

Strategy for Rapid Recovery of Simultaneous Sulfide and Nitrite Removal under High Substrate Inhibition

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Abstract

The paper deals with the strategy for a quick recovery of reactor treating sulfide and nitrite simultaneously under inhibition caused by high substrate concentration. For influent sulfide concentration of 360 mg S/L, respective sulfide and nitrite removal percentages dropped to 74.19% and 14.33% due to inhibition caused by high sulfide and nitrite concentrations. It was found that reduction in the influent substrate concentration (300 mg S/L) could not revive the nitrite removal performance in 4 days' operation, which still showed a declining tendency from 47.16–18.52%. Regulating the influent pH around 6.70 ± 0.10 , it only took 4 days to recover the performance for 300 mg S/L. Furthermore, at influent sulfide concentration increased to 360 mg S/L, respective sulfide and nitrite removal percentages were $99.76 \pm 0.27\%$ and 100%. The strategy of regulating influent pH could recover the process performance in a short term, which would provide great convenience for subsequent process research.

1 Introduction

Pulp production, pharmaceuticals, and oil refining industries generate large amounts of sulfur and nitrogen-containing wastewater (Show et al. 2013). Simultaneous sulfide and nitrite removal process is well-established autotrophic biological denitrification process, which can achieve sulfide and nitrite removal at the same time, and recovers elemental sulfur from wastewater, so this novel process has broad application prospects (Mahmood et al. 2007). High influent substrate concentration would pose an inhibitory effect on the process performance (Yuan et al. 2020). The process performance would be greatly deteriorated upon introduction of substrate concentration at certain level (Zhang et al. 2019), and even reach on the verge of irreversible collapse. Furthermore, sulfide and nitrite are both toxic to the microbial communities of the process, and have a serious inhibitory effect on the metabolic pathways (Xu et al. 2019; Zhou et al. 2007), which are prone to the performance deterioration.

However, little has been investigated about the recovery of the process performance once inhibited by high influent substrate. Generally, reducing the influent substrate concentration is an effective strategy to solve the problem, sometimes it takes a long time to be restrained by the strategy (Zheng et al. 2013). In case of failure to restore the process performance by reducing the influent substrate concentration failed to take effect on mitigate the deterioration of the performance, what strategy should be adopted? The objective of the present study was to answer the above-mentioned question. The sulfide and nitrite removal performance of was investigated at various sulfide concentrations (60–360 mg S/L), and the nature of products was investigated. After the process inhibition due to high influent sulfide concentration, two strategy were applied to recover the performance, which included the reduction of the influent substrate concentration and regulation of the influent pH. Based on the Principal Component Analysis (PCA) and multiple comparisons, the effectiveness of regulating the influent pH for the rapid performance recovery was validated.

2 Materials And Methods

2.1 Inoculum, reactor and synthetic wastewater

The inoculum was collected from the anaerobic reactor working at Municipal Wastewater Treatment Plant (WWTP) in Hangzhou city. Its volatile suspended solids (VSS) were 6.09 g/L.

A laboratory-scale EGSB reactor was operated during the experiment, which was made of perspex with an effective volume of 6 L. A peristaltic pump was used to inject the synthetic wastewater, and the other pump was used to control the reflux ratio of 1:2. The hydraulic retention time (HRT) was fixed at 13.33h, while the temperature was controlled at $25 \pm 2^\circ\text{C}$.

The synthetic wastewater contained $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$, NaNO_2 , NaHCO_3 (1 g/L), MgCl_2 (0.765 g/L), KH_2PO_4 (0.765 g/L), $(\text{NH}_4)_2\text{SO}_4$ (0.24 g/L) and trace element solution (1 mL/L). The trace element solution was formulated according to Mahmood et al. (Mahmood et al. 2007). The nitrite concentrations were fed according to a sulfide to nitrite molar ratio (3:2). The influent pH was maintained around 7.0 ± 0.1 , which was adjusted by 0.1M HCl solution.

2.2 Experimental procedure

The operation of the reactor was divided into three stages, which lasted for 62 d. Table 1 enlists the detailed operating conditions of the bioreactor during different stages. In Stage I, the influent sulfide concentration was increased from 60 mg S/L to 360 mg S/L with a gradient of 60 mg S/L. At influent sulfide concentration of 360 mg/L, the simultaneous sulfide and nitrite removal performance process was inhibited due to lower sulfide and nitrite removal percentages. During Stage II, decreasing the influent substrate concentration (300 mg S/L) was tried to recover the performance of the reactor; however, it did not work on short term basis. Finally, decreasing the influent pH (6.70 ± 0.10) was applied to recover the reactor performance, and the influent sulfide concentration was increased to 360 mg S/L.

Table 1
Operating conditions of the bioreactor at different stages

Stage	□			□			□		
Time(d)	1–8	9–16	17– 24	25– 31	32–38	39–45	46–49	50–57	58–62
HRT(h)	13.33								
S/N molar ratio	3:2								
Inf. pH	7.00 ± 0.10						7.00 ± 0.10	6.70 ± 0.10	
Inf. sulfide (mg S/L)	60	120	180	240	300	360	300	300	360
Inf. nitrite (mg N/L)	23.33	46.66	70.00	93.33	116.67	140.00	116.67	116.67	140.00

2.3 Analytical procedures

The influent and effluent pH, nitrate-nitrogen, nitrite-nitrogen, ammonia-nitrogen and sulfide-sulfur were analyzed according to standard methods (APHA et al. 1998). An ion chromatograph (DIONEX-900) analyzed thiosulfate-sulfur, sulfite-sulfur and sulfate-sulfur concentration after passing through a 0.45 µm filter. The elemental sulfur and nitrogen gas were estimated according to principle of mass conservation, which were calculated as Eq. (1) and Eq. (2).

$$[S_0] = [HS^-]_{inf} - [HS^-]_{eff} - [SO_4^{2-}]_{eff} - [S_2O_3^{2-}]_{eff} \quad (1)$$

$$[N_2] = [NO_2^-]_{inf} - [NO_2^-]_{eff} - [NO_3^-]_{eff} + [NH_4^+]_{Consmpt} \quad (2)$$

Where: $[S_0]$ and $[N_2]$ were elemental sulfur and nitrogen gas produced in the reactor; $[HS^-]_{inf}$ and $[NO_2^-]_{inf}$ were the influent concentration of corresponding substances, respectively (mg/L); $[HS^-]_{eff}$, $[SO_4^{2-}]_{eff}$, $[S_2O_3^{2-}]_{eff}$, $[NO_2^-]_{eff}$ and $[NO_3^-]_{eff}$ were the effluent concentration of corresponding substances (mg/L); $[NH_4^+]_{Consmpt}$ was the concentration of consumed ammonium, which was the derivative of the influent and effluent concentrations.

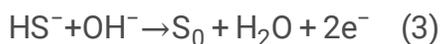
Multiple comparisons were made using SPSS 26.0 based on non-parametric tests. The statistical significance was estimated via Mann-Whitney U test at 0.05 level of significance. Principal Component Analysis (PCA) was also performed by SPSSAU v20.0.

3 Results And Discussion

3.1 Operation performance by elevating influent substrate concentration

Figure 1–3 showed the performance of simultaneous sulfide and nitrite removal process. When the influent sulfide concentration was gradually increased from 60 mg S/L to 300 mg S/L (Stage I), the respective sulfide and nitrite removal percentages were $92.65 \pm 5.94\%$ and $99.91 \pm 0.30\%$ (Fig. 2 and Fig. 3), with the influent pH controlled at 7.0 ± 0.1 (Fig. 1).

It was observed that there was a slope between influent concentration of 60–180 mg S/L (low concentration) and 240–300 mg S/L (high concentration). When the influent sulfide concentration was in the range of 60–180 mg S/L, the effluent pH showed a slight increasing trend $0.26\text{--}0.65(\Delta\text{pH})$. With the influent sulfide concentration in the range of 240–300 mg S/L, the effluent pH greatly went up with an increase of $1.52\text{--}2.11$. This phenomenon was coincided with the variation of final products of the process. Sulfate ($63.27 \pm 12.24\%$ accounted for oxidized sulfide) and nitrogen gas ($101.61 \pm 20.09\%$ accounted for reduced nitrate) were the main product of the influent sulfide concentration of 60–180 mg S/L. However, the elemental sulfur ($61.76 \pm 3.53\%$ accounted for oxidized sulfide) and nitrogen gas ($121.90 \pm 7.50\%$ accounted for reduced nitrite) were the main product at high sulfide concentration of 240–300 mg S/L. When sulfide was oxidized to element sulfur, it consumed 1 mol OH^- per mol sulfide (Eq. 3); when it was oxidized to sulfate, it consumed 9 mol OH^- per mol sulfide (Eq. 4). Hence, the variations of effluent pH between 60–180 mg S/L and 240–300 mg S/L were caused by the types of final product.



Furthermore, the ammonium consumption was significant at high influent concentration, which was 18.71 ± 2.96 mg N/L at 240 mg S/L, and 32.42 ± 4.88 mg N/L at 300 mg S/L. To date, it is well established that the sulfide-driven autotrophic denitrification was coupled with anaerobic ammonium oxidation (Anammox) (Deng et al. 2021), which was consistent with our experiment.

When the influent sulfide concentration was increased to 360 mg S/L (Stage I), there was a sudden reduction in the sulfide and nitrite removal performance, and the sulfide removal percentage decreased from $88.25\text{--}74.19\%$, while that of nitrite dropped from $42.58\text{--}14.33\%$. In the end, there were 93.66 mg S/L residual sulfide and 103.16 mg N/L residual nitrite in the effluent. According to literature, sulfide inhibited denitrification at the concentrations higher than 200 mg S/L (An et al. 2010). Cardoso et al. found that sulfide exerted an inhibitory effect on denitrification by approximately 21-folds, when its concentration increased from 80 mg S/L to 320 mg S/L (Cardoso et al. 2006). As previously mentioned, the process would be inhibited by 36–60 mg N/L nitrite, and a 50% inhibition was also found above 48 mg N/L (Fajardo et al. 2014). It suggested that the collapse of removal performance was induced by inhibition caused by sulfide and nitrite at high influent substrate concentration.

3.2 Rapid recovery strategy for high substrate inhibition

In order to recover the performance of the bioreactor, the strategy of reducing influent substrate concentration (Stage II) was primarily used. In 4 days' operation, sulfide removal percentage quickly recovered to $91.21 \pm 1.78\%$, however, nitrite removal percentage still showed a declining tendency, which descended from $47.16\text{--}18.52\%$. The results showed that reducing the influent substrate failed to recover the nitrite removal performance on a short-term basis (4 days). During a severe nitrite inhibition (its concentration was higher than inhibition parameter), Tang et al. also found that nitrite removal performance was hard to be recovered by decreasing substrate concentration, only achieved 89% after 39 days, which was due to high inhibition effect of nitrite (Tang et al. 2010).

Many studies have shown that pH has a significant effect on the denitrification process (Karanasios et al. 2010). PCA was used to analyze the relation of ΔpH and products of the stage I (60–300 mg S/L). Two principal components were extracted, and the equations related to principal components and ΔpH were established (Eqs. 3–5), which suggested an excellent linear relationship between the concentrations of products and ΔpH (Fig.S1, $R^2 = 0.9790$). It was supposed that the performance of the bioreactor at 360 mg S/L was as good as that at 300 mg S/L, ΔpH was as high as 2.41 according to Eq. (3), which meant that the effluent pH of 360 mg S/L reached 9.41, much higher than inhibited pH of 9.0 for denitrification (Chung et al. 2014; Fajardo et al. 2014).

$$\Delta\text{pH} = 0.02008 \times \text{PCA1} - 0.00322 \times \text{PCA2} \quad (3)$$

$$\text{PCA1} = -0.152 \times [\text{HS}]_{\text{eff}} - 0.014 \times [\text{SO}_4^{2-}]_{\text{eff}} + 0.149 \times [\text{S}_2\text{O}_3^{2-}]_{\text{eff}} + 0.152 \times [\text{S}_0] - 0.150 \times [\text{NO}_2^-]_{\text{eff}} + 0.144 \times [\text{NO}_3^-]_{\text{eff}} - 0.143 \times [\text{NH}_4^+]_{\text{Consmpt}} + 0.152 \times [\text{N}_2] \quad (4)$$

$$\text{PCA2} = -0.134 \times [\text{HS}]_{\text{eff}} + 0.808 \times [\text{SO}_4^{2-}]_{\text{eff}} - 0.204 \times [\text{S}_2\text{O}_3^{2-}]_{\text{eff}} + 0.029 \times [\text{S}_0] - 0.195 \times [\text{NO}_2^-]_{\text{eff}} - 0.205 \times [\text{NO}_3^-]_{\text{eff}} + 0.036 \times [\text{NH}_4^+]_{\text{Consmpt}} + 0.148 \times [\text{N}_2] \quad (5)$$

Hence, the strategy of regulating the influent pH was applied during Stage III. The influent pH was decreased to 6.70 ± 0.10 by keeping the influent sulfide concentration at 300 mg S/L as in Stage II. It only took 4 days to recover the process performance. On the 4th day of Stage III, sulfide and nitrate removal percentages were higher than 99.90% and 90.30%, respectively; furthermore, sulfide and nitrate removal percentages were even higher than 300 mg S/L in Stage I, without any detection of the effluent sulfide and nitrite. With the influent sulfide concentration going up to 360 mg S/L, the bioreactor still showed a good performance, which sulfide and nitrite removal percentages were $99.76 \pm 0.27\%$ and 100%, respectively. The results provided a strong evidence for the validity of strategy of influent pH regulation.

3.3 Comparison of both strategies

As shown in Table 2, there were significant differences in the removal performance (effluent sulfide and nitrite), main sulfur-containing products (elemental sulfur and Eff. sulfate) and main nitrogen-containing products (nitrogen gas) between 300 mg S/L in Stage I and that in Stage II, which suggested that

reduction in the concentration did not recover the process on short-term basis. No significant differences observed on removal performance when the influent pH was regulated at 6.70 ± 0.10 , and main products were intermediate nature of 300 mg S/L in Stage I and those in Stage III; however, significantly different products were found between 360 mg S/L in Stage I and those in Stage III. The results proved that regulating the influent pH effectively succeeded to recover the process on a short-term basis (4 days).

Table 2
Comparison of removal performance in different stages

Index	Removal performance		Sulfur-containing product		Nitrogen-containing product			
	Eff. HS ⁻ (mg S/L)	Eff. NO ₂ ⁻ (mg N/L)	S ⁰ (mg S/L)	Eff. SO ₄ ²⁻ (mg S/L)	Eff. S ₂ O ₃ ²⁻ (mg S/L)	N ₂ (mg N/L)	Eff. NO ₃ ⁻ (mg N/L)	Eff. NH ₄ ⁺ (mg N/L)
300 mg S/L in Stage I vs 300 mg S/L in Stage II	0.006**	0.006**	0.042*	0.006**	0.788	0.006**	0.109	0.109
300 mg S/L in Stage I vs 300 mg S/L in Stage III	0.073	0.315	1.000	0.315	0.042*	0.927	0.006**	0.230
360 mg S/L in Stage I vs 360 mg S/L in Stage III	0.003**	0.003**	0.018*	0.003**	0.003**	0.003**	0.639	0.003**
** represents extremely significant difference at the 0.01 level.								
* represents significant difference at the 0.05 level.								

It is worth noting that the variations of minor products (thiosulfate and nitrate) and consumed ammonium were a little different from those of removal percentage and main products, which inferred that regulating the influent pH would cause a slight impact on product types.

4 Conclusions

The two strategies of reducing influent substrate concentration and regulating influent pH were used to restore the process performance of simultaneous sulfide and nitrite removal after inhibition caused by

high substrate concentration. For a gradual increase in the influent sulfide concentration from 60 mg S/L to 300 mg S/L, the sulfide and nitrite removal percentages were $92.65 \pm 5.94\%$ and $99.91 \pm 0.30\%$, with influent pH controlled at 7.0 ± 0.1 . Upon increase in the influent sulfide concentration to 360 mg S/L, there was a sudden reduction of sulfide and nitrite removal performance, which dropped to 74.19% and 14.33%, respectively. Reducing the influent substrate concentration to 300 mg S/L, sulfide removal percentage quickly returned to $91.21 \pm 1.78\%$ in 4 days' operation; however, nitrite removal percentage still showed a declining tendency, descending from 47.16–18.52%. Upon regulating the influent pH around 6.70 ± 0.10 , the performance recovered in 4 days at 300 mg S/L. Furthermore, the sulfide and nitrite removal percentages were $99.76 \pm 0.27\%$ and 100%, respectively, with influent sulfide concentration going up to 360 mg S/L. Regulating the influent pH for the rapid performance recovery of was significant based on multiple comparisons ($p < 0.05$). The strategy of regulating influent pH for the process performance recovery in a short term (4 days) may be greatly convenient for subsequent research.

5. Declarations

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Authors' contributions

KW performed the experiment and wrote the initial draft. MQ was a major contributor in editing the manuscript. BC participated in the experiment. SL contributed to the conception of the study. YW and ZZ helped perform the analysis with constructive discussions. JC provided financial support for the experiment, and contributed significantly to analysis and manuscript preparation. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no conflict of interest.

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Figures

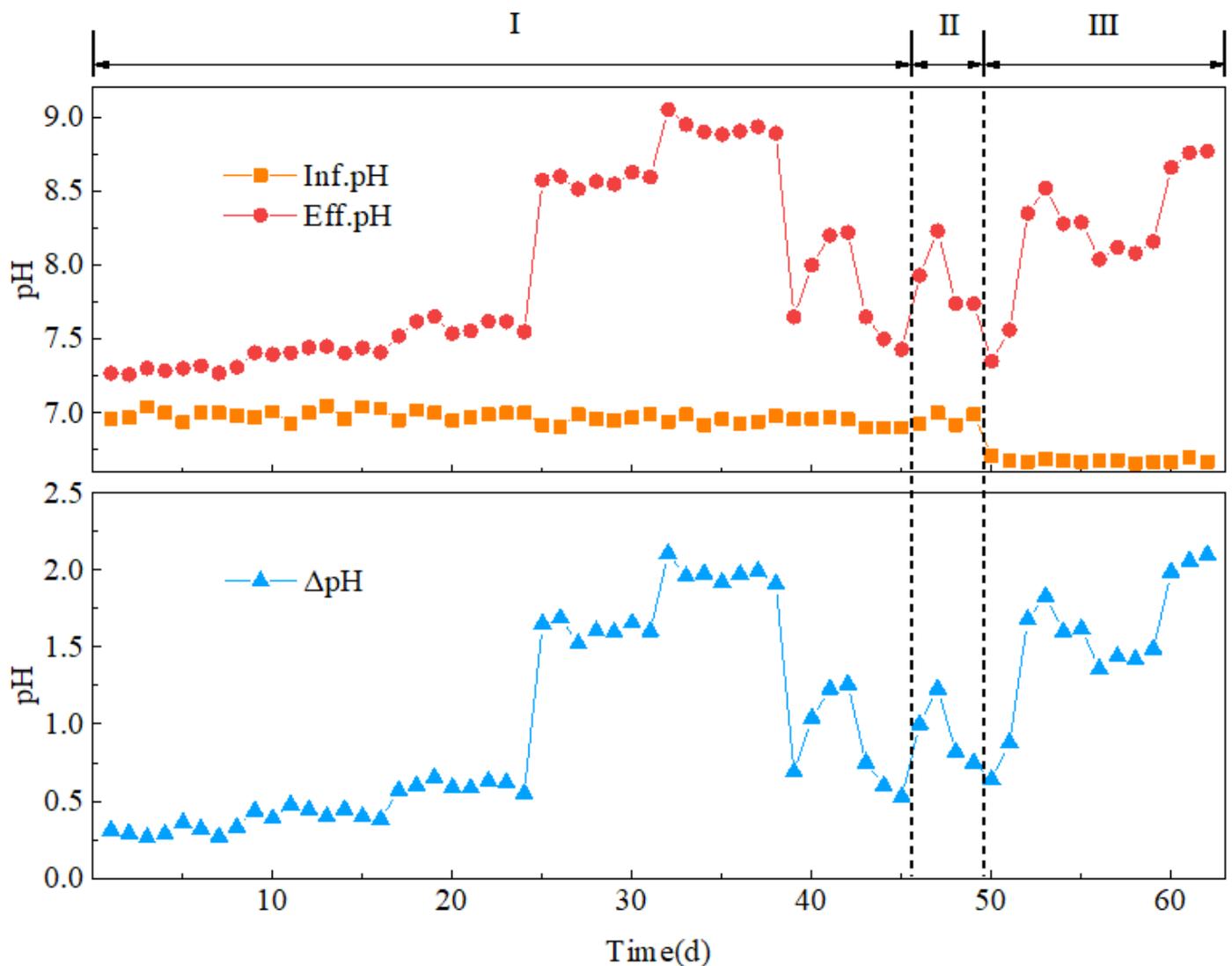


Figure 1

Variations of pH in the simultaneous sulfide and nitrite removal process ($\Delta pH = pH_{inf} - pH_{eff}$)

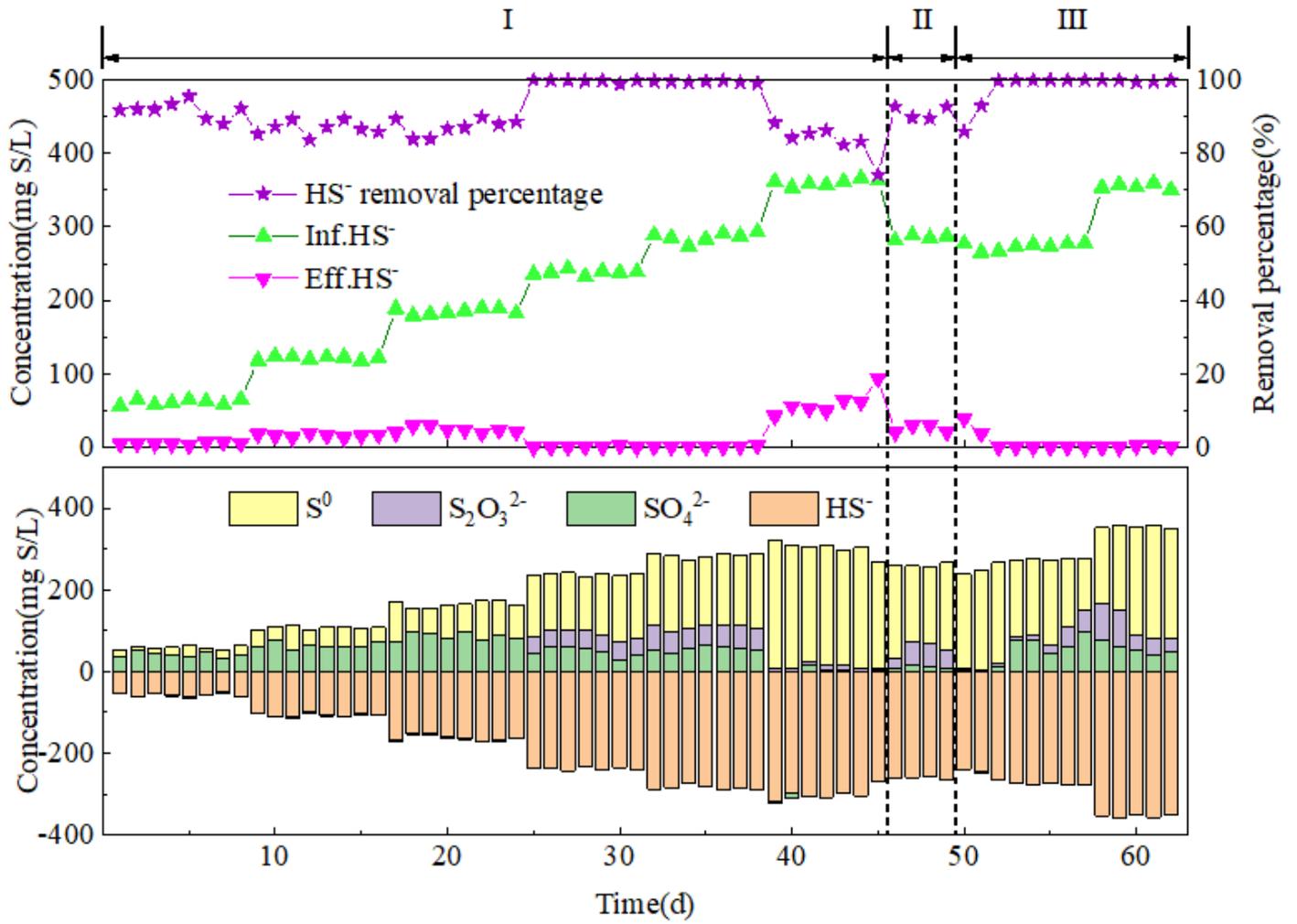


Figure 2

Sulfide removal performance in the simultaneous sulfide and nitrite removal process

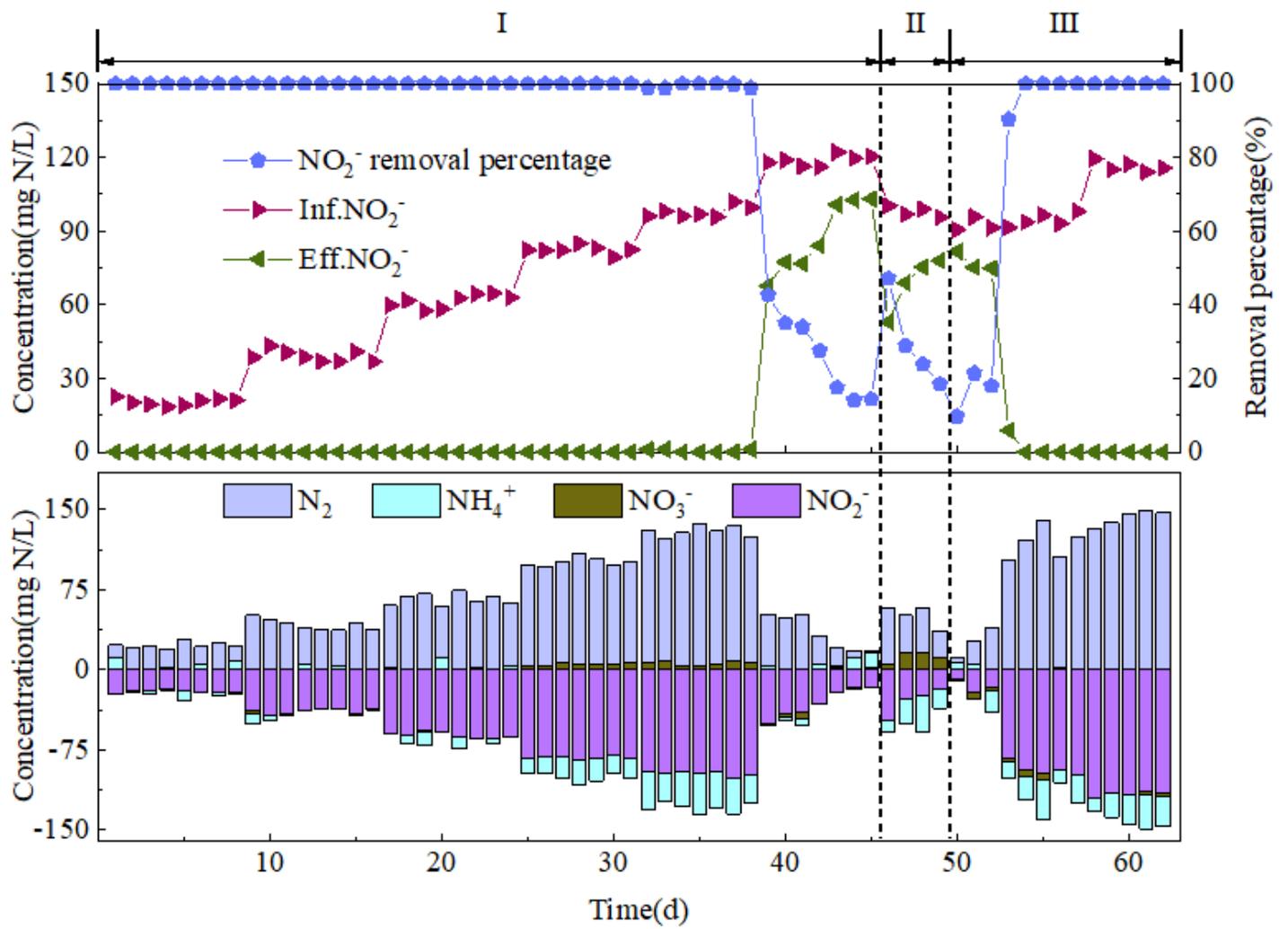


Figure 3

Nitrite removal performance in the simultaneous sulfide and nitrite removal process

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