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Study on the effect of sulfate in the treatment of high ammonia organic wastewater

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ABSTRACT

To obtain a better understanding of effect of sulfate in the treatment of high strength ammonia organic wastewater, we mainly studied the critical concentration of sulfate sludge could endure, the transformation of main microbial phases and the effluent parameters with the sulfate concentration of influent water increasing in anaerobic-aerobic (A/O) process. The results showed that the activated sludge could endure the sulfate concentration up to utmost 6500 mg/L as removal ratios of CODcr, NH_4^+ and TN attained 90%, 92% and 85%, respectively. With the increase of the sulfate concentration, SOUR of sludge weakened gradually due to inhibition of sulfate and sulfide, and main microbial phases changed from metazoan to protozoa, eventually to planktons. After innovatively analyzing the metabolic balance (metabolic pathways and intermediate products) of CODcr and NO_3^- with mathematical method, results indicated that heterotrophic sulfate reduction and autotrophic denitrification-desulfurization gradually strengthened and heterotrophic denitrification weakened. NO_3^- reduced via autotrophic denitrification-sulfurization increased from 28.76% in phase 1 all the way to 62.69% in phase 4, while NO_3^- reduced via heterotrophic denitrification decreased from 71.24% in phase 1 all the way to 37.31% in phase 4 simultaneously.

Keywords: Sulfate; High ammonia organic wastewater; Sulfate reduction; Autotrophic denitrification and desulfurization; Heterotrophic denitrification; Metabolic balance

1. Introduction

With the rapid development of industry and national economy, there are large quantities of untreated high ammonia industrial wastewater discharged from the synthetic ammonia industry, most of which have the characteristics of high COD_{cr}, high ammonia nitrogen and so forth [1–3]. It is imperatively necessary to assure the efficiency of microbial treatment processes. Nevertheless, there always exist large amounts of concomitant sulfate with the generation of high ammonia wastewater. It's reported that the emissions of high sulfate and ammonia (more than 150 mg/L) organic wastewater discharged from synthetic ammonia industry can account for one out of five of the total industrial wastewater each year [4–6].

Despite sulfate represents characteristics of stability and innocuity in water treatment, high-concentration sulfate will lead to a suppressive effect on microbes and bring about mass proliferation of sulfate-reducing bacteria (SRB), thus producing toxic H_2S and S^{2-} by reducing sulfate [7].

However, previous pertinent researches into effect of sulfate usually focused on the single aspect of salt tolerance acclimatization of activated sludge. Threshold about concentration of sulfate sludge can endure and specific microbial phase changes in high ammonia wastewater treatment are still inadequate [6,8].

As many researches denote, sulfate-reducing bacteria massively proliferates in anaerobic or an anoxic unit of industrial wastewater treatment under the condition of high sulfate concentration. In this section, SRB can reduce sulfate to sulfide while oxidizing suitable carbon source simultaneously needed by many other kinds of heterotrophic anaero-

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bic microbes such as acid-producing fermentation bacteria, methanogenic bacteria, denitrifying bacteria and etc. The dilemma will of course form microbial relationships of competition inhibiting the activity of denitrification organisms, while relevant concrete research is still insufficient [9].

According to many literatures, acid-producing fermentation bacteria hydrolyzes high molecular weight organics into glycerol, volatile fatty acids, ethanol and H_2 that are needed nutrients for SRB [10–12] and this fact illustrates that SRB and acid-producing fermentation bacteria form a relationship of cooperation and co-metabolism. Thereby SRB mainly competes with methanogenic bacteria and denitrifying bacteria [13–15]. Moreover, SRB reduces sulfate in a large amount to obtain energy and expel the resulting deleterious sulfide involves free hydrogen sulfide (H_2S), bisulfide (HS^-) and sulfide (S^{2-}), which are toxic and suppressive to most of microbes [12].

Countless studies of the treatment to high ammonia wastewater have been reported in literature [15,16]. Wastewater from synthetic ammonia industry usually contains substantial ammonia. In addition, it has many other negative peculiarities including poor biodegradation, low C/N ratio, and miscellaneous blend contaminations. In practice, biological nitrification-denitrification is the most common process for nitrogen removal of wastewater, in which anaerobic-aerobic process (A/O) is further the most widely applied industry in high ammonia wastewater treatment [7]. With regard to A/O, firstly ammonia nitrogen is oxidized to nitrite by Nitrosomonas species and nitrite is oxidized to nitrate by Nitrobacter species then, gaining energy for life activities respectively, which is usually called aerobic-autotrophic process. In secondary part of A/O,it's an anaerobic-heterotrophic process that nitrate is reduced to gas nitrogen by denitrifying organisms [17]. These two sections are usually setup in two separate units because of the different needs of operational condition in order to ensure the efficiency of treatment [18].

Under the high sulfate and ammonia wastewater condition, SRB has a strict need for anaerobic condition so that they cannot propagate massively in aerobic tank while they will proliferate rapidly in anaerobic tank with the complex interactions with acid-producing fermentation bacteria, methanogenic bacteria, denitrifying bacteria and etc.

Nevertheless, relevant researches about the specific competition and metabolism balance of carbon and nitrogen nutrients among microbes in treatment of high strength ammonia wastewater are still vacant.

Our systematic research elaborated the conditions of lab-scale experiments to investigate the effect of sulfate on the high ammonia organic wastewater. In this paper we studied: (a) the performance of sludge, the critical concentration of sulfate sludge could endure, (b) the transformation of main microbial phases and the removal of pollution by increasing the sulfate concentration, (c) and innovative analysis into the metabolic balance of COD_{cr} and NO_3^- in anaerobic tank to get the intensities of sulfate-reducing process and heterotrophic denitrifying sulfide-oxidizing process with the change of sulfate concentration.

2. Materials and methods

2.1 Characteristics of experimental wastewater

Wastewater samples of our research were artificially prepared simulating effluent water taken from Hubei Huaqiang Chemical Group Co., Ltd. Given the fact the effluent was from an ammonia synthetic industry, we heightened the concentration of NH_4^+-N with a proper degree temporarily when the reactor initiated.

The relevant parameters of our wastewater samples are as the following table shows.

The concentration of trace elements, stored in a refrigerator at around 4°C, was 1 mL/L. The relevant parameters are as follows.

2.2. Experimental devices and operational conditions

Our research adopted the Sequencing Batch Reactor, mainly made of organic glass, whose specific parameters were that internal diameter was 0.14 m, effective height was 0.20 m, total volume was 4.0 L and effective volume was 3.0 L, sealed up with a plastic cushion. Mechanical agitator and air pump were also applied to our SBR and we set up a thermal insulation system to ensure the temperature of SBR remained 25 ± 1°C. The main relevant devices are as follows: JP-022creeping pumps, RS-468B creeping pumps, XL-999 temperature heating rods, ACO Electromagnetic air pump and Guohua-78 magnetic stirrers.

The sequencing batch reactor worked 159 d in total automatically controlled by electronic controlled system, as we supplied experimental water and sampled regularly. Each circulation was about 36.5 h, involving 2 mins of water distribution, 24 h of aerobic treatment in which DO was controlled between 2~4 mg/L, 12 h of anaerobic treatment in

Table 1
The quality of influent and the used experimental reagents

Parameters of the influent	Concentration (mg/L)	Preparation reagents	Initial concentration (mg/L)
COD_{cr}	1000 ± 200	CH_3COONa	2550
NH_4^+-N	100 ± 15	NH_4Cl	200
TP	10 ± 2	KH_2PO_4	44
Alkalinity	1000 ± 350	$NaHCO_3$	1000
Ca^{2+}	18	$CaCl_2{\cdot}2H_2O$	136
Mn^{2+}	8	$MnCl_2{\cdot}2H_2O$	50
pH	7.0~8.5	/	/

Table 2
The composition of trace elements

Reagents	Concentration (mg/L)	Preparation reagents	Initial concentration (mg/L)
EDTA	2000	$FeSO_4$	3000
$ZnSO_4{\cdot}7H_2O$	430	$CuSO_4{\cdot}5H_2O$	250
H_3BO_4	14	$NiCl_2{\cdot}6H_2O$	190

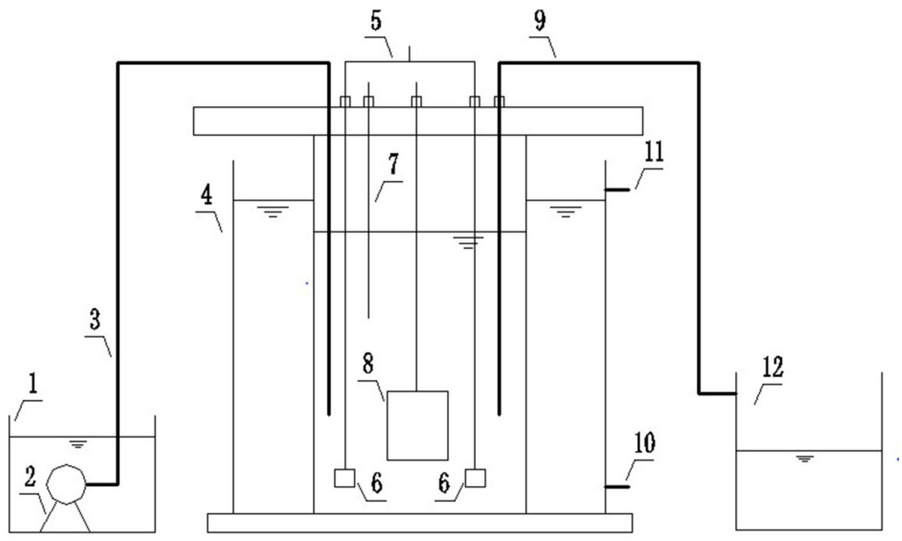

Fig. 1. The principle diagram of SBR. 1-feeding barrel, 2-feeding pump, 3-feeding pipe, 4-SBR, 5-aeration pipe, 6-aeration header, 7-thermometer, 8-stirring blade, 9-drainpipe, 10-feeding pipe of circulating water, 11-drainpipe of circulating water, 12-drain barrel.

which DO was controlled between 0.02~0.2 mg/L, 30 mins of precipitation and 2 mins of drain. Water replacement ratio in each cycle of the SBR accounted for nearly 1/2~2/3 effective reactor volume.

2.3. Sludge inoculation and acclimatization

Inoculum was derived from the secondary sedimentation tank return sludge of Hubei Huaqiang Chemical Group Co., Ltd, whose initial performance was too underactive to be applied to our research, observed unconsolidated structure (initial SVI was 187.4) and the existence of excess filamentous bacteria. Therefore, acclimatization of sludge was necessary to improve the capability of sludge [18].

At the beginning, we put the derived sludge on non-sulfate condition to rehabilitate till its performance reached an acceptable state (removal ratios of COD_{cr}, NH_4^+, TN were more than 92%). Then we added Na_2SO_4 to increase the concentration of sulfate gradient by gradient, each of which was 1500 mg/L thereof. It was performance of sludge and efficiency of nitrification and denitrification under the change of sulfate gradient that we wanted to monitor and investigate.

In our research, anaerobic sections in which DO was controlled between 0.02~0.2 mg/L and CH_3COONa was added as extra carbon resource were set after relevant aerobic sections. We also added $NaHCO_3$ to adjust influent alkalinity to nearly 300 mg/L in order that the whole system maintained buffer capacity and assured adequate inorganic carbon resource.

2.4. Detective and analytical methods

2.4.1. Conventional detective and analytical methods

Detective and analytical methods of our research depended on Standard methods for the Examination of Water and Wastewater.

The main experimental instruments applied included UV Spectrophotometer, LB-901COD_{cr} heating thermostat, ion chromatograph, scanning electron microscope, dissolved oxygen meter and etc.

2.4.2. Specific detective and analytical methods

We selected specific oxygen uptake rate (SOUR) as our analytical index of sludge activity. To detect SOUR of acclimatized sludge under different gradients of sulfate, we put the activated sludge into conical flasks after the prepared conditions that quantitative Na_2SO_4 was added in and aerated till DO was saturated in conical flasks, then set a DO probe, sealed conical flasks up and applied 25°C thermostatic water bath. By means of mathematic analysis, we arrived at SOURs calculated with formula of oxygen consumption per unit time divided by MLVSS.

By setting a control group, we compared SOUR of sludge in endogenous respiration and SOUR of sludge with organic load supplied. Simultaneously, we added propenyl thiourea to inhibit the effect of nitrification in control group.

Besides, microscope was applied to observe the microbial phases of sludge zooming in at the rate of 100× and 400×. Scanning electron microscopy (JSM-5610LV) was also applied with the intention to further figure out the micro structure of sludge in anaerobic sections [19], in which the device-wielding methods were quoted from relevant regulations [20].

3. Results and discussion

3.1. The effects of sulfate in aerobic section

3.1.1. Removal of COD_{cr} and NH_4^+-N

In phase 1~5, the concentrations of sulfate were 2000 mg/L, 3500 mg/L, 5000 mg/L, 6500 mg/L and

8000 mg/L, respectively, maintaining COD_{cr} load of 0.29 kg/(kgMLSS·d) simultaneously.

As was exhibited, we could find that a certain amount of impact took place,slightly as it was, in the beginning of phase 1. When the system became stable, the concentration and removal rate of effluent's COD_{cr} reached 132 mg/L and 88.9%. The system ran stably for 45 d, concentration of sulfate increased from 2000 mg/L to 3500 mg/L. The removal efficiency was as stable in spite of the increased sulfate load, which from our own perspective was attributable to acclimatization. Yet when it was 58th day, we observed the appearance of filamentous bacteria and the fact that effluent COD_{cr} rose to 112 mg/L. With regard to this situation, SRB accumulated in the anaerobic section between phase 1 and 2, producing a certain amount of sulfide that would be preferentially oxidized to prevent toxicity when back in aerobic section, performed as a rise of effluent COD_{cr}. By means of enhancing intensity of aeration and discharging sludge, we adjusted the operation condition so that the effluent COD_{cr} and the average removal rate attained 56 mg/L and 92.2% respectively. Compared with the conclusion in phase 2, we arrived at a same one in phase 3 (5000 mg/L sulfate).Overall, the average removal rate of COD_{cr} attained 91.6%.In phase 5, the effluent deteriorated gradually as the effluent's COD_{cr} rose to 180 mg/L and the average removal rate descended to 81.9%. We observed effluent water in this phase was full of white dense colloidal materials, namely affluent organisms' extracellular polymeric substance (EPS), which would be secreted by organisms under exterior stress. Detection of EPS could be obtained in supplementary information. Additionally, we further found that sludge flocs were small and loose, in which microbial phases decreased, by means of scanning electron microscope. Through 12 d adjustment and operation, effluent COD_{cr} looked up, maintaining at 85 mg/L, and the average removal rate of COD_{cr} attained 86.3% overall. When the concentration of sulfate exceeded 8000 mg/L, sludge system collapsed gradually. The effluent's COD_{cr} was beyond 498 mg/L, and capacity of sludge system was weak.

Those results indicated that an increase of sulfate concentration did impact sludge system in aerobic section, however, the sludge system could revive automatically under a sulfate concentration of 5000 mg/L or less. Moreover, the sludge system still could revive under our operation and adjustment at the sulfate concentration of 6500 mg/L.

Similarly, the whole system was impacted at the beginning due to the increase of sulfate concentration to 2000 mg/L and NH_4^+-N from 100 mg/L to 200 mg/L. The effluent NH_4^+-N ascended to 32 mg/L temporarily. Then with the sludge's gradual acclimatization to NH_4^+-N load (0.057 kg/(kg MLSS.d)), the effluent NH_4^+-N descended to 5.7 mg/L. In phase 2, we found there was sort of fluctuation of effluent NH_4^+-N while the removal efficiency of NH_4^+-N was still favorable. In phase 4, the fluctuation of effluent NH_4^+-N ended up with a gradual increase. The average concentration of effluent NH_4^+-N was 15.6 mg/L overall. On the 150th day, effluent NH_4^+-N rose to 72.9 mg/L rapidly.

As shown in Fig. 3, we could find that 6500 mg/L was the critical value of sulfate concentration [21].

3.1.2. The effects on specific oxygen uptake rates (SOURs)

In comparison with the studies above, this section focused on the activities of acclimatized sludge in different aerobic phases. We selected SOUR as our analytical index of sludge activity.

Curves under different concentration of sulfate followed a linear trend for a certain time when organic load wasn't involved, of which the slopes were namely the values of oxygen consumption per unit time. Furthermore, velocities of endogenous respiration weakened slightly with the increase of sulfate concentration.

Break points appeared when we added organic load in reactors represented as an increase of oxygen consumption per unit time till organic load was depleted.

To study the effect of sulfate in different phases, we took SOURs to get inhibition coefficients. The relevant formula is as follows.

$$y = \frac{SOUR_{(0)} - SOUR_{(i)}}{SOUR_{(0)}} \quad (1)$$

where y—inhibition coefficients; $SOUR_{(0)}$—SOUR (0 mg/L sulfate) (mg O_2/gVSS·h; $SOUR_{(i)}$—SOUR (mg O_2/gVSS·h)

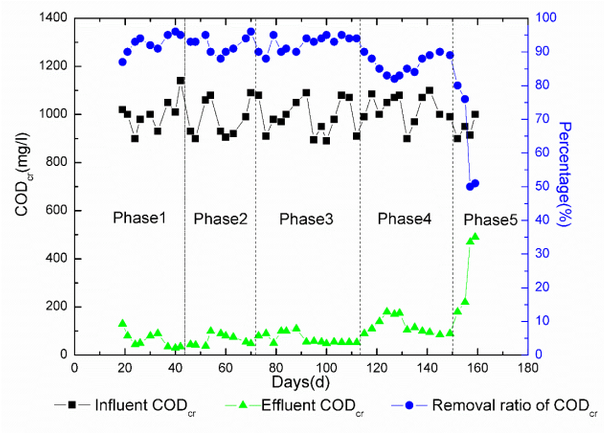

Fig. 2. The removal of COD_{cr} in the aerobic section.

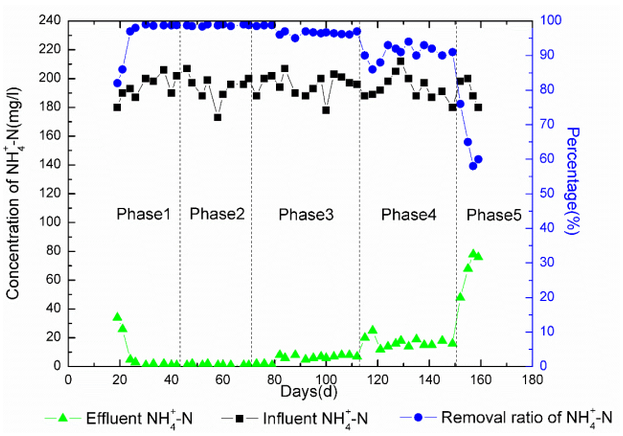

Fig. 3. Removal of NH_4^+-N in the aerobic section.

Inhibition coefficients was no more than 27% which meant the inhibition to sludge was acceptable when concentration of sulfate was less than 3500 mg/L. Nevertheless, inhibition coefficients reached 58% when concentration of sulfate increased to 6500 mg/L and sulfate strongly inhibit activity of sludge.

Velocity of aerobic respiration was almost two times as that of endogenous respiration. With the increase of sulfate, sludge activity weakened and inhibitory coefficient reached 60% when sulfate concentration came to 6500 mg/L.

According to many literatures, activated sludge performs well when its aerobic respiration velocity is between 20~40 mgO_2/g VSS·h. In contrast, activated sludge will be inhibited when aerobic respiration velocity is less than 20 mgO_2/gVSS·h [22].

Combined with our study,the fact further ensured 6500 mg/L was the critical value of sulfate concentration.

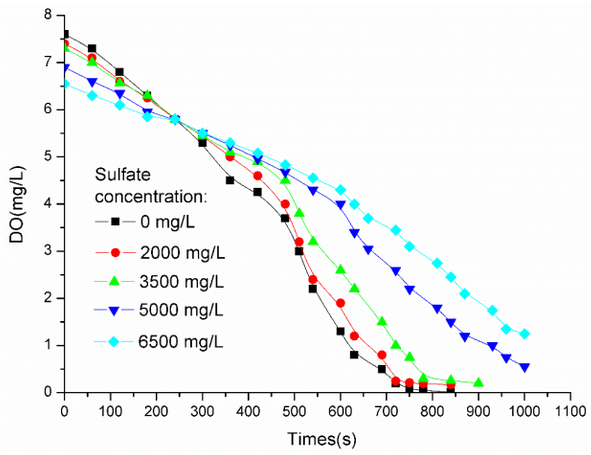

Fig. 4. Profiles of dissolved oxygen during endogenous respiration at different SO_4^{2-} concentrations of the sludge.

Table 3
Specific oxygen uptake of endogenous respiration and inhibitory coefficient at different SO_4^{2-} concentrations

Concentration of SO_4^{2-} (mg/L)	0	2000	3500	5000	6500
SOUR (mgO_2/gVSS·h)	30.17	25.60	21.89	16.56	12.82
Correlation coefficient-R^2	0.996	0.997	0.998	0.997	0.997
Inhibition coefficients-y	\	0.15	0.27	0.45	0.58

Table 4
Specific oxygen uptake and inhibitory coefficient at different SO_4^{2-} concentrations

Concentration of SO_4^{2-} (mg/L)	0	2000	3500	5000	6500
SOUR (mgO_2/gVSS·h)	68.22	57.60	46.44	34.38	27.07
Correlation coefficient-R^2	0.998	0.995	0.991	0.996	0.995
Inhibition coefficients-y	\	0.16	0.32	0.50	0.60

3.1.3. Microbial phases and micro structure

Initially sludge was derived from the secondary sedimentation tank return sludge of Hubei Huaqiang Chemical Group Co. In the beginning, sludge was loose and under active. After 18 days' culture, we observed sludge with microscopy finding that the structure became compact and the microbial phases turned diverse, full of protozoa in the company of a certain amount of metazoan such as rotifer (a). With the increase of sulfate concentration in phase 1 and phase 2, metazoan diminished while protozoa such as vorticella (b) still prospered largely, presented as favorable effluent parameters, which meant main microbes in activated sludge hadn't been affected. When it came to phase 3 and phase 4, multiplicity and mount of protozoa were becoming less, mainly paramecia (c), indicating that sulfate had been affecting bacteria leading to the decrease of microbial phases. In phase 5, we found sludge particle became loose and tiny, connected by filamentous bacteria (d). With the increase of sulfate and sulfide concentrations, former structure of sludge broke down and sulfur bacteria dominated.

We took scanning electron micro photographs of the micro structure of sludge in selective phases. It could be found that some parts of sludge was full of brevibacterium and coccus speculated to be bacteria reducing sulfate and nitrate. When sulfate concentration rose to 3500 mg/L, namely phase 2, most of microstructure was abundant with brevibacterium and coccus, indicating that colonies of bacteria matured (c). With the mounting concentration of sulfate, colonies of bacteria compacted due to secretion of EPS, helping to prevent toxicity and to adapt to the changing osmotic pressure. Microstructure of bacteria turned more irregular when sulfate concentration exceeded 6500 mg/L.

3.2. The effects of sulfate in anaerobic section

3.2.1. Changes of alkalinity and pH

Nitrate-reducing process and sulfate-reducing process were also in company with the production of alkalinity in anaerobic section. Therefore, monitor and investigation into alkalinity were necessary.

Concretely, pH and alkalinity of effluent water slightly fluctuated related to those of influent water and overall pH maintained between 7.8~8.0 in phase 1. However, alkalinity ascended substantially from 1100 mg/L to 1500 mg/L in phase 2 and phase 3. Alkalinity in phase 4 remained stable till it came to nearly the end of phase 4, eventually declined acutely. In phase 5, sludge system lost its capacity.

It could be concluded that bacterial colonies of sulfate-reducing bacteria, simultaneous nitrate-reducing and sulfide-oxidizing bacteria gradually became mature in phase 3 and phase 4. Alkalinity system consisted of HAc/Ac^-, S^{2-}/HS^-, H_2CO_3/HCO_3^-, HCO_3^-/CO_3^{2-} and etc., which ensured a higher effluent pH leading to less free H_2S.

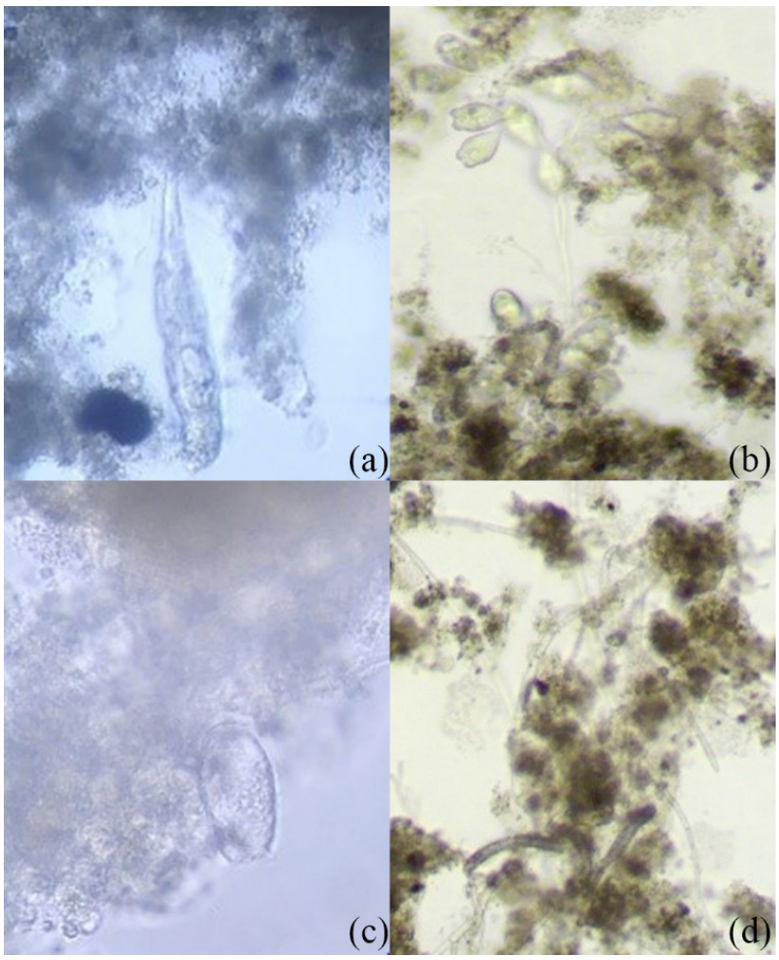

Fig. 5. Images of the micro structure and biological change. (a) 0 mg/L (×100); (b) 2000~3500 mg/L (×100); (c) 5000~6500 mg/L (×100); (d) 8000 mg/L (×100).

3.2.2. Changes of concentrations of sulfate and sulfide

Concentrations of sulfate and sulfide made a significant difference in the treatment of ammonia wastewater, thus we focused on the concentrations of sulfate and sulfide in anaerobic section.

Concentrations of influent and effluent sulfate almost coincided in phase 1, namely when concentration of sulfate began with 2000 mg/L, and removal ratio of sulfate increased from 0.5% to 4.7% with an effluent concentration of sulfide at 28.9 mg/L, which illustrated that this concentration of sulfide hadn't affected activity of sludge or deactivated microbes' enzymes [23]. In phase 2, removal ratio of sulfate increased slowly to 7.38% with effluent concentrations of sulfate and sulfide nearly at 3200 mg/L and 67.3 mg/L, respectively. In phase 3, removal ratio of sulfate increased continually to nearly 10.1~11.2%, colonies of SRB became mature but couldn't grow more due to insufficient carbon resource and competition among different heterotrophic microbes such as heterotrophic denitrifying bacteria. It's presented that concentrations of sulfate and sulfide was stably at 4450 mg/L and between 64.3~86.7 mg/L respectively. In phase 4, removal ratio of sulfate declined to 8.2~9.0% resulting from a lack of carbon resource, namely the reducing capacity of SRB reached threshold despite the excess sulfate's continual increase which presented as a descent of removal ratio of sulfate. In addition, effluent sulfide concentration fluctuated between 69.3~102 mg/L owing to impact of sulfide toxicity. When sulfate concentration rose to 8000 mg/L,sulfide effluent concentration reached 360 mg/L and declined sharply.

Heterotrophic nitrate-reducing and sulfide-oxidizing bacteria were partly inhibited with the increase of sulfate leading to its capacity of oxidizing sulfide weakening so that sulfide accumulated. When concentration of sulfide was above a certain amount, sulfide could easily enter inside of microbes to deactivated enzymes or react with metallic element of protein [22], impacting anabolism of microbes.

3.2.3. Consumption of carbon resource and removal of TN

3.2.3.1. Removal of TN

As was exhibited,in comparison with the influent TN in aerobic section, TN in anaerobic section decreased for

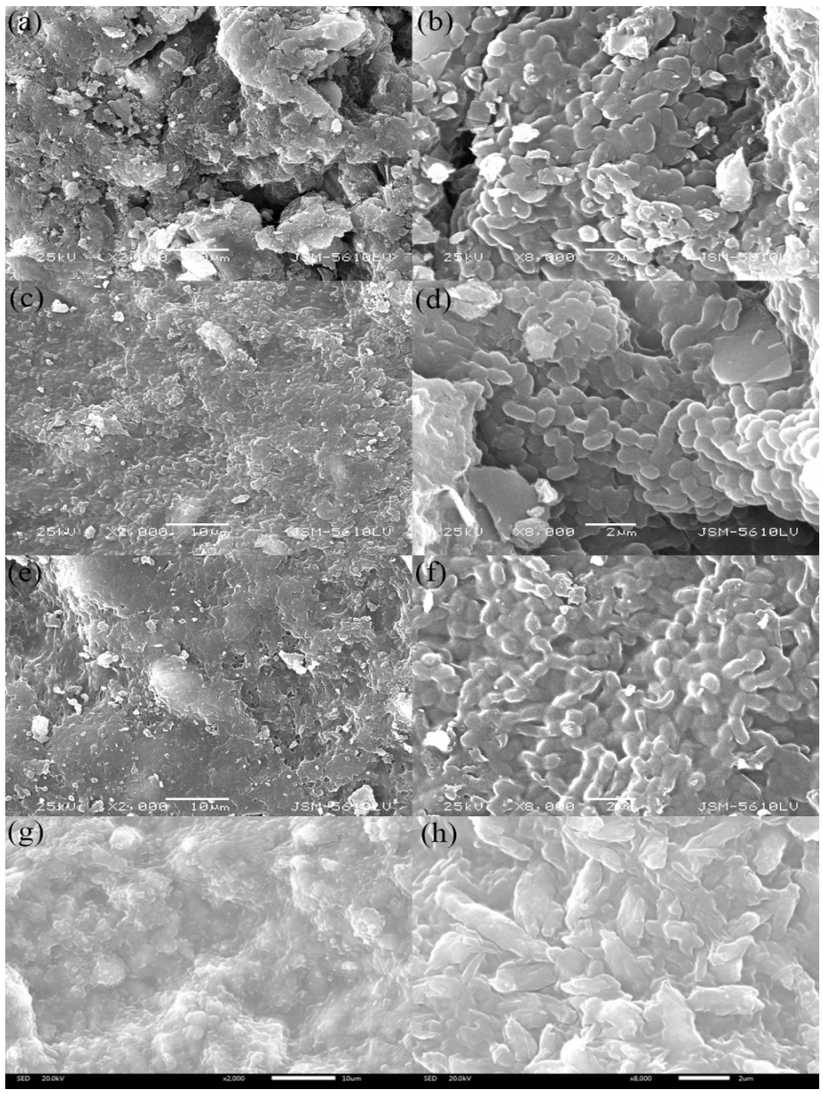

Fig. 6. Scanning electron microphotographs of the microstructure of sludge. (a) 2000 mg/L (×2000); (b) 2000 mg/L (×8000); (c) 3500 mg/L (×2000); (d) 3500 mg/L (×8000); (e) 5000 mg/L (×2000) ; (f) 5000 mg/L (×8000); (g) 6500 mg/L (×2000); (h) 6500 mg/L (×8000).

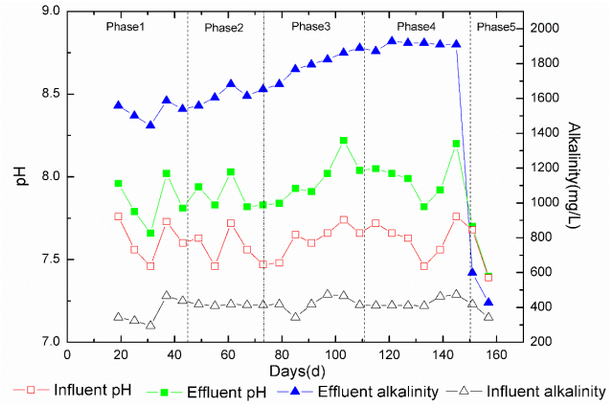

Fig. 7. Changes of pH and alkalinity of the influent and effluent in anaerobic tank of SBR.

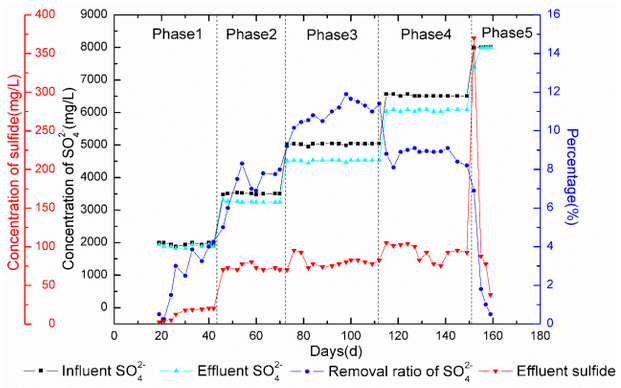

Fig. 8. Changes of the concentration of sulfide and SO_4^{2-} in the anaerobic tank.

10~20%. Aerobic denitrifying process and simultaneous sulfate-reducing and ammonia-oxidizing process worked in aerobic sections.

In phase 1, effluent TN and removal ratio reached 54.8 mg/L and 67.5% respectively. In phase 2, effluent TN continued slowly descending to 28.0 mg/L. In phase 3 despite the fact that effluent TN still could maintain at 18.4 mg/L, average effluent CODcr attained 54 mg/L, In phase 4, average effluent TN ascended to 27.4 mg/L, removal ratio of TN descended to 84.7% and average effluent CODcr ascended to 68.0 mg/L. In phase 5, the effluent TN and CODcr attained 89.8 mg/L and 230 mg/L in this section, respectively.

In conclusion, acclimatization process happened at the beginning of phase 1. Due to lack of carbon resource, effluent TN descended to 28.0 mg/L in phase 2. Though fluctuating in phase 3~4, TN removal was favorable as a whole. In phase 5, the effect of treatment turned bad. The fact above affirmed that 6500mg/L was the critical value of sulfate concentration.

3.2.3.1. Metabolic balance of CODcr and NO_3^-

According to many literatures, SRB mainly competes with methanogenic bacteria (MB) and denitrifying bacteria (DNB)[24]. Hydrogen (H_2) and CH_3COO^- are collective needed carbon resource of SRB and MB. The relevant thermodynamic and kinetic parameters of SRB and MB are as follows.

It was indicated that SRB had a slight advantage over MB in Gibbs free energy of substrates utilization, which meant SRB could take advantage of substrates before MB. Howbeit on the other hand MB's maximum specific substrate degradation rate was higher than that of SRB, illustrating the competition between SRB and MB was very complex. Concretely, COD_{cr} consumption via MB was regarded ignorable after analysis of relevant COD/SO_4^{2-}.

In terms of our research, effluent pH was higher than 8 all the way, which meant free H_2S's escape could be out of consideration.

There were two pathways to consume COD_{cr} concretely in our research, first of which was via heterotrophic denitrification, namely converting TN from nitrate to nitrogen by denitrifying bacteria. The second was autotrophic denitrification and desulfurization, namely sulfate-reducing and ammonia-oxidizing process producing sulfur and nitrogen in specific condition [25]. We added CH_3COONa as carbon resource which would be consumed through sulfate reduction and heterotrophic denitrification.

After calculation, we could find that Ymg/LNO_3^- reduced to nitrogen via heterotrophic denitrification needed Y*10*32/(8*14) = 2.86 Ymg/L COD_{cr} (CH_3COONa) at least, while Xmg/LNO_3^- reduced to nitrogen via autotrophic denitrification and desulfurization needed X*2.5*32/62 mg/L sulfide, which could be further converted that consumption of COD_{cr} (CH_3COONa), given effluent sulfide was equal to M mg/L and 1 g consumption of sulfate theoretically needed 2*32/86 = 0.67 g COD_{cr} (CH_3COONa), was nearly [X*2.5*(32/62)+M]*0.67 mg/L. The relevant formulas are as follows.

$$X + Y = a \tag{2}$$

$$\left[X \times 2.5 \times 32/62 + M\right] \times 0.67 + 2.86Y = b \tag{3}$$

After sampling in stable condition of phase 1~4, namely 34th day, 61st day, 100th day and 139th day, we aggregated relevant data.

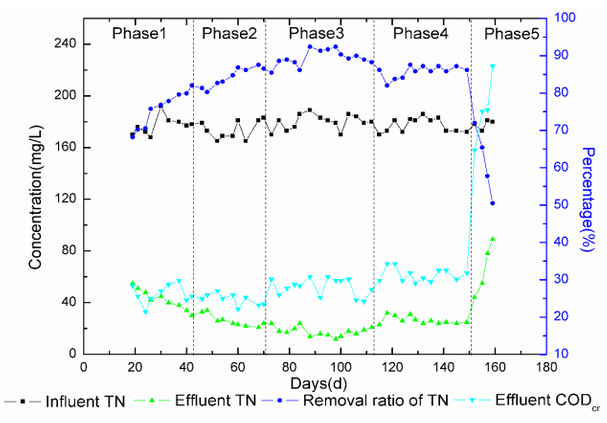

Fig. 9. Removal effect of TN in the anaerobic section.

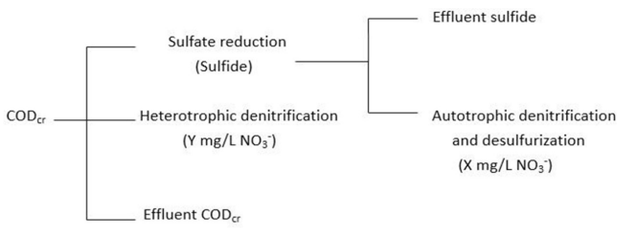

Fig. 10. Transformation and metabolism of organics in anaerobic section.

Table 5
Thermodynamic and kinetic parameters of SRB and MB [7]

Bacteria	Substrates	K_m/(mmol)	V_m/[mmol/(g VSS·d·L)]	Formulas	ΔG (kJ/mol)
SRB	H_2	0.001	112	$4H_2 + SO_4^{2-} + H^+ \rightarrow 4H_2O + HS^-$	–152.6
	acetic acid	3.0	15.4	$CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^-$	–71.7
MB	H_2	0.006	123	$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$	–135.6
	acetic acid	0.2	30.4	$CH_3COO^- + H_2O \rightarrow HCO_3^- + CH_4$	–31.0

Table 6
Operation conditions of four samplings

Running days (d)	a (mg/L)	b (mg/L)	M (mg/L)
34	141.0	338	21.89
67	156.2	366	68.69
100	153.7	356	83.27
139	158.7	362	88.57

Table 7
Nitrogen removal ratios of four samplings in the reactor

Running days (d)	NO_3^--X (mg/L)	NO_3^--Y (mg/L)	X/(X+Y) (%)	Y/(X+Y) (%)
34	40.55	100.95	28.76	71.24
61	75.98	80.22	48.64	51.36
100	90.25	63.45	58.72	41.28
139	96.35	62.35	62.69	37.31

X/(X+Y) gradually increased and Y/(X+Y) decreased simultaneously. It indicated that heterotrophic denitrification gradually weakened and autotrophic denitrification and sulfurization strengthened simultaneously corresponding to the data above, which meant that colonies of SRB and autotrophic denitrifying and sulfur-oxidizing bacteria became mature with the increase of sulfate concentration. Concretely, NO_3^- reduced via autotrophic denitrification and sulfurization increased from 28.76% in phase 1 all the way to 62.69% in phase 4, while NO_3^- reduced via heterotrophic denitrification decreased from 71.24% in phase 1 all the way to 37.31% in phase 4 in the same time. It was because SRB and autotrophic denitrifying and sulfur-oxidizing bacteria could acclimatize relatively high sulfate condition that they could took advantages of substrates efficiently with the increase of sulfate, to maintain stability of metabolism balance of sludge system,until sulfate concentration was too high.

Apparently, our formulas didn't take account of microbes' catabolism and by-products in sulfate reduction such as $S_2O_3^-$ (detected very little). To take specific consideration of more parameters, we need further study and investigation in the future.

4. Conclusions

We treated high ammonia organic wastewater simulating practical operation by our activated sludge system on the foundation of sequencing batch reactor. The reactor could perform well under a sulfate concertation of 6500 mg/L in conditions that influent COD_{cr} was 1000mg/L, influent NH_4^+-N was 200 mg/L, CH_3COONa was added as carbon resource, aerobic section lasted 24 h then anaerobic section lasted 12 h alternately. The removal ratio of COD_{cr}, NH_4^+-N and TN could attain 90%, 92% and 85% respectively. After analyzing specific oxygen uptake rates (SOURs) of sludge and relevant inhibitory coefficient at different sulfate concentrations in aerobic sections, we concluded that the inhibitory effect of sulfate on microbes' respiration and metabolism strengthened with the incremental sulfate concentration. Due to the anaerobic microbes' production of alkalinity in anaerobic section, effluent pH attained no less than 8, which ensured that produced sulfide wouldn't exist in a chemical speciation of free H_2S in large quantities. The fact above diminished the inhibitory effect of sulfide and protect sludge to a certain extent. After analyzing the metabolic balance of CODcr and NO_3^- in anaerobic tank, results indicated that heterotrophic sulfate reduction and autotrophic denitrification- desulfurization gradually strengthened and the heterotrophic denitrification weakened.

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Author Disclosure Statement

No competing financial interests exist.

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Supplementary information

Extraction and detection of EPS

40 mL extraction of sludge sample was put in sampling tube. After centrifugation under 6000 rpm in 4°C for 10 min, supernatant was collected to characterize EPS. EPS was characterized via FT-IR (Nicolet Magana-IR 750 spectrometer) between 400–4000 cm^{-1}wave number.

Analysis ofFT-IR to EPS:

As FT-IR demonstrated, there were 3 absorption peaks in figure, which were 3410, 1620 and 1100 cm^{-1}. The absorption peak around 3410 cm^{-1}came from stretching vibrations of –OH in polysaccharide and $–NH_2$ in protein [1]. The absorption peak around 1620 cm^{-1} came from stretching vibrations of C=O and C–N in amide structure. [2]. The absorption peak around 1100 cm^{-1} came from stretching vibrations of –OH in polysaccharide and C–O in aromatic compounds. The analysis above indicated that extraction mostly consisted of protein and polysaccharide with many functional groups. The fact ascertained that floating secretion was EPS of organisms.

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