

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

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1 Abstract:

2 A two-sludge denitrifying phosphorus removal process (A₂N-SBR), acclimatized
3 with propionate, was proposed as an efficient method for nitrous oxide (N₂O)
4 reduction during municipal wastewater treatment. Compared with the conventional
5 nitrification-denitrification process (AO-SBR) operated in parallel, the A₂N-SBR not
6 only significantly improved total nitrogen and soluble phosphorus removal
7 efficiencies by around 32.3% and 23.5%, respectively, but also greatly reduced N₂O
8 generation by around 31.5%. Moreover, like the anoxic stage of AO-SBR, nearly zero
9 N₂O (merely 0.054% of the removed nitrogen) was generated during the anoxic stage
10 of A₂N-SBR. The substantial N₂O reduction achieved in the proposed A₂N-SBR can
11 be reasonably explained by (i) the use of independent nitrification reactor resulting in
12 higher activity of nitrifying bacteria and no occurrence of heterotrophic denitrification
13 in aerobic stage, and (ii) the use of propionate as carbon source decreasing nitrite
14 accumulation in anoxic stage.

15

16 *Key words:* Nitrous oxide; reduction; denitrifying phosphorus removal; two-sludge
17 system; propionate

1 **1. Introduction**

2 As an important measure for eutrophication control, biological nutrient removal
3 (BNR) process has been increasingly applied worldwide for more effective removal
4 of nutrients, mainly nitrogen and phosphorus, in municipal wastewater. However, it
5 has been known that BNR process is an important nitrous oxide (N_2O) emission
6 source [1]. N_2O is a potent greenhouse gas, and its 100-year global warming potential
7 is approximately 300 times stronger than that of carbon dioxide (CO_2) [2]. It can also
8 react with oxygen (O_2) to produce nitric oxide (NO) in the stratosphere and
9 consequently contribute to the destruction of ozone layer [3]. Therefore, effective
10 control of N_2O emission from BNR process is of great importance and attracts
11 increasingly more attention.

12 Over the past decade, great efforts have been devoted to investigating the
13 characteristics of N_2O emission from BNR processes. It has been widely
14 acknowledged that most N_2O was generated in aerobic stages, mainly by autotrophic
15 nitrification and heterotrophic denitrification [4-6]. In autotrophic nitrification,
16 ammonium is oxidized to nitrite by ammonium-oxidizing bacteria (AOB), and the
17 nitrite is further oxidized to nitrate by nitrite-oxidizing bacteria (NOB). When nitrite
18 is accumulated, AOB denitrification occurs in which nitrite was reduced to N_2O and
19 N_2 [7]. In heterotrophic denitrification, reduction of nitrite/nitrate can be carried out
20 by denitrifier under low oxygen condition, and the end product is mainly N_2O rather
21 than N_2 [8].

22 Most BNR processes so far are single-sludge systems wherein nitrifying bacteria,

1 heterotrophic denitrifying bacteria, and phosphate-accumulating organisms (PAO)
2 co-exist with each other. For the optimal growth of heterotrophic denitrifying bacteria
3 and PAO, these systems normally cannot be operated at long sludge retention time
4 (SRT) and high dissolved oxygen (DO) concentration [9]. Unfortunately, under such
5 operating conditions, the high metabolic activity of NOB cannot be achieved,
6 resulting in more serious nitrite accumulation and consequently a larger amount of
7 N_2O generation [10, 11]. Besides, as previously mentioned, the low DO concentration
8 can also promote N_2O generation by favoring the occurrence of heterotrophic
9 denitrification in aerobic stages [11, 12]. It therefore appears that N_2O generation in
10 aerobic stages may be effectively reduced if the NOB can be separated into an
11 independent reactor, operated at desirable conditions (i.e., long SRT and high DO
12 concentration).

13 One promising solution to the inherent limitation of N_2O reduction in
14 single-sludge system turns out to be the use of two-sludge system, known as
15 anaerobic-anoxic/nitrifying sequencing batch reactor (A_2N -SBR). The two-sludge
16 system consists of an anaerobic–anoxic SBR (A_2 -SBR), and a separate nitrification
17 SBR (N-SBR) operated at the conditions favoring the growth of NOB [13-15]. It is
18 therefore hypothesized that N_2O generation in the N-SBR can be significantly reduced,
19 due to the enhanced NOB activity and no occurrence of heterotrophic denitrification.
20 However, no special efforts have yet been made to understand the potential reduction
21 of N_2O generation in the N-SBR of two-sludge system.

22 Although little is known about N_2O generation in A_2 -SBR, a large amount of

1 N₂O was measured by Wang et al. [16, 17], operating the A₂-SBR acclimatized with
2 acetate as carbon source. Carvalho et al. [18] reported that acetate would reduce the
3 denitrifying phosphate removal, while using propionate as carbon source for
4 acclimatization would be able to improve denitrifying phosphate removal.
5 Furthermore, compared with acetate, using propionate as carbon source significantly
6 decreased N₂O generation in anaerobic-aerobic (low dissolved oxygen) process [19,
7 20]. It appears that the use of propionate as carbon source may not only increase
8 phosphate removal performance but also decrease N₂O generation. It is therefore
9 hypothesized that N₂O reduction can be achieved if the A₂-SBR was acclimatized
10 with propionate.

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

20 **2. Materials and Methods**

21 *2.1. Experimental system setup and operation*

22 Two laboratory-scale systems, an A₂N-SBR and a conventional AO-SBR, were

1 operated in parallel to comparatively study their N_2O generation characteristics. They
2 were seeded with the same sludge, collected from the First Wastewater Treatment
3 Plant of Everbright Water (Jinan) Ltd. (Jinan, China). The A_2N -SBR consisted of two
4 reactors, an A_2 -SBR and an N-SBR. The former was operated under alternating
5 anaerobic, anoxic, and aerobic conditions, whereas the latter worked under aerobic
6 condition. The AO-SBR was performed in one single reactor and operated under
7 alternating anoxic and aerobic conditions. Each reactor has a working volume of 5 L.
8 The main operational parameters of the two systems are summarized in Table 1.

9 Fig.1A shows the operational scheme for the A_2N -SBR with a cycle length of 8
10 hours. In the initial feeding stage, 4 L of synthetic wastewater was fed into the
11 A_2 -SBR. After 90 min anaerobic reaction and 40 min settling, 4 L of supernatant was
12 flowed into the N-SBR where ammonium was completely oxidized to nitrate. After
13 settling, the nitrate enriched effluent of N-SBR was withdrawn and temporarily kept in
14 a storage tank. It was subsequently fed into the A_2 -SBR for further anoxic and
15 post-aerobic treatment. The DO concentration in the N-SBR was maintained around
16 2.0-2.5 mg/L.

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

21 After around four months of running, the effluent concentrations of nitrogen and
22 phosphorus of the two systems were stabilized, suggesting that the simultaneous

1 denitrification and phosphorus removal process was achieved. The investigations of
2 contaminant removal performance, N_2O generation characteristic as well as batch
3 tests were then conducted.

4 2.2. Synthetic wastewater

5 Table 2 lists the composition of the synthetic wastewater. The concentrations of
6 COD, NH_4^+-N , and TP were chosen to be 200, 40 and 5 mg/L, respectively, which
7 represented the typical influent quality of the First Wastewater Treatment Plant of
8 Everbright Water (Jinan) Ltd [21].

9 2.3. Batch tests

10 Three series of batch tests were conducted in order to further verify the
11 hypothesized mechanisms responsible for N_2O reduction in the two-sludge SBR
12 system acclimatized with propionate. Two reactors, each having a working volume of
13 1.4 L, were used in the batch tests. The temperature was controlled to be the same as
14 that in parent reactors.

15 2.3.1 N_2O reduction in N-SBR

16 Batch test 1, 2 were carried out to verify the lack of heterotrophic denitrification
17 and the enhanced metabolic activity of NOB in N-SBR. Batch test 1, a total of 2.8 L
18 of mixed liquor was taken from the parent N-SBR at the beginning of aerobic stage,
19 and divided equally into the two batch-test reactors (BT-1 and BT-2). Following the
20 method described by Tallec et al. [22], a certain amount of nitrite, allylthiourea (ATU),
21 and chlorate ($NaClO_3$) was added into BT-1 to give an initial $NO_2^- - N$, ATU, and
22 $NaClO_3$ concentration of 5, 10, and 1000 mg/L, respectively. In contrast, BT-2 was

1 only supplied with the same amount of nitrite. The two batch-test reactors were
2 aerated for 120 min, and the DO concentrations were maintained to be the same as
3 that in the parent N-SBR reactor.

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

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

20 2.3.2 N₂O reduction in A₂-SBR

21 Batch test 3 was carried out to further evaluate the advantage of propionate in the
22 carbon source for N₂O reduction in the anoxic stage of A₂-SBR. A total of 2.8 L of

1 mixed liquor was taken from the parent A₂-SBR at the end of post-aerobic stage, and
2 divided equally into the two batch-test reactors (BT-1 and BT-2). After the supernatant
3 was removed, 1.12 L synthetic wastewater was supplied to each reactor to give an
4 initial NH₄⁺-N and PO₄³⁻-P concentrations of 10 and 5 mg/L, respectively. One reactor
5 was fed with acetate, whereas the other was supplied with propionate. The COD
6 concentrations in two reactors were both 160 mg/L. After 90 min of anaerobic
7 reaction, KNO₃ solution was immediately added into each reactor, giving an initial
8 NO₃⁻-N concentration of 32 mg/L. The anoxic reaction lasted for 180 min.

9 *2.4. Analytical methods*

10 COD, NH₄⁺-N, NO₃⁻-N, NO₂⁻-N, PO₄³⁻-P and MLSS were analyzed in
11 accordance with Standard Methods [25]. DO was measured with DO meters
12 (HQ40d53LDO™, HACH, USA). The off-gas N₂O was collected and calculated
13 according to the methods of Yang et al. [6]. The N₂O concentrations in gas and liquid
14 phases were measured using gas chromatography (GC) (SP-3410, Beifen, China) and
15 N₂O microsensor (N₂O-100, Unisense A/S, Denmark), respectively.

16 **3. Results**

17 *3.1 General Performance*

18 The A₂N-SBR and AO-SBR reached steady state after running about four months,
19 indicated by the stable MLSS concentration and nitrogen and phosphorous removal
20 efficiencies. The sludge volume index (SVI) in A₂-SBR, N-SBR, and AO-SBR were
21 24.3-28.5, 31.4-35.5, and 77.6-83.2 mL/g SS, respectively. Compared to AO-SBR, the
22 sludge in A₂-SBR showed a better settleability. As A₂-SBR was mainly enriched with

1 denitrifying polyphosphate-accumulating organisms (DPAOs), the sludge may grow
2 more like granules rather than flocs observed in AO-SBR. Furthermore, N-SBR
3 operated at high DO concentration was mainly enriched with nitrifying bacteria, and
4 the sludge also showed a better settleability [13].

5 Table 3 summarizes the pollutant removal efficiencies of the two systems. It can
6 be seen that both A₂N-SBR and AO-SBR exhibited excellent COD and NH₄⁺-N
7 removal efficiencies, averaged over 87.1%. This is as expected since propionate is
8 readily biodegradable and no limiting factors are present for the nitrification occurring
9 in aerobic stages. In contrast, however, the TN and PO₄³⁻-P removal efficiencies of
10 A₂N-SBR (i.e. 92.3% and 92.0%, respectively) were found to be substantially higher
11 than those measured in AO-SBR (i.e. 60.2% and 68.5%, respectively). In other words,
12 compared with AO-SBR, A₂N-SBR significantly improved TN and PO₄³⁻-P removal
13 efficiencies by about 32.3% and 23.5%, respectively.

14 The excellent TN and PO₄³⁻-P removal efficiencies of A₂N-SBR may be
15 attributed to the existence of DPAOs, which could remove nitrogen and phosphorus
16 simultaneously using the same carbon source [13]. On the contrary, in single-sludge
17 AO-SBR systems, the removal of nitrogen and phosphorus were respectively carried
18 out by denitrifiers and polyphosphate-accumulating organisms (PAO), both of which
19 require carbon source. Therefore, the low influent carbon source could not meet the
20 demand of denitrifiers and PAO for simultaneous nitrogen and phosphorous removal
21 in AO-SBR.

22 *3.2 A typical cycle of A₂N-SBR system*

1 Fig. 2 shows the time profiles of COD, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{PO}_4^{3-}\text{-P}$
2 concentration as well as N_2O generation amount in a typical cycle of $\text{A}_2\text{-SBR}$ and
3 N-SBR . During the anaerobic stage of $\text{A}_2\text{-SBR}$, most of the COD was consumed
4 within 30 min, and this was accompanied by the release of $\text{PO}_4^{3-}\text{-P}$ (Fig. 2). After the
5 first settling, the supernatant rich in $\text{NH}_4^+\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$ was transferred into the
6 N-SBR , wherein most of the $\text{NH}_4^+\text{-N}$ was converted into $\text{NO}_3^-\text{-N}$ after 90 min aerobic
7 operation, and $\text{PO}_4^{3-}\text{-P}$ concentration remained almost constant (Fig. 2).

8 The effluent of N-SBR was subsequently returned back to $\text{A}_2\text{-SBR}$, wherein
9 simultaneous denitrification and phosphorus uptake occurred. The $\text{NO}_3^-\text{-N}$
10 concentration was gradually decreased to zero after 180 min of anoxic operation.
11 Only 0.57 mg/L $\text{NO}_2^-\text{-N}$ was generated at 160 min, after which it was rapidly reduced
12 to zero. With the reduction of $\text{NO}_3^-\text{-N}$, $\text{PO}_4^{3-}\text{-P}$ concentration decreased gradually to
13 2.82 mg/L. In the subsequent post-aerobic stage, half of the residual $\text{NH}_4^+\text{-N}$ was
14 converted to $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$, and the $\text{PO}_4^{3-}\text{-P}$ concentration was further
15 decreased to 0.09 mg/L.

16 From Fig. 2, it was found that N_2O generation occurred in the anoxic and
17 post-aerobic stage of $\text{A}_2\text{-SBR}$ and the aerobic stage of N-SBR . During the whole
18 anaerobic stage, N_2O generation was negligible. When the NH_4^+ rich supernatant
19 transferred into the N-SBR , N_2O generation amount increased rapidly, reaching the
20 highest value of 0.15 mg/L at 60 min, and then leveled off. At the beginning of anoxic
21 stage, once NO_3^- rich supernatant was added to the reactor, N_2O generation amount
22 increased rapidly to 0.31 mg/L in the first 10 min and decreased drastically to around

1 zero thereafter. This was because the generated N_2O was mainly dissolved which was
2 denitrified by DPAOs in subsequent anoxic reaction. In the post-aerobic stage, N_2O
3 generation amount constantly increased reaching a maximum of 0.72 mg/L at 360
4 min.

5 3.3 A typical cycle of AO-SBR system

6 Fig. 3 illustrates the variations of COD, NH_4^+-N , $NO_3^- -N$, $NO_2^- -N$, and $PO_4^{3-} -P$
7 concentration as well as N_2O generation amount in one typical cycle of AO-SBR. It
8 can be seen that, in anoxic stage, the concentration of COD and $NO_3^- -N$ rapidly
9 decreased to around 25.36 mg/L and 0 mg/L, respectively, whereas $PO_4^{3-} -P$
10 concentration slowly increased and reached to 7.69 mg/L at 90 min. Like the case in
11 A_2 -SBR, no $NO_2^- -N$ accumulation was observed during this stage.

12 In aerobic stage, $NH_4^+ -N$ was completely depleted after 60 min. Moreover, the
13 $NO_2^- -N$ concentration rapidly increased to 2.42 mg/L at 120 min and gradually
14 reduced thereafter. The $NO_3^- -N$ concentration increased from the beginning of the
15 aerobic stage, and finally reached up to 16.07 mg N/L. The $PO_4^{3-} -P$ concentration
16 decreased gradually to 1.94 mg/L. The obtained results suggest that denitrification
17 and phosphorus release mainly occurred in anoxic stage, whereas nitrification and
18 phosphorus uptake mainly occurred in aerobic stage.

19 Fig. 3 shows that N_2O generation in anoxic stage was very low, and the
20 maximum N_2O generation amount was only 0.01 mg/L. This result was different from
21 the studied A_2 -SBR where the maximum N_2O generation amount up to 0.32 mg/L.
22 The main explanation is that AO-SBR used propionate as carbon source for

1 denitrification, but A₂-SBR using poly-b-hydroxyalkanoates (PHA) for denitrification.
2 Some researchers also reported that the consumption of PHA for denitrification could
3 increase the N₂O generation [26-28]. In the aerobic period, N₂O generation amount
4 increased rapidly and reached to the highest value of 0.20 mg/L at 150 min.

5 **4. Discussion**

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

21 Table 5 compares the generation amount and conversion rate of N₂O in the
22 studied A₂N-SBR system with AO-SBR and some other A₂-SBR systems in literature.

1 Compared with other systems, total N_2O generation amount in our A_2N -SBR was very
2 low, and it only accounted for 0.69% of the influent TN. This was attributed partly to
3 the low amount of N_2O generation in N-SBR, and partly to the limited amount of N_2O
4 generation in A_2 -SBR.

5 *4.1 Causes of low N_2O generation in N-SBR*

6 It can be seen from Table 5 that the N_2O generation amount during aerobic stage
7 of N-SBR accounted for 0.47% of the influent nitrogen load, which was much lower
8 than those in AO-SBR and other systems. Moreover, N_2O generation in AO-SBR was
9 also found to be lower than that in the study of Jia et al. [30]. This may be attributed
10 to the difference of carbon source. In this study propionate was used as carbon source,
11 whereas Jia et al. [30] using acetate and glucose. It has been reported that the use of
12 propionate could decrease N_2O generation [19, 20]. Zhu and Chen [20] used sludge
13 alkaline fermentation liquid enriched in propionate as carbon source, but the N_2O
14 generation appeared to be much higher than that in our study. The main reason was
15 probably that they used an anaerobic-aerobic process operated at low DO
16 concentration.

17 There are two possible explanations for the low N_2O generation in N-SBR, one is
18 no occurrence of heterotrophic denitrification, and the other is the higher activity of
19 NOB. Firstly, autotrophic nitrification and heterotrophic denitrification can occur
20 simultaneously in aerobic stage of single-sludge system, both of them can contribute
21 to N_2O generation [22, 30]. However, unlike the single-sludge system, autotrophic
22 nitrification was the only source of N_2O generation in N-SBR. The lack of

1 heterotrophic denitrification in N-SBR was clearly evidenced by the batch test results
2 shown in Fig. 4. It was noted that autotrophic nitrification was the main source in
3 N-SBR, accounting for more than 95.4% of total N_2O generation. However, both
4 autotrophic nitrification and heterotrophic denitrification contributed to N_2O
5 generation in the aerobic stage of AO-SBR, and heterotrophic denitrification
6 accounted for about 30.6%. With the use of inhibitors, Tallec et al. [22] reported that
7 heterotrophic denitrification represents from 17% to 42% of total N_2O generation
8 amount during aerobic stage of urban wastewater treatment.

9 The other explanation is that the activity of NOB in N-SBR was much higher
10 than that in A/O-SBR or other single-sludge systems. As previously hypothesized,
11 since there was only nitrification occurring in N-SBR, the SRT and DO concentration
12 could be controlled at higher level favoring the growth of NOB. The higher activity of
13 NOB would decrease NO_2^- accumulation, and would consequently reduce N_2O
14 generation. This hypothesis can be verified by comparing the NOR in N-SBR and
15 A/O-SBR. According to the results of batch test 2, the NOR of N-SBR was $13.36 \pm$
16 1.46 mg NO_2^- /h/g VSS, which was significantly higher than that (i.e. 9.23 ± 0.82 mg
17 NO_2^- /h/g VSS) in AO-SBR. This suggests that the amount of NO_2^- accumulated and
18 N_2O generated in N-SBR would be much less than those in AO-SBR. Fukumoto et al.
19 [31] also found that the N_2O emission was decreased in a laboratory-scale composting
20 experiment with the addition of NOB, and the main reason was supposed to be the
21 added NOB preventing NO_2^- accumulation.

22 *4.2 Causes of low N_2O generation in anoxic stage of A_2 -SBR*

1 Although N_2O generation amount in anoxic stage of A_2 -SBR was much higher
2 than that in AO-SBR (Table 5), it only contributed 7.17% of the total N_2O generation
3 of A_2 -SBR. Moreover, it was noteworthy that the presented A_2 -SBR produced much
4 less N_2O than other A_2 -SBR systems reported in literature. More specifically, the
5 anoxic N_2O generation amount in the presented A_2 -SBR was only 0.05% of the
6 influent nitrogen load, which was much lower than those (2.34%~21.6%) reported in
7 previous A_2 -SBR systems [16, 17].

8 The less anoxic N_2O generation amount in the presented A_2 -SBR may be
9 attributed to the use of propionate as carbon source, which is unlike other studies
10 using acetate as carbon source [16, 17]. The positive effect of propionate on N_2O
11 reduction was partially proved by the results of batch test 3. It was observed from
12 Fig.5 that the use of propionate significantly decreased N_2O generation amount.
13 Furthermore, nearly no NO_2^- -N accumulation occurred in the anoxic stage when using
14 propionate as carbon source. In contrast, NO_2^- -N accumulation during this stage
15 reached the maximum of 6.09 mg/L when using acetate as carbon source. NO_2^- can
16 promote N_2O generation by inhibiting the activity of N_2O reductase [32, 33]. Other
17 A_2 -SBR systems reported in literature were always operated with acetate as the sole
18 carbon source, and the N_2O generation in these systems would be higher due to the
19 high accumulation of NO_2^- .

20 Although Wang et al. [16] investigated N_2O generation in A_2 -SBR using
21 propionate as carbon source, the observed nitrite accumulation and N_2O generation
22 were much higher than our results. One possible reason was that the sludge used in

1 their study was not acclimated by propionate but acetate, and the effect of propionate
2 shock on N_2O generation was investigated in one cycle. On the contrary, however, the
3 sludge in our study was acclimated solely by propionate. The species of DPAOs in the
4 two studies might be different. A detailed and in-depth analysis of the microbial
5 community is desired to better elucidate the underlying mechanisms via which
6 propionate decreases N_2O generation in denitrifying phosphorus removal systems.

7 It therefore appears that the long-term use of propionate as carbon source could
8 not only improve nitrogen and phosphorous removal efficiencies, but also
9 significantly decrease N_2O generation during denitrifying phosphorus removal system.
10 Therefore, propionate or propionate-riched liquid (i.e. fermentation liquid of biowaste)
11 can be recommended as a promising external carbon source for effective control of
12 N_2O generation during biological wastewater treatment processes.

13 **5. Conclusion**

14 In summary, this study suggested that the two-sludge SBR system acclimatized
15 with propionate remarkably reduced N_2O generation and increased the nitrogen and
16 phosphorus removal efficiencies. N_2O generation amount during aerobic stage of
17 N-SBR was lower than that in single-sludge system, because the independent
18 nitrification reactor resulted in higher activity of nitrifying bacteria and no occurrence
19 of heterotrophic denitrification. Compared with the anoxic stage of denitrifying
20 phosphorus removal process reported in literature , N_2O generation in A_2 -SBR was
21 very low, because the use of propionate as carbon source decreased nitrite
22 accumulation.

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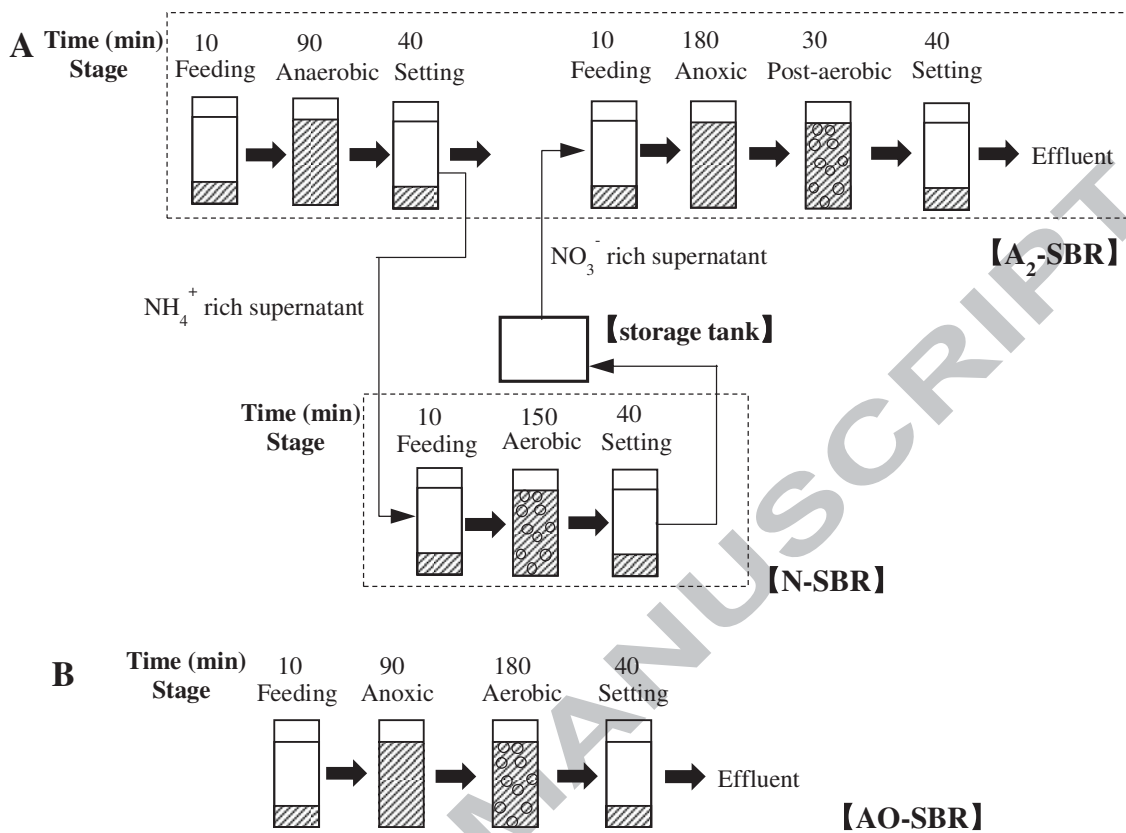
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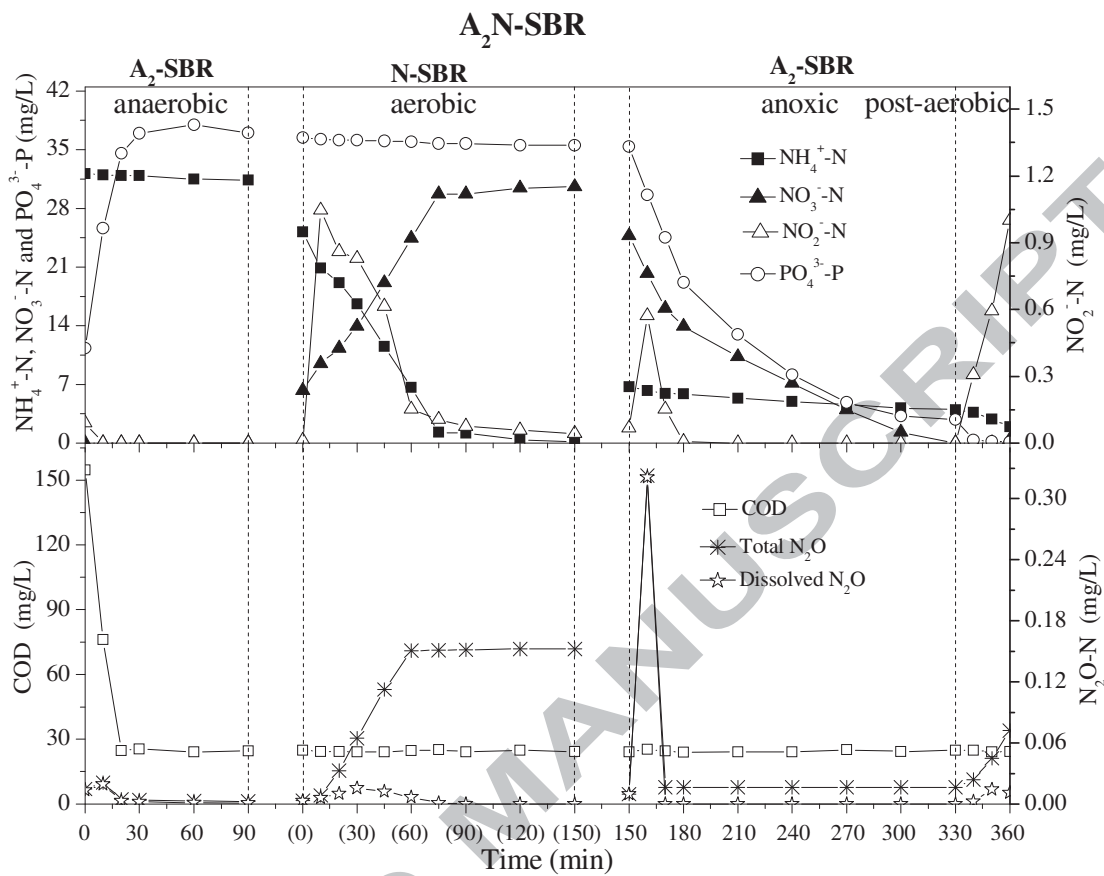
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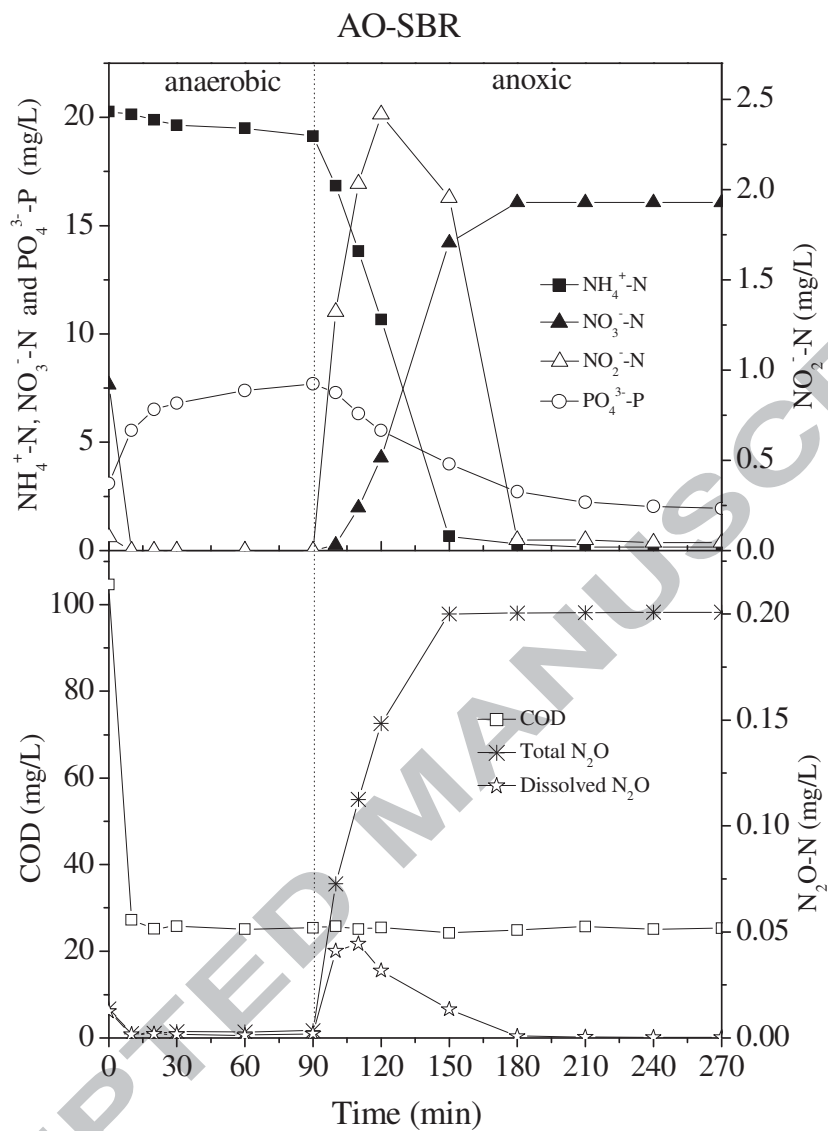
2 **Fig. 1.** Schematic diagrams of the operation of (A) A₂N-SBR system and (B)

3 AO-SBR system.



1

2 **Fig. 2.** Variations of COD, nitrogen, and phosphorus in a typical cycle of A₂N-SBR.

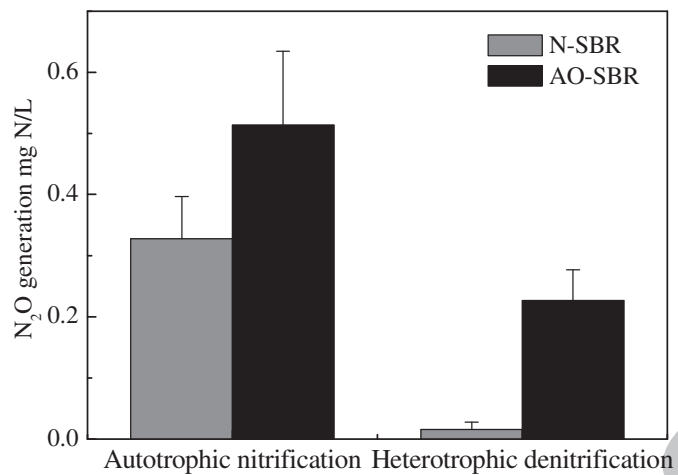


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2 **Fig. 3.** Variations of COD, nitrogen, and phosphorus concentration in a typical cycle

3 of AO-SBR.

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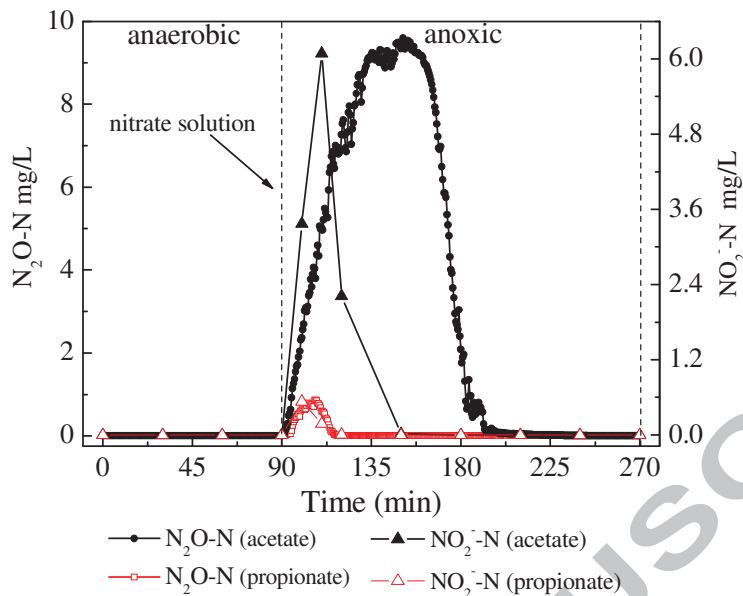


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2 **Fig. 4.** N₂O generation by autotrophic nitrification and heterotrophic denitrification in

3 the N-SBR and AO-SBR.

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1

2 **Fig. 5.** N_2O and NO_2^- generation profiles during two batch experiments with acetate
3 and propionate as carbon source, respectively.

4

5 **Table 1**

6 The operating parameters of different reactors

Parameter	$\text{A}_2\text{N-SBR}$		AO-SBR
	$\text{A}_2\text{-SBR}$	N-SBR	
F/M ratio (g COD/g SS/d)	0.65-0.71	0.07-0.08	0.63-0.69
Nitrogen loading rate (g N/g SS/d)	0.13-0.14	0.11-0.12	0.12-0.13
Temperature ($^{\circ}\text{C}$)	$25 \pm 1^{\circ}\text{C}$	$25 \pm 1^{\circ}\text{C}$	$25 \pm 1^{\circ}\text{C}$
SRT (d)	25	50	20
MLSS (g/L)	3.4-3.7	3.1-3.3	2.9-3.2

7

1 **Table 2**

2 Composition of the synthetic wastewater

Components	Concentration (mg/L)	Components	Concentration (mg/L)
CH ₃ CH ₂ COONa	172	H ₃ BO ₃	0.15
NH ₄ Cl	153	CuSO ₄ ·5H ₂ O	0.03
NaHCO ₃	200	KI	0.18
KH ₂ PO ₄	11	MnCl ₂ ·4H ₂ O	0.12
K ₂ HPO ₄ ·3H ₂ O	18	Na ₂ MoO ₄ ·2H ₂ O	0.06
MgSO ₄ ·7H ₂ O	10	ZnSO ₄ ·7H ₂ O	0.12
FeSO ₄ ·7H ₂ O	10	CoCl ₂ ·6H ₂ O	0.15
CaCl ₂ ·2H ₂ O	10	EDTA	10

1 **Table 3**2 Removal performance of two systems for COD, $\text{NH}_4^+\text{-N}$, TN and $\text{PO}_4^{3-}\text{-P}^a$

Parameter	Influent concentration (mg/L)	A ₂ N-SBR		AO-SBR	
		Effluent concentration (mg/L)	Removal Efficiency (%)	Effluent concentration (mg/L)	Removal Efficiency (%)
COD	194.4±20.12	25.08±9.36	87.13±5.73	24.71±12.64	87.28±7.12
$\text{NH}_4^+\text{-N}$	40.78±2.03	1.86±0.95	95.47±2.17	0.28±0.35	99.31±0.82
TN	40.78±2.03	3.16±1.15	92.28±2.69	16.27 ±1.31	60.02±3.65
$\text{PO}_4^{3-}\text{-P}$	5.05±0.28	0.39±0.20	92.04±4.35	1.64±0.21	68.52±4.25

3 ^a Sample mean ± standard deviation, number of measurements: n = 25.

1 **Table 4**

2 Comparison of nitrogen and phosphorous removal efficiencies of A₂N-SBR and
 3 single-sludge SBR

Parameter	A ₂ N-SBR (this study)	Single-sludge SBR (Ji and Chen, 2009)
Influent COD (mg/L)	200	265
Influent TN (mg/L)	40	38
Influent PO ₄ ³⁻ -P (mg/L)	5	15
Effluent TN (mg/L)	3.16	0.49
Effluent PO ₄ ³⁻ -P (mg/L)	0.39	0.36
TN removal efficiency (%)	92.0	98.7
PO ₄ ³⁻ -P removal efficiency (%)	92.3	97.6

4

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1 **Table 5**2 N_2O generation amount per cycle in different reactors.

Reactor	Carbon source for sludge acclimatization	Stage	N_2O generation amount ^a (%)	N_2
A ₂ N-SBR	propionate	anaerobic stage in A ₂ -SBR	0.0076	
		anoxic stage in A ₂ -SBR	0.050	
		post-aerobic stage in A ₂ -SBR	0.17	
		aerobic stage in N-SBR	0.47	
		total generation	0.69	
AO-SBR	propionate	anoxic stage	0.017	
		aerobic stage	1.00	
		total generation	1.01	
A ₂ -SBR	acetate	anoxic stage	2.34-21.63	
AO (low DO)-SBR	acetate and glucose	aerobic stage	7.05	
AO-SBR			2.12	
AO (low DO)-SBR	sludge alkaline fermentation liquid	aerobic stage	10.46	
	acetate		28.21	

3 ^a N_2O generation amount = (total N_2O -N generation)/(influent TN) *100%.4 ^b N_2O conversion rate = (total N_2O -N generation)/(TN removed) *100%.5 **Highlights**

- 6 • Compared with AO-SBR, A₂N-SBR reduced N_2O generation by around
7 31.5%.
- 8 • Only 0.054% of the removed nitrogen in anoxic stage of A₂N-SBR was
9 converted to N_2O .
- 10 • Autotrophic nitrification contributed to 95.4% of total N_2O generation in
11 N-SBR.
- 12 • The separate nitrification reactor reduced N_2O generation in aerobic stage.
- 13 • The use of propionate as carbon source reduced N_2O generation in anoxic
14 stage.

15