

Effect of Propionic to Acetic Acid Ratio on Denitrifying Phosphorus Removal and Nitrous Oxide Production

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Keywords: Denitrifying phosphorus removal, Propionic to acetic acid ratio, PHA, N₂O

Abstract. In this paper, four lab-scale sequencing batch reactors (SBRs) operated with anaerobic/anoxic configuration were long-term cultured, respectively with different ratios of propionic to acetic acid as carbon sources. Effects of propionic to acetic acid ratio on polyhydroxyalkanoates (PHA) formation, denitrifying phosphorus removal and nitrous oxide (N₂O) production were investigated. Results showed that with the propionic to acetic acid ratio increased, the anaerobic synthesis of PHA reduced, and (PHV+PH2MV)/PHA ratio increased, furthermore, the rate of PHA degradation in the anoxic phase decreased, ultimately leading to more N₂O production. System running efficiency with the phosphorus removal rate of 93.33%, nitrogen removal rate of 77.48% and anoxic N₂O-N production to denitrified nitrogen rate of 4.15% was best when acetic acid was the sole carbon source.

Introduction

Denitrifying phosphorus removal, which can achieve simultaneous denitrification and phosphorus removal, is a novel biological nutrient removal technology. Denitrifying phosphorus removal occurs due to the capacity of denitrifying phosphorus accumulating organisms (DPAOs) to use nitrate and/or nitrite as an electron acceptor for phosphorus removal instead of oxygen. When compared with conventional enhanced biological phosphorus removal (EBPR) processes, the denitrifying phosphorus removal process can reduce the need for aeration by 30%, as well as reduce sludge production and the demand for carbon sources by 50%^[1]. These characteristics benefit significantly for the municipal wastewater treatment, especially for the low influent C/N municipal wastewater treatment where the lack of carbon sources effects the nutrient removal efficiency. However, it was reported that a significant amount of N₂O was produced in this process^[2], which is of significant environmental concern due to the high global warming potential of N₂O. This finding significantly diminishes the overall benefits of the denitrifying phosphorus removal and limits the prospect of implementing this process in wastewater treatment plants.

N₂O is a by-product of microbial denitrification in denitrifying phosphorus removal, complete denitrification from nitrate (NO₃⁻) to molecular nitrogen (N₂) consists of four reduction steps, with nitrite (NO₂⁻), nitric oxide (NO) and N₂O as reaction intermediates. However, under certain conditions, N₂O has been found to be the final product instead of an intermediate^[3-6]. The mechanism for N₂O production in the denitrifying phosphorus removal process may not have been described properly to date, the possible reasons are as follows: ① PHA have been reported to be a possible inducer of N₂O emissions when utilized as growth substrate and the availability of external growth substrate is limited^[7-10]. Itokawa et al. suggested that at relatively low COD/N ratios, a higher N₂O concentration could be observed due to incomplete denitrification caused by the lack of external carbon sources^[11]. However, Adouani et al. found a lack of correlation between the types of carbon source with the amount of N₂O produced^[12]. It has also been observed that COD-limited conditions did not necessarily increase N₂O accumulation^[13]. ② In denitrifying phosphorus removal systems, the composition of PHA (including poly-β-hydroxybutyrate (PHB), poly-β-hydroxyvalerate (PHV) and poly-β-hydroxy-2-methylvalerate (PH2MV)) produced by DPAOs during the anaerobic period varies based on the carbon sources. However, DPAOs are unable to metabolize these different types

of PHA at the same rate in the subsequent anoxic phase^[14-15]. Therefore, the use of different carbon sources may lead to different properties of phosphorus metabolism and N₂O emission. At present, few have considered the impact of intracellular PHA on N₂O accumulation in denitrifying phosphorus removal process, a large number of research are need to be done.

Little is known about the long-term effects of carbon sources on N₂O production in DPAOs dominant systems. In this study, the effects of different ratio of propionic to acetic acid on N₂O production were investigated using four lab-scale anaerobic/anoxic (A/An) reactors to reveal the characteristics of N₂O production during denitrification phosphorus removal using different carbon sources, as well as the relationship between carbon sources and the amount and composition of PHA synthesized. Aiming at providing a process control strategy to minimize N₂O production while maintaining effective nitrogen and phosphorus removal.

Materials and methods

Reactor set-up and operation. Four sealed laboratory-scale SBRs with a working volume of 4.5 L were seeded with activated sludge from the WWTP in Tianjin, which routinely achieved EBPR. The SBRs in this study were maintained at 20±1°C and operated under anaerobic-anoxic conditions. It was operated with four 6 hr cycles per day. Each cycle consisted of an anaerobic period (90 min), and anoxic period (210 min), with the remainder of the cycle time (60 min) for settling, decanting, and idle. During the filling phase 3.15 L of simulated domestic wastewater was introduced into the reactor using peristaltic pumps on timers. At the beginning of anoxic phase, 0.2 L nitrate solution (1.24 g N L⁻¹) was pumped into the reactor with a constant flow rate. SBRs was mixed using stirrer in the anaerobic and anoxic stage. Wastewater was poured into jars for the SBRs per day. Sludge retention time (SRT) was controlled at approximately 15 days by drawing the sludge from the reactor at the end of the anoxic period. The MLSS was kept at 3000 mg/L. It took about three months before a stable nitrogen and phosphorus removal was achieved in four SBRs. Thereafter, the experiments reported below were conducted.

Synthetic wastewater. The four SBRs were supplied with different propionic to acetic ratios synthetic wastewater (as shown in Tab.1) containing VFA (220 mg/L as COD basis), NH₄Cl (15 mg/L as NH₄⁺-N basis), KH₂PO₄ (10 mg/L as PO₄³⁻-P basis), KNO₃ (55 mg/L as NO₃⁻-N in the anoxic phase) and trace element solution. The trace element solution consisted of the following compounds per liter: 0.1mg ZnCl₂, 0.5mg MgSO₄, 0.5mg FeCl₃ · 6H₂O, 0.1mg MnSO₄ · H₂O, 0.1mg KI, 0.5mg CaCl₂, 0.1mg H₂BO₄, 0.1mg NiCl₂.

Tab.1 Composition of main carbon sources and their concentrations in four SBRs

Reactor	Acetic (mmol-C·L ⁻¹)	Propionic (mmol-C·L ⁻¹)	Propionic/Acetic (mol-C mol-C ⁻¹)
R-A	6.875	0	—
R-B	4.342	2.171	1/2
R-C	2.063	4.125	2/1
R-D	0	5.893	—

Analytical methods. The liquid samples were immediately filtered through Millipore filter units (0.45µm pore size) for the analysis of NH₄⁺-N, NO₃⁻-N, NO₂⁻-N, PO₄³⁻-P, MLSS and MLVSS which were measured in accordance with Standard Methods for the Examination of Water and Wastewater^[16]. Total nitrogen (TN) was calculated as the sum of NH₄⁺-N, NO₃⁻-N and NO₂⁻-N.

The N₂O concentrations in the gas samples were analyzed using a gas chromatograph (GC) (Perkin Elmer Clarus 500, USA) equipped with an electron capture detector (ECD). The GC was equipped with a main column (Porapak Q 80/100 mesh, 6m×1/8 inch). A 1mL sample loop and a 6 port valve were used to inject the gas samples. The temperatures of the columns and the ECD were 60°C and 300°C, respectively. A mixture of 95% Ar + 5% CH₄ was used as the carrier gas. The dissolved N₂O was measured by GC using a headspace method. The equilibrium temperature and

time were 70°C and 1h, respectively. N₂O was quantified using the standard curves generated from certified standard gases (National Institute of Metrology, PR China).

Acetic acid and Propionic acid were measured using an Perkin Elmer Clarus 500 gas GC equipped with a 300×0.32 mm×0.5µm(length×ID×film) Elite-FFAP column and a flame ionization detector (FID) at 220°C. PHB, PHV and PH2MV were measured using an PERSEE GC1100 gas GC equipped with a 30×0.32 mm×0.25 µm(length×ID×film) SE-54 col-umn and a flame ionization detector (FID) at 250°C according to the method described by Oehmen et al [17]. The total PHA in the samples was calculated as the sum of the measured PHB, PHV and PH2MV.

Results and discussion

Effects of different carbon sources on denitrifying phosphorus removal and N₂O production.

Comparison of N and P removal performance in four SBRs. As showed in Fig.1, of the four SBRs, the higher the propionic/acetic acid ratio the less the phosphorus release were observed. This coincides well with other studies that have suggested that an increase in the propionic/acetic ratio led to less phosphorus release [18].

After the four reactors were fed with KNO₃ solution, denitrification and phosphorus uptake occurred simultaneously. The highest phosphorus removal was obtained in R-A, while the lowest was observed in R-D, which were 88.99%, 85.45%, 80.95%and 74.51% respectively. Moreover, the added NO₃⁻-N was rapidly reduced and this reduction was accompanied by the accumulation and reduction of NO₂⁻-N, which resulted in corresponding TN removal efficiencies of 79.71%, 78.57% 77.71% and 76.00%, respectively. These findings indicated that, as the amount of propionate acid added increased, the denitrifying phosphorus removal decreased.

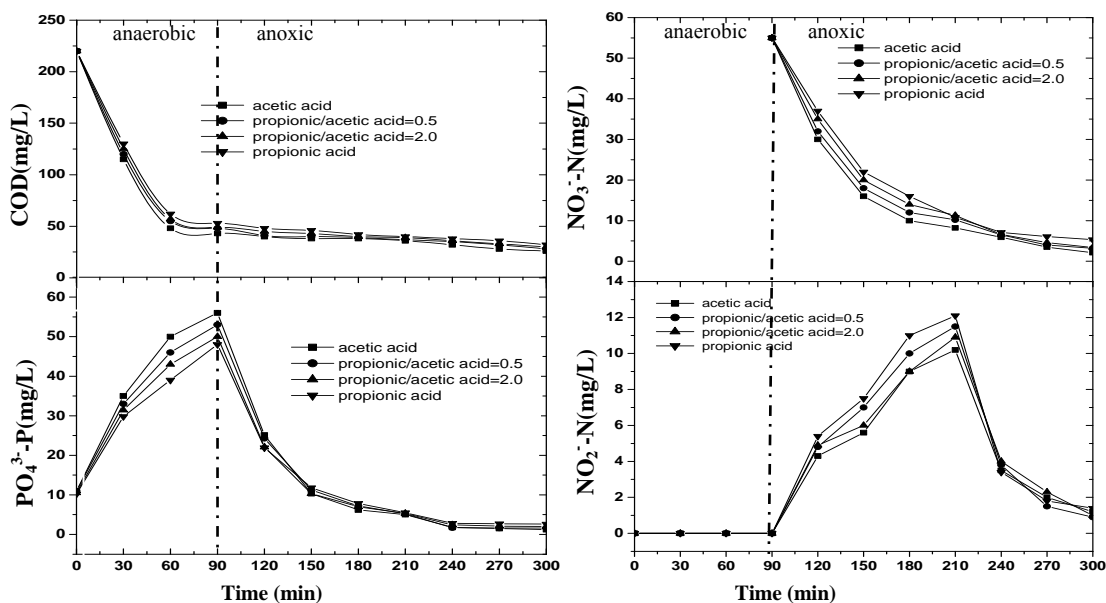


Fig.1 Variations in COD, nitrogen, phosphorus during one cycle in four SBRs

Effects of carbon sources on the transformation of PHA and N₂O production. Fig.2 showed the transformations of PHA in the four SBRs. During the anaerobic phase, as the percentage of added propionate increased, the PHA synthesis decreased from 97.0, 84.4, 76.0 to 58.0 mg/g-MLSS. Moreover, a decrease and an increase occurred in the fractions of formed PHB and PHV+PH2MV respectively (as shown in Tab.2). During the anoxic phase, the amounts of PHA degradation in the four SBRs were 106.8, 98.2, 86.0 and 68.0 mg/g-MLVSS respectively, suggesting that increasing propionic acid content results in decreased PHA synthesis and oxidation.

Tab.2 Comparison of N and P removal and N₂O production in the four SBRs

Item	R-A	R-B	R-C	R-D
Anaerobic PHA synthesis (mg/g-MLVSS)	97.00	84.40	76.00	58.00
Anaerobic PHB synthesis (mg/g-MLVSS)	77.31	60.29	35.76	12.45
Anaerobic PHV+PH ₂ MV synthesis (mg/g-MLVSS)	19.69	24.11	40.24	45.55
(PHV+PH ₂ MV)/PHA (%)	20.30	28.57	52.95	78.53
P removal efficiency (%)	88.99	85.45	80.95	74.51
Denitrification efficiency (%)	79.71	78.57	77.71	76.00
Anoxic N ₂ O production (mg N/L)	2.23	2.50	2.60	2.90
Ratio of anoxic N ₂ O-N production to denitrified nitrogen (%)	4.15	5.00	5.78	6.30

Fig.2 also showed the variations in total N₂O concentrations in the four SBRs. During the anaerobic phase, no N₂O production was detected in any of the reactors. However, after the KNO₃ solution was pulse added at the end of the anaerobic period, a remarkable increase in the N₂O concentration occurred in all reactors. Consequently, the ratios of N₂O–N production to the TN removal were 4.15%, 5.00%, 5.78% and 6.30% respectively. Obviously, the ratio of N₂O–N production to TN removal increased with the increasing of propionate/acetate acid ratio.

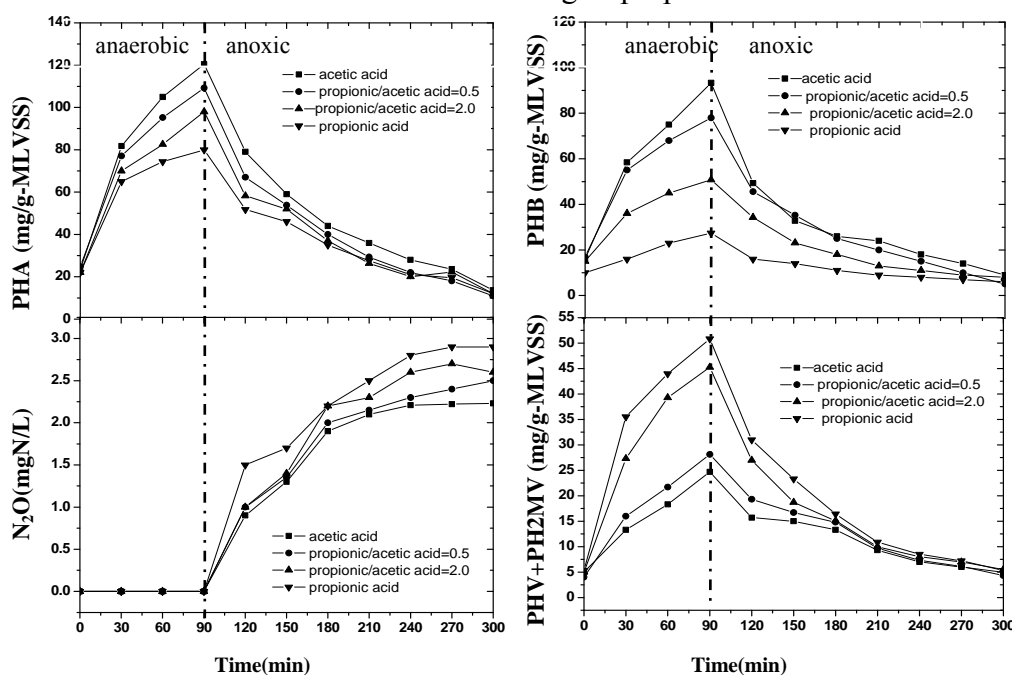


Fig.2 Variations of PHA and N₂O in four SBRs with different carbon sources

From the above mentioned study we can see that the amount of anaerobically synthesized PHA had a significant effect on denitrification phosphorus removal efficiency as well as N₂O production. The reduction of N₂O is the last step of denitrification, which makes it more difficult for N₂O reductase (Nos) to compete for electrons than other enzymes, particularly when the amount of the anaerobically synthesized PHA is low. Therefore, if more PHA is synthesized, more carbon sources are available for denitrification and the PHA degradation rate can be improved. This ultimately leads to a lower ratio of N₂O-N production to denitrified nitrogen.

Internal carbon source type may also be an important factor influencing N₂O production. With the increasing of (PHV+PH₂MV)/PHA ratio in the four SBRs, the corresponding N₂O generation rate increases. These findings suggest that more PHV+PH₂MV leads to higher N₂O production. As the carbon source, PHB and PHV+PH₂MV denitrification rate is different, this may have different effects on the activity of denitrification enzymes, eventually also affected the N₂O generation.

PHV+PH2MV denitrification rate was slower than that of PHB, PHA containing higher proportion of PHB may be more suitable as a denitrification internal carbon source, thereby producing a low content of N₂O.

Conclusion

In the anaerobic-anoxic sequencing batch reactors, the effects of different ratios of propionic to acetic acid on the efficiency of phosphorus and nitrogen removal and the transformations of PHA and N₂O were investigated. In the anaerobic stage, the increase of propionic fraction caused less PHB, more PHV and PH2MV, and less total PHA synthesis. This directly stimulated N₂O production. The N₂O–N production to TN removal ratios were 1.20, 1.39 or 1.52 times higher, respectively, when biomass acclimatized with acetate/propionate or propionate than when acetate alone was used. In addition, the TP removal efficiency decreased from 88.99% to 85.45%, 80.95% or 74.51%, respectively, and the TN removal efficiency declined from 79.71% to 78.57%, 77.71% or 76.00%, respectively, after the carbon sources was switched from acetate to acetate/propionate or propionate.

Acknowledgements

This work was financially supported by the outstanding young teacher support program (03140602).

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