N$_2$O reduction during denitrifying phosphorus removal with propionate as carbon source

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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 nitrous oxide (N$_2$O) production were investigated, and the factors affecting N$_2$O 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. N$_2$O 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 N$_2$O 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 N$_2$O production.

Keywords Nitrous oxide · Denitrifying phosphorus removal · Propionate · PHAs · Nitrite accumulation

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) were 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$_2$O) emission during DPR process has raised increasing concerns in the last decade (Gan et al. 2017; Li et al. 2013b; Liu et al. 2015; Wisniewski et al. 2018). N$_2$O is considered 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$_2$O. During the denitrifying phosphorus removal systems, the N$_2$O 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$_2$O production during denitrifying phosphorus removal process.

During denitrification, N$_2$O can accumulate due to the imbalance in the reduction activities of key denitrifying enzymes (N$_2$O reductase (N$_2$OR), nitrate reductase (NAR), and nitrite
the DPR process, the production of N₂O mainly arises from the N₂O emission characteristics. Wang et al. (2011) reported carbon source on PHAs synthesis and utilization also affect posed to propionate (Miao et al. 2016). The effects of the is higher when acetate acts as the organic substance as op-

d to PHAs than propionate, and the ratio of wastewater. Acetate is more likely to be taken up and convert-

Acetate and propionate are the most common volatile fatty acids (VFAs) that serve as biodegradable carbon sources in the environment. Denitrifying phosphorus removal.

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 was approximately 3.8 g/L. To enrich DPAOs, the SBRs were operated at 24°C ± 1°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 KNO₃ 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 NO₃⁻−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 days, respectively. The SBRs were operated over 4 months.

**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 CH₃COONa for RA, 172 mg of CH₃CH₂COONa for RP, or 77 mg of CH₃COONa and 120 mg of CH₃CH₂COONa for RM; 0.058 g NH₄Cl; 200 mg of NaHCO₃; 11 mg of KH₂PO₄; 18 mg of K₂HPO₄·3H₂O; 10 mg of MgSO₄·7H₂O; 10 mg of FeSO₄·7H₂O; 10 mg of CaCl₂·2H₂O; and 1.0 mL of the nutrient solution described by Li et al. (2013a). The concentrations of COD, NH₄⁺−N, and total phosphorous were approximately 200, 15, and 5 mg/L, respectively.

**Batch experiments**

Three batch experiments were carried out to investigate the influence of the carbon source on N₂O 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.

**Materials and methods**

**Experimental system**

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 PHAs synthesis and utilization also affect the N₂O emission characteristics. Wang et al. (2011) reported that N₂O 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 N₂O emission during denitrifying phosphorus removal remain unclear.

In the present study, three anaerobic/anoxic/oxic sequencing batch reactors (SBRs) with different influent carbon substances (acetate, propionate, or a mixture of acetate and propionate, respectively) were established to realize the DPR process. The long-term influences of organic substances on N₂O production were investigated, and the mechanisms were further explored from the perspectives of the synthesis of PHAs, nitrite accumulation, and the activities of denitrification enzymes.
Batch I: Effects of carbon source on nitrate, nitrite, and N₂O 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 KNO₃, NaNO₂, or N₂O stock solution was rapidly added to the reactor at the beginning of each test, to obtain an initial concentration of 15 mg/L of NO₃⁻–N or NO₂⁻–N and 3 mg/L of N₂O–N, respectively. The reactors were operated for 30 min, and 5-mL liquor samples were collected every 5 min for NO₃⁻–N and NO₂⁻–N analyses. N₂O production was monitored online using a microsensor.

Batch II: Effects of carbon source on nitrite and N₂O reduction in the presence of nitrate

To investigate the competition between denitrifying enzymes, the reductions of NO₂⁻–N and N₂O–N were investigated in the presence of NO₃⁻–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. KNO₃ solution was added into the two reactors to achieve an initial NO₃⁻–N concentration of 15 mg/L. Subsequently, NaNO₂ or N₂O stock solution was added to the reactor to obtain an initial NO₂⁻–N concentration of 15 mg/L or N₂O–N concentration of 3 mg/L. The batch experiments lasted for 30 min, and samples were collected and analyzed as described for batch I.

Batch III: N₂O generation in the propionate system with nitrate or nitrite as the electron acceptor

The effect of nitrite on N₂O 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. KNO₃ or NaNO₂ solution was added into the reactor to obtain an initial NO₃⁻–N concentration of 40 mg/L or NO₂⁻–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.

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). Briefly, sludge sample collected from the reactors was centrifuged to remove the supernatant and washed with the phosphate buffer solution. After re-centrifuged, the sludge sample was lyophilized at −60°C and 0.1 mbar. Then the sample was mixed with 2 mL of chloroform and 2 mL of an acidified methanol solution and digested in tightly sealed vials for 5 h at 100°C. When the digestion liquid cooled to room temperature, 1 mL of distilled water was added and mixed vigorously. After 1 h of settling, the chloroform phase was transferred to another vial and dried with granular sodium sulfate. Then 2 μL of chloroform phase was analyzed by gas chromatography with a flame ionization detection. To extract the glycogen, the lyophilized sludge was mixed with 1 M NaOH solution and heated at 60°C for 2 h. After cooling to room temperature, the samples were centrifuged, and 0.5 mL of the supernatant was collected to measure the glycogen concentration using the phenol–sulfuric acid method (Chen et al. 2019). The dissolved N₂O concentration was measured using a N₂O microsensor (Unisense, Denmark).

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.

Results and discussion

Nutrient removal and N₂O production

After running for over 4 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% ± 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.
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 NO$_3^−$–N and NO$_2^−$–N concentrations differed among the three SBRs. In the anoxic phase, the nitrate concentration decreased gradually, and the reduction rate of NO$_3^−$–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 PO$_4^{3−}$–P was similar in the three SBRs, the temporal variation in PO$_4^{3−}$–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 PO$_4^{3−}$–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 PO$_4^{3−}$–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\textsubscript{2}O production was calculated in the three SBRs with different carbon sources (Table 1). The N\textsubscript{2}O 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\textsubscript{2}O production and emission factor were much lower than those in the RA system. When propionate was used as the carbon source (RP), the N\textsubscript{2}O 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\textsubscript{2}O production during DPR process was reduced when propionate was used as the carbon source instead of acetate.

The temporal variations in N\textsubscript{2}O concentration during the typical running cycle in the three SBRs are shown in Fig. 2. In all systems, N\textsubscript{2}O production mainly occurred in the anoxic phase, during which simultaneous nitrogen and phosphorus removal occurred; nearly no N\textsubscript{2}O was produced in the anaerobic phase. The carbon source had a significant effect on the temporal variation in N\textsubscript{2}O concentration. In the RA system, the N\textsubscript{2}O concentration increased rapidly from 150 min on, 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 N\textsubscript{2}O concentration was close to zero during the entire running cycle. The production of N\textsubscript{2}O 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 $N_2O$ 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 lead to a low denitrifying rate and the accumulation of $NO_2^-$, further inhibiting $N_2O$R activity and resulting in the $N_2O$ accumulation (Kampschreur et al. 2009). Moreover, the low biodegradation rate of PHAs aggravates the competition between denitrifying enzymes for electrons, and $N_2O$R may be unable to reduce $N_2O$ to $N_2$ (Lenhart et al. 2019). In this study, the synthesis of PHAs in the anaerobic phase and the high $NO_2^-$ accumulation in the three systems were the key factors leading to the differences in $N_2O$ production during DPR process. The production of $N_2O$ was reported to be governed predominantly by the presence of $NO_2^-$ in the reactor regardless of the carbon source and the C/N ratio (Wisniewski et al. 2018). Therefore, the mechanisms of $N_2O$ production during the DPR process with different carbon sources were explored.

Factors leading to low $N_2O$ 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 N₂O reduction was not affected by the PHAs degradation rate, and the electron supply generated by PHAs degradation could satisfy the electron demand for all denitrification steps during the DPR process (Wei et al. 2014). Therefore, the low N₂O production in the RP system resulted from other factors.

To further explore these factors leading to low N₂O production when propionate was used as the carbon source, the reduction rates of NO₃⁻–N, NO₂⁻–N, and N₂O–N in the three systems were investigated in batch experiments (Table 2). The reduction rates of NO₃⁻–N, NO₂⁻–N, and N₂O–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 NO₂⁻–N was significantly lower than that of NO₃⁻–N (p < 0.01), which would lead to the accumulation of NO₂⁻–N in these two systems. Although the denitrification rates in the RP system were lower than those in the other two systems, the NO₂⁻–N reduction rate was similar to the NO₃⁻–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 and Chen 2011); thus, the high R1/R2 values in RA and RM indicate substantial nitrite accumulation in these two systems. NO₂⁻ has a toxic effect on the denitrification process and can affect the production of N₂O during denitrification; Miao et al. (2016) reported that the accumulation of NO₂⁻ contributed more to the production of N₂O than other factors during denitrifying phosphorus removal.

In the batch II experiments, the NO₂⁻ and N₂O 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 NO₂⁻ and N₂O 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 NO₂⁻ and N₂O

| Parameters                  | RA       | RM       | RP       |
|-----------------------------|----------|----------|----------|
| Reduction rate of NO₃⁻–N (R₁) | 0.49 (0.04) | 0.38 (0.03) | 0.12 (0.03) |
| Reduction rate of NO₂⁻–N (R₂) | 0.16 (0.02) | 0.14 (0.02) | 0.13 (0.02) |
| Reduction rate of N₂O–N (R₃)   | 0.24 (0.03) | 0.21 (0.02) | 0.15 (0.04) |
| R₁/R₂ ratio                  | 3.06 (0.25) | 2.64 (0.25) | 0.92 (0.25) |
| R₂/R₃ ratio                  | 0.67 (0.06) | 0.67 (0.06) | 0.87 (0.06) |

Fig. 4 Effects of nitrate on the reductions of nitrite and N₂O in each reactor: a without nitrate and b with nitrate
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 N₂OR 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 N₂OR 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 N₂O production in the RP system. This conclusion was confirmed by the results of the batch III experiments, in which N₂O 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 N₂O production in the RP system compared to nitrate.

**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. N₂O production was significantly affected by the carbon source; both N₂O 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 N₂O production in this system.

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