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A novel integrated thiosulfate-driven denitritation (TDD) and anaerobic ammonia oxidation (anammox) process for biological nitrogen removal

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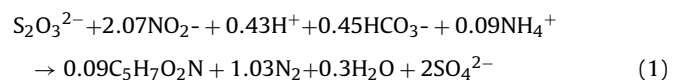
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ABSTRACT

An integrated system by combining the thiosulfate-driven denitritation (TDD) and anaerobic ammonia oxidation (anammox) was established for high-rate biological nitrogen removal (BNR) and less N$_2$O generation under 35 °C. The anammox contribute to 27% nitrogen removal in the system. The anammox activity was enhanced by four times as temperature increased from 20 to 35 °C. The less N$_2$O emission at 35 °C compared with that at 20 °C is attributed to the involvement of anammox (characterized as no N$_2$O generation) function rather than higher N$_2$O reducing rate under the thermophilic condition. The produced NO$_3^-$ in anammox can be readily reduced by thiosulfate. The results of this study could enlighten us how to induce a high nitrogen degradation activity with lower sludge yield, in the meanwhile to minimize the greenhouse gas emission and the nitrogenous residues in the BNR process.

1. Introduction

Various biological nitrogen removal (BNR) processes have been developed for wastewater reclamation [1–4]. However, the conventional heterotrophic denitrification technology in BNR was characterized as high sludge yield and greenhouse gas (i.e. N$_2$O) emissions [5–7]. It had been reported that the reduced sulfur-based denitrification could play a critical role in BNR with the benefits of minimized biomass production, no need of organic carbon [8]. Among the three commonly used sulfur species, i.e. sulfide (HS$^-$), elemental sulfur (S$^\circ$) and thiosulfate (S$_2$O$_3^{2-}$), S$_2$O$_3^{2-}$ has been proven to be the most favorable electron donor for autotrophic denitrification (AD) due to its high bioavailability and non-toxicity to denitrifying microorganisms [9]. The specific denitrification activity on thiosulfate was found to be 4.6 and 9.5 times of those on sulfide and elemental sulfur, respectively in batch cultures

[9], while much higher nitrogen removal rate could be achieved with thiosulfate (e.g. 0.24 kg N/day/m^3) than that with sulfide (e.g. 0.11 kg N/day/m^3) [10]. In addition, thiosulfate could accelerate the denitrifying sludge granulation which in turn improved the biomass settleability [11]. These all indicate that the thiosulfate-driven denitrification/denitritation (Eq. (1)) is a promising process for high-rate BNR. However, it should be noted that the potential emission of N$_2$O from this AD process has been poorly understood so far, which would be a major concerns for the practical application of AD.

$$S_2O_3{}^{2-} + 2.07NO_2- + 0.43H^+ + 0.45HCO_3- + 0.09NH_4{}^+$$
$$\rightarrow 0.09C_5H_7O_2N + 1.03N_2 + 0.3H_2O + 2SO_4{}^{2-} \quad (1)$$

Anaerobic ammonia oxidation (anammox) plays an essential part in biological nitrogen cycle and is also involved in BNR (Eq. (2)) [12–16]. The nitrate production in anammox (as shown in Eq. (2)) may deteriorate the BNR performance and could not always meet the current stringent discharge limit, such as the first class (level A) requirement of the National Municipal Wastewater Discharge Standards of China (i.e. total nitrogen, i.e. TN < 15 mg/L), though N$_2$O
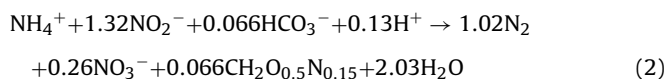
Table 1
Main conditions and performances of the TDD-UASB reactor's operation.

	Stages			
	I (Day 1–31)	II (Day 32–62)	III (Day 63–96)	IV (Day 97–158)
HRT (hrs)	4	4	4	4
Reactor pH	7.0	7.0	8.5	8.5
Influent $S_2O_3^{2-}$ conc. (mg S/L)	150	150	150	150
Influent NO_2^- conc. (mg N/L)	25	50	50	50
Influent NH_4^+ conc. (mg N/L)	48	48	48	48
Temperature (°C)	20	20	20	35
Influent FNA conc. (mg N/L)	0.0064	0.0128	0.0004	0.0003
Average NO_2^- removal efficiency (%)	83.6	86.3	89.1	94.1
Average NO_2^- removal rate (kg N/d/m^3)	0.13	0.25	0.27	0.28
N_2O emission (% of NO_2^-–N load)	0.84	0.80	0.78	0.53
Average $\triangle S_2O_3^{2-}$-S/$\triangle NO_2$–N	2.27	2.15	2.10	1.71
Average effluent NO_3^- conc. (mg N/L)	Undetectable			

generation could be mitigated. It has been well known that anammox bacteria are unable to compete with heterotrophic denitrifying bacteria in the presence of sufficient COD. However, it should be realized that almost no information is available on the possible competition between autotrophic dentrification and anammox in the literature. Therefore, this study attempted to develop a novel integrated thiosulfate-driven denitrition (TDD) and anammox process for high-efficient and cost-effective nitrogen removal from wastewater, with the aims to reduce N_2O emission and sludge minimization. And the generated NO_3^- in anammox could be readily degraded with thiosulfate as the electron donor, thus improving the effluent quality of BNR in terms of TN level [17].

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2$$
$$+ 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \qquad (2)$$

Herein, we mainly aim to establish a combined system for the high BNR capacity and low N_2O emissions with the coordination of thiosulfate-driven denitrition (TDD) and anammox. The factors affecting the successful establishment of this combined system were explored.

2. Materials and methods

2.1. Experimental setup and operation

A lab-scale UASB reactor with a working volume of 1.2 L was used in this study. Detailed configuration can be found in Fig. S1. The anoxic sludge was taken from a sequential batch reactor (SBR) fed with a synthetic wastewater containing sodium thiosulfate, sodium nitrite and ammonia chloride for more than 8 months. After cultivation, the sludge was enriched with *Thiobacillus*-like autotrophic denitrifiers and anammox-related bacteria (data not shown here). 500 mL of the concentrated mixed liquor taken the SBR was seeded into the UASB reactor, resulted in the initial biomass concentration of about 2800 mg/L.

During 158-day continuous operation of the UASB reactor, the hydraulic retention time (HRT) was set at 4 h and an internal recirculation flow of 4Q (Q is influent flow) was practiced to provide adequate mixing in the UASB reactor. The influent contained 150 mg S/L of $Na_2S_2O_3$. The detail composition of the influent fed to the UASB reactor can be found elsewhere [11]. In addition, 48 mg N/L of ammonium was also supplied with the aim to promote anammox in the UASB reactor. The whole operation of the UASB reac-

tor was divided into four stages and the corresponding operation conditions were all summarized in Table 1.

During the UASB reactor's operation, both the influent and effluent samples were withdrawn frequently, followed by the immediate filtration through the disposable Millipore filters (0.22 mm pore size) before water quality analysis. Nitrate, nitrite, ammonia, thiosulfate and sulfate were subsequently measured within four hours. The gas samples collected from the steady-stated UASB reactor were analyzed for N_2O quantification.

2.2. Batch tests

Two sets of batch tests were performed in the 500 mL-flasks to further evaluate the N_2O reducing rates and anammox activities under different temperatures. The sludge was taken from UASB reactor to perform the following tests. For each batch test, the sludge was washed, using the synthetic wastewater for three times to remove the background materials. Analytical-graded helium gas was purged into each batch reactor before the assay for half an hour to exclude residual oxygen. The sludge in each batch reactor was acclimated to each required temperature (20 or 35 °C) for at least four hours before the assay to avoid the temperature shock to the microorganisms. The biomass concentrations in each reactor were 450 ± 20 mg MLVSS/L. Afterwards, all the flasks were sealed tightly with butyl rubber stoppers and aluminum crimp seals. The reactors were well mixed by the magnetic stirrers at 150 rpm. The reported results of the batch tests were the average values in triplicate.

2.2.1. Batch Test I: N_2O reduction under different ambient temperatures

Batch Reactors 1 (under 20 °C) and 2 (under 35 °C) were performed with nitrous oxide as the direct electron acceptor. pH in the two batch reactors was maintained at 8.5 by Na_2HPO_4-NaH_2PO_4 buffer solution. The fresh stock nitrous oxide solution was prepared each time by sparging 99.9% N_2O gas into oxygen-free Milli-Q water for more than 1 h prior to the test. And N_2O concentration in the saturated stock solution is about 700 mg N_2O-N/L. Approximately 10.7 mL as-prepared stock solution was spiked into each 500 mL-flask, resulted in the same initial N_2O of 15 mg N_2O-N/L. $Na_2S_2O_3$ at initial concentration of 50 mg S/L was used as the electron donor, ensuring the N_2O could be fully reduced to N_2. The batch reactors was well mixed by using a heating magnetic stirrer (JOANLAB HS-17) at 150 rpm under the desired temperature (20 °C in Batch Reactor 1 and 35 °C in Batch Reactor 2). The test lasts for 3 h.

2.2.2. Batch Test II: Anammox activities under different ambient temperatures

Two batch reactors (Batch Reactors 3 and 4) were set up in Batch Test II. The fresh nitrite stock solution (1.5 g NO_2^--N/L) was prepared by dissolving 7.393 g analytic grade sodium nitrite into 1 L ultrapure waster, followed by pipetting 20 mL stock solution in 500 mL-flasks of the two batch reactors, obtaining the initial NO_2^- concentration of 60 mg N/L in both reactors. Besides NO_2^-, ammonia was another nitrogen source, originated from the nutrient stock solution, feeding the biomass in both reactors. The initial NO_2^- and NH_4^+ concentrations were 60 and 48 mg N/L, respectively (see Table 2). pH in both reactors was controlled at 8.5 by Na_2HPO_4-NaH_2PO_4 buffer solution. The temperature was controlled at 20 °C in Batch Reactor 3 and 35 °C in Batch Reactor 4. The test lasts for 36 h.

2.3. Sampling and chemical/physical analysis

Mixed liquor samples from the abovementioned batch reactors were taken at a specific time interval using a 10-mL syringe and these were immediately filtered through disposable Millipore filters (0.22 um pore size). Nitrate, nitrite, sulfate and thiosulfate were detected with an ion chromatograph (DIONEX-900). Ammonia (NH_4^+-N + NH_3-N) was determined according to the colorimetric method of Standard Method [18]. MLSS and MLVSS were measured according to the Standard Method [18]. Total organic carbon (TOC) and total nitrogen (TN) were determined by a TOC analyzer (Shimadzu 5000A). The gaseous N_2O was detected by a gas chromatograph (PE Clarus600) with an electrical conductivity detector). Dissolved N_2O in the Batch Reactors 1 and 2 in Batch Test I was continuously monitored using a calibrated real-time inline N_2O micro-sensor probe (N_2O-100, Unisense A/S, Aarhus, Denmark).

Biomass-specific N_2O reducing activities in Batch Test I and biomass-specific NO_2^- reducing activities in Batch Test II were derived from the linear regression of N_2O or NO_2^- concentrations versus time (day) and normalized by the biomass concentration (g MLVSS/L). The units of the presented results were mg N_2O/g MLVSS/h and mg NO_2^--N/g MLVSS/h.

3. Results and discussion

3.1. Establishment of TDD for BNR

The nitrogen removal performance was illustrated in Fig. 1a. After acclimation, the nitrite removal performance could achieve as high as above 93% with the influent nitrogen loading rate of 0.3 kg N/day/m³. The influent and effluent thiosulfate concentrations were presented in Fig. 1b. Corresponding to the nitrite degradation, thiosulfate was oxidized by the denitrifying biomass in the UASB reactor. The average ratios of thiosulfate consumption to nitrite degradation (i.e. $\triangle S_2O_3^{2-}$-S/$\triangle NO_2^-$-N) were between 2.10 and 2.27 in the first three stages (see Table 1). These values are very closed to the theoretical $\triangle S_2O_3^{2-}$-S/$\triangle NO_2^-$-N mass ratio of 2.2 according to Eq. (1), showing that nitrite reduction in the UASB reactor was only coupled with thiosulfate oxidation at 20 °C. Thus the nitrite removal in the first stages was almost via the TDD pathway. But this ratio dropped to 1.71 in Stage IV when temperature raised to 35 °C. Actually, this ratio could be even lower. The thiosulfate could also be chemically or biologically oxidized when the dissolved oxygen was involved in the UASB reactor through pumping the influent. So nitrite must be degraded via other pathways than TDD.

3.2. Establishment of anammox for BNR

The concentration profile of ammonia was shown in Fig. 1c. In the UASB reactor studied, it is reasonable to consider that ammo-
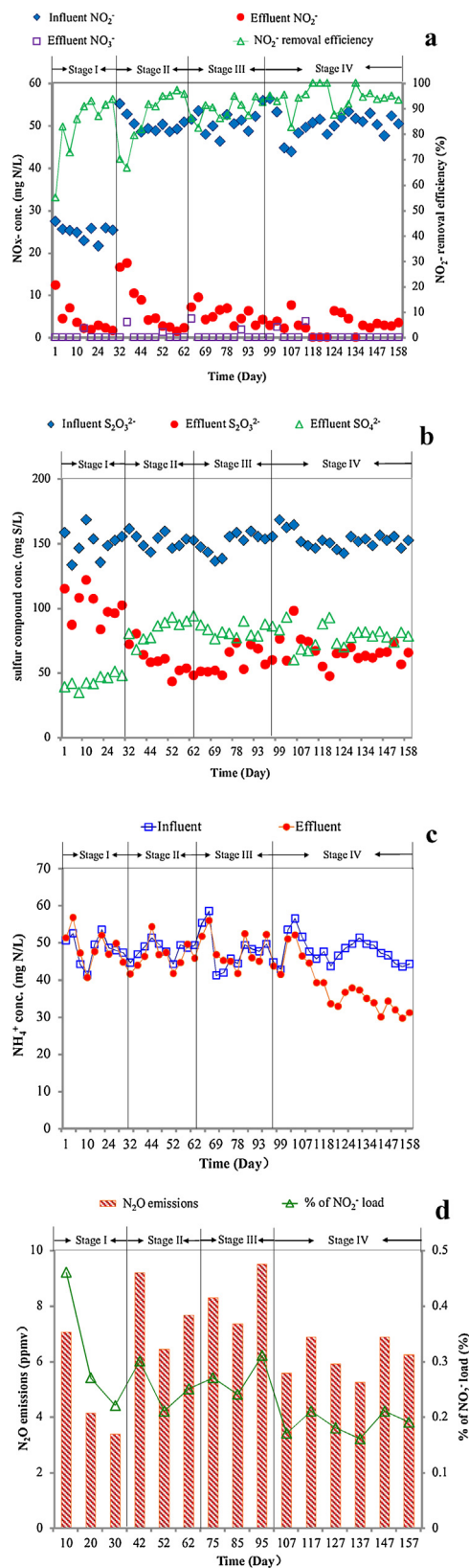

Fig. 1. Profile of NO_2^- removal (a), $S_2O_3^{2-}$ oxidation (b), NH_4^+ consumption (c), N_2O emissions (d) during the entire period of TDD-UASB reactor's operation.

Table 2
Conditions and results of the Batch Tests I and II.

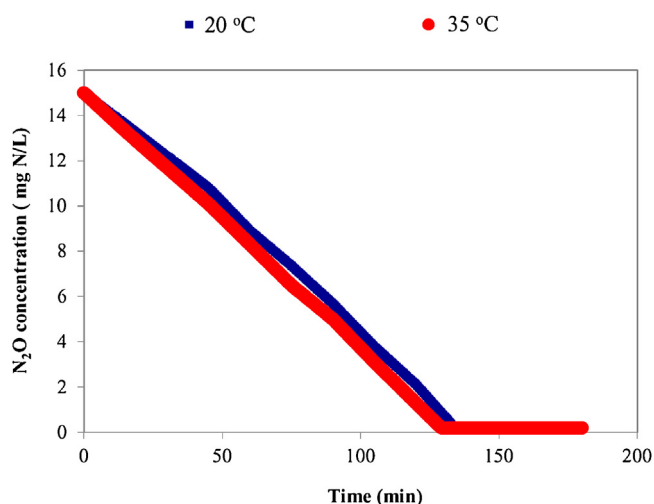|	Batch Test I		Batch Test II	
	Batch Reactor 1	Batch Reactor 2	Batch Reactor 3	Batch Reactor 4
pH	8.5	8.5	8.5	8.5
Temperature (°C)	20	35	20	35
Electron donor	$S_2O_3^{2-}$	$S_2O_3^{2-}$	NH_4^+	NH_4^+
Electron acceptor	N_2O	N_2O	N_2O	N_2O
Conc. of electron donor	50 mg $S_2O_3^{2-}$-S/L		48 mg NH_4^+-N/L	
Conc. of electron acceptor	15 mg N_2O-N/L		60 mg NO_2^--N/L	
Biomass-specific N_2O reducing rate (mg N/g MLVSS/h)	15.3	14.6	NA[*]	NA
Biomass-specific NO_2^- reducing rate (mg N/g MLVSS/h)	NA	NA	5.61	1.32
Biomass-specific NH_4^+ oxidation rate (mg N/g MLVSS/h)	NA	NA	3.98	1.06

[*] NA means not available.

Fig. 2. Results of Batch Test I for N_2O reduction with thiosulfate as the electron donor at 20 °C (a) and 35 °C (b).

nia in the influent may be utilized by (i) microbial growth in TDD as shown in Eq. (1) and anammox reactions illustrated in Eq. (2). The ammonia removal was not observed in Stages I to III when the reactor temperature was controlled at 20 °C, while nitrite was mainly removed via TDD, evidenced by the determined ratio of $\triangle S_2O_3^{2-}$-S/$\triangle NO_2$–N close to the stoichiometric value. These suggested that the anammox activity was not yet established in the reactor operated at 20 °C. However, ammonia started to be utilized when the reactor temperature was increased from 20 °C to 35 °C (Table 1), i.e. the anammox activity was gradually established in the reactor. According to the results In Fig. 1c, it was estimated that about 27% nitrite was removed via anammox, and the remaining by TDD. Consequently, it appears that temperature played a decisive role towards the successful establishment of the integrated TDD & Anammox system.

To further verify the temperature effect on anammox activity, the batch experiments with the sludge taken from the UASB reactor were conducted without the presence of thiosulfate at 20 and 35 °C, respectively (Fig. 2). It was found that the utilization ratio of ammonia over nitrite was in good agreement with the stiochiometric ratio for anammox reaction (Eq. 2). Meanwhile, the anammox activity at 35 °C was found to be 4-fold higher than that at 20 °C. These clearly confirmed that high temperature favored the establishment of anammox activity in the integrated TD-anammox system. In fact, the optimum temperature for anammox had been reported to be in the range of 30 and 40 °C [19,20], while the anammox performance could be deteriorated at the temperature below 25 °C [21]. On the

contrary, the denitrification on thiosulfate was stabilized at the temperature 20 °C and above [22]. In this study, it was shown that autotrophic denitrification with thiosulfate remained comparable at 20 °C and 35 °C (Fig. 1).

3.3. N_2O emission/accumulation and reduction

The N_2O generation in the tightly-sealed UASB reactor was recorded. Fig. 1d shows N_2O emissions at the steady-state of each stage. The N_2O emissions (% of NO_2^- load) were comparable among the first three stages (without anammox involvement) under different influent NO_2^- concentrations and pH. In the final stage when the temperature raised from 20 to 35 °C, the N_2O generation decreased by 29.6%, i.e. the N_2O emission is 0.19% of NO_2^- load. Therefore, with the nitrogen removal rate not changed, the reduction of N_2O generation was confirmed in the integrated system of TDD with anammox.

Fig. 3 displays the N_2O reduction with thiosulfate as the electron donor under 20 (Batch Reactor 1) and 35 °C (Batch Reactor 2) respectively. Directly using the N_2O as the electron acceptor, the N_2O was completely reduced by thiosulfate within 140 min in both reactors (see Fig. 3). The biomass-specific nitrogen removal activities were similar under each temperature (15.3 and 14.6 mg N_2O-N/g MLVSS/h, see Table 2). Accordingly, the temperature rarely affected the N_2O reducing rate under the TDD condition. Under the similar ambient temperature and with the N_2O as the sole electron acceptor, the autotrophic thiosulfate-driven N_2O reducing activity (at 35 °C) is similar to the autotrophic sulfide-driven N_2O reducing activity (30 °C) [23]. Both of them are higher compared with the heterotrophic N_2O reducing activity of 11.3 mg N/g MLVSS/h (35 °C) [24]. Theoretically, the denitrifying-related microorganisms could assimilate organics as the organic carbon source to favor the bacterial anabolism and induce a high denitrification activity [25]. The unexpected higher autotrophic N_2O reducing activities in AD could be attributed to the different inoculum used in each study. The well-cultivated granular sludge was seeded in the batch reactor in the two autotrophic tests (Yang et al. [23] and this study), but the raw sludge withdrawn from bioreactor of the MLE process after simple washing was used in the heterotrophic N_2O reducing test in Poh et al. [24].

3.4. Implication of this study

Thiosulfate-based denitrification has been demonstrated to be a holistic approach for BNR in municipal sewage treatment [10,26]. It is characterized as the high nitrogen degradation activity which is comparable with HD but with much less sludge production [11]. Integrating the short-cut bioreactions and
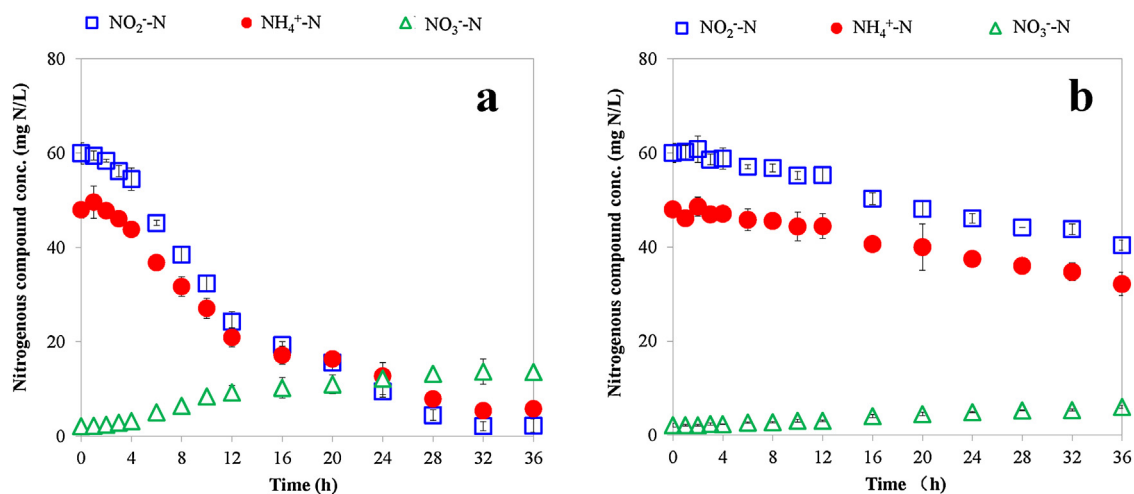
Fig. 3. Results of Batch Test II for anammox activities at 35 °C (a) and 20 °C (b).

thiosulfate-driven denitrification, BNR over nitrite driven by thiosulfate (i.e. Nitritation-TDD) was developed [27], as a result of total nitrogen removal capacity of 0.43 kg N/d/m^3. In this study, a combined system by integrating the TDD with ananmox was developed at 35 °C under the nitrite-limiting condition with a high BNR capacity, to further lessen the N$_2$O emissions. Pertaining to the anammox, one of the drawbacks of its application is the excessive NO$_3^-$ production (see the results of Batch Test III in Figs. 3a and b), i.e. 11% total nitrogen is converted to nitrate, hindering to meet the stringent discharge standard with regards to TN [28], such as the first class (level A) requirement of the National Municipal Wastewater Discharge Standards of China i.e. TN < 15 mg/L (GB18918-2002). Nevertheless, nitrate was almost undetectable all the time in our UASB reactor's effluent, owning to the high NO$_3^-$ reducing rate with thiosulfate as the electron donor [9]. Hence the combined process coordinated by TDD and anammox could ensure low GHG emission and high effluent quality simultaneously, in addition to the advanced nitrogen biodegradation activity and low sludge yield. Taking the high global warming potential of N$_2$O (310 times of CO$_2$ IPCC [29]) into consideration, the N$_2$O reduction by 29.6% in the integrated system (see Section 3.2) could mitigate the global warming issue to a large extent. And the involvement of anammox may also reduce electron donor consumption. For example, sodium thiosulfate could be saved by 20% under 35 °C with the nitrite removal efficiency of above 94% (see Section 3.1). And the sulfate levels in the BNR effluent could be minimized in this integrated process (with the involvement of anammox for BNR) to decrease the potential secondary contamination caused by sulfate as much as possible, as shown in Fig. 1b that the effluent sulfate concentrations were always below 80 mg S/L. These advantages could be highlighted to an even huger extent in the future larger-scale application in practice.

4. Conclusions

The combined system of thiosulfate-driven denitritation coupled with anammox was established under the temperature of 35 °C, in which 27% nitrogen was removed via anammox. Temperature plays a decisive role in the successful establishment of the system, as the anammox activities could be enhanced by four times when temperature raised from 20 to 35 °C. N$_2$O emissions were reduced by 29.6% in the integrated system compared with TDD alone. The NO$_3^-$ produced from anammox could be readily reduced by thiosulfate in the combined system. The co-concurrence

of anammox and TDD ensures the less GHG emission and high BNR effluent quality.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi: https://doi.org/10.1016/j.bej.2018.07.025.

References

[1] G. Tchobanoglous, F. Burton, H.D. Stensel, Wastewater Engineering: Treatment and Reuse, McGraw-Hill Inc, 2003.
[2] Q. Wang, H. Duan, W. Wei, B. Ni, A. Laloo, Z. Yuan, Achieving stable mainstream nitrogen removal via the nitrite pathway by sludge treatment using free ammonia, Environ. Sci. Technol. 51 (2017) 9800–9807.
[3] G. Yang, D. Wang, Q. Yang, J. Zhao, Y. Liu, Q. Wang, G. Zeng, X. Li, H. Li, Effect of acetate to glycerol ratio on enhanced biological phosphorus removal, Chemosphere 196 (2018) 78–86.
[4] D. Wang, Q. Fu, Q. Xu, Y. Liu, H.H. Ngo, Q. Yang, G. Zeng, X. Li, B.J. Ni, Free nitrous acid-based nitrifying sludge treatment in a two-sludge system enhances nutrient removal from low-carbon wastewater, Bioresour. Technol. 244 (2017) 920–928.
[5] D. Wang, Q. Wang, A.E. Laloo, Z. Yuan, Reducing N$_2$O emission from a domestic-strength nitrifying culture by free nitrous acid-based sludge treatment, Environ. Sci. Technol. 50 (2016) 7425–7433.
[6] W. Wei, X. Zhou, D. Wang, J. Sun, Q. Wang, Free ammonia pre-treatment of secondary sludge significantly increases anaerobic methane production, Water Res. 118 (2017) 12–19.
[7] W. Wei, X. Zhou, G. Xie, H. Duan, Q. Wang, A novel free ammonia based pretreatment technology to enhance anaerobic methane production from primary sludge, Biotechnol. Bioeng. 114 (2017) 2245–2252.
[8] M.F. Shao, T. Zhang, H.H.P. Fang, Sulfur-driven autotrophic denitrification: diversity, biochemistry, and engineering applications, Appl. Microbiol. Biotechnol. 88 (2010) 1027–1042.
[9] R.B. Cardoso, R. Sierra-Alvarez, P. Rowlette, E.R. Flores, J. Gomez, J.A. Field, Sulfide oxidation under chemolithoautotrophic denitrifying conditions, Biotechnol. Bioeng. 95 (2006) 1148–1157.

[10] I. Manconi, A. Carucci, P. Lens, Combined removal of sulfur compounds and nitrate by autotrophic denitrification in bioaugmented activated sludge system, Biotechnol. Bioeng. 98 (2007) 551–560.

[11] J. Qian, L. Wei, Y. Wu, Q. Wang, X. Fu, X. Zhang, X. Chang, L. Wang, X. Pei, A comparative study on denitrifying sludge granulation with different electron donors: sulfide, thiosulfate and organics, Chemosphere 186 (2017) 322–330.

[12] Z.Z. Zhang, Y.F. Cheng, L.Z.J. Xu, Y.H. Bai, R.C. Jin, Anammox granules show strong resistance to engineered silver nanoparticles during long-term exposure, Bioresour. Technol. 259 (2018) 10–17.

[13] Q.Q. Zhang, G.F. Yang, K.K. Sun, G.M. Tian, R.C. Jin, Insights into the effects of bio-augmentation on the granule-based anammox process under continuous oxytetracycline stress: performance and microflora structure, Chem. Eng. J. 348 (2018) 503–513.

[14] Z.J. Shi, Q. Guo, Y.Q. Xu, D. Wu, S.M. Liao, F.Y. Zhang, Z.Z. Zhang, R.C. Jin, Mass transfer characteristics, rheological behavior and fractal dimension of anammox granules: the roles of upflow velocity and temperature, Bioresour. Technol. 244 (2017) 117–124.

[15] H. Chen, H.Y. Hu, Q.Q. Chen, M.L. Shi, R.C. Jin, Successful start-up of the anammox process: influence of the seeding strategy on performance and granule properties, Bioresour. Technol. 211 (2016) 594–602.

[16] J. Qian, M. Zhang, Y. Wu, J. Niu, X. Chang, H. Yao, S. Hu, X. Pei, A feasibility study on biological nitrogen removal (BNR) via integrated thiosulfate-driven denitratation with anammox, Chemosphere 208 (2018) 793–799.

[17] J. Qian, R. Liu, L. Wei, H. Lu, G. Chen, System evaluation and microbial analysis of a sulfur cycle-based wastewater treatment process for Co-treatment of simple wet flue gas desulfurization wastes with freshwater sewage, Water Res. 80 (2015) 189–199.

[18] APHA, Standard Methods for the Examination of Water and Wastewater, 20th ed., United Book Press, USA, 2005.

[19] M. Strous, J.G. Kuenen, M.S.M. Jetten, Key physiology of anaerobic ammonium oxidation, Appl. Environ. Microbiol. 65 (1999) 3248–3250.

[20] K. Isaka, Y. Date, Y. Kimura, T. Sumino, S. Tsuneda, Nitrogen removal performance using anaerobic ammonium oxidation at low temperatures, FEMS Microbiol. Lett. 282 (2008) 32–38.

[21] B.S. Xing, Q. Guo, G.F. Yang, J. Zhang, T.Y. Qin, P. Li, W.M. Ni, R.C. Jin, The influences of temperature, salt and calcium concentration on the performance of anaerobic ammonium oxidation (anammox) process, Chem. Eng. J. 265 (2015) 58–66.

[22] C. Trouve, P. Chazal, B. Gueroux, N. Sauvaitre, Denitrification by new strains of Thiobacillus denitrificans under non-standard physicochemical conditions. Effect of temperature, pH, and sulphur source, Environ. Technol. 19 (1998) 601–610.

[23] W. Yang, Q. Zhao, H. Lu, Z. Ding, L. Meng, G.H. Chen, Sulfide-driven autotrophic denitrification significantly reduces N_2O emissions, Water Res. 90 (2016) 176–184.

[24] L.S. Poh, X. Jiang, Z. Zhang, Y. Liu, W.J. Ng, Y. Zhou, N_2O accumulation from denitrification under different temperatures, Appl. Microbiol. Biotechnol. 99 (2015) 9215–9226.

[25] S. Otte, J.G. Kuenen, L.P. Nielsen, H.W. Paerl, J. Zopfi, H.N. Schulz, A. Teske, B. Strotmann, V.A. Gallardo, B.B. Jorgensen, Nitrogen, carbon, and sulfur metabolism in natural Thioploca samples, Appl. Environ. Microbiol. 65 (1999) 3148–3157.

[26] R. Yamamoto-Ikemoto, T. Komori, M. Nomura, Y. Ide, T. Matsukami, Nitrogen removal from hydroponic culture wastewater by autotrophic denitrification using thiosulfate, Water Sci. Technol. 42 (2000) 369–376.

[27] J. Qian, J. Zhou, Z. Zhang, R. Liu, Q. Wang, Biological nitrogen removal through nitritation coupled with thiosulfate-driven denitritation, Sci. Rep. 6 (2016) 27502.

[28] R. Du, Y. Peng, S. Cao, C. Wu, D. Weng, S. Wang, J. He, Advanced nitrogen removal with simultaneous Anammox and denitrification in sequencing batch reactor, Bioresour. Technol. 162 (2014) 316–322.

[29] IPCC, Climate Change 2007: The Scientific Basis, Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, New York, NY, USA, 2007.