Effect of nitrite and nitrate on sulfate reducing ammonium oxidation

Dandan Zhang, Li Cui, Rayan M. A. Madani, Hui Wang, Hao Zhu and Jiyan Liang

ABSTRACT

The effects of nitrite and nitrate on the integration of ammonium oxidization and sulfate reduction were investigated in a self-designed reactor with an effective volume of 5 L. An experimental study indicated that the ammonium oxidization and sulfate reduction efficiencies were increased in the presence of nitrite and nitrate. Studies showed that a decreasing proportion of N/S in the presence of NO_2^- at 30 mg·L^{-1} would lead to high removal efficiencies of NH_4^+-N and SO_4^{2-}-S of up to 78.13% and 46.72%, respectively. On the other hand, NO_3^- was produced at approximately 26.89 mg·L^{-1}. *Proteobacteria*, *Chloroflexi*, *Bacteroidetes*, *Chlorobi*, *Acidobacteria*, *Planctomycetes* and *Nitrospirae* were detected in the anaerobic cycle growth reactor. *Proteobacteria* was identified as the dominant functional bacteria removing nitrogen in the reactor. The nitritation reaction could promote the sulfate-reducing ammonium oxidation (SRAO) process. NH_4^+ was converted to NO_2 and other intermediates, for which the electron acceptor was SO_4^{2-}. These results showed that nitrogen was converted by the nitrification process, the denitrification process, and the traditional anammox process simultaneously with the SRAO process. The sulfur-based autotrophic denitration and denitrification in the reactor were caused by the influent nitrite and nitrate.

Key words | anammox process, denitrification process, nitrification process, sulfate-reducing ammonium oxidation

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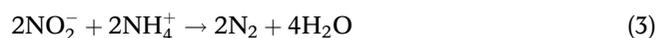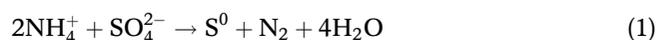**Hui Wang**
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INTRODUCTION

Industrial and domestic sewage with ammonium and sulfate are of major environmental concern (Zhao *et al.* 2006). High levels of ammonium lead to eutrophication of surface waters, while sulfate can leach into secondary contaminations because sulfide reduction under anaerobic conditions is hazardous to aquatic plants and affects environmental quality (Hao *et al.* 2014).

Sulfate reducing ammonium oxidation (SRAO) technology that achieves the simultaneous removal of ammonium and sulfate in a single bioreactor was reported by Fdz-Polanco *et al.* (2001a). The ammonium and sulfate were converted to elemental sulfur and dinitrogen gas without secondary pollution. The SRAO technology is a cost-effective, environmentally friendly process and has become the focus of recent research (Liu *et al.* 2015a). Fdz-Polanco *et al.* (2001a) proposed Equation (1) through Equations (2)–(4), which involve the two-step ammonium oxidizing processes. The first step is the removal of ammonium and sulfate

(Equation (2)), and the second step is the anammox process, which occurs synchronously (Equation (3)).

$$2NH_4^+ + SO_4^{2-} \rightarrow S^0 + N_2 + 4H_2O \qquad (1)$$

$$3SO_4^{2-} + 4NH_4^+ \rightarrow 3S^{2-} + 4NO_2^- + 4H_2O + 8H^+ \qquad (2)$$

$$2NO_2^- + 2NH_4^+ \rightarrow 2N_2 + 4H_2O \qquad (3)$$

$$3S^{2-} + 2NO_2^- + 8H^+ \rightarrow 3S + N_2 + 4H_2O \qquad (4)$$

With further studies, during the anammox process the reaction between extra electron acceptors, such as NO_2^-, SO_4^{2-} and Fe^{3+}, and ammonium was thought to be facilitated by bacteria (Chamchoi *et al.* 2008). The removal of ammonium observed to occur in the denitrifying process was carried out by bacteria using ammonium as an electron donor for nitrate reduction (Graaf *et al.* 1995). NO_2^- and NO_3^- could also act as potential electron acceptors for

sulfur oxidation. Re-oxidation of elemental sulfur or sulfide to SO_4^{2-} could readily take place via sulfur-utilizing denitrification (Rikmann et al. 2012). Another researcher observed that as the reaction proceeded, the SO_4^{2-} concentration did not remain steady, and the NO_2^- concentration also decreased and promoted SO_4^{2-} resynthesis via sulfur-utilizing denitritation (Liu et al. 2015b). Most other research concerning SRAO has shown that disproportionally higher removal of NO_2^- and NH_4^+ was based on the effluent (Fdz-Polanco et al. 2001b; Liu et al. 2008; Yang et al. 2009; Jing et al. 2010). At the same time, it should be noted that the effluent contained more NO_3^- in previous studies (Strous et al. 2002; Mahmood 2007; Sabumon 2008; Rikmann et al. 2012). Therefore, the nature of the electron acceptor was a key factor for determining the ammonium oxidation pathway in SRAO.

Strous et al. (2002) believed that some intermediates, such as NO_2^-, NO_3^-, S^{2-} and S, might affect the removal efficiencies of ammonium and sulfate in the SRAO process. Liu et al. (2008) isolated and described a new autotrophic Planctomycete bacterium and thought this bacterium was an anammox bacterium. Anammox bacteria preferentially utilize nitrite and nitrate as the electron acceptor to compete with sulfate, thus showing a significant decrease in sulfate reduction (Lai & Zhou 2010; Rikmann et al. 2012). The SRAO process was heavily suppressed by increasing NO_2^- and NO_3^- concentrations.

Dissolved O_2 (range: 0–0.2 mg·L^{-1}) and NO_3^- present in the influent could be consumed in the SRAO process (De et al. 2016). Rikmann et al. (2014) believed that NO_2^- was transformed to NO_3^- under anaerobic conditions due to O_2 entry from the tank provided in the reactor. Schrum et al. (2009) described possible involvement in the nitrate producing stage of SRAO, and the ammonium could also be oxidized to NO_3^-. An organic compound could be used as an electron donor to couple with a subsequent heterotrophic denitrification reaction at 30° and under normal pressure:

$$NH_4^+ + SO_4^{2-} \rightarrow NO_3^- + HS^- + 2H_2O + H^+ \qquad (5)$$
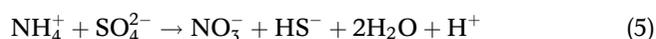
These discoveries generated great interest in research regarding the role of nitrite and nitrate in the SRAO process. Many researchers detected different NO_3^- and NO_2^- concentrations, which made the mechanism of the SRAO process unclear and complex (Zhang et al. 2013). Few studies have focused on the metabolic pathways and microbial community changes. SRAO is a multi-step reaction that includes the production of NO_2^- and NO_3^- compounds.

This work used a self-designed anaerobic SRAO reactor, using different concentrations of nitrite and nitrate as environmental factors, to explore the performance of wastewater treatment. The anaerobic end products were tested during the experiment to evaluate the synergistic effects of nitrite and nitrate. The microbial community was analysed to study the mechanism.

MATERIALS AND METHODS

Anaerobic cycle growth bioreactor and synthetic wastewater

As shown in Figure 1, the experiment was conducted in two anaerobic cycle growth reactors. These reactors were designed independently by our laboratory. Non-woven fabric filler was used as the biological carrier to enhance the attachment performance for good adsorption characteristics. The laboratory-scale reactors were made of organic glass with an effective volume of 5 L and were located in a water bath. The flow of water was circulated clockwise and could effectively contact microorganisms. To maintain anoxic conditions, the reactor was flushed continuously with nitrogen gas and covered to protect the bacteria from light and algal growth. The temperature inside the reactor was adjusted to $35 \pm 1\,°C$.

Predetermined amounts of ammonium and sulfate (using $(NH_4)_2SO_4$) were added as requirements of each experiment. Trace elements, including EDTA (in mg·L^{-1}) 5,000, $ZnSO_4·7H_2O$ 430, $CoCl_2·6H_2O$ 240, $NaMoO_4·H_2O$ 220, $NiCl_2·6H_2O$ 190, $NaSeO_4·10H_2O$ 210, and H_3BO_4 14, were added to the water as mineral media (Sliekers et al. 2002). In addition, the synthetic wastewater trace element solution contained (in g/L) KH_2PO_4 0.027, $CaCl_2·2H_2O$ 0.136, and $MgCl_2$ 0.2 (Zhang et al. 2019). With the addition of $NaHCO_3$, the pH within the reactors varied between 8.1 and 8.3.

Analytical methods

The concentrations of nitrate, nitrite, and sulfate in the collected liquor samples following 0.45 μm filtration were measured by ion chromatography (ICS-1100, Thermo Fisher). HS^-, S^{2-} and H_2S in the liquid were analysed by Standard Methods. Temperature and pH were measured using a HQ30d portable multi-parameter measuring meter (HQ30d, USA).
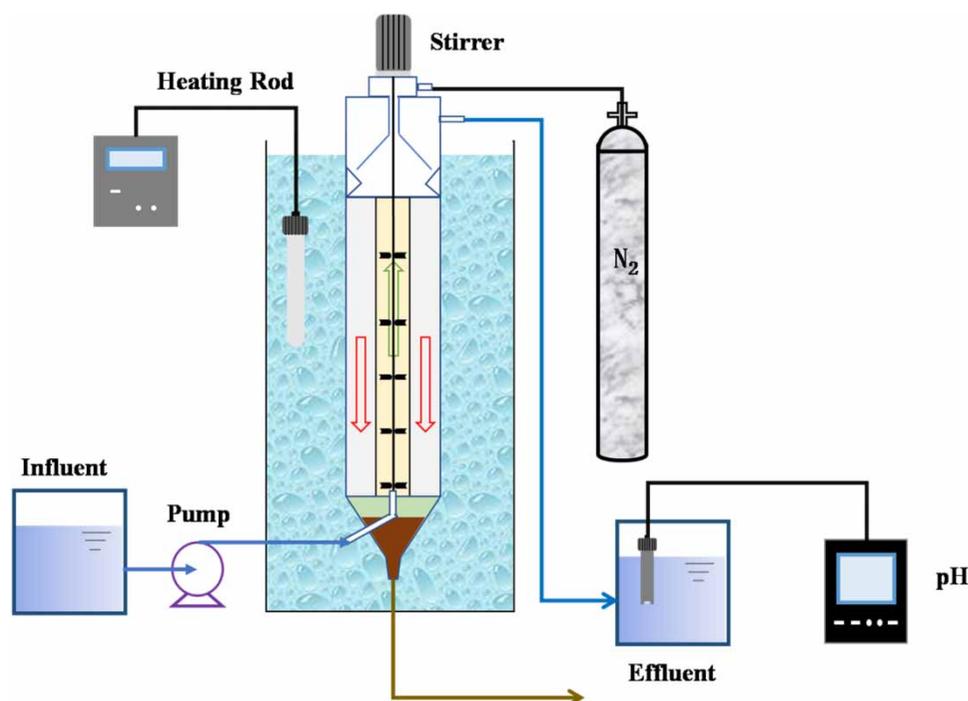
Figure 1 | Schematic of the laboratory-scale SRAO.

Adding influencing factors nitrite and nitrate

The synthetic wastewater contained different initial concentrations of $(NH_4)_2SO_4$ supplemented with NO_2^--N (30 mg·L^{-1}). Table 1 summarizes the characteristics of the substrate.

To explore the effects of nitrate on the removal of ammonium and sulfate, three different concentrations of nitrate, ammonium, and sulfate were used, as shown in Table 2.

Table 1 | Influent concentrations for the SRAO process in the presence of nitrite

Parameter	NH_4^+-N (mg·L^{-1})	SO_4^{2-}-S (mg·L^{-1})	NO_2^--N (mg·L^{-1})
1	119.50 ± 10.43	182.69 ± 9.86	0
2	159.86 ± 2.01	216.05 ± 11.71	33.98 ± 0.86
3	109.53 ± 5.85	115.78 ± 12.76	30.02 ± 2.56
4	80.23 ± 3.74	100.43 ± 8.29	27.64 ± 2.61

Table 2 | Influent concentrations for the SRAO process in the presence of nitrate

Time (days)	NH_4^+-N (mg·L^{-1})	SO_4^{2-}-S (mg·L^{-1})	NO_3^--N (mg·L^{-1})
1–23	120.27 ± 9.95	182.71 ± 11.26	0
24–42	160.44 ± 5.03	216.03 ± 6.57	30.29 ± 1.97
43–55	159.81 ± 2.49	216.11 ± 10.91	60.14 ± 6.53
56–75	89.59 ± 10.74	133.00 ± 11.73	89.71 ± 8.48

RESULTS AND DISCUSSION

Effects of nitrite on the integration of ammonium and sulfate

Performance of the reactor

The effect of nitrite on the integration of ammonium and sulfate was investigated for 97 days. A blank group without NO_2^- addition was also included for comparison. The SO_4^{2-}-S and NH_4^+-N profiles, as the function of the reaction time, are shown in Figure 2(a).

From 24 to 49 days, the influent NH_4^+-N loading rate was 160 mg·$(L·d)^{-1}$, and the SO_4^{2-}-S loading rate was 216 mg·$(L·d)^{-1}$; the loading rate of NO_2^--N was maintained at 30 mg·$(L·d)^{-1}$. The hydraulic retention time (HRT) during the experiment was 24 h. The significant substance removal was initially observed after 48 days. The NH_4^+-N removal efficiency gradually increased from 39% to 60% with a corresponding NH_4^+-N removal rate of 81.46 mg·$(L·d)^{-1}$. The removal efficiency of NO_2^--N increased, but the SO_4^{2-}-S removal efficiency decreased (Figure 2(b)). In addition, complete removal of NO_2^--N was obtained with 48 days.

Figure 2(c) shows the high loading rate of NH_4^+-N and SO_4^{2-}-S when the influent NO_2^--N was kept constant. In the blank group, the SO_4^{2-}-S removal rate was approximately
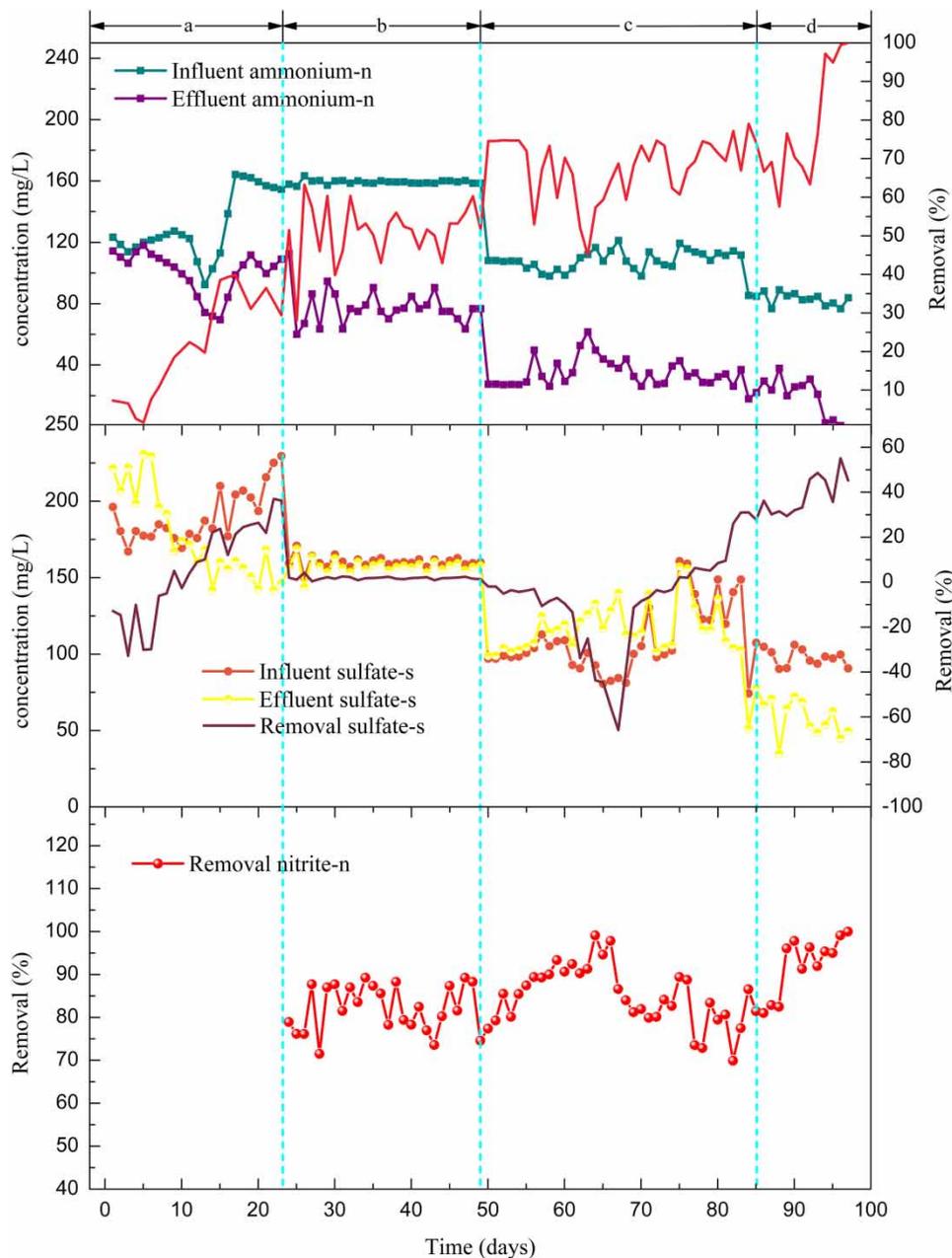
Figure 2 │ Concentrations and removal efficiencies of NH_4^+-N, SO_4^{2-}-S and NO_2^--N: (a) in the presence of a blank group without NO_2^-; (b) with the addition of NO_2^--N (34 mg·L^{-1}); (c) with the addition of NO_2^--N (30 mg·L^{-1}) and (d) with the addition of NO_2^--N (27 mg·L^{-1}).

70.46 mg·$(L·d)^{-1}$. With the addition of NO_2^--N (30 mg·L^{-1}), the concentration of SO_4^{2-}-S in the effluent was higher than the influent concentration. SO_4^{2-}-S removal was strongly related to the NO_2^--N removal level in this stage. In addition, the yellow colour of the effluent was lighter than that of the water in the previous stage. The yellow matter was determined to be S^0 by using carbon as an organic solvent as described in Liu *et al.* (2015b). The result showed that S^0 was produced during the process.

This result indicated that the accumulated amounts of sulfur decreased with time during 50–74 days.

The NH_4^+-N loading rate gradually decreased to 80.0 mg·$(L·d)^{-1}$ while maintaining an N/S of 2 (Figure 2(d)). Approximately no NH_4^+-N was detected in the effluent, and the removal rate of SO_4^{2-}-S was 45.29 mg·$(L·d)^{-1}$. The effluent NO_3^--N was increased to 30 mg·$(L·d)^{-1}$, which was higher than the previous phase. This phenomenon continued for approximately one week and was stably maintained.
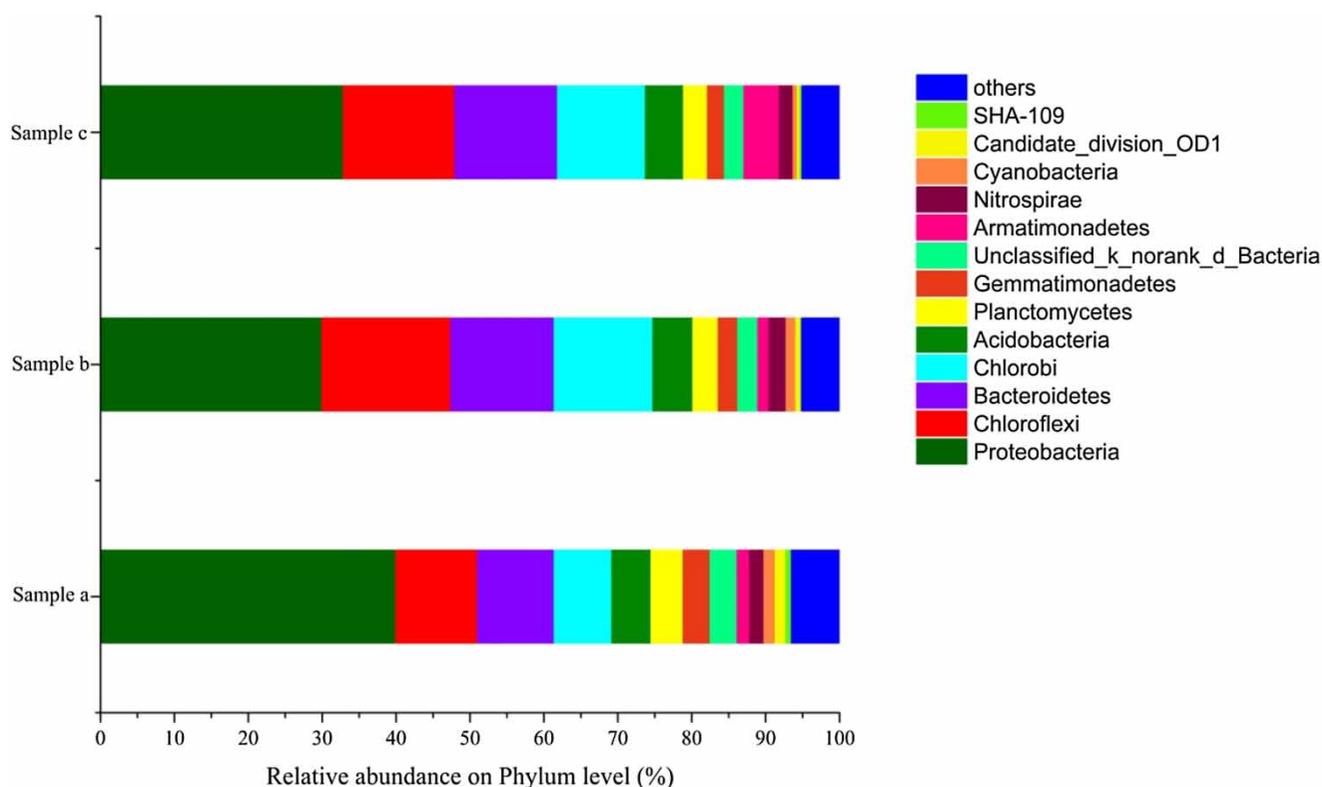
Figure 3 | Charts showing the microbial community composition at the level of phylum: (sample a) the microbial community associated with the blank group; (sample b) the microbial community associated with the addition of nitrite; (sample c) the microbial community associated with the addition of nitrate.

Microbial community structure in the presence of NO_2^-

The phylogenetic relationships and classifications of the bacterial 16S rRNA sequences, with relative abundance above 1%, in the sludge samples extracted from the blank group and the group with the addition of nitrite are illustrated on a phylum basis in Figure 3(a) and 3(b). *Planctomycetes* (4.35% in the blank group and 3.43% in the nitrite group) provided evidence that SRAO was occurring. The increasing relative abundance of anammox bacteria (*Proteobacteria* (39.97% in the blank group and 29.97% in the nitrite group) and *Nitrospirae* (1.94% in the blank group and 2.31% in the nitrite group)) and nitrifying bacteria (*Bacteroidetes* (10.36% in the blank group and 13.98% in the nitrite group), *Chloroflexi* (11.07% in the blank group and 17.45% in the nitrite group) and *Chlorobi* (7.79% in the blank group and 13.35% in the nitrite group)) were investigated and could indicate the role of the observed consortium bacteria. *Proteobacteria* was identified as the most domain functional bacteria thought to oxidize NH_4^+ into NO_2^-. The large proportion of *Bacteroidetes, Chloroflexi* and *Chlorobi* present in this reactor contributed to the oxidation of NO_2^- into NO_3^-; only

SO_4^{2-} provided an electron acceptor during the process (Lai & Zhou 2010). *Acidobacteria* (5.31% in the blank group and 5.41% in the nitrite group) accounted for 5.41% with a few observations and was considered as playing a critical role in the NO_2^- and SO_4^{2-} removal (Figure 2(d)), which was a clear indicator of the simultaneous achievement of SRAO and nitrification.

Sulfur-based autotrophic denitritation

The re-oxidation of elemental sulfur into sulfate could readily take place via sulfur-utilizing denitritation during the SRAO process (Rikmann *et al.* 2012). The loading rates of SO_4^{2-}-S and NH_4^+-N were maintained at 110 mg·(L·d)$^{-1}$ and 116.7 mg·(L·d)$^{-1}$, respectively. A NO_2^--N loading rate up to 30 mg·(L·d)$^{-1}$ was applied to the bioreactor, which changed the sulfur balance and resulted in the partial recovery of SO_4^{2-} (Sun & Nemati 2012). The bioreactor for this experiment had been successfully started and run for some time, and elemental sulfur was attached to the non-woven fabric. The removal efficiency of SO_4^{2-} was lower during this phase, and sulfur could only donate electrons without any possibility of accepting electrons under anaerobic

conditions (Nanda et al. 2013). According to a previous report, the equation for SO_4^{2-} resynthesis via sulfur-utilizing denitritation is shown as below (Equation (6)) (Liu et al. 2008). Few studies have focused on the related functional microbial communities. In the study, the presence of a high abundance of Chlorobi (7.79% in the blank group and 13.35% in the nitrite group) in the seeding sludge provided evidence in favour of this mechanism.

$$S + 2NO_2^- \rightarrow N_2 + SO_4^{2-} \qquad (6)$$

Effects of nitrate on the integration of ammonium and sulfate

Performance of the reactor

As shown in Figure 4(b), when the loading rate of NO_3^- in influent was 30 mg·(L·d)^{-1}, the removal of NH_4^+-N and SO_4^{2-}-S in the anaerobic reactor decreased from 30% to 40%, respectively, to 11% simultaneously. The SRAO process could not be promoted with the NO_3^--N concentration 30 mg·L^{-1}.

According to Figure 4(c), the NO_3^-N loading rate increasing from 30 mg·(L·d)^{-1} to 60 mg·(L·d)^{-1} resulted in the immediate utilization of NH_4^+ and SO_4^{2-}, although part of the NO_3^- was generated during SRAO. This result indicated that moderate NO_3^- concentration could stimulate the removal of NH_4^+ and SO_4^{2-}.

The average NO_3^--N loading rate was gradually increased to 90 mg·(L·d)^{-1}. The steady state profiles of NO_3^--N, NH_4^+-N, and SO_4^{2-}-S concentrations are shown as Figure 4(d). When the feed NO_3^- concentration was further increased, the NH_4^+-N and SO_4^{2-}-S removal efficiencies were greatly increased. The average NH_4^+-N and SO_4^{2-}-S removal efficiencies were 90% and 85%, respectively. The removal rates for NH_4^+-N and SO_4^{2-}-S reached $81.67 \text{ mg·(L·d)}^{-1}$ and $113.67 \text{ mg·(L·d)}^{-1}$, respectively.

Microbial community structure in the presence of NO_3^-

Fdz-Polanco et al. (2001a) proposed a summary equation describing the SRAO process (Equation (1)) and concluded that the N/S ratio was 2:1. In cultures containing different concentrations of NO_3^-, the N/S conversion ratios were lower than 2:1 (Table 3).

High-throughput 16S rRNA gene sequencing technology was used to identify the microbial communities in the blank group and the group with the addition of nitrate (Figure 3(a) and 3(c)). The results showed that Proteobacteria (39.9% in the blank group and 32.86% in the nitrite group) were the most abundant, followed by Chloroflexi (11.07% in the blank group and 15.1% in the nitrite group), Bacteroidetes (10.36% in the blank group and 13.88% in the nitrite group), Chlorobi (7.79% in the blank group and 11.9%in the nitrite group), Acidobacteria (5.31% in the blank group and 5.15% in the nitrite group) and Planctomycetes (4.35% in the blank group and 3.24% in the nitrite group) in the two samples. Proteobacteria and Armatimonadetes observed in the sample could indicate that ammonium was directly oxidized to NO_2^- under anoxic conditions (Teeseling et al. 2015; Mi et al. 2017). Notably, the effluent contained more NO_3^-, but NO_2^- was not present. Bacteroidetes, Chlorobi, and Chloroflexi were the most abundant compared with the blank group, which indicated NO_2^- could be oxidized to NO_3^- in the SRAO process (Mulder et al. 1995). NH_4^+ was converted to NO_2 and other intermediates, in which the electron acceptor was SO_4^{2-} (Xu et al. 2014). The optimum removal of NH_4^+ and SO_4^{2-} was achieved in the system.

Sulfur-based autotrophic denitrification

Autotrophic denitrification utilizing elemental sulfur was an easy reaction for water contaminated NO_3^- (Huang et al. 2018). Rikmann et al. (2012) thought that successfully starting and operating SRAO for a period would accumulate sulfur. Under the condition of 30 mg·L^{-1} NO_3^--N, the effluent concentration of SO_4^{2-} increased and NO_3^- decreased, indicating that elemental sulfur was used as an electron donor (Pokorna & Zabranska 2015). The presence of a high abundance of Chlorobi (7.79% in the blank group and 11.9% in the nitrite group) in the seeding sludge provided evidence for the oxidation of sulfur. During the process of reduction, a little NO_2^- was detected in the effluent. At the same time, the NO_2^- in the liquid began to decrease during the further reduction of the remaining sulfate. One could speculate that at this stage, the system was experiencing sulfur-based autotrophic denitrification and, with sufficient time, NO_3^- was reduced to NO_2^- and other nitrogenous compounds, possibly N_2 (Yin et al. 2015). The reduction of NO_3^- in the presence of elemental sulfur could be represented by reaction (Equation (7)):
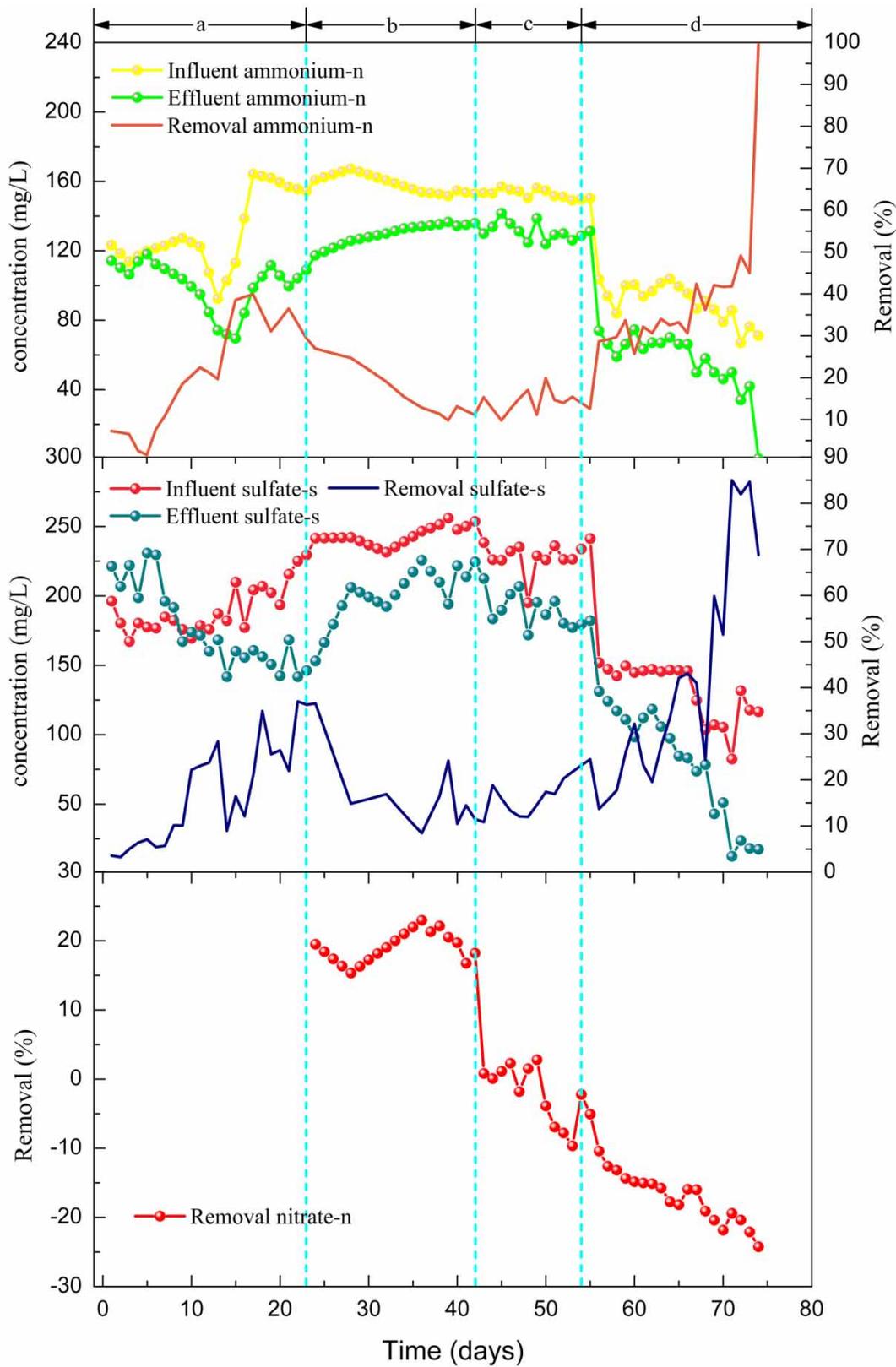
$$S + 2NO_3^- \rightarrow N_2 + SO_4^{2-} \qquad (7)$$

Figure 4 │ Concentrations and removal efficiencies of NH_4^+-N, SO_4^{2-}-S and NO_3^--N: (a) in the presence of a blank group without NO_3^-; (b) with the addition of NO_3^--N (30 mg·L^{-1}); (c) with the addition of NO_3^--N (60 mg·L^{-1}) and (d) with the addition of NO_3^--N (90 mg·L^{-1}).

Table 3 │ Effects of nitrate on SRAO activity

Time (d)	$(NO_3^--N)_{in}$	$(NO_3^--N)_{eff}$	$(NH_4^+-N)_{in}$	$(NH_4^+-N)_{eff}$	$(SO_4^{2-}-S)_{in}$	$(SO_4^{2-}-S)_{eff}$	N/S
24–42	30	24.27	158.81	129.78	243.31	201.25	1.57
43–55	60	63.55	152.82	131.78	228.5733	189.47	1.23
56–75	85	101.61	90.29	31.56	112.09	22.64	1.50

All units in $mg \cdot L^{-1}$; N/S meaning is $NH_4^+-N/SO_4^{2-}-S$; (in) in the presence of (influent); (eff) in the presence of (effluent).
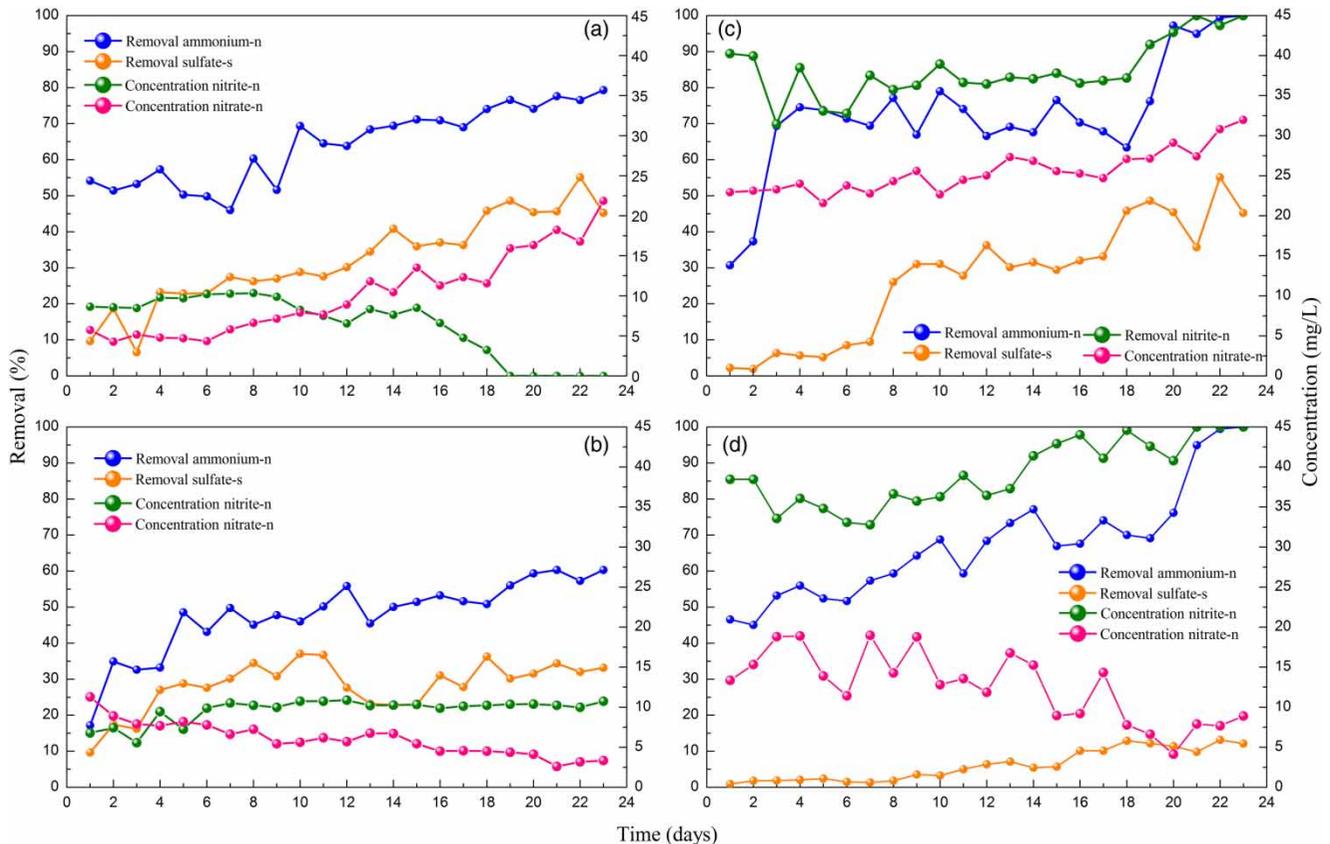
Figure 5 │ Concentrations profiles of various ions with different N/S ratios. (a) The $NH_4^+-N/SO_4^{2-}-S$ ratios of 2:1; (b) the $NH_4^+-N/SO_4^{2-}-S$ ratios of 4:1; (c) the $NH_4^+-N/SO_4^{2-}-S$ ratios of 2:1 in the presence of nitrite; (d) the $NH_4^+-N/SO_4^{2-}-S$ ratios of 4:1 in the presence of nitrite.

Impacts of the ammonium to sulfate ratio

In this study, the N/S ratios of 2:1 and 4:1 in the presence of NO_2^- and four levels of $SO_4^{2-}-S$ concentrations were investigated in the reactor (Figure 5). The $SO_4^{2-}-S$ removal efficiency at an N/S of 4:1 was 33.2%, which was lower than that of an N/S of 2:1. Approximately $16.8 \ mg \cdot L^{-1}$ of NO_3^- was produced when the N/S ratio was 2:1, and the NH_4^+-N removal efficiency was enhanced. The NO_3^- yield was $7.8 \ mg \cdot L^{-1}$ when the N/S ratio was 4:1. Figure 5(c) and 5(d) show two different N/S ratios in the presence of NO_2^-. At an influent NO_2^--N concentration of approximately

$30 \ mg \cdot L^{-1}$, a regular pattern that reduced the influent N/S ratio enhanced the NH_4^+ and SO_4^{2-} removal efficiencies but accumulated more NO_3^-.

In contrast with these two situations, with NO_2^- in the influent and an N/S ratio of 2:1, the NH_4^+ and SO_4^{2-} of the conversion ratio was higher, and the NO_3^- formation was higher. However, the NO_3^- formation was lower. This result indicated that NH_4^+ could be oxidized into NO_3^- or NO_2^- depending on the $NH_4^+-N/SO_4^{2-}-S$ ratios.

The data presented in Table 4 further highlight this distinction.

Table 4 | The effect of influent N/S ratio

$(NH_4^+\text{-}N)_{in}$	$(NO_2^-\text{-}N)_{in}$	$(SO_4^{2-}\text{-}S)_{in}$	$(N/S)_{in}$	$(NH_4^+\text{-}N)_{removal}$	$(SO_4^{2-}\text{-}S)_{removal}$	$(NO_3^-\text{-}N)_{eff}$
83.76	0	112.27	2	71.13	38.80	13.54
83.02	0	40.25	4	52.65	30.48	6.42
83.23	29.16	96.64	2	78.13	46.72	26.89
87.13	29.79	52.86	4	74.77	8.91	5.41

All units in mg·L^{-1} except removal; N/S meaning is NH_4^+-N/SO_4^{2-}-S; (in) in the presence of influent; (eff) in the presence of effluent.

During the SRAO process, ammonium oxidation and sulfate reduction proceeded by a three-step process, with the end-products dependent on the ratio of elemental N and S. The high-throughput 16S rRNA gene sequencing experiments clearly showed that nitrite was the intermediate of the ammonium oxidation. In the case of high influent N/S, the SRAO bacteria produced nitrite from ammonium, and subsequently, the reaction of nitrite to nitrogen gas namely, the anammox process, occurred. In the case of low influent N/S, anammox bacteria could make use of the nitrification pathway to directly produce nitrate. The appropriate ratio of N/S led to the shift in the nitrite and nitrate reduction pathways from anammox to sulfur-utilizing denitritation and denitrification, in which the electron donor was elemental sulfur. Future studies will concentrate on the mechanism of nitrite and nitrate reduction.

CONCLUSIONS

Injections of a certain amounts of nitrite and nitrate had positive effects on the SRAO process performance; the sulfate removal efficiency was 85%, and approximately no NH_4^+-N was detected in the effluent. Compared with the blank group, the relative abundances of microbial communities showed increased Proteobacteria, Chloroflexi, Bacteroidetes, Chlorobi, Nitrospirae Planctomycetes and Armatimonadetes after the reactor ran for 97 days. Since nitrifying bacteria and traditional anammox activity were positively impacted, and nitrification-denitrification and anammox occurred simultaneously with SRAO. This experiment was able to integrate NH_4^+-N, NO_2^--N and SO_4^{2-}-S cycles to maintain optimal electron flow for the SRAO process. Sulfur-based autotrophic denitritation and denitrification occurring in the reactor were caused by nitrite and nitrate.

Studying the role of nitrite and nitrate was important for the SRAO process. Many researchers have reached different conclusions while studying the NO_3^- and NO_2^- produced during the SRAO process, which indicated the mechanism of SRAO process was unclear and complex (Zhang et al. 2013). Future work will focus on isolating the functional bacteria and reducing the production of excess nitrate and nitrite.

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