N₂O Reduction During Denitrifying Phosphorus Removal With Propionate as Carbon Source

Li Cong

Liaocheng University

Qian Wang

Jiangsu Normal University

Wenlin Jia (✉ jiawenlin@jsnu.edu.cn)

Jiangsu Normal University

Research Article

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Posted Date: April 19th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-375078/v1>

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Title:

N₂O reduction during denitrifying phosphorus removal with propionate as carbon source

Author names:

Li Cong^a, Qian Wang^b, Wenlin Jia^{b,*}

Author affiliations:

^a *School of Environmental and Programming, Liaocheng University, Liaocheng 252059, Shandong, China*

^b *School of Chemistry and Materials Science, Jiangsu Normal University, Xuzhou 221116, Jiangsu, China*

Corresponding author: Wenlin Jia, Tel: +86 516 83403165, Email address: jiawenlin@jsnu.edu.cn

Abstract

Denitrifying phosphorus removal was realized in sequencing batch reactors using different carbon sources (acetate, propionate, and a mixture of acetate/propionate). Nutrient removal and N₂O production were investigated, and the factors affecting N₂O production were explored. Nitrogen removal was 40.6% lower when propionate was used as the carbon source instead of acetate, while phosphorus removal was not significantly different. N₂O production was greatly reduced when propionate was used as the carbon source instead of acetate. The emission factor in the propionate system was only 0.43%, while those in the acetate and mixed-carbon source system were 16.3% and 1.9%, respectively. Compared to the propionate system, ordinary heterotrophic organisms (i.e., glycogen-accumulating organisms) were enriched in the acetate system, explaining the higher N₂O production in the acetate system. The lower nitrite accumulation in the propionate system compared to the acetate system was the dominant factor leading to the lower N₂O production.

Keywords: Nitrous oxide; Denitrifying phosphorus removal; Propionate; PHAs; Nitrite accumulation

31 **Declarations**

32 **Ethical approval:** Not applicable.

33 **Consent to participate:** Not applicable.

34 **Consent to publish:** Not applicable.

35 **Availability of data and materials:** Not applicable.

36 **Conflict of interest:** The authors declare that they have no conflict of interest.

37 **Funding:** This work was supported by the Natural Science Foundation of the Jiangsu
38 Higher Education Institutions of China (20KJA610003), and the Xuzhou Technology
39 & Science Foundation (KC20165).

40 **Authors' contributions:** C. Li: conceptualization, methodology, statistical analysis,
41 data analysis, writing original data preparation; Q. Wang: data collection; W. Jia:
42 supervision, reviewing, and editing.

43

1. Introduction

In order to control the eutrophication in surface water, nitrogen and phosphorus are continuously removed from wastewaters in many countries. Biological nutrient removal (BNR) is a widely applied process to simultaneously remove nitrogen and phosphorus through a combination of nitrification/denitrification and enhanced biological phosphorus removal (EBPR). However, the competition for carbon sources between the denitrification and EBPR processes limits the efficiency of nutrient removal and increases the cost (Wang et al. 2015). Currently, denitrifying phosphorus removal (DPR) is attracting considerable attention as a novel BNR process. In this process, denitrifying phosphorus-accumulating organisms (DPAOs) was enriched. It can use nitrite/nitrate instead of oxygen as the electron acceptor for phosphorous removal to achieve the simultaneous removal of nitrogen and phosphorus (Wang et al. 2011). Compared to the conventional BNR process, DPR process exhibits reduced sludge production and lower demand for oxygen and carbon, making it particularly suitable for treating wastewater with a low ratio of chemical oxygen demand (COD) to nitrogen.

However, nitrous oxide (N_2O) emission during DPR process have raised increasing concerns in the last decade (Gan et al. 2017, Li et al. 2013b, Liu et al. 2015, Wisniewski et al. 2018). N_2O is considered as an important greenhouse gas, and its 100-year global warming potential is 265 times higher than that of carbon dioxide (Chen et

al. 2019). During the biological nitrogen removal, it can be produced as an intermediate or byproduct. Therefore, biological wastewater treatment is regarded as an important anthropogenic source of N_2O . During the denitrifying phosphorus removal systems, the N_2O emission factors range from 2.3% to 21.6% of the influent nitrogen load (Liu et al. 2015). Therefore, to control the greenhouse gases release along with the overall carbon and nitrogen footprints of the wastewater treatment system, it is important to understand N_2O production during denitrifying phosphorus removal process.

During denitrification, N_2O can accumulate due to the imbalance in the reduction activities of key denitrifying enzymes [N_2O reductase (N_2OR), nitrate reductase (NAR), and nitrite reductase (NIR)]; this imbalance may result from either the acceleration of the reduction step (i.e., NO_2^- -N reduction) or the inhibition of N_2OR (Wisniewski et al. 2018). N_2O can be induced by specific operational conditions such as low dissolved oxygen concentration (Xie et al. 2012) or the influent compositions of the bioreactors (Massara et al. 2017). During the DPR process, the production of N_2O mainly arises from the following phenomena: the enrichment of denitrifying glycan bacteria, which can produce N_2O ; NO_2^- accumulation caused by the low denitrifying metabolic rate using endogenous substances as electron donors in DPAOs; and the inhibition of N_2O reduction to N_2 caused by the low degradation rate of endogenous substances (Dai et al. 2021). Internally stored substances such as polyhydroxyalkanoates (PHAs) can be used as carbon sources to drive denitrification when the external substrate is limited. The degradation rate of PHAs was reported to be 6–20 times slower than that of the

external carbon source, inhibiting the reduction of N_2O to N_2 (Lenhart et al. 2019). The low PHA degradation rate can lead to the competition for electrons between enzymes and results in N_2O accumulation. During the denitrifying phosphorus removal process, DPAOs can be enriched under alternate anaerobic/anoxic conditions. Under anaerobic conditions, DPAOs can consume external organic carbon and store it as PHAs. Alternately, under anoxic conditions, DPAOs oxidize intracellular PHAs to absorb phosphate using NO_x^- as an electron acceptor. Therefore, the synthesis and utilization of intracellular PHAs have critical effects on N_2O emissions during denitrifying phosphorus removal.

The synthesis and utilization of intracellular PHAs depend on the extracellular organic substances in the environment. Acetate and propionate are the most common volatile fatty acids (VFAs) that serve as biodegradable carbon sources in wastewater. Acetate is more likely to be taken up and converted to PHAs than propionate, and the ratio of polyhydroxybutyrate (PHB) to polyhydroxyvalerate (PHV) is higher when acetate acts as the organic substance as opposed to propionate (Miao et al. 2016). The effects of the carbon source on PHA synthesis and utilization also affect the N_2O emission characteristics. Wang et al. (2011) reported that N_2O production increased by 1.72 or 0.77 times in the short term when the carbon source switched from acetate to a mixture of acetate and propionate or propionate, respectively. However, the long-term effects of the carbon source on N_2O emission during denitrifying phosphorus removal remain unclear.

In this study, we investigated the long-term influences of organic substances on N_2O accumulation during DPR process in lab-scale reactors. Three anaerobic/anoxic/oxic sequencing batch reactors (SBRs) were established, and acetate, propionate, or a mixture of acetate and propionate was used as the carbon source, respectively. The characteristics of N_2O production were studied in the different reactors, and the mechanisms of N_2O production were investigated from the perspectives of the amount of synthesized PHAs, nitrite accumulation, and the activities of denitrification enzymes.

2. Materials and methods

2.1. Experimental system

Three SBRs with working volumes of 5 L were established in the laboratory to carry out the experiments. The seed sludge in the SBRs were collected from the secondary sedimentation tank of a local municipal wastewater treatment plant. The concentration of mixed liquor suspended solids (MLSS) in each reactor were approximately 3.8 g/L. To enrich DPAOs, the SBRs were operated at $24^\circ\text{C} \pm 1^\circ\text{C}$ with a cycle time of 6 h consisting of 10 min of feeding, a 90-min anaerobic phase, a 180-min anoxic phase, a 30-min aerobic phase, 20 min of setting, 10 min of decanting, and a 20-min idle phase. During the feeding phase, 4 L of wastewater was pumped into the reactor using a peristaltic pump. Then 0.1 L of KNO_3 solution with the concentration of 2 g N/L was added directly into the reactor at the beginning of the anoxic phase, to

obtain an initial NO_3^- -N concentration of 40 mg/L. The sludge was kept suspended in the reactor with a magnetic stirrer. The hydraulic retention time and sludge retention time were 7.5 h and 25 d, respectively. The SBRs were operated over four months.

2.2. Synthetic wastewater

The reactors with acetate, propionate, and a mixture of acetate and propionate as the carbon source were abbreviated as RA, RP, and RM, respectively. The compositions of the synthetic wastewaters (per liter) were as follows: 257 mg of CH_3COONa for RA, 172 mg of $\text{CH}_3\text{CH}_2\text{COONa}$ for RP, or 77 mg of CH_3COONa and 120 mg of $\text{CH}_3\text{CH}_2\text{COONa}$ for RM; 0.058 g NH_4Cl ; 200 mg of NaHCO_3 ; 11 mg of KH_2PO_4 ; 18 mg of $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$; 10 mg of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 10 mg of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; 10 mg of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$; and 1.0 mL of the nutrient solution described by Li et al. (2013a). The concentrations of COD, NH_4^+ -N and total phosphorous were approximately 200, 15, and 5 mg/L, respectively.

2.3. Batch experiments

Three batch experiments were carried out to investigate the influence of the carbon source on N_2O production. All batch experiments were carried out in sealable reactors with a working volume of 0.5 L. The operation parameters for all batch experiments were the same as those of the parent SBRs.

2.3.1. Batch I: Effects of carbon source on nitrate, nitrite, and N_2O reduction

At the end of the anaerobic phase, 1.5 L of sludge was taken from each parent SBR and divided equally into three batch reactors. Then KNO_3 , NaNO_2 , or N_2O stock

solution was rapidly added to the reactor at the beginning of each test, to obtain an initial concentration of 15 mg/L of NO_3^- -N, NO_2^- -N, or N_2O -N, respectively. The reactors were operated for 30 min, and 5-mL liquor samples were collected every 5 min for NO_3^- -N and NO_2^- -N analyses. N_2O production was monitored online using a microsensor.

2.3.2. Batch II: Effects of carbon source on nitrite and N_2O reduction in the presence of nitrate

To investigate the competition between denitrifying enzymes, the reductions of NO_2^- -N and N_2O -N were investigated in the presence of NO_3^- -N. For each test, 1.0 L of sludge was collected from the parent SBR at the end of the anaerobic phase and divided equally into two batch reactors. KNO_3 solution was added into the two reactors to achieve an initial NO_3^- -N concentration of 15 mg/L. Subsequently, NaNO_2 or N_2O stock solution was added to the reactor to obtain an initial NO_2^- -N or N_2O -N concentration of 15 mg/L, respectively. The batch experiments lasted for 30 min, and samples were collected and analyzed as described for batch I.

2.3.3. Batch III: N_2O generation in the propionate system with nitrate or nitrite as the electron acceptor

The effect of nitrite on N_2O generation in the propionate system was investigated. One liter of sludge was taken from the RP system at the end of the anaerobic phase and divided equally into two batch reactors. KNO_3 or NaNO_2 solution was added into the reactor to obtain an initial NO_3^- -N concentration of 40 mg/L or NO_2^- -N concentration

of 30 mg/L in the reactor, respectively. The reactors were then operated for 180 min, and the N₂O concentration was monitored in situ using a microsensor.

2.4 Analytical methods

The analyses of COD, NH₄⁺-N, NO₂⁻-N, NO₃⁻-N, PO₄³⁻-P, MLSS, and mixed liquid volatile suspended solids (MLVSS) were conducted using standard methods (APHA-AWWA-WEF 2012). PHAs, including PHB, PHV, and poly-3-hydroxy-2-methylvalerate (PH2MV), were extracted from the activated sludge and analyzed by gas chromatography according to the method reported by Oehmen et al. (2005). Glycogen was extracted and analyzed using the phenol-sulfuric acid method (Chen et al. 2019). The dissolved N₂O concentration was measured using a N₂O microsensor (Unisense, Denmark).

2.5 Statistical analysis

The differences in the data were tested by *t*-test using the statistical program SPSS 19.0 (SPSS Inc., Chicago, USA). For all tests, differences were considered significant if $p < 0.01$.

3. Results and discussion

3.1. Nutrient removal and N₂O production

After running for over four months, all SBRs achieved stable nitrogen and phosphorus removal, and granular sludge with good settling performance was forming. The nutrient removal performance and N₂O production of each SBR were calculated

(Table 1). In all SBRs, the COD removal efficiency was above 87%; acetate and propionate were both easily biodegraded, and the effect of the carbon source on COD removal was insignificant ($p = 0.758$). However, the nitrogen removal varied with carbon source. In the RA system, the total nitrogen (TN) removal efficiency reached $71.4\% \pm 4.7\%$, which was 20.8% and 68.4% higher than those of the RM and RP systems, respectively. Compared to the RA system, nitrogen removal was weakened significantly by the addition of propionate ($p < 0.01$), in agreement with the findings of other studies (Miao et al. 2016, Wang et al. 2011). All SBRs exhibited high phosphorus removal efficiency ($> 87\%$), with no significant difference observed among the SBRs. The effects of carbon source on phosphorus removal in this study differed from the results of Wang et al. (2011), who found that a higher propionate content led to lower phosphorus removal in short-term anaerobic/oxic experiments. Compared to acetate, propionate is more difficult for microorganisms to take up, even though propionic acid is a typical short-chain VFA (Miao et al. 2016). After long-term acclimation, the microorganisms adapted to the different carbon sources, and DPAOs were enriched in all systems in this study, leading to high phosphorus removal efficiency. However, ordinary heterotrophic organisms (OHOs) have been reported to play an important role in the denitrifying phosphorus removal process (Wisniewski et al. 2018). We hypothesize that OHO denitrification led to the higher nitrogen removal efficiencies in the RA and RM systems in this study.

The nitrogen and phosphorus transformations in each SBR during one typical

running cycle are shown in Fig. 1. All SBRs were operated in anaerobic/anoxic/aerobic conditions, and the carbon source had no significant influence on the transformation of NH_4^+-N ($p = 0.844$). However, the temporal variations in the $\text{NO}_3^- - \text{N}$ and $\text{NO}_2^- - \text{N}$ concentrations differed among the three SBRs. In the anoxic phase, the nitrate concentration decreased gradually, and the reduction rate of $\text{NO}_3^- - \text{N}$ was highest in the RA system and lowest in the RP system. The effluent nitrate concentration in the RA system was only 1.5 ± 0.68 mg N/L, while those in RM and RP were 6.2 ± 1.3 and 22.1 ± 2.5 mg N/L, respectively. Correspondingly, nitrite accumulated in the RA and RM systems, while nearly no nitrite accumulated in RP. When acetate was used as the carbon source, the maximum nitrite concentration in RA reached 21.3 ± 1.9 mg N/L at 150 min, and the effluent nitrite concentration was 11.3 ± 1.6 mg N/L. In the RM system with the mixed carbon source, the maximum nitrite concentration was 14.5 ± 1.8 mg N/L at 120 min, and the effluent concentration was 7.7 ± 1.4 mg N/L. However, when propionate was used as the carbon source (RP), the nitrite concentration remained very low, and its concentration in the effluent was only 1.0 ± 0.52 mg N/L. This indicated that the reduction of nitrate to nitrite was inhibited in the RP system with propionate as the carbon source, leading to poor nitrogen removal. Although the removal efficiency of $\text{PO}_4^{3+} - \text{P}$ was similar in the three SBRs, the temporal variation in $\text{PO}_4^{3+} - \text{P}$ concentration during the running cycle varied among the SBRs. In the anaerobic phase, phosphorus was released from the DPAOs, and the phosphorous concentration in the liquid increased rapidly. Compared to the RA and RM systems, the amount of

phosphorus released was higher in RP with propionate as the carbon source. The maximum $\text{PO}_4^{3+}\text{-P}$ concentration in the RP system was 49.7 ± 3.8 mg/L at 60 min, 97.2% and 63.5% higher than those in RA and RM, respectively. The $\text{PO}_4^{3+}\text{-P}$ concentration decreased gradually during the anoxic phase in all systems, verifying that denitrifying phosphorus removal occurred. In the RP system, phosphorus was absorbed by DPAOs at a rate of 0.20 mg P/L/min during the anoxic phase; this rate of absorption was 1.9 and 1.4 times higher than those in the RA and RM systems, respectively. Meanwhile, unlike in the RA and RM systems, some phosphate was still taken up in the aerobic phase in the RP system. This indicated that aerobic phosphorus removal occurred, and that ordinary phosphorus-accumulating organisms were enriched in RP.

N_2O production was calculated in the three SBRs with different carbon sources (Table 1). The N_2O production in RA was 4.6 ± 0.18 mg N/L, accounting for 16.3% of the removed nitrogen (emission factor). However, in both RM and RP, the N_2O production and emission factor were much lower than those in the RA system. When propionate was used as the carbon source (RP), the N_2O production was only 0.07 ± 0.04 mg N/L, and the emission factor was 0.43%, both significantly lower than in the RA system ($p < 0.001$). Thus, N_2O production during DPR process was reduced when propionate was used as the carbon source instead of acetate.

The temporal variations in N_2O concentration during the typical running cycle in the three SBRs are shown in Fig. 2. In all systems, N_2O production mainly occurred in the anoxic phase, during which simultaneous nitrogen and phosphorus removal

occurred; nearly no N₂O was produced in the anaerobic phase. The carbon source had a significant effect on the temporal variation in N₂O concentration. In the RA system, the N₂O concentration increased rapidly from 150 min on, reaching 3.3 mg N/L at the end of the anoxic phase. The N₂O concentration then decreased in the aerobic phase. When the mixed carbon source was used (RM), the N₂O concentration increased in the beginning of the anoxic phase, reaching a maximum value of 1.6 mg N/L at 135 min. The concentration then decreased rapidly to zero at 210 min. In RP with propionate as the carbon source, the N₂O concentration was close to zero during the entire running cycle. The production of N₂O during DPR process is affected by many factors. In denitrifying phosphorus removal systems, the enrichment of ordinary heterotrophic denitrification organisms, particularly glycogen-accumulating organisms (GAOs), leads to high N₂O emission (Wisniewski et al. 2018). In addition, the limited electron donors supplied by intracellular substances (mainly PHAs) in the anoxic phase of the DPR process leads to a low denitrifying rate and the accumulation of NO₂⁻, further inhibiting N₂OR activity and resulting in the N₂O accumulation (Kampschreur et al. 2009). Moreover, the low biodegradation rate of PHAs aggravates the competition between denitrifying enzymes for electrons, and N₂OR may be unable to reduce N₂O to N₂ (Lenhart et al. 2019). In this study, the synthesis of PHAs in the anaerobic phase and the high NO₂⁻ accumulation in the three systems were the key factors leading to the differences in N₂O production during DPR process. The production of N₂O was reported to be governed predominantly by the presence of NO₂⁻ in the reactor regardless

of the carbon source and the C/N ratio (Wisniewski et al. 2018). Therefore, the mechanisms of N₂O production during the DPR process with different carbon sources were explored.

3.2. Factors leading to low N₂O production in the RP system

During denitrifying phosphorus removal process, intracellular substances (i.e., PHAs and glycogen) have the dual functions of nitrogen and phosphorus removal (Dai et al. 2021). The DPAOs conduct denitrification using intracellular PHAs as the electron donor. PHA synthesis is closely related to the metabolism of the microorganisms. The amount of synthesized PHAs, including PHB, PHV, and PH₂MV, and the amount of glycogen consumed in the anaerobic phase were detected in each system (Fig. 3). The amount of synthesized PHAs at the end of the anaerobic phase decreased as the portion of propionate in the system increased. In the RA system, the amount of synthesized PHAs was 3.0 mmol-C/g VSS; PHB accounted for 66.1% of the PHAs, while PHV accounted for 30.6%. The proportion of PH₂MV was only 3.3%. In the RM and RP systems, the amounts of synthesized PHAs were 29.2% and 32.6% lower than in the RA system, respectively. Meanwhile, the proportion of PHB decreased to 21.8% in the RM system and 10.0% in the RP system. In the RM system, PHV was the primary PHA, accounting for 56.0% of all PHAs, whereas PH₂MV accounted for 22.1%. When propionate was used as the carbon source in the RP system, PH₂MV was the dominant PHA, accounting for over 63% of all PHAs. As the proportion of propionate in the reactor increased, the fraction of PHB decreased, while the fraction of PHV increased,

in agreement with the findings of other studies (Miao et al. 2016, Wang et al. 2011). However, a large proportion of PH2MV was detected in the RP system, contradicting the findings of Wang et al. (2011), who reported that negligible amounts of PH2MV were anaerobically synthesized in a short-term RP system. Acetyl-CoA, which is primarily derived from acetate activation and glycolysis, was reported to be the precursor of PHB and PHV; meanwhile, the precursor of PHV and PH2MV is propionyl-CoA, which is mainly derived from propionate activation and pyruvate conversion (Miao et al. 2016). PHV monomers are generated by the condensation of acetyl-CoA and propionyl-CoA in a 1:1 ratio, while PHB or PH2MV monomer is composed of two molecules of acetyl-CoA or propionyl-CoA, respectively (Zhang et al. 2008).

In this study, long-term acclimation using different carbon sources resulted in different microbial community structures, particularly with respect to the relative abundances of phosphate-accumulating organisms (PAOs) and GAOs, which were the dominant organisms in denitrification. GAOs and PAOs had similar metabolic processes in the anaerobic phase (i.e., both took up VFAs and accumulated them as PHAs). However, the energy source and reducing power of GAOs are derived from glycogen degradation without poly-P involvement (Zhang et al. 2008). Lv et al. (2014) reported that propionate is superior to acetate for denitrifying phosphorus removal because it encourages the accumulation of PAOs and the elimination of GAOs. We speculate that more GAOs were enriched simultaneously with PAOs in the RA system

compared to the other two systems. The amounts of synthesized PHAs and consumed glycogen in the three systems during the anaerobic phase confirm the above conclusion. Among the reactors, RA exhibited the highest amount of consumed glycogen and the lowest amount of released phosphorus (Figs. 1 and 3), indicating the presence of GAOs in the RA system. Compared to PAOs, GAOs lead to increased N_2O emission (Ni et al. 2015)

PHAs are considered to be the electron donor in the denitrification process; the presence of PHAs leads to competition with denitrifying enzymes, resulting in the accumulation of N_2O . In this study, the amount of synthesized PHAs decreased as the proportion of propionate in the system increased. However, the N_2O production in the RP system, which had the lowest content of synthesized PHAs, was much lower than in the other two systems. In fact, the competition for electrons between nitrite reduction and N_2O reduction was not affected by the PHA degradation rate, and the electron supply generated by PHA degradation could satisfy the electron demand for all denitrification steps during the DPR process (Wei et al. 2014). Therefore, the low N_2O production in the RP system resulted from other factors.

To further explore these factors leading to low N_2O production when propionate was used as the carbon source, the reduction rates of NO_3^- -N, NO_2^- -N, and N_2O -N in the three systems were investigated in batch experiments (Table 2). The reduction rates of NO_3^- -N, NO_2^- -N, and N_2O -N decreased as the proportion of propionate increased, indicating that the change of carbon source from acetate to propionate inhibited

denitrification and decreased the nitrogen removal efficiency, as shown in Table 1. In the RA and RM systems, the reduction rate of NO_2^- -N was significantly lower than that of NO_3^- -N ($p < 0.01$), which would lead to the accumulation of NO_2^- -N in these two systems. Although the denitrification rates in the RP system were lower than those in the other two systems, the NO_2^- -N reduction rate was similar to the NO_3^- -N reduction rate. The ratio of the nitrate reduction rate to the nitrite reduction rate (R1/R2) in the RP system was only 0.92, much lower than in the other two systems ($p < 0.01$). The R1/R2 value was reported to be closely related to nitrite accumulation (Zhu & Chen 2011) ; thus, the high R1/R2 values in RA and RM indicate substantial nitrite accumulation in these two systems. NO_2^- has a toxic effect on the denitrification process and can affect the production of N_2O during denitrification; Miao et al. (2016) reported that the accumulation of NO_2^- contributed more to the production of N_2O than other factors during denitrifying phosphorus removal.

In the batch II experiments, the NO_2^- and N_2O reduction rates in the three systems were evaluated in the presence and absence of nitrate to determine the effects of carbon source on enzyme activity (Fig. 4). When the experiments were conducted without nitrate, both NO_2^- and N_2O were gradually reduced over time in all three systems, although the reduction rate was lower in RP than in the other two systems (Fig. 4a). When nitrate was added, the reductions of NO_2^- and N_2O were blocked in the RA and RM systems, whereas nitrate had little effect in the RP system (Fig. 4b). This indicates that the activities of NIR and N_2OR were not inhibited during the anoxic phase of

denitrifying phosphorus removal when propionate was used as the carbon source. The competition for electrons between NIR and N_2OR was not noticeable. The above analysis suggests that the lower nitrite accumulation in RP compared to in the other two systems was the dominant reason for the low N_2O production in the RP system. This conclusion was confirmed by the results of the batch III experiments, in which N_2O production in the RP system with nitrate or nitrite as the electron acceptor was measured (Fig. 5). Nitrite as an electron acceptor resulted in more N_2O production in the RP system compared to nitrate.

4. Conclusion

Using propionate as the carbon source for denitrifying phosphorus removal rather than acetate resulted in decreased nitrogen removal but did not affect phosphorus removal. N_2O production was significantly affected by the carbon source; both N_2O production and the emission factor decreased greatly when acetate was replaced with propionate as the carbon source. During long-term acclimation, the carbon source affected the microbial community. Compared to when propionate was used as the carbon source, the use of acetate resulted in the enrichment of GAOs, which were responsible for the high nitrogen removal. The lower nitrite accumulation in the system with propionate as the carbon source was the dominant reason for the low N_2O production in this system.

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445 **Figure captions**

446 Fig. 1 Variations in the concentrations of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{PO}_4^{3-}\text{-P}$
447 during a running cycle in the RA, RM and RP systems.

448 Fig. 2 Variation in $\text{N}_2\text{O-N}$ production during a running cycle in the RA, RM, and RP
449 systems.

450 Fig. 3 Amounts of synthesized PHAs and glycogen consumed during the anerobic phase
451 in the RA, RM, and RP systems.

452 Fig. 4 Effects of nitrate on the reductions of nitrite and N_2O in each reactor: (a) without
453 nitrate and (b) with nitrate.

454 Fig. 5 N_2O production in the RP system when nitrate or nitrite served as the electron
455 acceptor.

Figures

Figure 1

Variations in the concentrations of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{PO}_4^{3-}\text{-P}$ during a running cycle in the RA, RM and RP systems.

Figure 2

Variation in $\text{N}_2\text{O-N}$ production during a running cycle in the RA, RM, and RP systems.

Figure 3

Amounts of synthesized PHAs and glycogen consumed during the anaerobic phase in the RA, RM, and RP systems.

Figure 4

Effects of nitrate on the reductions of nitrite and N_2O in each reactor: (a) without nitrate and (b) with nitrate.

Figure 5

N₂O production in the RP system when nitrate or nitrite served as the electron acceptor.