Title: N₂O reduction during municipal wastewater treatment using a two-sludge SBR system acclimatized with propionate

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Abstract:

A two-sludge denitrifying phosphorus removal process (A$_2$N-SBR), acclimatized with propionate, was proposed as an efficient method for nitrous oxide (N$_2$O) reduction during municipal wastewater treatment. Compared with the conventional nitrification-denitrification process (AO-SBR) operated in parallel, the A$_2$N-SBR not only significantly improved total nitrogen and soluble phosphorus removal efficiencies by around 32.3% and 23.5%, respectively, but also greatly reduced N$_2$O generation by around 31.5%. Moreover, like the anoxic stage of AO-SBR, nearly zero N$_2$O (merely 0.054% of the removed nitrogen) was generated during the anoxic stage of A$_2$N-SBR. The substantial N$_2$O reduction achieved in the proposed A$_2$N-SBR can be reasonably explained by (i) the use of independent nitrification reactor resulting in higher activity of nitrifying bacteria and no occurrence of heterotrophic denitrification in aerobic stage, and (ii) the use of propionate as carbon source decreasing nitrite accumulation in anoxic stage.

Key words: Nitrous oxide; reduction; denitrifying phosphorus removal; two-sludge system; propionate
1. Introduction

As an important measure for eutrophication control, biological nutrient removal (BNR) process has been increasingly applied worldwide for more effective removal of nutrients, mainly nitrogen and phosphorus, in municipal wastewater. However, it has been known that BNR process is an important nitrous oxide (N$_2$O) emission source [1]. N$_2$O is a potent greenhouse gas, and its 100-year global warming potential is approximately 300 times stronger than that of carbon dioxide (CO$_2$) [2]. It can also react with oxygen (O$_2$) to produce nitric oxide (NO) in the stratosphere and consequently contribute to the destruction of ozone layer [3]. Therefore, effective control of N$_2$O emission from BNR process is of great importance and attracts increasingly more attention.

Over the past decade, great efforts have been devoted to investigating the characteristics of N$_2$O emission from BNR processes. It has been widely acknowledged that most N$_2$O was generated in aerobic stages, mainly by autotrophic nitrification and heterotrophic denitrification [4-6]. In autotrophic nitrification, ammonium is oxidized to nitrite by ammonium-oxidizing bacteria (AOB), and the nitrite is further oxidized to nitrate by nitrite-oxidizing bacteria (NOB). When nitrite is accumulated, AOB denitrification occurs in which nitrite was reduced to N$_2$O and N$_2$ [7]. In heterotrophic denitrification, reduction of nitrite/nitrate can be carried out by denitrifier under low oxygen condition, and the end product is mainly N$_2$O rather than N$_2$ [8].

Most BNR processes so far are single-sludge systems wherein nitrifying bacteria,
heterotrophic denitrifying bacteria, and phosphate-accumulating organisms (PAO) co-exist with each other. For the optimal growth of heterotrophic denitrifying bacteria and PAO, these systems normally cannot be operated at long sludge retention time (SRT) and high dissolved oxygen (DO) concentration [9]. Unfortunately, under such operating conditions, the high metabolic activity of NOB cannot be achieved, resulting in more serious nitrite accumulation and consequently a larger amount of N₂O generation [10, 11]. Besides, as previously mentioned, the low DO concentration can also promote N₂O generation by favoring the occurrence of heterotrophic denitrification in aerobic stages [11, 12]. It therefore appears that N₂O generation in aerobic stages may be effectively reduced if the NOB can be separated into an independent reactor, operated at desirable conditions (i.e., long SRT and high DO concentration).

One promising solution to the inherent limitation of N₂O reduction in single-sludge system turns out to be the use of two-sludge system, known as anaerobic-anoxic/nitrifying sequencing batch reactor (A₂N-SBR). The two-sludge system consists of an anaerobic–anoxic SBR (A₂-SBR), and a separate nitrification SBR (N-SBR) operated at the conditions favoring the growth of NOB [13-15]. It is therefore hypothesized that N₂O generation in the N-SBR can be significantly reduced, due to the enhanced NOB activity and no occurrence of heterotrophic denitrification. However, no special efforts have yet been made to understand the potential reduction of N₂O generation in the N-SBR of two-sludge system.

Although little is known about N₂O generation in A₂-SBR, a large amount of
N₂O was measured by Wang et al. [16, 17], operating the A₂-SBR acclimatized with acetate as carbon source. Carvalho et al. [18] reported that acetate would reduce the denitrifying phosphate removal, while using propionate as carbon source for acclimatization would be able to improve denitrifying phosphate removal. Furthermore, compared with acetate, using propionate as carbon source significantly decreased N₂O generation in anaerobic-aerobic (low dissolved oxygen) process [19, 20]. It appears that the use of propionate as carbon source may not only increase phosphate removal performance but also decrease N₂O generation. It is therefore hypothesized that N₂O reduction can be achieved if the A₂-SBR was acclimatized with propionate.

As an initial attempt, our study presents an A₂N-SBR system acclimatized with propionate for N₂O reduction during municipal wastewater treatment. The characteristics of N₂O generation in A₂N-SBR were investigated in details and were compared with the parallel conventional nitrification/denitrification process (AO-SBR) and some other representatives in literature. The hypothesized N₂O reduction mechanisms were analyzed in-depth and further verified with the additional experimental evidence obtained from the specially designed batch tests. Our results may lay an important foundation for future application of A₂N-SBR for N₂O reduction.

2. Materials and Methods

2.1. Experimental system setup and operation

Two laboratory-scale systems, an A₂N-SBR and a conventional AO-SBR, were
operated in parallel to comparatively study their \( \text{N}_2\text{O} \) generation characteristics. They were seeded with the same sludge, collected from the First Wastewater Treatment Plant of Everbright Water (Jinan) Ltd. (Jinan, China). The \( \text{A}_2\text{N-SBR} \) consisted of two reactors, an \( \text{A}_2\text{-SBR} \) and an \( \text{N-SBR} \). The former was operated under alternating anaerobic, anoxic, and aerobic conditions, whereas the latter worked under aerobic condition. The \( \text{AO-SBR} \) was performed in one single reactor and operated under alternating anoxic and aerobic conditions. Each reactor has a working volume of 5 L. The main operational parameters of the two systems are summarized in Table 1.

Fig.1A shows the operational scheme for the \( \text{A}_2\text{N-SBR} \) with a cycle length of 8 hours. In the initial feeding stage, 4 L of synthetic wastewater was fed into the \( \text{A}_2\text{-SBR} \). After 90 min anaerobic reaction and 40 min settling, 4 L of supernatant was flowed into the \( \text{N-SBR} \) where ammonium was completely oxidized to nitrate. After settling, the nitrate enriched effluent of \( \text{N-SBR} \) was withdrawn and temporally kept in a storage tank. It was subsequently fed into the \( \text{A}_2\text{-SBR} \) for further anoxic and post-aerobic treatment. The DO concentration in the \( \text{N-SBR} \) was maintained around 2.0-2.5 mg/L.

Fig.1B shows the operational scheme of the \( \text{AO-SBR} \) with a cycle length of 6 hours. In the feeding stage, 2.5 L of synthetic wastewater was pumped into the reactor, while 2.5 L of supernatant was removed after settling. The DO concentration was maintained around 1.0-1.5 mg/L.

After around four months of running, the effluent concentrations of nitrogen and phosphorus of the two systems were stabilized, suggesting that the simultaneous
denitrification and phosphorus removal process was achieved. The investigations of contaminant removal performance, N₂O generation characteristic as well as batch tests were then conducted.

2.2. Synthetic wastewater

Table 2 lists the composition of the synthetic wastewater. The concentrations of COD, NH₄⁺-N, and TP were chosen to be 200, 40 and 5 mg/L, respectively, which represented the typical influent quality of the First Wastewater Treatment Plant of Everbright Water (Jinan) Ltd [21].

2.3. Batch tests

Three series of batch tests were conducted in order to further verify the hypothesized mechanisms responsible for N₂O reduction in the two-sludge SBR system acclimatized with propionate. Two reactors, each having a working volume of 1.4 L, were used in the batch tests. The temperature was controlled to be the same as that in parent reactors.

2.3.1 N₂O reduction in N-SBR

Batch test 1, 2 were carried out to verify the lack of heterotrophic denitrification and the enhanced metabolic activity of NOB in N-SBR. Batch test 1, a total of 2.8 L of mixed liquor was taken from the parent N-SBR at the beginning of aerobic stage, and divided equally into the two batch-test reactors (BT-1 and BT-2). Following the method described by Tallec et al. [22], a certain amount of nitrite, allylthiourea (ATU), and chlorate (NaClO₃) was added into BT-1 to give an initial NO₂⁻-N, ATU, and NaClO₃ concentration of 5, 10, and 1000 mg/L, respectively. In contrast, BT-2 was
only supplied with the same amount of nitrite. The two batch-test reactors were aerated for 120 min, and the DO concentrations were maintained to be the same as that in the parent N-SBR reactor.

Since ATU [23] and NaClO3 [24] are well known nitrification inhibitors, the N2O generation in BT-1 can be reasonably assumed to be mainly from heterotrophic denitrification, which is expected to be around zero. The N2O generated by autotrophic nitrification was equal to the difference between BT-1 and BT-2. Moreover, for comparison, an additional batch test was performed for AO-SBR following the same procedure as that applied to N-SBR.

In batch test 2, the metabolic activity of NOB, indicated by nitrite oxidation rate (NOR), in N-SBR was evaluated and compared with that in AO-SBR. 1.4 L of sludge was withdrawn at the beginning of aerobic stage from the parent N-SBR and AO-SBR, respectively. Each sludge was firstly washed three times with 0.9% NaCl solution. The supernatant was then removed and the settled sludge was transferred into batch test reactor. Thereafter, a certain amount of tap water and nitrite were supplied to each reactor to give an initial NO2--N concentrations of 10 mg/L. The aerobic reaction was conducted for 90 min in which the NOR in each reactor was determined based on the change in nitrite concentration (mg NO2--N) over time (h), normalized to MLVSS concentration (g VSS).

2.3.2 N2O reduction in A2-SBR

Batch test 3 was carried out to further evaluate the advantage of propionate in the carbon source for N2O reduction in the anoxic stage of A2-SBR. A total of 2.8 L of
mixed liquor was taken from the parent A2-SBR at the end of post-aerobic stage, and divided equally into the two batch-test reactors (BT-1 and BT-2). After the supernatant was removed, 1.12 L synthetic wastewater was supplied to each reactor to give an initial NH$_4^+$-N and PO$_4^{3-}$-P concentrations of 10 and 5 mg/L, respectively. One reactor was fed with acetate, whereas the other was supplied with propionate. The COD concentrations in two reactors were both 160 mg/L. After 90 min of anaerobic reaction, KNO$_3$ solution was immediately added into each reactor, giving an initial NO$_3^-$-N concentration of 32 mg/L. The anoxic reaction lasted for 180 min.

2.4. Analytical methods

COD, NH$_4^+$-N, NO$_3^-$-N, NO$_2^-$-N, PO$_4^{3-}$-P and MLSS were analyzed in accordance with Standard Methods [25]. DO was measured with DO meters (HQ40d53LDO™, HACH, USA). The off-gas N$_2$O was collected and calculated according to the methods of Yang et al. [6]. The N$_2$O concentrations in gas and liquid phases were measured using gas chromatography (GC) (SP-3410, Beifen, China) and N$_2$O microsensor (N$_2$O-100, Unisense A/S, Denmark), respectively.

3. Results

3.1 General Performance

The A$_2$N-SBR and AO-SBR reached steady state after running about four months, indicated by the stable MLSS concentration and nitrogen and phosphorous removal efficiencies. The sludge volume index (SVI) in A$_2$-SBR, N-SBR, and AO-SBR were 24.3-28.5, 31.4-35.5, and 77.6-83.2 mL/g SS, respectively. Compared to AO-SBR, the sludge in A$_2$-SBR showed a better settleability. As A$_2$-SBR was mainly enriched with
denitrifying polyphosphate-accumulating organisms (DPAOs), the sludge may grow more like granules rather than flocs observed in AO-SBR. Furthermore, N-SBR operated at high DO concentration was mainly enriched with nitrifying bacteria, and the sludge also showed a better settleability [13].

Table 3 summarizes the pollutant removal efficiencies of the two systems. It can be seen that both A2N-SBR and AO-SBR exhibited excellent COD and NH$_4^+$-N removal efficiencies, averaged over 87.1%. This is as expected since propionate is readily biodegradable and no limiting factors are present for the nitrification occurring in aerobic stages. In contrast, however, the TN and PO$_4^{3-}$-P removal efficiencies of A2N-SBR (i.e. 92.3% and 92.0%, respectively) were found to be substantially higher than those measured in AO-SBR (i.e. 60.2% and 68.5%, respectively). In other words, compared with AO-SBR, A2N-SBR significantly improved TN and PO$_4^{3-}$-P removal efficiencies by about 32.3% and 23.5%, respectively.

The excellent TN and PO$_4^{3-}$-P removal efficiencies of A2N-SBR may be attributed to the existence of DPAOs, which could remove nitrogen and phosphorus simultaneously using the same carbon source [13]. On the contrary, in single-sludge AO-SBR systems, the removal of nitrogen and phosphorus were respectively carried out by denitrifiers and polyphosphate-accumulating organisms (PAO), both of which require carbon source. Therefore, the low influent carbon source could not meet the demand of denitrifiers and PAO for simultaneous nitrogen and phosphorous removal in AO-SBR.

3.2 A typical cycle of A2N-SBR system
Fig. 2 shows the time profiles of COD, NH$_4^+$-N, NO$_3^-$-N, NO$_2^-$-N, and PO$_4^{3-}$-P concentration as well as N$_2$O generation amount in a typical cycle of A$_2$-SBR and N-SBR. During the anaerobic stage of A$_2$-SBR, most of the COD was consumed within 30 min, and this was accompanied by the release of PO$_4^{3-}$-P (Fig. 2). After the first settling, the supernatant rich in NH$_4^+$-N and PO$_4^{3-}$-P was transferred into the N-SBR, wherein most of the NH$_4^+$-N was converted into NO$_3^-$-N after 90 min aerobic operation, and PO$_4^{3-}$-P concentration remained almost constant (Fig. 2).

The effluent of N-SBR was subsequently returned back to A$_2$-SBR, wherein simultaneous denitrification and phosphorus uptake occurred. The NO$_3^-$-N concentration was gradually decreased to zero after 180 min of anoxic operation. Only 0.57 mg/L NO$_2^-$-N was generated at 160 min, after which it was rapidly reduced to zero. With the reduction of NO$_3^-$-N, PO$_4^{3-}$-P concentration decreased gradually to 2.82 mg/L. In the subsequent post-aerobic stage, half of the residual NH$_4^+$-N was converted to NO$_2^-$-N and NO$_3^-$-N, and the PO$_4^{3-}$-P concentration was further decreased to 0.09 mg/L.

From Fig. 2, it was found that N$_2$O generation occurred in the anoxic and post-aerobic stage of A$_2$-SBR and the aerobic stage of N-SBR. During the whole anaerobic stage, N$_2$O generation was negligible. When the NH$_4^+$ rich supernatant transferred into the N-SBR, N$_2$O generation amount increased rapidly, reaching the highest value of 0.15 mg/L at 60 min, and then leveled off. At the beginning of anoxic stage, once NO$_3^-$ rich supernatant was added to the reactor, N$_2$O generation amount increased rapidly to 0.31 mg/L in the first 10 min and decreased drastically to around
zero thereafter. This was because the generated N$_2$O was mainly dissolved which was
denitrified by DPAOs in subsequent anoxic reaction. In the post-aerobic stage, N$_2$O
generation amount constantly increased reaching a maximum of 0.72 mg/L at 360
min.

3.3 A typical cycle of AO-SBR system

Fig. 3 illustrates the variations of COD, NH$_4^+$-N, NO$_3^-$-N, NO$_2^-$-N, and PO$_4^{3-}$-P
concentration as well as N$_2$O generation amount in one typical cycle of AO-SBR. It
can be seen that, in anoxic stage, the concentration of COD and NO$_3^-$-N rapidly
decreased to around 25.36 mg/L and 0 mg/L respectively, whereas PO$_4^{3-}$-P
concentration slowly increased and reached to 7.69 mg/L at 90 min. Like the case in
A$_2$-SBR, no NO$_2^-$-N accumulation was observed during this stage.

In aerobic stage, NH$_4^+$-N was completely depleted after 60 min. Moreover, the
NO$_2^-$-N concentration rapidly increased to 2.42 mg/L at 120 min and gradually
reduced thereafter. The NO$_3^-$-N concentration increased from the beginning of the
aerobic stage, and finally reached up to 16.07 mg N/L. The PO$_4^{3-}$-P concentration
decreased gradually to 1.94 mg/L. The obtained results suggest that denitrification
and phosphorus release mainly occurred in anoxic stage, whereas nitrification and
phosphorus uptake mainly occurred in aerobic stage.

Fig. 3 shows that N$_2$O generation in anoxic stage was very low, and the
maximum N$_2$O generation amount was only 0.01 mg/L. This result was different from
the studied A$_2$-SBR where the maximum N$_2$O generation amount up to 0.32 mg/L.
The main explanation is that AO-SBR used propionate as carbon source for
denitrification, but A2-SBR using poly-b-hydroxyalkanoates (PHA) for denitrification.

Some researchers also reported that the consumption of PHA for denitrification could increase the N₂O generation [26-28]. In the aerobic period, N₂O generation amount increased rapidly and reached to the highest value of 0.20 mg/L at 150 min.

4. Discussion

As a suitable carbon source for DPAOs, propionate could maintain the stability of denitrifying phosphorous removal process [18]. Therefore, the nitrogen and phosphorous removal efficiencies in the studied A₂N-SBR appeared to be above 90% all the time. With the use of sludge fermentation liquid enriched in propionate, improved nitrogen and phosphorous removal was observed in a single-sludge denitrifying phosphorous removal via nitrite system [29]. The nitrogen and phosphorous removal efficiencies (98.7% and 97.6%) were higher than those of the presented A₂N-SBR (Table 4). The lower phosphorous removal efficiency of A₂N-SBR may be partly attributed to the lower influent phosphorous concentration (5 mg/L), whereas the lower nitrogen removal efficiency in A₂N-SBR may be due to the inevitable discharge of some residual ammonia. However, it was noteworthy that nitrite rather nitrate was the intermediate between nitrification and denitrification in the single-sludge SBR [29], and the accumulation of nitrite could cause N₂O generation. It is therefore assumed that the N₂O generation in the single-sludge SBRs would possibly be higher, justifying further research needs.

Table 5 compares the generation amount and conversion rate of N₂O in the studied A₂N-SBR system with AO-SBR and some other A₂-SBR systems in literature.
Compared with other systems, total N$_2$O generation amount in our A$_2$N-SBR was very low, and it only accounted for 0.69% of the influent TN. This was attributed partly to the low amount of N$_2$O generation in N-SBR, and partly to the limited amount of N$_2$O generation in A$_2$-SBR.

4.1 Causes of low N$_2$O generation in N-SBR

It can be seen from Table 5 that the N$_2$O generation amount during aerobic stage of N-SBR accounted for 0.47% of the influent nitrogen load, which was much lower than those in AO-SBR and other systems. Moreover, N$_2$O generation in AO-SBR was also found to be lower than that in the study of Jia et al. [30]. This may be attributed to the difference of carbon source. In this study propionate was used as carbon source, whereas Jia et al. [30] using acetate and glucose. It has been reported that the use of propionate could decrease N$_2$O generation [19, 20]. Zhu and Chen [20] used sludge alkaline fermentation liquid enriched in propionate as carbon source, but the N$_2$O generation appeared to be much higher than that in our study. The main reason was probably that they used an anaerobic-aerobic process operated at low DO concentration.

There are two possible explanations for the low N$_2$O generation in N-SBR, one is no occurrence of heterotrophic denitrification, and the other is the higher activity of NOB. Firstly, autotrophic nitrification and heterotrophic denitrification can occur simultaneously in aerobic stage of single-sludge system, both of them can contribute to N$_2$O generation [22, 30]. However, unlike the single-sludge system, autotrophic nitrification was the only source of N$_2$O generation in N-SBR. The lack of
heterotrophic denitrification in N-SBR was clearly evidenced by the batch test results shown in Fig. 4. It was noted that autotrophic nitrification was the main source in N-SBR, accounting for more than 95.4% of total N₂O generation. However, both autotrophic nitrification and heterotrophic denitrification contributed to N₂O generation in the aerobic stage of AO-SBR, and heterotrophic denitrification accounted for about 30.6%. With the use of inhibitors, Tallec et al. [22] reported that heterotrophic denitrification represents from 17% to 42% of total N₂O generation amount during aerobic stage of urban wastewater treatment.

The other explanation is that the activity of NOB in N-SBR was much higher than that in A/O-SBR or other single-sludge systems. As previously hypothesized, since there was only nitrification occurring in N-SBR, the SRT and DO concentration could be controlled at higher level favoring the growth of NOB. The higher activity of NOB would decrease NO₂⁻ accumulation, and would consequently reduce N₂O generation. This hypothesis can be verified by comparing the NOR in N-SBR and A/O-SBR. According to the results of batch test 2, the NOR of N-SBR was 13.36 ± 1.46 mg NO₂⁻/h/g VSS, which was significantly higher than that (i.e. 9.23 ± 0.82 mg NO₂⁻/h/g VSS) in AO-SBR. This suggests that the amount of NO₂⁻ accumulated and N₂O generated in N-SBR would be much less than those in AO-SBR. Fukumoto et al. [31] also found that the N₂O emission was decreased in a laboratory-scale composting experiment with the addition of NOB, and the main reason was supposed to be the added NOB preventing NO₂⁻ accumulation.

4.2 Causes of low N₂O generation in anoxic stage of A₂-SBR
Although $\text{N}_2\text{O}$ generation amount in anoxic stage of $\text{A}_2\text{-SBR}$ was much higher than that in AO-SBR (Table 5), it only contributed 7.17% of the total $\text{N}_2\text{O}$ generation of $\text{A}_2\text{N}-\text{SBR}$. Moreover, it was noteworthy that the presented $\text{A}_2\text{-SBR}$ produced much less $\text{N}_2\text{O}$ than other $\text{A}_2\text{-SBR}$ systems reported in literature. More specifically, the anoxic $\text{N}_2\text{O}$ generation amount in the presented $\text{A}_2\text{-SBR}$ was only 0.05% of the influent nitrogen load, which was much lower than those (2.34%~21.6%) reported in previous $\text{A}_2\text{-SBR}$ systems [16, 17].

The less anoxic $\text{N}_2\text{O}$ generation amount in the presented $\text{A}_2\text{-SBR}$ may be attributed to the use of propionate as carbon source, which is unlike other studies using acetate as carbon source [16, 17]. The positive effect of propionate on $\text{N}_2\text{O}$ reduction was partially proved by the results of batch test 3. It was observed from Fig.5 that the use of propionate significantly decreased $\text{N}_2\text{O}$ generation amount. Furthermore, nearly no $\text{NO}_2^-\text{-N}$ accumulation occurred in the anoxic stage when using propionate as carbon source. In contrast, $\text{NO}_2^-\text{-N}$ accumulation during this stage reached the maximum of 6.09 mg/L when using acetate as carbon source. $\text{NO}_2^-$ can promote $\text{N}_2\text{O}$ generation by inhibiting the activity of $\text{N}_2\text{O}$ reductase [32, 33]. Other $\text{A}_2\text{-SBR}$ systems reported in literature were always operated with acetate as the sole carbon source, and the $\text{N}_2\text{O}$ generation in these systems would be higher due to the high accumulation of $\text{NO}_2^-$. Although Wang et al. [16] investigated $\text{N}_2\text{O}$ generation in $\text{A}_2\text{-SBR}$ using propionate as carbon source, the observed nitrite accumulation and $\text{N}_2\text{O}$ generation were much higher than our results. One possible reason was that the sludge used in
their study was not acclimated by propionate but acetate, and the effect of propionate shock on N\textsubscript{2}O generation was investigated in one cycle. On the contrary, however, the sludge in our study was acclimated solely by propionate. The species of DPAOs in the two studies might be different. A detailed and in-depth analysis of the microbial community is desired to better elucidate the underlying mechanisms via which propionate decreases N\textsubscript{2}O generation in denitrifying phosphorus removal systems.

It therefore appears that the long-term use of propionate as carbon source could not only improve nitrogen and phosphorous removal efficiencies, but also significantly decrease N\textsubscript{2}O generation during denitrifying phosphorus removal system. Therefore, propionate or propionate-riched liquid (i.e. fermentation liquid of biowaste) can be recommended as a promising external carbon source for effective control of N\textsubscript{2}O generation during biological wastewater treatment processes.

5. Conclusion

In summary, this study suggested that the two-sludge SBR system acclimatized with propionate remarkably reduced N\textsubscript{2}O generation and increased the nitrogen and phosphorus removal efficiencies. N\textsubscript{2}O generation amount during aerobic stage of N-SBR was lower than that in single-sludge system, because the independent nitrification reactor resulted in higher activity of nitrifying bacteria and no occurrence of heterotrophic denitrification. Compared with the anoxic stage of denitrifying phosphorus removal process reported in literature, N\textsubscript{2}O generation in A\textsubscript{2}-SBR was very low, because the use of propionate as carbon source decreased nitrite accumulation.
Acknowledgements

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References


with minimal cod requirement by integration of denitrifying dephosphatation and

[14] Y. Zhou, M. Pijuan, Z.G. Yuan, Development of a 2-sludge, 3-stage system for
nitrogen and phosphorous removal from nutrient-rich wastewater using granular

hydraulic retention time (HRT) on simultaneous phosphorus and nitrogen
100 (2009) 3506-3512.

[16] Y.Y. Wang, J.J. Geng, G. Guo, C. Wang, S.H. Liu, N_2O production in
anaerobic/anoxic denitrifying phosphorus removal process: The effects of carbon

of anaerobic reaction time on denitrifying phosphorus removal and N_2O

[18] G. Carvalho, P.C. Lemos, A. Oehmen, M.A.M. Reis, Denitrifying phosphorus
removal: Linking the process performance with the microbial community

on nitrous oxide emission in anaerobic-aerobic (low dissolved oxygen)


Fig. 1. Schematic diagrams of the operation of (A) A$_2$N-SBR system and (B) AO-SBR system.
Fig. 2. Variations of COD, nitrogen, and phosphorus in a typical cycle of A2N-SBR.
Fig. 3. Variations of COD, nitrogen, and phosphorus concentration in a typical cycle of AO-SBR.
Fig. 4. N\textsubscript{2}O generation by autotrophic nitrification and heterotrophic denitrification in the N-SBR and AO-SBR.
Fig. 5. N₂O and NO₂⁻ generation profiles during two batch experiments with acetate and propionate as carbon source, respectively.

Table 1

The operating parameters of different reactors

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<th>Parameter</th>
<th>A₂N-SBR</th>
<th>A₂-SBR</th>
<th>N-SBR</th>
<th>AO-SBR</th>
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<td>F/M ratio (g COD/g SS/d)</td>
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Table 2

Composition of the synthetic wastewater

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<th>Concentration (mg/L)</th>
<th>Components</th>
<th>Concentration (mg/L)</th>
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<td>NaHCO$_3$</td>
<td>200</td>
<td>KI</td>
<td>0.18</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
<td>11</td>
<td>MnCl$_2$·4H$_2$O</td>
<td>0.12</td>
</tr>
<tr>
<td>K$_2$HPO$_4$·3H$_2$O</td>
<td>18</td>
<td>Na$_2$MoO$_4$·2H$_2$O</td>
<td>0.06</td>
</tr>
<tr>
<td>MgSO$_4$·7H$_2$O</td>
<td>10</td>
<td>ZnSO$_4$·7H$_2$O</td>
<td>0.12</td>
</tr>
<tr>
<td>FeSO$_4$·7H$_2$O</td>
<td>10</td>
<td>CoCl$_2$·6H$_2$O</td>
<td>0.15</td>
</tr>
<tr>
<td>CaCl$_2$·2H$_2$O</td>
<td>10</td>
<td>EDTA</td>
<td>10</td>
</tr>
</tbody>
</table>
Table 3

Removal performance of two systems for COD, NH$_4^+$-N, TN and PO$_4^{3-}$-P$^a$

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Influent concentration (mg/L)</th>
<th>A$_2$N-SBR</th>
<th>AO-SBR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effluent concentration (mg/L)</td>
<td>Removal Efficiency (%)</td>
<td>Effluent concentration (mg/L)</td>
</tr>
<tr>
<td>COD</td>
<td>194.4±20.12</td>
<td>25.08±9.36</td>
<td>87.13±5.73</td>
</tr>
<tr>
<td>NH$_4^+$-N</td>
<td>40.78±2.03</td>
<td>1.86±0.95</td>
<td>95.47±2.17</td>
</tr>
<tr>
<td>TN</td>
<td>40.78±2.03</td>
<td>3.16±1.15</td>
<td>92.28±2.69</td>
</tr>
<tr>
<td>PO$_4^{3-}$-P</td>
<td>5.05±0.28</td>
<td>0.39±0.20</td>
<td>92.04±4.35</td>
</tr>
</tbody>
</table>

$^a$ Sample mean ± standard deviation, number of measurements: n = 25.
Comparison of nitrogen and phosphorous removal efficiencies of A₂N-SBR and single-sludge SBR

<table>
<thead>
<tr>
<th>Parameter</th>
<th>A₂N-SBR (this study)</th>
<th>Single-sludge SBR (Ji and Chen, 2009)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent COD (mg/L)</td>
<td>200</td>
<td>265</td>
</tr>
<tr>
<td>Influent TN (mg/L)</td>
<td>40</td>
<td>38</td>
</tr>
<tr>
<td>Influent PO₄³⁻-P (mg/L)</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Effluent TN (mg/L)</td>
<td>3.16</td>
<td>0.49</td>
</tr>
<tr>
<td>Effluent PO₄³⁻-P (mg/L)</td>
<td>0.39</td>
<td>0.36</td>
</tr>
<tr>
<td>TN removal efficiency (%)</td>
<td>92.0</td>
<td>98.7</td>
</tr>
<tr>
<td>PO₄³⁻-P removal efficiency (%)</td>
<td>92.3</td>
<td>97.6</td>
</tr>
</tbody>
</table>
Table 5

N$_2$O generation amount per cycle in different reactors.

| Reactor       | Carbon source for sludge acclimatization | Stage                          | N$_2$O generation amount (%) | N$_2$
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A$_2$N-SBR</td>
<td>propionate</td>
<td>anaerobic stage in A$_2$-SBR</td>
<td>0.0076</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>anoxic stage in A$_2$-SBR</td>
<td>0.050</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>post-aerobic stage in A$_2$-SBR</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>aerobic stage in N-SBR</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>total generation</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>AO-SBR</td>
<td>propionate</td>
<td>anoxic stage</td>
<td>0.017</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>aerobic stage</td>
<td>1.00</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>total generation</td>
<td>1.01</td>
<td></td>
</tr>
<tr>
<td>A$_2$-SBR</td>
<td>acetate</td>
<td>anoxic stage</td>
<td>2.34-21.63</td>
<td></td>
</tr>
<tr>
<td>AO (low DO)-SBR</td>
<td>acetate and glucose</td>
<td>aerobic stage</td>
<td>7.05</td>
<td>2.12</td>
</tr>
<tr>
<td>AO-SBR</td>
<td>acetate and glucose</td>
<td>aerobic stage</td>
<td>2.12</td>
<td></td>
</tr>
<tr>
<td>AO (low DO)-SBR</td>
<td>sludge alkaline fermentation liquid</td>
<td>aerobic stage</td>
<td>10.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>acetate</td>
<td></td>
<td>28.21</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ N$_2$O generation amount = (total N$_2$O-N generation)/(influent TN) $\times$100%.

$^b$ N$_2$O conversion rate = (total N$_2$O-N generation)/(TN removed) $\times$100%.

Highlights

- Compared with AO-SBR, A$_2$N-SBR reduced N$_2$O generation by around 31.5%.
- Only 0.054% of the removed nitrogen in anoxic stage of A$_2$N-SBR was converted to N$_2$O.
- Autotrophic nitrification contributed to 95.4% of total N$_2$O generation in N-SBR.
- The separate nitrification reactor reduced N$_2$O generation in aerobic stage.
- The use of propionate as carbon source reduced N$_2$O generation in anoxic stage.