



Start-up of simultaneous removal of ammonium and sulfate from an anaerobic ammonium oxidation (anammox) process in an anaerobic up-flow bioreactor

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ABSTRACT

A laboratory testing of simultaneous removal of ammonium and sulfate (SRAS) was studied from an anammox process in an anaerobic bioreactor filled with granular activated carbon. Two different phases of experiment were investigated to start up the SRAS process, and final batch tests were performed to analyze the SRAS process. The experiment included an anammox process and an SRAS process. During the anammox process, the highest removal efficiency of ammonium and nitrite was up to 97 and 98%, respectively. After 160 days in the stationary phase of anammox process, the ratio of ammonium to nitrite consumption was approximately 1:1.15, which is much higher than 1:1.32 in the traditional anammox process. The extra electron acceptor, such as sulfate, was thought to react with ammonium by bacteria. Synthetic wastewater containing ammonium chloride and sodium sulfate was used as the feed for the bioreactor in the second phase of experiment. During the SRAS process, the influent concentrations of ammonium and sulfate were controlled to be 50–60 and 210–240 mg L⁻¹ respectively. After start-up and acclimatization of this process for 60 days, the average effluent concentrations of ammonium and sulfate were 30 and 160 mg L⁻¹, respectively. The simultaneous ammonium and sulfate removal was detected in the reactor. In order to further validate the biochemical interaction between ammonium and sulfate, batch tests was carried out. Abiotic tests were carried out to demonstrate that the pure chemical action between ammonium and sulfate without microorganism was not possible. Biotic assays with different ammonium and sulfate concentrations were further investigated that high concentrations of ammonium and sulfate could promote simultaneous removal of ammonium and sulfate. And elemental sulfur and nitrogen gas as the products measured in the SRAS process helped to demonstrate the occurrence of new interaction between nitrogen and sulfur. The new process of SRAS in the inorganic condition, including simultaneous removal of ammonium and sulfate, and the appearance of elemental sulfur and nitrogen gas as the terminal products, widened the cycle approach between nitrogen and sulfur.

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1. Introduction

The contaminations by ammonium and sulfate compounds in wastewaters and water bodies are critical problems. Some of the problems that ammonium can cause include eutrophication of rivers [1,2], potential hazard to human or animal health and deterioration of water quality [3]. And high sulfate concentrations can unbalance the natural sulfur cycle [4,5]. The accumulation of sulfate-rich sediments in lakes, rivers and sea may cause the release of toxic sulfides that can provoke damages to the environment, such as odor, corrosion [6,7]. Therefore, it is necessary that

both ammonium and sulphate-rich wastewaters require treatment before being released into the environment.

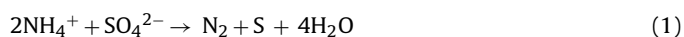
In order to remove ammonium and sulfate in wastewater, biological removal technologies are commonly used due to lower energy consumption and operation cost. At present, the treatment processes for the wastewater contained ammonium and sulfate, mainly focus on the removal of ammonium and sulfate separately. As for ammonium, with more research into the mechanisms of nitrogen cycle, more and more sustainable processes have appeared, including shortcut nitrification–denitrification (SND), the single reactor high activity ammonium removal over nitrite (SHARON) process, oxygen-limited autotrophic nitrification–denitrification (OLAND), anaerobic ammonium oxidation (ANAMMOX) and completely autotrophic nitrogen removal over nitrite (CANON) processes [8,9]. As for sulphate-rich wastewaters, anaerobic biological treatment has been widely used. Under anaerobic conditions, sulfate reducing bacteria (SRB) respire sulfate

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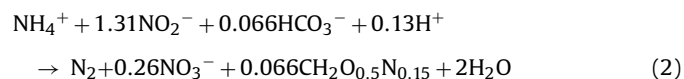
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as a terminal electron acceptor at the expense of the oxidation of electron donors. Depending on the strain and species, these SRB can utilize electron donors such as hydrogen, low molecular weight fatty acids and alcohols, and a variety of environmental contaminants to support their metabolism [10,11]. Therefore, SRB are commonly found in anaerobic processes treating sulfate-rich wastewater [12,13]. The biological sulfate reduction has been recognized as an efficient method for removing sulfate from wastewater. Various aspects of this anaerobic process have been studied [14,15].

When more and more pollutants like ammonium, sulfate are discharged together in wastewaters from pharmacy, food, paper making industry and so on, a renewable process that might be capable of simultaneously removing ammonium and sulfate was received a primary attention. A process for treating vinasse from an ethanol distillery of sugar beet molasses was reported by Fdz-Polanco [16] to show the possibility of removing ammonium and sulfate in wastewater simultaneously. In their study, an anaerobic fluidized-bed reactor was operated to show that sulfate and nitrogen removal seemed to convert into the elemental sulfur and nitrogen gas. They think that sulfate as acceptor of the electrons was produced in the ammonium oxidation to nitrogen or nitrite. A novel anaerobic ammonium oxidation (ANAMMOX) with sulfate as acceptor of the electrons was predicted and a new biochemical reaction was postulated in Eq. (1). Under the organic condition, some researches continued to investigate the fate of the nitrogenous and sulfurous compounds in the process [17,18]. The studies for the mechanism of anoxic removal of ammonium in the presence of sulfate help to develop the bio-treatment in wastewater contained ammonium and sulfate together.



Although some studies of the simultaneous nitrogen and sulfate removal under organic condition has been presented, few investigations have been focused on the autotrophic process of the synchronously ammonium and sulfate removal. As an autotrophic process, the anammox was usually using nitrite as the inorganic electron acceptors. The stoichiometric conversion of nitrite and ammonium to nitrogen gas with production of cell material and nitrate was considered in Eq. (2) [19,20]. However, recently, some different inorganic electron acceptors like nitrate and sulfate have been reported for anammox [16,21]. The new anaerobic ammonium and sulfate process can offer a great future potential for an energy-saving, environmentally sound, and efficient nitrogen/sulfate removal from wastewater.



The objective of this work was to carry out anaerobic ammonium and sulfate removal simultaneously in the inorganic condition. First, an autotrophic anammox was described, in which the high removal efficiency of ammonium and nitrite was acquired. After the anammox process in bioreactor reached steady state, sulfate instead of nitrite was fed to the bioreactor. Only ammonium and sulfate were supplied to the reactor to evaluate the performance of the bioreactor. The SRAS process was operated successfully in the continuous bioreactor. Finally, batch experiments were conducted measuring the specific removal rates in different concentrations of ammonium and sulfate. An attempt was also made to understand the underlying mechanism of anaerobic ammonium and sulfate removal.

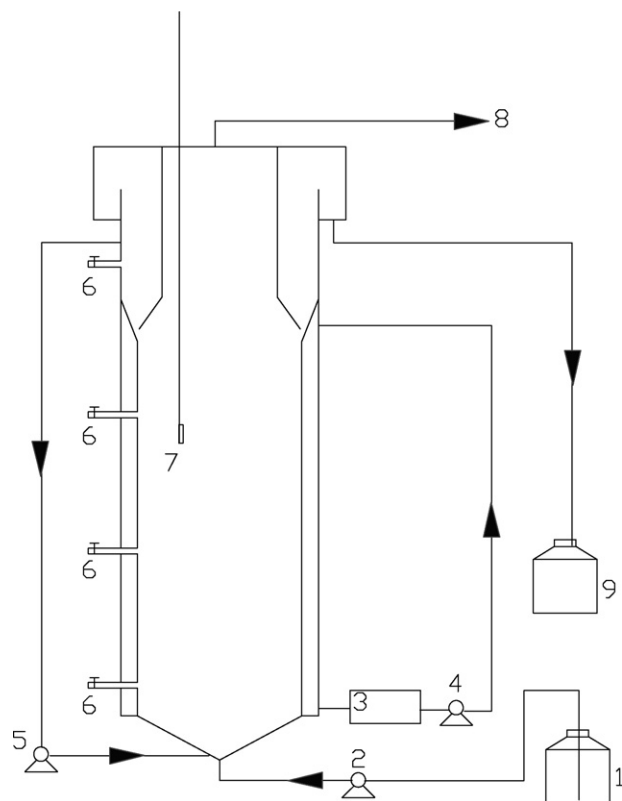


Fig. 1. UASB reactor configuration for anaerobic ammonium oxidation process. 1. Influent tank, 2. metering pump, 3. thermostated water tank, 4. hot water recycle pump, 5. reflux pump, 6. sampling port, 7. thermometer, 8. gas out, 9. effluent water.

2. Materials and methods

2.1. Experimental setup

The bioreactor was a reformative reactor from the up-flow anaerobic sludge bioreactor. As illustrated in Fig. 1, the laboratory-scale up-flow anaerobic bioreactor comprised of a reaction zone and a gas-liquid-solid separator. The column of the reaction zone was fabricated with an internal diameter of 100 mm and a height of 500 mm. An inner cylinder (70 mm internal diameter, 500 mm height) for the main reaction zone was located in the middle of the reactor. It was encircled by the outer annulus (15 mm width) to be kept in the thermostat (under $35 \pm 1^\circ\text{C}$). The reactor had a temperature sensor in the centre of reactor to monitor the temperature of water treatment system. On the top of the reactor was a 1.2 L gas-liquid-solid (GLS) separator. The effective reactor volume, excluding the GLS separator was 2 L.

The bioreactor was operated in up-flow mode in which the influent was pumped from the bottom. Effluent was recycled to obtain various up-flow velocities. Some granular activated carbon was filled into the reactor with an averaged particle diameter between 0.43 and 0.5 mm. The reactor was covered with black cloth to protect the bacteria from light and continuously fed with synthetic wastewater at a hydraulic retention time of 1.5 days. The feed was flushed with nitrogen gas for 10 min before it was used to maintain anaerobic condition in the reactors. Influent pH varied in a range of 7.5–8.5. The up-flow bioreactor filled with active carbon was selected due to its high solid retention and mass transfer rate which would minimize sludge washout during the initial stage and allow good substrate conversions.

2.2. Inoculum and synthetic water

The activated culture used in this study was obtained from nitrifying sludge in a municipal wastewater treatment plant. The initial biomass concentration in the reactor was about $1.56 \text{ g VSS L}^{-1}$.

Two individual phases of experiment were carried out. During the first phase, ammonium and nitrite were supplemented to mineral medium as needed in the form of NH_4Cl , NaNO_2 . But the second phase, the ammonium and sulfate in the form of NH_4Cl and Na_2SO_4 were used as main influent substrates. The amounts of these concentrations varied depending on the applied load. The concentration range of ammonium and nitrite in the first phase were kept at 60–120 and 90–140 mg L^{-1} , respectively. And the concentration range of ammonium and sulfate in the second phase were kept at 50–60 and 210–240 mg L^{-1} , respectively. Besides, the mineral medium composition used throughout this study was (in g): NaHCO_3 , 1.25; KH_2PO_4 , 0.027; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.3; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.3; dissolved in 1 L of distilled water. One milliliter per liter of trace element solution was added to the above medium. The composition of trace element 1 solution was (in g) (based on van de Graf et al. [25]): EDTA, 15.0; ZnCl_2 , 0.20; $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 0.17; $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, 0.19; H_3BO_3 , 0.014; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.24; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.99; $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$, 0.22; $\text{NaSeO}_4 \cdot 10\text{H}_2\text{O}$, 0.21; dissolved in 1 L of distilled water. The composition of trace element 2 solution was (in g): EDTA, 15.0; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 5.0. Predetermined amount of ammonium (using NH_4Cl), nitrite (using NaNO_2) or sulfate (using Na_2SO_4) were added as per requirement of each experiment. All chemicals were analytical reagent (AR) grade (China).

2.3. Batch experiments

Two type of batch tests (biotic and abiotic) were conducted out in order to measure the substrate consumption. Fresh mineral medium with different concentrations of ammonium and sulfate was added to 120 ml serum bottles. For the biotic test, 15 ml of sludge from bioreactor during the SRAS process was transferred into serum bottles containing 100 ml of mineral medium as described above. After the mediums and the inoculums were added, the serum bottles were flushed with Ar gas for 3–5 min. Then, each bottle was sealed by septum cap to maintain anaerobic conditions. The bottles were incubated at 35°C for anaerobic analysis. The conditions for abiotic tests were similar but no inoculum was added. The bottles in all batch assays were shaken for 1.5 days in a constant temperature oscillator at a speed of 150 rpm at a temperature of 35°C . After deposition for another 0.5 day, the Gas samples preserved in sealed bottle were monitored by gas chromatography, and liquid samples were collected using syringes with needles for monitoring the ammonium and nitrite concentrations over times. The initial pH value was adjusted at 7.5 by addition of NaOH or HCl. All incubations were repeated at three different places to calculate the average value.

2.4. Analytical techniques

Ammonium, nitrate, nitrite and sulfate were measured using the standard methods recommended by US Environmental Protection Agency (EPA) [22]. The ammonium and nitrite were measured by using the different colorimetric method and nitrate was analyzed by using the UV spectrophotometric method. Sulfate was measured by Ion chromatograph (ICS-1000, DIONEX). N_2 was analyzed by gas chromatography (HP 6890). Elemental sulfur analysis was made by a modification of the method described by Bartlett and Skoog [23]. A sludge sample dried at 80°C for 2 h was mixed with petroleum ether to dissolve sulfur which was analyzed by spectrophotometer (UV-2100, UNICO) method. A calibration curve was prepared using dilutions of a 50 ppm of elemental sulfur dissolved in petroleum

ether and measuring absorbance at 465 nm. The pH was measured by pH meter model (pHS-21, China). Statistical analysis of data was performed using Microsoft Excel.

In order to analyze the bio-reaction of anammox and SRAS process, the ratio of ammonium to nitrite conversion and the ratio of N/S molar were calculated. These calculation procedures were as follows:

The ratio of ammonium to nitrite conversion = $(\text{influent concentration of } \text{NH}_4^+ - \text{N} - \text{effluent concentration of } \text{NH}_4^+ - \text{N}) / (\text{influent concentration of } \text{NO}_2^- - \text{N} - \text{effluent concentration of } \text{NO}_2^- - \text{N})$.

The ratio of N/S molar = $(\text{influent concentration of } \text{NH}_4^+ - \text{N} - \text{effluent concentration of } \text{NH}_4^+ - \text{N}) / 14 / ((\text{influent concentration of sulfate} - \text{effluent concentration of sulfate}) / 96)$.

3. Results and discussion

3.1. Anammox process under steady state

The bioreactor could start up the process of anammox successfully as illustrated in Fig. 2a and b. By analyzing the behavior of nitrogen removal efficiency, the start-up period of anammox process could be divided into four phases, including an adaptive phase (0–30 days), a lag phase (31–80 days), an increasing phase (81–120 days) and a stationary phase (121–220 days). In order to start up the process of anammox quickly, the low influent concentrations of ammonium (70 mg L^{-1}) and nitrate (90 mg L^{-1}) were applied on

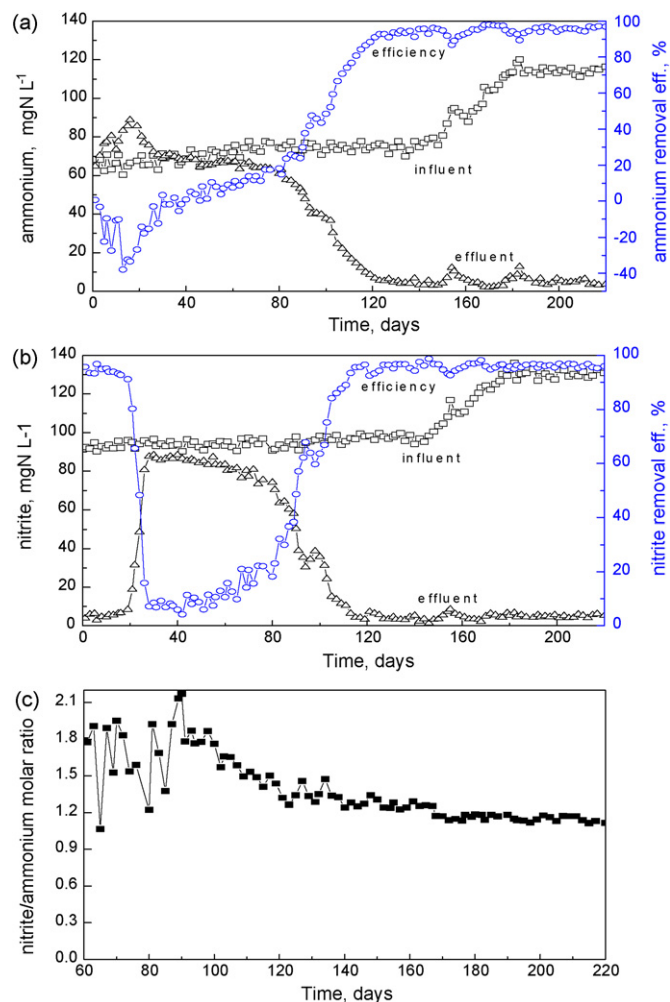


Fig. 2. Influent and effluent ammonium and nitrite concentrations, efficiencies and ratio.

the bioreactor. In the adaptive phase of operation, the ammonium concentrations were higher than the influent concentrations. It was the change in environment of seed sludge that might cause the turnover of bacteria. The former dormant bacteria (such as nitrifying bacteria) might be killed, causing cell lysis and breakdown of organic nitrogen to ammonium [24]. As a result, ammonium concentration increased. During the first phase, nitrite was removed by the denitrifying bacteria in presence of the endogenous organic matters. After 20 days operation, the removal efficiency of nitrite was weakened, because the denitrifying bacteria were restrained by the lack of organic matters. In the lag phase, sludge digestion activity was reduced for the exhaustion of organic substrate from cell lysis, and the anammox population was increased in favoring of the provided substrate. The removal efficiency of ammonium and nitrite was improving during this phase. For the increasing phase, a significant removal of ammonium and nitrite was initially observed after 80 days and a near complete removal of nitrite was obtained with 120 days. The last stationary phase, the optimum removal of ammonium and nitrite was achieved in the system. The highest removal efficiency of ammonium and nitrite was up to 97% and 98%, respectively.

During the different phase of anammox process, the ratio of ammonium to nitrite conversion was changing. From 69 to 100 days, the average ratio was 1:1.62. From 101 to 120 days, the average ratio was 1:1.48. From 120 to 160 days, the average ratio was 1:1.37, similar to the stoichiometric ratio given in Eq. (2) (1:1.32). From 150 days, the influent ammonium and nitrite loading rate was increasing up to about 115 and 130 mg L⁻¹, respectively. The anammox process could keep good condition under a certain high load of ammonium and nitrite. But, after days 160 in the stationary phase of anammox process, an anomalous behavior of anammox was found. The ratio of ammonium to nitrite consumption was approximately 1:1.15 in Fig. 2c, which is much higher than 1:1.32 reported by van de Graaf et al. [20]. Consequently, there might be another anaerobic ammonium oxidation reaction and extra electron acceptor in the anammox process. By analyzing the composition of synthetic wastewater, sulfate was thought as a possible electron acceptor. Further investigation was needed to promote this anaerobic ammonium oxidation reaction with sulfate as electron acceptor.

3.2. Simultaneous ammonium and sulfate removal under inorganic condition

In order to analyze the feasibility of simultaneous ammonium and sulfate removal, Na₂SO₄, instead of NaNO₂, was added into the synthetic water to start up the SRAS process. The influent concentrations of ammonium and sulfate were 50–60 and 210–240 mg L⁻¹. During the experiment of 120 days, the process of SRAS was achieved.

The experiment data for testing the SRAS process in the bioreactor was illustrated in Fig. 3. Fig. 3a shows the influent and effluent ammonium concentration in the reactor and its removal efficiency. Initially, ammonium removal efficiency was high, because the inoculum included much anammox microorganism. Due to the electron acceptor change, the anammox process was limited gradually by the lack of nitrite. The removal efficiency of ammonium was decreased from 75 to 8% in the first 10 days. In this bioreactor inoculated with synthetic water, only N³⁻ from NH₄Cl could be electron donor and S⁶⁺ from Na₂SO₄ could be electron acceptor. It promoted the microorganism to acquire energy by making use of the reaction between N³⁻ and S⁶⁺. From 10 to 60 days, during a propagation phase, the effluent ammonium concentrations decreased with time from 55 to 30 mg L⁻¹. After 60 days, a stationary phase was appeared. The removal efficiency was kept at about 40%. During the stationary phase, the minimum concentration achieved in the effluent was 27 mg L⁻¹ on 81 days, the maximum removal obtained

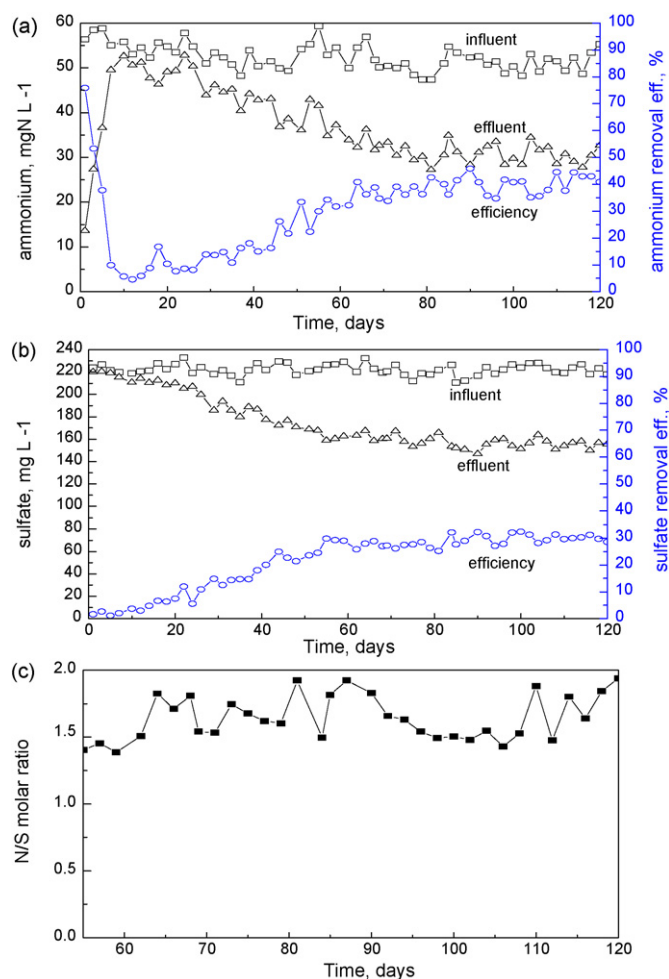


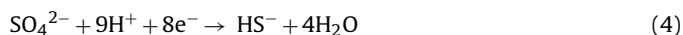
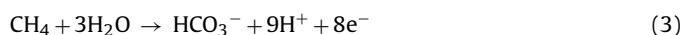
Fig. 3. Influent and effluent ammonium and sulfate concentrations, efficiencies and ratio.

being 45% on 90 days. The removal of ammonium was efficient after about 60-day operation. The variation of sulfate concentration and sulfate removal efficiencies in the bioreactor were also present in Fig. 3b. After about 60-day operation, the average sulfate effluent concentration decreased to 160 mg L⁻¹. During the stationary phase after 60 days, the simultaneous removal of ammonium and sulfate was acquired clearly.

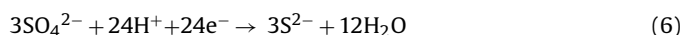
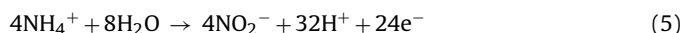
The molar ratio of ammonium to sulfate consumption was changed with the variation of influent wastewater, and came into stabilization at about 1.8:1 finally, which is closed to 2:1 listed in Eq. (1). During the initial 10 days, the anaerobic ammonium oxidation reaction dominated in the reactor, probably due to anammox process consuming ammonium with some surplus nitrite in the bioreactor. With the components change in synthetic water, the conventional anammox process with nitrite as electron acceptor was decreased. The simultaneous removal process of ammonium and sulfate reacted highly. After 60 days, the average ratio of ammonium conversion to sulfate conversion was closed to 2:1 presented in Fig. 3c. The ammonium removal was coupled to sulfate removal, which is a clear indicator of simultaneous removal of ammonium and sulfate. It demonstrated that the new biochemical reaction listed as Eq. (1) is feasible in this bioreactor.

Although the reaction (ammonium removal coupled to sulfate removal) is feasible in the bioreactor, the removal efficiencies of ammonium and sulfate might be affected by some especial biotransformation processes of ammonium and sulfate. The microbially mediated anaerobic oxidation of methane (AOM) with sulfate

[25,26] was reported by Nauhaus et al. They think half-reactions for AOM with sulfate would be as follows:



The bio-reaction might be completed by Methane-utilizing archaea and the SRB. Because the chemical structure of ammonium is similar to that of methane, the transformation of SRAS process would also be similar to Eqs. (3) and (4). The possible half-reactions for SASR would be as follows:



The removal efficiency during the SRAS process might be limited by the intermediate hydrogen shuttle, ammonium-utilizing archaea activity and so on.

3.3. Batch experiment

3.3.1. Abiotic test

Three abiotic experiments contained different concentrations of ammonium and sulfate in synthetic water were carried out in serum bottles under anoxic condition. The ammonium concentration in the three abiotic experiments was about 50, 100 and 200 mg L⁻¹, and the sulfate concentration was about 150, 340 and 680 mg L⁻¹. Experiments were conducted under 35 °C for 2 days. The removal changes about ammonium and sulfate were less than 1 mg L⁻¹. So the pure chemical action between ammonium and sulfate without microorganism was not possible.

3.3.2. Biotic test

In order to further investigate the biochemical interaction between ammonium and sulfate, three biotic assays were performed to study the influence of ammonium and sulfate loading rate on the bio-reaction of nitrogen and sulfur. The experiments were carried out on the same conditions of temperature, but with variable ammonium and sulfate concentrations in the wastewater. Samples were taken after 2 days and ammonium and sulfate concentrations were measured, obtaining the results shown in Fig. 4b. As can be seen, when the influent concentrations of ammonium and sulfate are about 28 and 76 mg L⁻¹, the removal efficiency is very low. With the increasing loading concentration of ammonium and sulfate, the decreasing extent of concentration of it was enlarged. When the average influent concentrations of ammonium and sulfate were up to 92 and 307 mg L⁻¹, the removal amounts of ammonium and sulfate concentrations was 40, 130 mg L⁻¹. It indicates that high concentrations of ammonium and sulfate could promote simultaneous removal of ammonium and sulfate.

The increase of sulfur and nitrogen gas production in the batch experiment help to demonstrate the occurrence of the new interaction between N-S listed in Eq. (1). In the batch experiment, elemental sulfur and N₂ were also measured as shown in Fig. 4a. With increasing amount of sulfate removal, the sulfur production among the sludge solid was increased from 0 to 4.0 mg. At the same time, the sulfate consumption was increased from 1 to 130 mg L⁻¹. It indicated that some sulfate was efficiently converted to sulfur. The emission rate of N₂ collected in the bottle from the batch experiment was also showed an augmentative trend in Fig. 4a. N₂ emission was increased from 0 to 3.7 mg, while ammonium consumption varied from 0 to 40 mg L⁻¹. It also indicated that some ammonium was efficiently converted to N₂.

The SRAS process, including simultaneous removal of ammonium and sulfate, and the appearance of elemental sulfur and nitrogen gas as the terminal products, indicated the possibility of

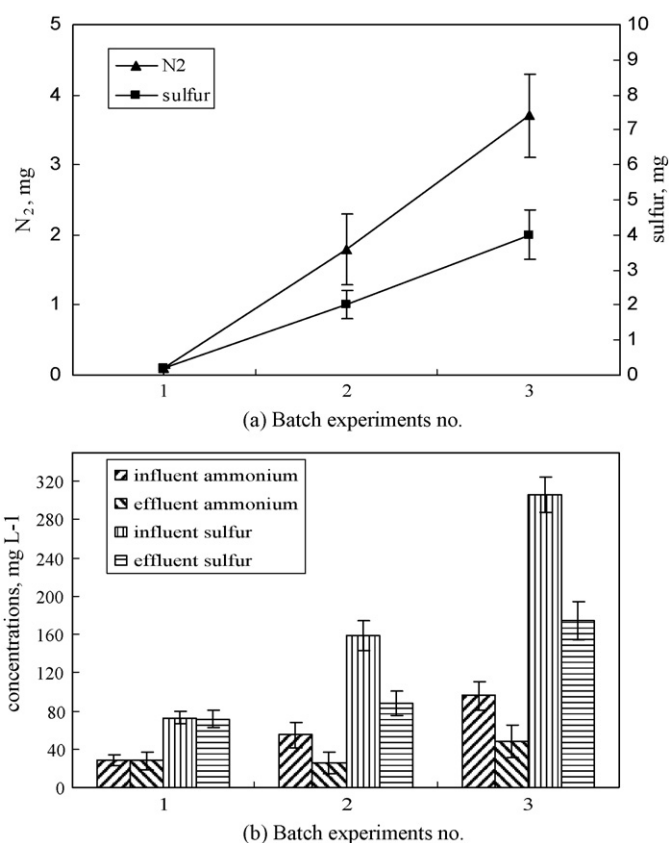
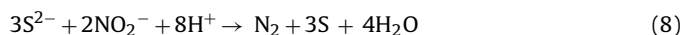
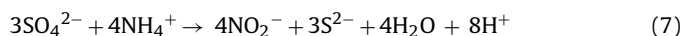


Fig. 4. Batch tests in various concentration of ammonium and sulfate (mg L⁻¹).

Eq. (1). Furthermore, Fdz-Polanco reported the Eq. (1) could be obtained by Eqs. (7)–(9). The half-reactions (5) and (6) combined into the Eq. (7), which showed the possibility of Eq. (7). The nitrite could be intermediate product during the SRAS process. Some denitrification process can be operated by the reduction of nitrites to nitrogen accompanied by the oxidation of sulfide by autotrophic denitrifiers [27], listed in Eq. (8). Eq. (9) was the reaction in the traditional anammox process. The three anaerobic ammonium oxidation reactions could be achieved during the SRAS process.



The removal efficiencies of ammonium and sulfate might be affected by some middle medium, such as nitrite, H₂S and sulfur. Strous et al. [28] reported a complete loss of anammox activity when the nitrite concentration remained above 5 mM (70 g NO₂⁻-N m⁻³) for a long period (12 h). Sulfide may be toxic to microorganisms [29]. The coverage of sludge by sulfur might limit the sufficient contact among reactants. In order to improve removal efficiency, further works might focus on reduction of the amounts of nitrite production, sound release of sulfured hydrogen and collection of sulfur from reaction.

All the biochemical processes involved can be represented by an oxidation–reduction or electron donor–acceptor scheme. ΔG^0 is the increment of free energy for the reaction under standard conditions, which are 25 °C and a pressure of 1 atm. Thauer et al. [30] believe that it is permissible to use ΔG^0 as the mean of $\Delta G'_{\text{initial}}$ and $\Delta G'_{\text{final}}$ for the assessment of the thermodynamics of the overall process in cultures of non-H₂-forming bacteria. The ΔG^0 value for Eq. (1) amounts to -44.3 kJ/mol calculated from Gibbs free energies of formation from the elements for compounds

of biological interest [31]. Taken together, these results, including experimental data and negative ΔG^0 of Eq. (1), showed the feasibility of simultaneous ammonium and sulfate bio-reaction.

4. Conclusions

The SRAS process was started up successfully in this experiment by changing nitrite into sulfate as electron acceptor after the stationary phase of anammox process. During the anammox process, anammox bacteria oxidized ammonium with nitrite as the electron acceptor to get energy, and the highest removal efficiency of ammonium and nitrite was up to 97 and 98%, respectively. In order to start up the SRAS process, the ammonium and sulfate in form of NH_4Cl and NaSO_4 was added into the synthetic water. The simultaneous removal of ammonium and sulfate was detected in the bioreactor after 60 days operation in the SRAS process. And the average effluent concentrations of ammonium and sulfate were 30 and 160 mg L^{-1} . The removal efficiencies of ammonium and sulfate were kept at about 40% and 30%, respectively. The molar ratio of ammonium to sulfate consumption was close to 2:1. The studies on the SRAS process helps to improve the application of wastewater treatment, and also might widen the cycle approach between elemental nitrogen and sulfur.

Acknowledgements

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