treating the separated liquid fraction of anaerobically digested pig manure. Water, Air, & Soil Pollution, **223** (5), 1973, **2012**.
23. DING X., ZHAO J., HU B., LI X., GE G., GAO K., CHEN Y. Mathematical modeling of nitrous oxide (N_2O) production in anaerobic/anoxic/oxic processes: Improvements to published N_2O models. Chemical Engineering Journal, **325**, 386,**2017**.
24. WANG Y., GENG J., REN Z., HE W., XING M., WU M., CHEN S. Effect of anaerobic reaction time on denitrifying phosphorus removal and N_2O production. Bioresource Technology, **102** (10), 5674, **2011**.
25. MANSER R., GUJER W., SIEGRIST H. Decay processes of nitrifying bacteria in biological wastewater treatment systems. Water Research **40** (12), 2416,**2006**.
26. RODRÍGUEZ-ESCALES P., VAN BREUKELEN B.M., VIDAL-GAVILAN G., SOLER A., FOLCH A. Integrated modeling of biogeochemical reactions and associated isotope fractionations at batch scale: A tool to monitor enhanced biodenitrification applications. Chemical Geology, **365**, 20,**2014**.
27. LI C., WANG T., ZHENG N., ZHANG J., NGO H.H., GUO W., LIANG S. Influence of organic shock loads on the production of N_2O in denitrifying phosphorus removal process. Bioresource Technology, **141**, 160, **2013**.
28. PAN Y., NI B.J., BOND P.L., YE L., YUAN Z. Electron competition among nitrogen oxides reduction during methanol-utilizing denitrification in wastewater treatment. Water Research, **47** (10), 3273, **2013**.
29. PAN Y., YE L., NI B.J., YUAN Z. Effect of pH on N_2O reduction and accumulation during denitrification by methanol utilizing denitrifiers. Water Research, **46** (15), 4832, **2012**.
30. KIM D.-J. Effect of Aeration on Nitrous Oxide (N_2O) Emission from Nitrogen-Removing Sequencing Batch Reactors. Journal of Microbiology and Biotechnology, **23** (1), 99,**2013**.
31. KIM D.-J. Effect of Ammonium Concentration on the Emission of N_2O Under Oxygen-Limited Autotrophic Wastewater Nitrification. Journal of Microbiology and Biotechnology, **21** (9), 988, **2011**.
32. WANG Y., GENG J., GUO G., WANG C., LIU S. N_2O production in anaerobic/anoxic denitrifying phosphorus removal process: The effects of carbon sources shock. Chemical Engineering Journal, **172** (2-3), 999, **2011**.
33. DING X., ZHAO J., HU B., CHEN Y., GE G., LI X., WANG S., GAO K., TIAN X. Mathematical modeling of nitrous oxide production in an anaerobic/oxic/anoxic process. Bioresource Technology, **222**, 39, **2016**.
34. ZHOU Y., PIJUAN M., ZENG R.J., YUAN Z. Free nitrous acid inhibition on nitrous oxide reduction by a denitrifying-enhanced biological phosphorus removal sludge. Environmental Science & Technology, **42**(22), 8260,**2008**.
35. ZHOU Y., OEHMEN A., LIM M., VADIVELU V., NG W.J. The role of nitrite and free nitrous acid (FNA) in wastewater treatment plants. Water Research, **45** (15), 4672, **2011**.
36. ANTHONISEN A.C., LOEHR R.C., PRAKASAM T.B.S., SRINATH E.G. Inhibition of nitrification by ammonia and nitrous acid. Water Pollution Control Federation, **48** (5), 835, **1976**.
37. WEI Y., WANG S., MA B., LI X., YUAN Z., HE Y., PENG Y. The effect of poly-beta-hydroxyalkanoates degradation rate on nitrous oxide production in a denitrifying phosphorus removal system. Bioresource Technology, **170**, 175, **2014**.
38. JOHNSTON H. Reduction of stratospheric ozone by nitrogen oxide catalysts from supersonic transport exhaust. Science, **173** (3996), 517, **1971**.
39. KAMPSCHREUR M.J., VAN DER STAR W.R., WIELDERS H.A., MULDER J.W., JETTEN M.S., VAN LOOSDRECHT M.C. Dynamics of nitric oxide and nitrous oxide emission during full-scale reject water treatment. Water Research, **42** (3), 812, **2008**.
40. STARKENBURG S.R., ARP D.J., BOTTOMLEY P.J. Expression of a putative nitrite reductase and the reversible inhibition of nitrite-dependent respiration by nitric oxide in Nitrobacter winogradskyi Nb-255. Environmental microbiology, **10** (11), 3036, **2008**.
41. KARTAL B., TAN N.C., VAN DE BIEZEN E., KAMPSCHREUR M.J., VAN LOOSDRECHT M.C., JETTEN M.S. Effect of nitric oxide on anammox bacteria.

Applied and environmental microbiology, **76** (18), 6304, **2010**.
42. RODRIGUEZ-CABALLERO A., PIJUAN M. N_2O and NO emissions from a partial nitrification sequencing batch reactor: exploring dynamics, sources and minimization mechanisms. Water Research, **47** (9), 3131, **2013**.
43. ZHAO J., HUANG N., HU B., JIA L., GE G. Potential of nitrous oxide recovery from an aerobic/oxic/anoxic SBR process. Water Science and Technology, **73** (5), 1061, **2016**.