



Fine-tuning key parameters of an integrated reactor system for the simultaneous removal of COD, sulfate and ammonium and elemental sulfur reclamation



Ye Yuan^a, Chuan Chen^{a,*}, Bin Liang^a, Cong Huang^a, Youkang Zhao^a, Xijun Xu^a, Wenbo Tan^a, Xu Zhou^a, Shuang Gao^a, Dezhi Sun^a, Duujong Lee^{a,b}, Jizhong Zhou^{c,d,e}, Aijie Wang^{a,*}

^a State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, China

^b Department of Chemical Engineering, National Taiwan University of Science and Technology, Taipei 106, Taiwan

^c Institute for Environmental Genomics and Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK 73019, USA

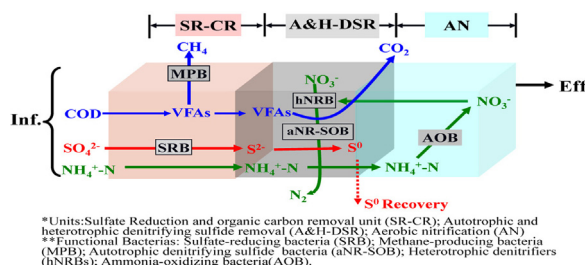
^d State Key Joint Laboratory of Environment Simulation and Pollution Control, School of Environment, Tsinghua University, Beijing 100084, China

^e Earth Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

HIGHLIGHTS

- The system achieved simultaneous removal of 98% COD, 98% sulfate and 78% nitrogen.
- The HRTs and reflux ratios were key parameters for performance and S⁰ recovery.
- 60% S⁰ reclaimed from effluent, 30% deposited in reactor S⁰ (S⁰ production ≈ 90%).
- Characteristics of bio-S⁰ were targeted to acquire high-rate S⁰ recovery.
- Microbial community succession and function were discussed at different stage.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 13 September 2013

Received in revised form

24 November 2013

Accepted 10 December 2013

Available online 16 December 2013

Keywords:

Sulfate reduction and organic carbon removal

Denitrifying sulfide removal

Elemental sulfur production

Aerated nitrification

Sulfur reclamation

ABSTRACT

In this paper, we proposed an integrated reactor system for simultaneous removal of COD, sulfate and ammonium (integrated C-S-N removal system) and investigated the key parameters of the system for a high level of elemental sulfur (S⁰) production. The system consisted of 4 main units: sulfate reduction and organic carbon removal (SR-CR), autotrophic and heterotrophic denitrifying sulfide removal (A&H-DSR), sulfur reclamation (SR), and aerated filter for aerobic nitrification (AN). In the system, the effects of key operational parameters on production of elemental sulfur were investigated, including hydraulic retention time (HRT) of each unit, sulfide/nitrate (S²⁻-S/NO₃⁻-N) ratios, reflux ratios between the A&H-DSR and AN units, and loading rates of chemical oxygen demand (COD), sulfate and ammonium. Physico-chemical characteristics of biosulfur were studied for acquiring efficient S⁰ recovery. The experiments successfully explored the optimum parameters for each unit and demonstrated 98% COD, 98% sulfate and 78% nitrogen removal efficiency. The optimum HRTs for SR-CR, A&H-DSR and AN were 12 h, 3 h and

* Corresponding authors at: State Key Laboratory of Urban Water Resource and Environment, School of Municipal and Environmental Engineering, Harbin Institute of Technology, P.O. Box 2650, 73 Huanghe Road, Nangang District, Harbin, Heilongjiang Province 150090, China. Tel.: +86 451 86282195; fax: +86 451 86282195.

E-mail addresses: yuan_ye_19840915@163.com, yuan_ye_19840915@aliyun.com (Y. Yuan), echo110244@126.com (C. Chen), liangbin1214@163.com (B. Liang), hengyue5257@163.com (C. Huang), zhaoyoukang@gmail.com (Y. Zhao), xuxijun3220@sina.com (X. Xu), tanwenbo1@163.com (W. Tan), x.zhou@awmc.uq.edu.au (X. Zhou), localinna.1990@163.com (S. Gao), sundezhi@bjfu.edu.cn (D. Sun), djlee@ntu.edu.tw (D. Lee), jzhou@ou.edu (J. Zhou), waj0578@hit.edu.cn (A. Wang).

0304-3894/\$ – see front matter © 2013 Elsevier B.V. All rights reserved.

<http://dx.doi.org/10.1016/j.jhazmat.2013.12.014>

3 h, respectively. The reflux ratio of 3 could provide adequate S^{2-} - S/NO_3^- -N ratio (approximately 1:1) to the A&H-DSR unit for obtaining maximum sulfur production. In this system, the maximum production of S^0 reached 90%, but only 60% S^0 was reclaimed from effluent. The S^0 that adhered to the outer layer of granules was deposited in the bottom of the A&H-DSR unit. Finally, the microbial community structure of the corresponding unit at different operational stage were analyzed by 16S rRNA gene based high throughput Illumina MiSeq sequencing and the potential function of dominant species were discussed.

© 2013 Elsevier B.V. All rights reserved.

Abbreviation

Reactors

Integrated C-S-N removal system	integrated reactor system for the simultaneous removal of COD, sulfate and ammonium
SR-CR unit	sulfate reduction and organic carbon removal unit (the first unit of system)
A&H-DSR unit	autotrophic and heterotrophic denitrifying sulfide removal unit (the second unit of system)
SR unit	sulfur reclamation (the third unit of system)
AN unit	aerobic nitrification filter (the fourth unit of system)
EGSB	up-flow anaerobic sludge bed
BAF	biological aerated filter

Parameters

COD	chemical oxygen demand
TOC	total organic carbon
HRT	hydraulic retention time
VVS/TSS	volatile suspended solids/total suspended solids
S^{2-} - S/NO_3^- -N ratios	sulfide-S/nitrate-N ratios
Org-C/ S^{2-} -S ratios	TOC/sulfide-S (molar concentration ratio)
S^0	bio-produced elemental sulfur
DS	dissolved sulfide
CLR	organic loading rate
SLR	sulfate loading rate
VFA	volatile fatty acid
FA	free ammonia

Bacteria

SRB	sulfate-reducing bacteria
MPB	methane-producing bacteria
aNR-SOB	autotrophic denitrifying sulfide bacteria
hNRB	heterotrophic denitrifiers
AOB	ammonia-oxidizing bacteria

1. Introduction

Many industries (pharmaceutical companies, food-making factories, etc.) usually have high concentrations of sulfate, ammonium and carbon compounds in their effluents, which can contaminate the receiving waters, cause eutrophication and endanger life if these emissions not well-treated before discharge [1–3].

Conventional treatment of wastewater containing sulfur and nitrogen focus mainly on the removal of ammonium and sulfate in separate processes [4–6]. Recent years, some investigations have been focused on the autotrophic processes for simultaneous removal of ammonium and sulfate in a single bioreactor [7–9]. In their studies, they found that sulfate as acceptor of the electrons involved in the anaerobic ammonium oxidation (ANAM-MOX) process under inorganic condition. However, it also has been reported that presence of organic matter adversely affected ANAM-MOX process and could slow down anaerobic ammonium removal [10,11].

However, chemical oxygen demand (COD), ammonium and sulfate are often discharged together in industrial wastewater. Therefore, an innovative process capable of simultaneously removing ammonium and sulfate under organic condition is worthy of consideration. Wang et al. [12] developed an integrated sulfate-reduction, autotrophic denitrification and nitrification system (SANI) to treat the sulfate, ammonium and COD-containing saline sewage. In their study, COD (lower concentration) was fully used by sulfate-reducing bacteria (SRB) to produce sulfide in the first anaerobic zone, an autotrophic denitrification zone subsequently converted sulfide and nitrate (recycled from an aerobic zone) to sulfate and N_2 . A combined nitrifying/autotrophic denitrifying system was used by Carmen et al. [13] for the post-treatment of an effluent (containing sulfide and ammonium) coming from an anaerobic digester treating the wastewater produced in a fish canning industry. In this system, sulfide in autotrophic denitrifying unit was used as electron donor to reduce the nitrate generated in the nitrification step. However, besides sulfide, a lot of organic acids (such as acetic and propionic) are often present in the effluent of sulfate reduction.

Therefore, a subsequent unit that can simultaneous removal of sulfide, nitrate, and COD will widen its application. Reyes-Avila et al. [14], Manconi et al. [15] and Chen et al. [16] developed a denitrifying sulfide removal (DSR) process which could simultaneously convert sulfide, nitrate, and COD into elemental sulfur (S^0), nitrogen gas (N_2), and carbon dioxide (CO_2) based on the cooperative work of the autotrophic and heterotrophic denitrifiers. The bio-produced elemental sulfur, in the form of colloidal micro-particles in the effluent, is easy to recover by precipitation [17,18]. The production of alkalinity in the DSR process can be supplemented for the post-nitrification process to lower the operational cost. In addition, alkalinity can keep suspension pH high so to minimize release of hydrogen sulfide (H_2S) from the SR-CR and DSR units. Meanwhile, the DSR process requires a special influent quality due to TOC/ NO_3^- and S^{2-}/NO_3^- ratio limitations [14,16].

Based on the above characteristics of the DSR process, we proposed a novel integrated reactor system for simultaneous removal of COD, sulfate and ammonium (the description of the system in Section 2.1). In this system, we can adjust operational parameters of each unit to provide appropriate S/N/C ratios for the DSR process and widen its practical application. Sulfate reduction can lead to a low sludge yield because the growth yield of SRB is only 0.2 g VSS g^{-1} reduced sulfate [6,19,20]. There is no need to add a sludge return setup after the integrated system because autotrophic nitrifying biofilm have low growth yields. All these will help decrease the operational cost.

The work was divided into two phases: first, the synthetic wastewater was treated by the integrated system on the laboratory scale. Second, the pilot test of the system treating food industry wastewater will be carried out at the Daoli food factory in Harbin, China. This paper reports on the laboratory study. The main objectives of this study were to (1) find a balance between sulfate-reducing and methane-producing efficiency of the SR-CR unit, (2) adjust the reflux ratios between A&H-DSR and AN units, (3) recover elemental sulfur effectively from the system, and (4) analyze the microbial community structure and function of dominant species of the corresponding unit at different operational stage.

2. Materials and methods

2.1. Experimental setup and startup

Fig. 1 shows the laboratory-scale integrated C-S-N removal system. It consists of 4 units: (a) sulfate reduction and organic carbon removal unit (SR-CR), using expanded granular sludge bed (EGSB) reactor to reduce sulfate to sulfide and remove COD by methane-producing bacteria (MPB) and sulfate-reducing bacteria (SRB); (b) autotrophic and heterotrophic denitrifying sulfide removal unit (A&H-DSR), also with EGSB reactor for denitrifying sulfide removal. As the center, the autotrophic denitrifiers (aNR-SOB) converts sulfide to elemental sulfur using nitrate recirculated from the effluent of AN unit as an electron acceptor (NO_3^- to NO_2^-), while the heterotrophic denitrifiers (hNRB) utilize the residual fatty acids generated (VFA) from the SR-CR unit for denitrification (NO_2^- to N_2); (c) sulfur reclamation unit (SR), a sedimentation tank to collect elemental sulfur before the effluent of the A&H-DSR unit enters into the AN unit; (d) aerobic nitrification (AN) unit, biological aerated filter (BAF) was used to nitrify ammonia to nitrate and then recirculated to the A&H-DSR unit for denitrifying sulfide removal.

The plexiglass EGSB reactor was a modified version of the reactor developed by Chen et al. [21]. The EGSB for A&H-DSR unit was identical in appearance and size with the SR-CR unit. The reactor was 50 mm in diameter and 120 cm high with a working volume of 5.0 L. A thermostat (SHINKO model no. PCD-33A) automatically ensured stable measuring temperatures at $30 \pm 1^\circ\text{C}$. A peristaltic pump (Longer model no. BT100-2J) introduced influent into the reactor at the column bottom. A gas-washing device collected the H_2S gas that was generated at the column top. The gas production rate was measured in a wet type of gas meter (SHINAGAWA model no. WS-1A). The biological aerated filter (BAF) was 20 cm in diameter and 30 cm high with a working volume of 5.0 L. As support for immobilization of the biomass, 10 mm polyurethane foam cubes with an apparent density of 23 kg m^{-3} and porosity near 95% were used. The supporting materials were packed to a filtration layer depth of 15 cm in the reactor. Wastewater was fed to the reactor in the up-flow mode using a peristaltic pump (Longer model no. BT100-2J).

2.2. Inoculations and synthetic wastewater

2.2.1. Inoculations

All reactors were initially inoculated with 3 L of activated sludge with biomass VSS of 1.75 g L^{-1} (VVS/TSS (Volatile suspended solids/Total suspended solids)=0.72). The activated sludge was obtained from Taiping Wastewater Treatment Plant in Harbin, China. Seed sludge was screened using 0.2 mm Tyler mesh to remove most grids.

2.2.2. Synthetic wastewater

The wastewater of Daoli food factory (in Harbin, China) mainly contain organic matter (average COD concentration of 2000 mg L^{-1}), sulfate (average SO_4^{2-} concentration of 1500 mg L^{-1}) and ammonium (average $\text{NH}_4^+\text{-N}$ concentration of 300 mg L^{-1}). During food processing and storage, sodium lactate is used as anti-septic, flavoring and freshening agent, which form main organic matter in the wastewater.

But, the unstable wastewater is not suitable for the initial study. To simulate the characteristics of the wastewater in terms of COD, sulfate and ammonium concentration, sodium lactate, sodium sulfate and ammonium chloride was added to tap water to achieve desired influent concentrations of COD, sulfate and ammonium ($2000 \text{ mg COD L}^{-1}$, $1500 \text{ mg SO}_4^{2-} \text{ L}^{-1}$ (500 mg S L^{-1}) and $300 \text{ mg N L}^{-1} \text{ NH}_4^+\text{-N}$). This stable wastewater can help preliminary exploration for operational parameters.

During the period of start-up of SR-CR unit, the sulfate-laden synthetic wastewater consisting of SO_4^{2-} at 1500 mg L^{-1} and COD at 2000 mg L^{-1} was used as the influent to cultivate the functional bacteria. Bicarbonate was employed to maintain the influent pH at 8.5 ± 0.3 . During the period of start-up of A&H-DSR unit, the sulfide-laden synthetic wastewater was fed into the A&H-DSR reactor, including the following composition: 500 mg L^{-1} of S^{2-} , 218 mg N L^{-1} of NO_3^- , 256 mg C L^{-1} of acetate. During the period of start-up of AN unit, tap water with $(\text{NH}_4)\text{HCO}_3$ and $\text{K}_2\text{H}(\text{PO}_4)$ added was used as the ammonium synthetic wastewater. The main water quality indicators were pH of 6.8–8.5, $\text{NH}_4^+\text{-N}$ concentration of 300 mg L^{-1} , initial dissolved oxygen (DO) of $1.5\text{--}2.0 \text{ mg L}^{-1}$, and temperature of $25 \pm 1^\circ\text{C}$. During the stage of integrative operation of SR-CR, A&H-DSR and AN units, the synthetic wastewater containing COD, sulfate and ammonium ($2000 \text{ mg COD L}^{-1}$, $1500 \text{ mg SO}_4^{2-} \text{ L}^{-1}$ and $300 \text{ mg N L}^{-1} \text{ NH}_4^+\text{-N}$) as the influent was fed into the system.

2.3. Reactor system operating procedures

2.3.1. Stage I: separate operation of SR-CR, A&H-DSR and AN unit

At stage I (1–70 days), the SR-CR and A&H-DSR units were respectively inoculated by feeding the sulfate-laden and sulfide-laden synthetic wastewater at HRT of 24 h. Internal circulating fluid was applied to suspend the sludge and make well mixing conditions in the reactor. In the same period, the AN unit was continuously fed with the ammonium synthetic wastewater at HRT of 24 h. In this stage, when the effluents of the three units became stable, the HRT would further reduced to improve the operational loading rate. The operation conditions and performance of the three units in these runs are shown in Table 1.

2.3.2. Stage II: integrative operation of SR-CR and A&H-DSR unit

At stage II (70–120 days), as the SR-CR and A&H-DSR unit obtained a stable performance, the A&H-DSR unit was joined to the SR-CR unit at HRT of 12 h and received its effluent with the addition of $220 \text{ mg NO}_3^-\text{-N L}^{-1}$. When the integration became stable, the HRT would be further reduced to maximize its loading rate. Meanwhile, the cultivation of the AN unit was also continued, the HRT of the unit was gradually reduced from 12 h to 6 h to increase the ammonium loading rate.

2.3.3. Stage III: integrative operation of SR-CR, A&H-DSR and AN units

The AN unit biomass cultivation lasted for 120 days before the full nitrification capability was obtained and the unit was then connected to the integrated processes of the SR-CR and A&H-DSR units to establish a completely integrated C-S-N removal system (see Fig. 1). The complete system was continuously fed with the synthetic wastewater containing COD, sulfate and ammonium. In this stage, to provide maximum dissolved sulfide for the A&H-DSR unit, the SR-CR unit was operated with a fixed HRT of 12 h which was determined during the stage II. The overall performance of the complete system in terms of sulfide and nitrogen removal was investigated at different reflux ratios between the A&H-DSR unit and AN unit for improving the nitrogen removal and elemental sulfur production of the system.

2.4. Analytical methods

The concentrations of nitrate, nitrite, ammonium, sulfate, and thiosulfate in the collected liquor samples following $0.45\text{-}\mu\text{m}$ filtration were measured by ion chromatography (Dionex model no. ICS-3000) equipped with a conductivity detector and an Ion-Pac AG4A AS4A-SC 4 mm analytical column. The total dissolved sulfide ($\text{H}_2\text{S}_{(\text{aq})}$, HS^- and S^{2-}), COD, total suspended solids (TSS)

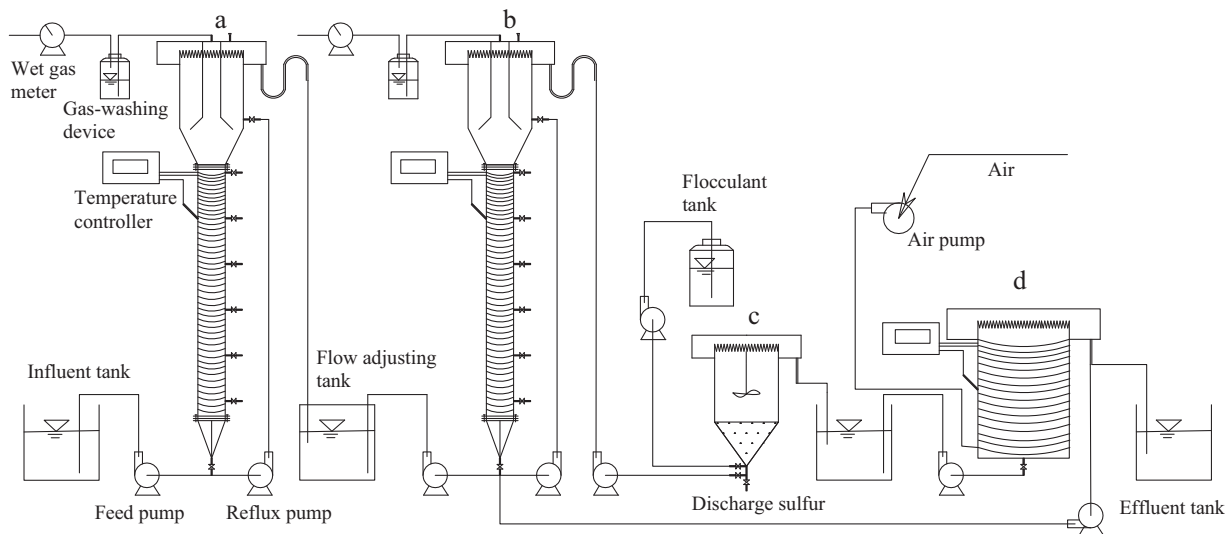


Fig. 1. A schematic diagram of the experimental setup of the C-N-S system. (a) Sulfate reduction and organic carbon removal (SR-CR), (b) autotrophic and heterotrophic denitrifying sulfide removal (A&H-DSR), (c) sulfur reclamation (SR), and (d) aerobic nitrification filter (AN).

Table 1
The operating conditions of each unit under different stage.

Unit	Run	Stage I	Stage II	Stage III
SR-CR	HRT (h)	24 → 18 → 12	12 → 8 → 12	12
	Organic loading rate (kg TOC m ⁻³ d ⁻¹)	0.8 → 1.07 → 1.6	1.6 → 2.44 → 1.6	1.6
	Sulfate loading rate (kg SO ₄ ²⁻ -S m ⁻³ d ⁻¹)	0.5 → 0.67 → 1	1 → 1.5 → 1	1
A&H-DSR	HRT (h)	24 → 18 → 12	12 → 8 → 12	See Table 3
	Organic loading rate (kg TOC m ⁻³ d ⁻¹)	0.19 → 0.25 → 0.38	0.46 → 1.6 → 0.46	
	Sulfide loading rate (kg S ²⁻ m ⁻³ d ⁻¹)	0.5 → 0.66 → 1.0	0.9 → 1 → 0.9	
	Nitrate loading rate (kg N m ⁻³ d ⁻¹)	0.22 → 0.3 → 0.44	0.44 → 0.66 → 0.44	
AN	HRT (h)	24 → 18 → 12	12 → 6	See Table 3
	Ammonium loading rate (kg N m ⁻³ d ⁻¹)	0.3 → 0.4 → 0.6	0.6 → 1.2	

and volatile suspended solids (VSS) were determined according to standard methods [22], while available alkalinity was determined by direct titration method [23,24]. Total organic carbon (TOC) was determined with a TOC analyzer (Shimadzu model no. TOC-VCPH). Reducing agents (such as dissolved sulfide ions) interfere with the COD measurements. TOC can truly reflect the organic strength of the samples. Volatile fatty acid (VFA) concentrations and species were determined by an HPLC system (Shimadzu model no. LC-10A). A Ph/ORP meter (METTLER TOLEDO model no. FE20) determined the pH and oxidation–reduction potential (ORP) of the liquid samples. Dissolved oxygen (DO) was measured with a DO meter (Hanna model no. HI9146N). The compositions of gas (N₂, N₂O, CO₂ and CH₄) were analyzed using a gas chromatography (Agilent, model no. 6890). Elemental sulfur in the effluent analysis was determined by the method described by Jiang et al. [25]. Elemental sulfur in the sludge was determined by the method mentioned by Ravishankar et al. [26]. The S⁰ conversion rate was calculated according to the following equation (Eq. (1)) [27]:

$$[S^0] = [\text{Influent S}] - [\text{SO}_4^{2-}] - 2 * [\text{S}_2\text{O}_3^{2-}] - [\text{HS}^-] \quad (1)$$

The equation is used to calculate S⁰ concentration according to sulfur balance.

$$\begin{aligned} \text{Produced } S^0 \text{ mg}^{-1} \text{ L} \\ = [\text{Total S mg}^{-1} \text{ L}]_{\text{Influent}} - [\text{SO}_4^{2-} \text{ S mg}^{-1} \text{ L} \\ + 2 * (\text{S}_2\text{O}_3^{2-}) \text{ S mg}^{-1} \text{ L} + \text{HS}^- \text{ S mg}^{-1} \text{ L}]_{\text{Effluent}} \end{aligned}$$

2.5. Illumina MiSeq sequencing and data analysis

The details for the DNA extraction, 16S rRNA gene PCR amplification and Illumina MiSeq sequencing, and data analysis are available in the Supplemental Material (part 1). Sampling information is shown in Table 2.

3. Results and discussion

3.1. Separate operation of each unit in the integrated C-S-N removal system

3.1.1. SR-CR

The performance of the SR-CR unit was reported in Fig. 2a–c. The SR-CR unit was initially operated at HRT of 24 h (0–30 days) and achieved 40% of COD removal rate and 49% of TOC removal rate. 93% of the sulfate was reduced to dissolved sulfide (DS). The HRT was then reduced to 18 h (31–50 days), and the COD loading rate was increased from 2.00 to 2.67 kg COD m⁻³ d⁻¹. The COD and TOC removal increased to 48% and 60%, respectively. The sulfate loading rate (SLR) was increased from 0.5 to 0.67 kg SO₄²⁻-S m⁻³ d⁻¹, and 90% of sulfate was reduced to dissolved sulfide.

Table 2
The sampling conditions in different stage.

Unit	Sample name	Sampling time	HRT	Operation
SR-CR	SR-CR1	Stage I (day 30)	24 h	Separate
	SR-CR2	Stage II (day 120)	12 h	Integrative
A&H-DSR	DSR1	Stage I (day 30)	24 h	Separate
	DSR2	Stage II (day 120)	12 h	Integrative

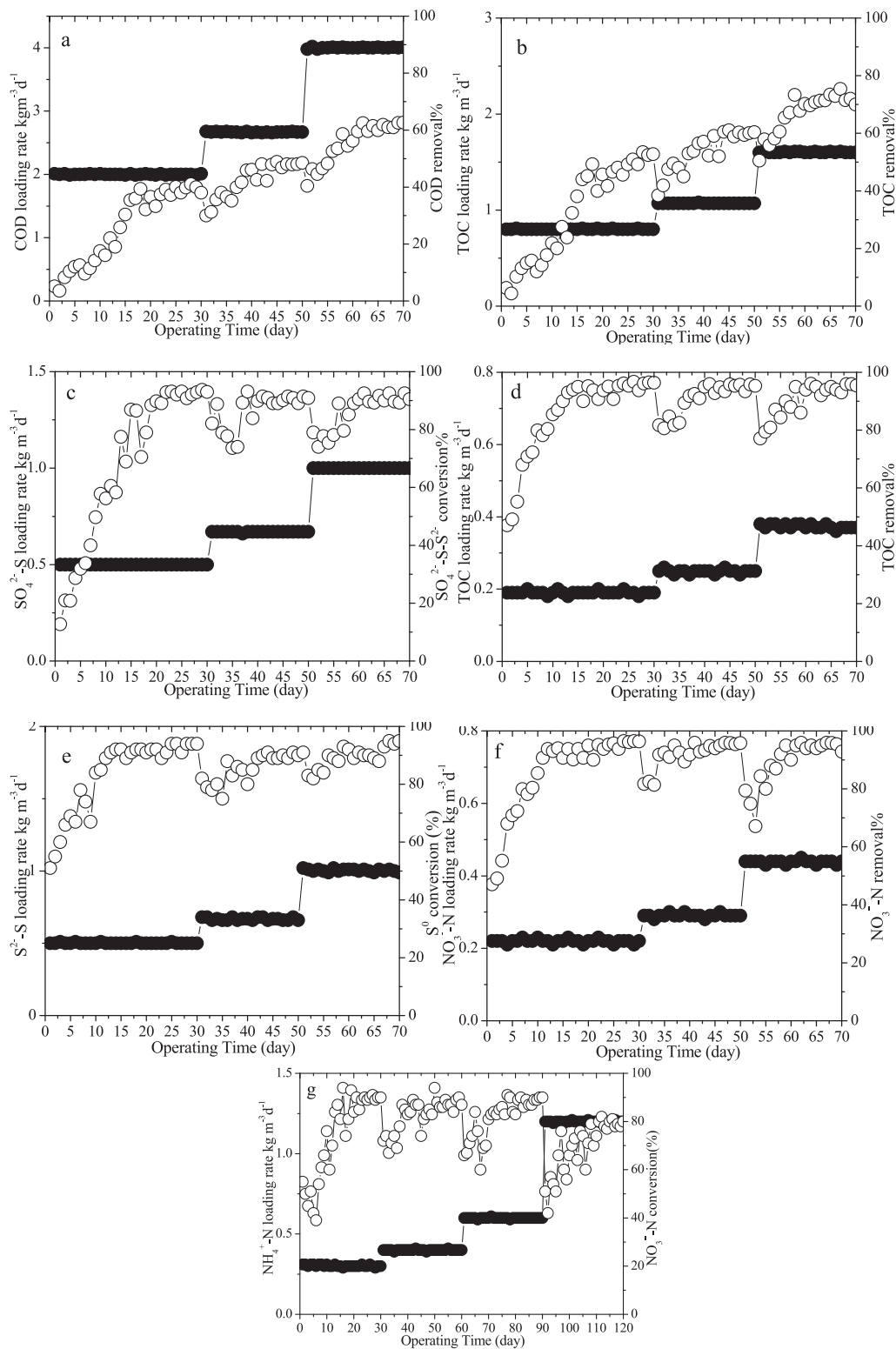


Fig. 2. The performance of separate operation of the SR-CR, A&H-DSR and AN unit. SR-CR: (a) COD removal, (b) TOC removal, (c) $\text{SO}_4^{2-}\text{-S} \rightarrow \text{S}^{2-}$ conversion rate; A&H-DSR: (d) TOC removal, (e) $\text{S}^{2-}\text{-S} \rightarrow \text{S}^0$ conversion rate, (f) $\text{NO}_3^- \text{-N}$ removal; AN: (g) $\text{NH}_4^+ \text{-N} \rightarrow \text{NO}_3^- \text{-N}$ conversion rate.

When the HRT of the SR-CR unit was further reduced to 12 h (51–70 days), the COD and TOC loading rate increased to $4.00 \text{ kg COD m}^{-3} \text{ d}^{-1}$ and $1.60 \text{ kg TOC m}^{-3} \text{ d}^{-1}$, respectively. The reactor gradually began to produce methane, and the production of methane reached a maximum of $0.31 \text{ m}^3 \text{ m}^{-3} \text{ d}^{-1}$. Therefore, the COD and TOC removal increased to 63% and 70%. Lactate as the

organic carbon source could be degraded to acetate easily by acid-forming bacteria. VFAs in the effluent of the SR-CR consisted mainly of acetate, which accounted for 90% of the total organic carbon (figure not show). MPB was also found to outcompete SRB gradually at a volumetric loading of approximately $1.3 \text{ kg Ac}^- \text{-C m}^{-3} \text{ d}^{-1}$ [28].

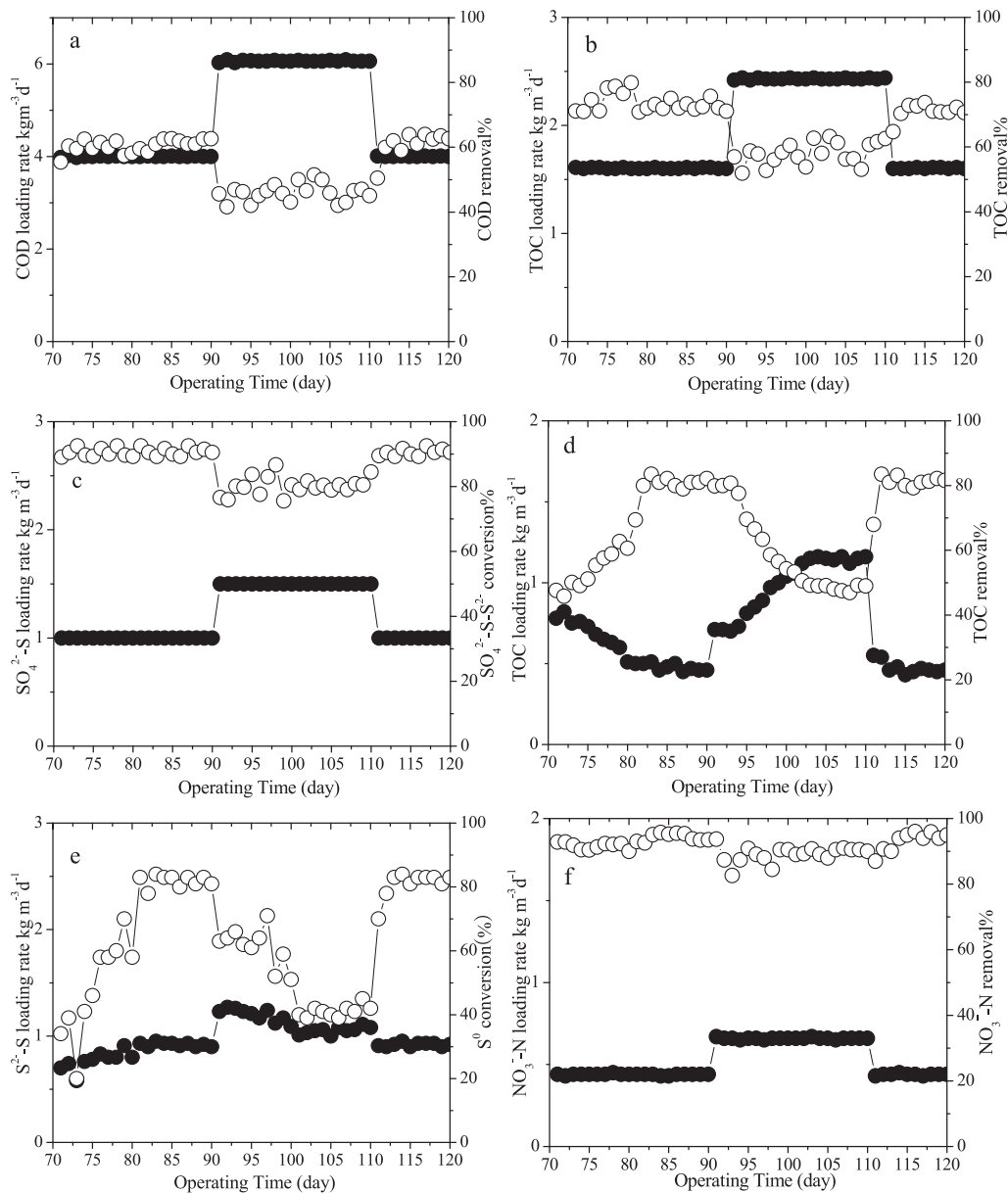


Fig. 3. The performance of integrative operation of SR-CR and A&H-DSR unit. SR-CR: (a) COD removal, (b) TOC removal, (c) $\text{SO}_4^{2-}\text{-S} \rightarrow \text{S}^0$ conversion; A&H-DSR: (d) TOC removal, (e) $\text{S}^{2-}\text{-S} \rightarrow \text{S}^0$ conversion rate, (f) $\text{NO}_3^- \text{-N}$ removal.

Meanwhile, the SLR was increased from 0.67 to $1 \text{ kg SO}_4^{2-}\text{-S m}^{-3} \text{ d}^{-1}$, the SR-CR unit still maintained 90% of sulfate reduction. In this study, converting the majority of the sulfate into dissolved sulfide in the SR-CR unit could ensure high S^0 production rate in the A&H-DSR unit. The $\text{Org-C/S}^{2-}\text{-S}$ ratio in the effluent of the SR-CR unit was the critical parameter for obtaining the maximum S^0 conversion rate from the A&H-DSR unit. Sulfide detected in the SR-CR unit contained three species (H_2S , HS^- , and S^{2-}), and pH adjustment would influence the dominant type of the three species. The undissociated sulfide (H_2S) could cause the inhibition of SRB and MPB activity [29]. When lowering the H_2S concentration to the threshold in the anaerobic reactor, the activity of methanogens could be protected [30]. The toxicity of the sulfide depended critically on the pH value because only the free H_2S is able to pass through the cell membrane [31]. When the pH was between 8 and 9, virtually all dissolved sulfide was in the ionized state. At a neutral pH value, approximately 20% to 50% of the dissolved sulfide was present as the free H_2S .

3.1.2. A&H-DSR

The performance of the A&H-DSR unit was reported in Fig. 2d–f. The DSR-EGSB reactor performed well at HRT of 24 h (0–30 days) and 18 h (31–50 days) because the sulfide removal reached 96% at loading rates of 0.50 and $0.67 \text{ kg S}^{2-} \text{ m}^{-3} \text{ d}^{-1}$. At this time, this unit performed by a synergistic relationship of both autotrophic and heterotrophic denitrifiers. The TOC and nitrate removal reached 96% both at HRT of 24 h and 18 h. 93% of the dissolved sulfide was converted to elemental sulfur (S^0). On day 70, mature granules were formed in the reactor and developed to $0.5\text{--}2.0 \text{ mm}$ in diameter, being covered with a layer of elemental sulfur.

TOC, sulfide and nitrate removal reached over 96% even when the HRT reduced to 12 h (51–70 days). CO_2 and N_2 were noted in the gas phase of the unit. Meanwhile, negligible amounts of N_2O and H_2S were detected in the GC spectra.

3.1.3. AN

The performance of the AN unit was reported in Fig. 2g. The unit was initially subjected to a continuous feed of ammonium

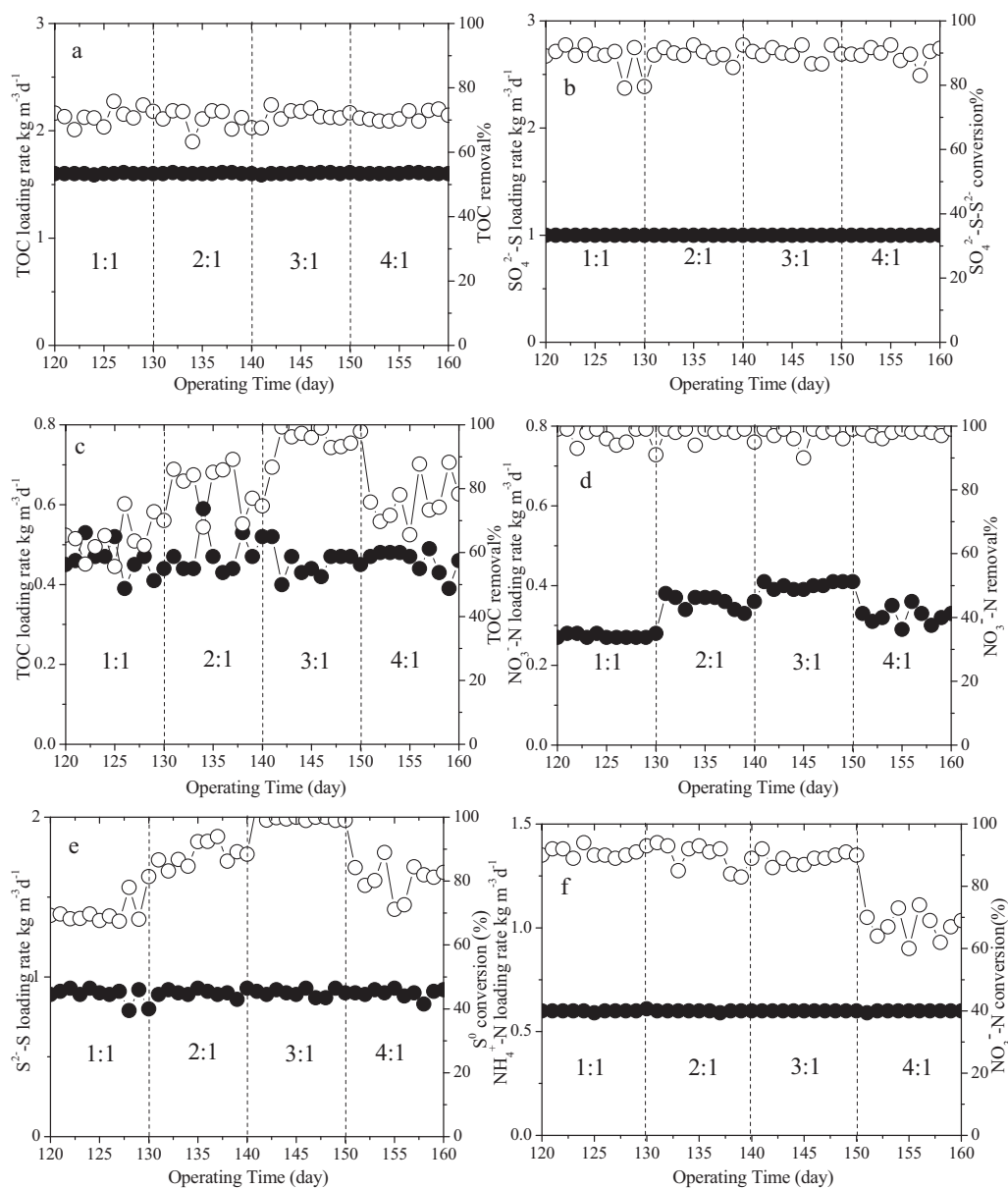


Fig. 4. The performance of the integrated C-S-N removal system under various recirculation ratios. SR-CR: (a) TOC removal, (b) SO_4^{2-} -S \rightarrow S^{2-} conversion; A&H-DSR: (c) TOC removal, (d) NO_3^- -N removal, (e) S^{2-} -S \rightarrow S^0 conversion rate; AN: (f) NH_4^+ -N \rightarrow NO_3^- -N conversion rate.

synthetic wastewater with initial HRT of 24 h (0–30 days) and gradually reduced to 18 h (31–60 days) to increase the NH_4^+ -N loading. The unit performed well during the cultivation period as the nitrification rate reached more than 90% at a loading rate of 0.6 kg NH_4^+ -N $\text{m}^{-3} \text{d}^{-1}$ (61–90 days) When the pH value in the reactor was adjusted to 7, the concentration of free ammonia (FA) in the reactor was less than 5 mg L^{-1} , and nitrification was not inhibited. When HRT was reduced to 8 h (91–120 days), the production of nitrate decreased from 90% to 80%.

3.2. Combination of each unit in the integrated C-N-S removal system

3.2.1. Integrative operation of SR-CR and A&H-DSR unit

During period of start-up, high sulfate-reducing and denitrifying sulfide removal efficiency were obtained in the SR-CR and A&H-DSR units, respectively. From day 71 (Fig. 3a–f), the A&H-DSR unit was joined to the SR-CR unit and received its effluent with the addition of 218 mg NO_3^- -N L^{-1} . As the integrated operation continued

(71–90 days), the removal rate for nitrate and TOC in the A&H-DSR unit increased from 92% and 48% to 96% and 82%, respectively. The S^0 conversion rate increased considerably from 34% to 81%. When the HRT of the SR-CR and A&H-DSR units was shortened from 12 h to 8 h (91–110 days), the TOC removal and sulfate reduction rate in the SR-CR decreased from 70 to 50% and 93 to 80%. Meanwhile, the corresponding removal for nitrate and TOC in the A&H-DSR unit decreased from 96% and 82% to 90% and 49%, respectively. The S^0 conversion rate decreased considerably from 80% to 42%. The optimum HRT for the SR-CR and A&H-DSR unit was therefore 12 h (111–120 days). The SR-CR unit could provide appropriate concentrations of TOC and dissolved sulfide to realize a maximum S^0 conversion rate in the A&H-DSR unit.

Chen et al. [16,21] argued that factors affecting the removal of TOC, sulfide and nitrite and S^0 conversion rate in the DSR process included the S^{2-} -S/ NO_3^- -N ratio, TOC/ NO_3^- -N ratio and pH. The optimum parameters for the maximum S^0 conversion rate were as follows: pH near 7.5, S^{2-} -S/ NO_3^- -N ratio near 1:1 and TOC/ NO_3^- -N ratio near 1.26:1. Chen et al. [32] argued that

Table 3
The performance of integrated C-S-N removal system under various recirculation ratios.

Parameters	I	II	III	IV
Reflux ratios of nitrification	1:1	2:1	3:1	4:1
HRT of each unit of the system (h)	12 h; 6 h; 6 h	12 h; 4 h; 4 h	12 h; 3 h; 3 h	12 h; 2.4 h; 2.4 h
SR-CR; A&H-DSR; AN				
Influent TOC of SR-CR unit ($\text{kg TOC m}^{-3} \text{d}^{-1}$)	1.6 ± 0.01	1.6 ± 0.09	1.6 ± 0.07	1.6 ± 0.05
Effluent TOC of AN unit ($\text{kg TOC m}^{-3} \text{d}^{-1}$)	0.13	0.04	0.004	0.1
TOC removal efficiency in system (%)	92 ± 1.4	97 ± 0.7	98 ± 3.1	90 ± 5.6
COD removal efficiency in system (%)	93 ± 2.9	97 ± 1.2	98 ± 2.7	90 ± 7.9
Influent SO_4^{2-} -S of SR-CR unit ($\text{kg SO}_4^{2-}\text{-S m}^{-3} \text{d}^{-1}$)	1	1	1	1
Effluent SO_4^{2-} -S of AN unit ($\text{kg SO}_4^{2-}\text{-S m}^{-3} \text{d}^{-1}$)	0.23	0.14	0.02	0.25
SO_4^{2-} -S removal efficiency in system (%)	77 ± 4.4	86 ± 2.7	98 ± 1.8	75 ± 2.4
S^0 conversion rate in DSR unit (%)	80 ± 1.4	89 ± 2.7	99 ± 0.06	78 ± 3.5
Influent TN of SR-CR unit ($\text{kg N m}^{-3} \text{d}^{-1}$)	0.6	0.6	0.6	0.6
Effluent TN of AN unit ($\text{kg N m}^{-3} \text{d}^{-1}$)	0.31	0.2	0.16	0.13
TN removal efficiency in system (%)	49 ± 4.1	66 ± 2.9	74 ± 4.2	79 ± 3.7
Nitrification efficiency in AN (%)	91 ± 5.4	90 ± 7.1	90 ± 4.7	69 ± 9.1
Nitrate removal in A&H-DSR (%)	94 ± 5.6	96 ± 7.8	96 ± 3.7	95 ± 2.1

$\text{TOC}/\text{NO}_3^- \text{-N} = 0.75:1$ (in mol) was the limiting ratio. At $\text{TOC}/\text{NO}_3^- \text{-N} > 2:1$, the nitrate removal rate remained at 100%. However, the TOC removal declined from 95% to 60%, and the S^0 conversion rate declined from 0.46 to $0.17 \text{ kg S m}^{-3} \text{d}^{-1}$. Our study also confirmed that when the $\text{TOC}/\text{NO}_3^- \text{-N}$ ratio increased to over 2.0 (HRT = 8 h, 91–110 days), the S^0 conversion rate decreased from 80% to 40%. When TOC was present in excess, heterotrophic denitrifiers dominated the autotrophic denitrifiers for the reduction of nitrate as electron donors, thereby yielding a low S^0 production rate [13,32].

3.2.2. Integrative operation of SR-CR, A&H-DSR and AN units

After 120 days of startup, the AN unit could provide sufficient nitrate to the A&H-DSR unit which was subsequently joined to the integrated processes of the SR-CR and A&H-DSR units to establish a completely integrated C-S-N removal system. In the system, the optimum influent loading of TOC and sulfate was $1.6 \text{ kg TOC m}^{-3} \text{d}^{-1}$ and $1 \text{ kg SO}_4^{2-}\text{-S m}^{-3} \text{d}^{-1}$ in the SR-CR unit (HRT = 12 h). The A&H-DSR unit simultaneously removed dissolved sulfide produced in the SR-CR unit and nitrate recycled from the AN unit.

Ammonium could not be degraded in the former two anaerobic units and was indirectly introduced into the AN unit to generate nitrate via nitrification. Therefore, part of the treated wastewater from the AN unit needed to be returned to the A&H-DSR unit. The different recirculation ratios between the A&H-DSR and AN units would directly affect the removal of sulfide and nitrate and the S^0 conversion rate in the A&H-DSR unit. The effects of different reflux ratios on the performance of the system were examined, and the results are shown in Fig. 4 and summarized in Table 3 (reflux ratio (n) = recirculation flow (nQ)/influent flow of the system (Q)).

As shown in Fig. 4, the reflux ratio variation had little influence on the nitrate removal of the system. The TOC removal improved

from 65% to 99% in the A&H-DSR unit when R was from 1 to 3Q. Meanwhile, S^0 production rate increased from 69% to 99% in the A&H-DSR unit. However, when R was improved from 3 to 4Q, the TOC removal and S^0 production rate were reduced to 78 and 79%, respectively, resulting in a low total nitrogen removal of 62%. The reason of reduction of total nitrogen removal could be related to enhanced oxygen input in the AN unit or the occurrence of a short-cut flow at the higher flow velocity. Hence, $\text{S}^{2-}\text{-S}/\text{NO}_3^- \text{-N}$ ratio would be affected by reflux ratios and led to a low S^0 production.

The optimum reflux ratio between the A&H-DSR and AN units was 3:1, and both HRT of the A&H-DSR and AN units were 3 h. Under these operational parameters, the system successfully demonstrated 98% COD, 98% sulfate and 78% nitrogen removal efficiency. At reflux ratio of 3:1, $\text{S}^{2-}\text{-S}/\text{NO}_3^- \text{-N}$ ratio in the A&H-DSR unit achieved 1:1 which was the optimum $\text{S}^{2-}\text{-S}/\text{NO}_3^- \text{-N}$ ratio for obtaining maximum S^0 production.

3.3. Nitrogen, carbon and sulfur balance

Nitrogen, carbon, and sulfur balance and S^0 production rate were investigated under optimal operating parameters mentioned above. In the system (Fig. 5), an average 90% of $\text{SO}_4^{2-}\text{-S}$ in the influent of the SR-CR was reduced to dissolved $\text{S}^{2-}\text{-S}$, and 99% of the $\text{S}^{2-}\text{-S}$ produced in the SR-CR was converted to elemental sulfur in the A&H-DSR. However, only 60% of elemental sulfur was detected in the effluent of the A&H-DSR on average because portions (30%) of elemental sulfur were attached to the granules and deposited in the reactor. The granules covered with elemental sulfur are shown in Fig. 6. Fig. 7 showed that the sizes of most of the elemental sulfur particles were between 1 and 101 μm with a mean size of 27 μm . The zeta potential value was approximately -19 mV , between $\pm 30 \text{ mV}$, indicating that the sulfur colloid was not stable;

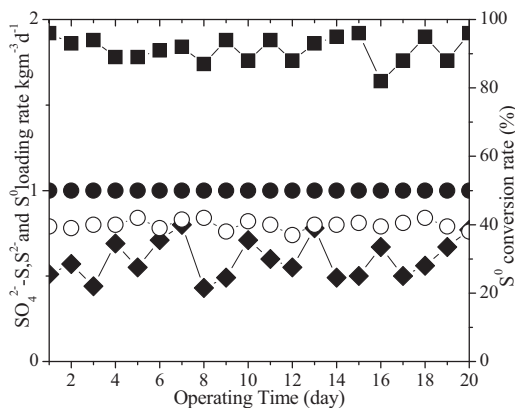


Fig. 5. Performance of S^0 production rate in the integrated C-S-N removal system. Influent SO_4^{2-} -S loading rate (●), influent S^{2-} -S loading rate (○) of A&H-DSR, effluent S^0 loading rate (◆) of A&H-DSR, and S^0 production rate (■).

therefore, the biological sulfur was easily coagulated and precipitated. After analysis of various forms of biological sulfur particles, we found that the biological sulfur particles contained 60% S, 30% C, 9% O, and 1% Mg.

Elemental sulfur in the effluents was collected by using 5 mg L⁻¹ of polyaluminum chloride (PAC) as a flocculant. After treatment

by flocculation, nearly 98% of the elemental sulfur was recovered from the effluent of the A&H-DSR unit. The sulfur balance is shown in Table 4. The elemental sulfur production rate was 90%, on the average (the elemental sulfur production efficiency (%) = effluent S^0 loading rate / influent SO_4^{2-} -S loading rate * 100). Hence, the sulfur reclamation efficiency was approximately 60% and the loading rate for S^0 reclamation was approximately 0.6 kg S^0 m⁻³ d⁻¹. Reyes-Avila et al. [14] operated an anaerobic continuous stirred tank reactor at loadings of 0.29 kg Acetate-C m⁻³ d⁻¹, 0.2 kg NO_3^- -N m⁻³ d⁻¹ and 0.294 kg S^{2-} -S m⁻³ d⁻¹ and obtained 99% of S^0 production (0.291 kg S^0 m⁻³ d⁻¹) which lower than our work. Chen et al. [16] achieved simultaneous removal for nitrate, sulfide, and COD from a single EGSB of 1.45 kg N m⁻³ d⁻¹, 3.1 kg S m⁻³ d⁻¹, and 2.77 kg C m⁻³ d⁻¹, respectively. Approximately 90% of sulfide was converted to S^0 (2.79 kg S^0 m⁻³ d⁻¹) which higher than our work. These works focused on the process in which nitrate, sulfide, and COD were converted to N_2 , S^0 and CO_2 . But, our work mainly focused on the process in which ammonium, sulfate, and COD were converted to N_2 , S^0 and CO_2 , that was poorly reported.

As shown in Table 5, nitrate was reduced to nitrogen gas with a yield of 0.9 g N_2 / (g NO_3^- -N being consumed). Accumulation of Org-C and nitrite was not observed in the effluent. Carbon dioxide (CO_2) and nitrogen gas (N_2) were noted in the gas phase of the A&H-DSR reactor. Meanwhile, negligible amounts of N_2O gas and H_2S gas were detected.

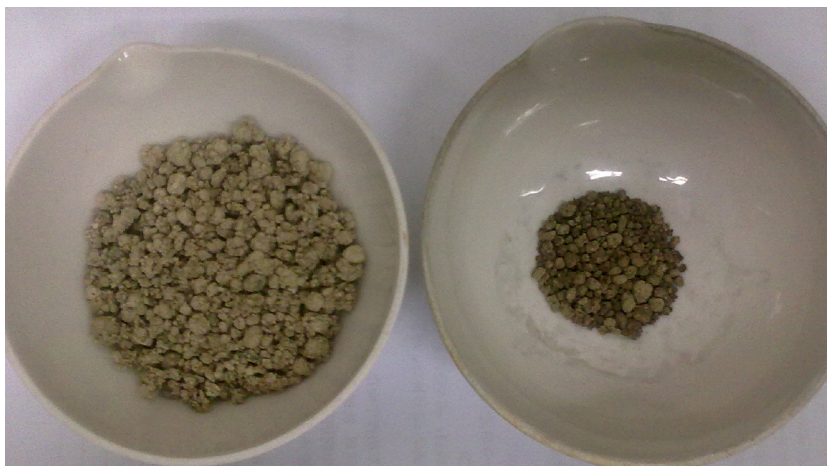


Fig. 6. Granules in the DSR unit.

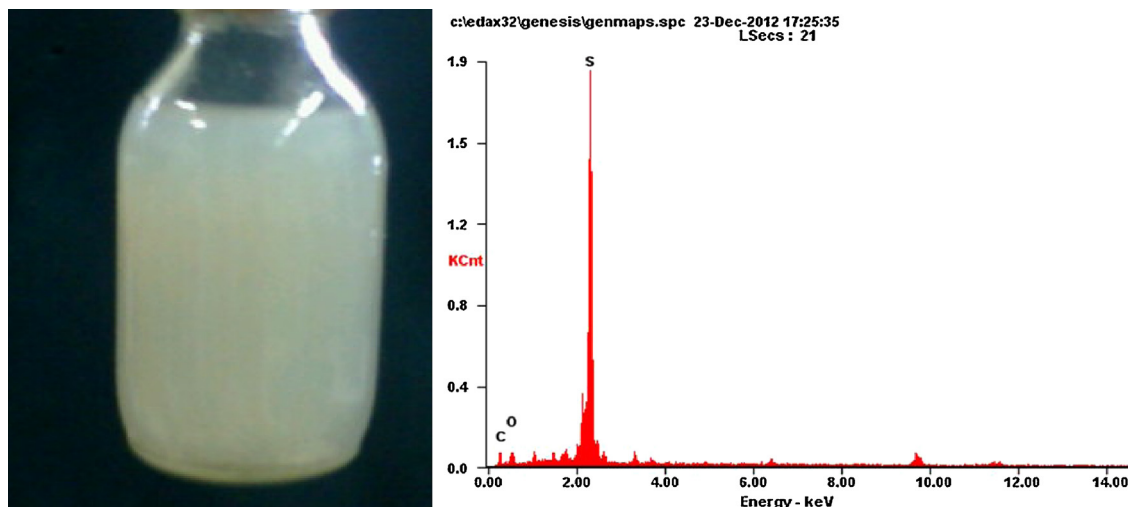


Fig. 7. Particle analysis of elemental sulfur in the effluent of the DSR unit.

Table 4
Sulfur balance in the integrated C-S-N removal system.

Units	SO ₄ ²⁻ -S loading (kg m ⁻³ d ⁻¹)		S ₂ O ₃ ²⁻ -S loading (kg m ⁻³ d ⁻¹)	H ₂ S _{gas} -S loading (kg m ⁻³ d ⁻¹)	S ²⁻ -S loading (kg m ⁻³ d ⁻¹)		S ⁰ loading (kg m ⁻³ d ⁻¹)			S ⁰ production rate (%)
	Influent	Effluent			Influent	Effluent	Influent	Effluent	Sludge	
SR-CR	1 ± 0.01	0.02	0	0.5 ± 0.03	0	0.9 ± 0.03	0	0	0	0
A&H-DSR	0	0	0	0.5 ± 0.03	0.92 ± 0.03	0	0	0.6 ± 0.12	0.3 ± 0.07	90
AN	0	0	0	0	0	0	0	0.012	0	0

Table 5
Nitrogen and carbon balance in the integrated C-S-N removal system.

Units	TOC loading (kg m ⁻³ d ⁻¹)		TOC removal (%)	HN ₄ ⁺ -N (kg m ⁻³ d ⁻¹)		NO ₃ ⁻ -N (kg m ⁻³ d ⁻¹)		N ₂ prod. (kg m ⁻³ d ⁻¹)	N removal (%)
	Influent	Effluent		Influent	Effluent	Influent	Effluent		
SR-CR	1.61	0.64 ± 0.1	60	0.55 ± 0.1	0.54 ± 0.3	/	/	/	2%
A&H-DSR	0.64 ± 0.1	0.27 ± 0.11	23	0.54 ± 0.1	0.53 ± 0.1	0.41 ± 0.2	0	0.43	71%
AN	0.27 ± 0.11	0.11 ± 0.2	10	0.53 ± 0.1	0.01	0.52 ± 0.1	0.11	0.054	1%
Whole system	1.61	0.11 ± 0.2	93	0.55	0.01	/	0.11	0.43	78%

3.4. Microbial community structure and dominant species analysis

To understand the microbial community structure and succession in the SR-CR and A&H-DSR units at different stages, the classified OTUs were analyzed at family level (Fig. 8). Meanwhile, to explain the key role of the dominant functional species in different stages, the classified OTUs were also analyzed at genus level (Table 6).

In stage I (separate incubation), the SR-CR unit achieved approximately 90% removal of 0.5 kg S m⁻³ d⁻¹ for sulfate and 49% removal of 0.8 kg C m⁻³ d⁻¹ for TOC. In stage II (integrative operation), this unit obtained approximately 90% removal of sulfate and 70%

removal of TOC with loadings increased to 1 kg S m⁻³ d⁻¹ for sulfate and 1.6 kg C m⁻³ d⁻¹ for TOC. The relative abundances for *Desulfomicrobiaceae* (from 6.21 to 12.69%), *Desulfobulbaceae* (from 6.55 to 10.13%) and *Methanobacteriaceae* (from 0.93 to 14.20%) families were increased with increased loadings. *Desulfomicrobium* (from 2.75 to 7.18%) and *Desulfobulbus* (from 3.15 to 8.01%) species were capable of reducing sulfate to sulfide [33,34], and gradually enriched with the increased loadings from stage I to stage II. *Methanobacterium* species was capable of converting VFAs to CH₄ [35] and its enrichment (from 0.93 to 14.20%) likely led to the further degradation of COD in the stage II. All these mentioned reasons together could explain why the SR-CR unit can provide appropriate concentration of TOC and sulfide for the A&H-DSR unit

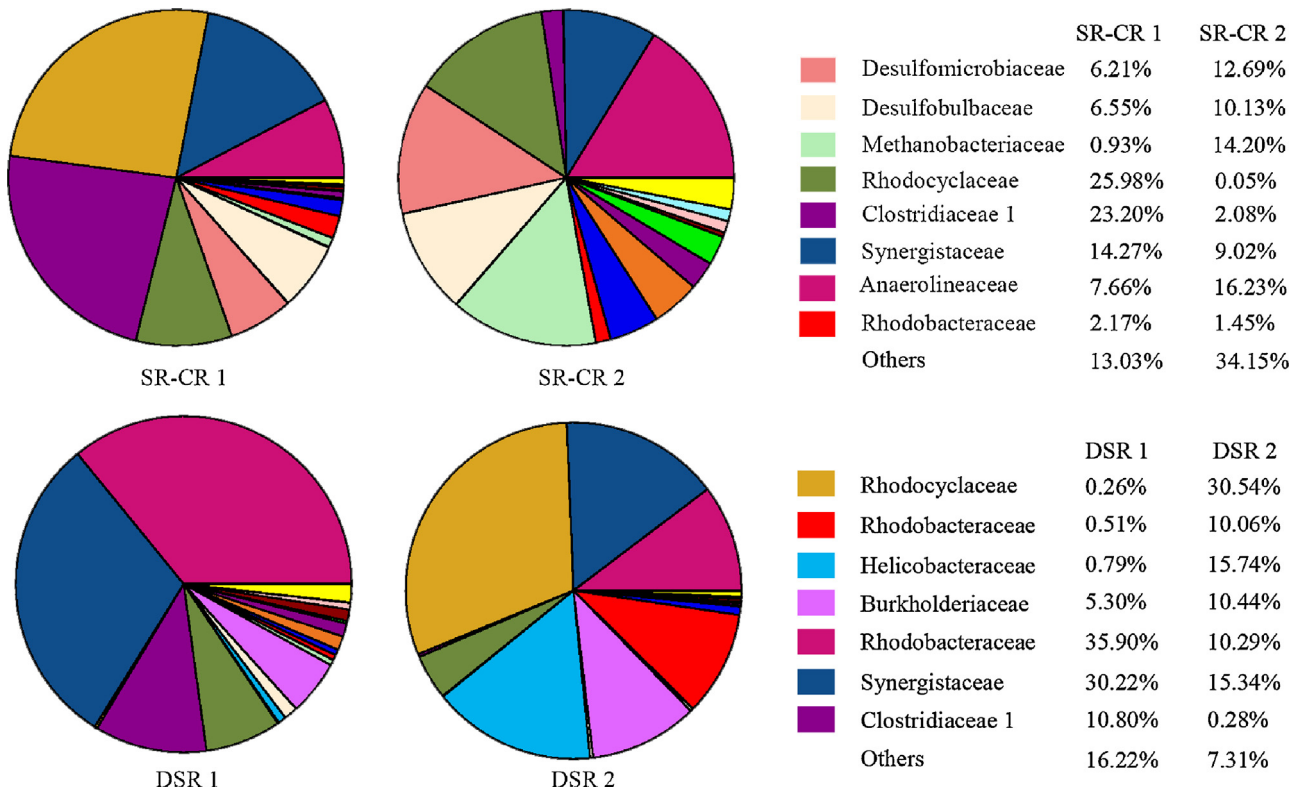


Fig. 8. Microbial community structure of SR-CR and DSR unit at family level in different stages. Relative abundance of each family was defined as the percentage of the same family to the corresponding total sequences for each sample.

Table 6
Phylogenetic classification of the 16S rRNA gene sequences at genus level (relative abundance >1%) in the SR-CR1, SR-CR2, DSR1 and DSR2.

Phylum	Family	Genus (%)	SR-CR1	SR-CR2	DSR1	DSR2
Proteobacteria	Desulfomicrobiaceae	Desulfomicrobium	6.09	11.87	0.01	0.03
Proteobacteria	Desulfobulbaceae	Desulfobulbus	6.22	9.18	0.98	0.19
Euryarchaeota	Methanobacteriaceae	Methanobacterium	0.87	12.88	0.31	0.11
Proteobacteria	Rhodocyclaceae	Azoarcus	17.02	0.02	0.21	20.21
		<i>Thauera</i>	5.3	0.01	0.01	7.67
Proteobacteria	Rhodobacteraceae	Paracoccus	1.76	0.65	0.45	9.74
Proteobacteria	Helicobacteraceae	Sulfurovum	0	0	0.63	14.98
Proteobacteria	Burkholderiaceae	Thermothrix	0.03	0.03	4.98	9.45
Chloroflexi	Anaerolineaceae	<i>Bellilinea</i>	5.06	10.08	20.98	6.55
		<i>Longilinea</i>	1.05	2.11	13.33	2.09
		<i>Levilina</i>	1.09	2.98	0.67	0.01
Synergistetes	Synergistaceae	<i>Cloacibacillus</i>	10.43	5.44	17.66	9.89
		<i>Synergistes</i>	2.18	3.02	11.01	4.43
		<i>Thermovirga</i>	1.80	0.78	2.09	0.22
Firmicutes	Clostridiaceae 1	<i>Proteinclasticum</i>	15.64	1.02	8.03	0.00
		<i>Clostridium</i>	3.98	0.32	1.09	0.01
Firmicutes	Clostridiales_Incertae Sedis XI	<i>Tissierella</i>	0.78	1.89	0.21	0.31
Bacteroidetes	Porphyromonadaceae	<i>Petrimonas</i>	0.01	3.67	0.13	0.01
Firmicutes	Peptostreptococcaceae	<i>Acetoanaerobium</i>	0.26	1.21	1.01	0
Proteobacteria	Comamonadaceae	<i>Albidiferax</i>	0.09	2.81	0.26	0.12
Firmicutes	Eubacteriaceae	<i>Acetobacterium</i>	0.25	0.24	1.10	0.29
Planctomycetes	Planctomycetaceae	<i>Planctomyces</i>	0.23	1.13	0.63	0.21

at this loading rate ($1 \text{ kg S m}^{-3} \text{ d}^{-1}$ for sulfate and $1.6 \text{ kg C m}^{-3} \text{ d}^{-1}$ for TOC). Importantly, these indicated that functional communities were well established at appropriate operational conditions.

At the same period of the stage I, the A&H-DSR unit achieved approximately 96% removal of $0.5 \text{ kg S m}^{-3} \text{ d}^{-1}$ for sulfide, $0.22 \text{ kg N m}^{-3} \text{ d}^{-1}$ for nitrate and $0.19 \text{ kg C m}^{-3} \text{ d}^{-1}$ for TOC. In the stage II, this unit was joined to the A&H-DSR unit still maintained 96%, 96% and 82% removal of $0.9 \text{ kg S m}^{-3} \text{ d}^{-1}$ for sulfide, $0.44 \text{ kg N m}^{-3} \text{ d}^{-1}$ for nitrate and $0.46 \text{ kg C m}^{-3} \text{ d}^{-1}$ for TOC, respectively. The relatively abundances for *Rhodocyclaceae* (from 0.26 to 30.54%), *Rhodobacteraceae* (from 0.51 to 10.06%), *Helicobacteraceae* (from 0.79 to 15.74%) and *Burkholderiaceae* families (from 5.3 to 10.44%) were obviously increased after loadings of sulfide, nitrate and TOC increased. At genus level, *Azoarcus* (from 0.21 to 20.21%), *Paracoccus* (from 0.45 to 9.74%), *Sulfurovum* (from 0.63 to 14.98%) and *Thermothrix* (from 4.98 to 9.45%) species were gradually enriched from stage I to stage II. *Sulfurovum* and *Thermothrix* species are chemolithotrophic bacteria that can use sulfur, sulfide, or thiosulfate as electron donors and nitrate as an electron acceptor [36,37]. *Azoarcus* species was capable of reducing nitrate via heterotrophic pathways [38]. *Paracoccus* species was capable of converting nitrite to N_2 [39]. *Azoarcus* and *Paracoccus* species likely acted as the heterotrophs to play a key role, whereas species from *Sulfurovum* and *Thermothrix* are the autotrophs that required to generate a high-rate A&H-DSR system. In stage III, the integrated SR-CR and A&H-DSR unit was connected with AN unit at the operational conditions obtained in stage II. Hence, the microbial community structures and dominant functional species in stage III were highly similar with the stage II (data not shown).

4. Conclusions

This study successfully established operating parameters for the integrated C-S-N removal system and demonstrated 98% COD, 98% sulfate and 78% nitrogen removal efficiency. The optimum HRTs for SR-CR, A&H-DSR and AN were 12 h, 3 h and 3 h, respectively. The optimum reflux ratio between the A&H-DSR and AN units was 3. In the system, sulfur balance analyses indicated the S^0 production rate was 90%. Approximately, 60% of S^0 presented in the effluent and 30% attached to the granules and deposited in the A&H-DSR unit. Physico-chemical characteristics of biosulfur in the effluent indicating that the sulfur colloid was not stable and was easily coagulated

and precipitated. The S^0 reclamation efficiency was approximately $0.6 \text{ kg S}^0 \text{ m}^{-3} \text{ d}^{-1}$. This study also provided insights into microbial community succession and the functional role of dominate species in the integrated system in response to different operational stages. The A&H-DSR unit requires appropriate operational parameters, such as TOC/NO_3^- and $\text{S}^{2-}/\text{NO}_3^-$ ratios. The studies on adjustment of key parameters of this system help to improve the application of the DSR process. In the future work, integrated C-S-N removal system will be applied to treating wastewater from food-making factory.

Acknowledgements

We gratefully acknowledge the support by the National High-tech R&D Program of China (863 Program, Grant No. 2011AA060904), by National Science Foundation for Distinguished Young Scholars (Grant No. 51225802), by Science Fund for Creative Research Groups of the National Natural Science Foundation of China (Grant No. 51121062), by National Key Technology Research and Development Program of the Ministry of Science and Technology of China (2010BAC67B02), and by the National Natural Science Foundation of China (Grant Nos. 51176037 and 51308147).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jhazmat.2013.12.014>.

References

- [1] G. Klein, P. Perera, *Eutrophication Health*, World Health Organization, European Commission, 2002.
- [2] US Environmental Protection Agency (US EPA), *Drinking water advisory: consumer acceptability advice and health effects analysis and health effects analysis on sulfate*, EPA 822-R-03-007, Washington, DC, 2003.
- [3] Ministry of Environmental Protection of the People's Republic of China, *Emission standard of pollutants for sulfuric acid industry*, GB 26132-2010, Beijing, 2010.
- [4] D. Paredes, P. Kuschk, T.S.A. Mbwette, F. Stange, R.A. Muller, H. Koser, *New aspects of microbial nitrogen transformations in the context of wastewater treatment – a review*, *Eng. Life Sci.* 7 (2007) 13–25.
- [5] P.N.L. Lens, A. Visser, A.J.H. Janssen, P.L.W. Hulshoff, G. Lettinga, *Biotechnological treatment of sulfate-rich wastewaters*, *Crit. Rev. Environ. Sci. Technol.* 28 (1998) 41–88.

- [6] G. Muyzer, A.J.M. Stams, The ecology and biotechnology of sulphate-reducing bacteria, *Nat. Rev. Microbiol.* 6 (2008) 441–454.
- [7] F. Fdz-Polanco, M. Fdz-Polanco, N. Fernandez, M.A. Uruena, P.A. Garcia, S. Villaverde, New process for simultaneous removal of nitrogen and sulphur under anaerobic conditions, *Water Res.* 35 (2001) 1111–1114.
- [8] S.T. Liu, F.L. Yang, Z. Gong, F.G. Meng, H.H. Chen, Y. Xue, K. Furukawa, Application of anaerobic ammonium-oxidizing consortium to achieve completely autotrophic ammonium and sulfate removal, *Bioresour. Technol.* 99 (2008) 6817–6825.
- [9] Z.Q. Yang, S.Q. Zhou, Y.B. Sun, Start-up of simultaneous removal of ammonium and sulfate from an anaerobic ammonium oxidation (anammox) process in an anaerobic up-flow bioreactor, *J. Hazard. Mater.* 169 (2009) 113–118.
- [10] J. Wang, J. Kang, The characteristics of anaerobic ammonia oxidation (ANAM-MOX) by granular sludge from an EGSB reactor, *Process Biochem.* 40 (2005) 1973–1978.
- [11] Y.H. Ahn, Sustainable nitrogen elimination biotechnologies: a review, *Process Biochem.* 41 (2006) 1709–1721.
- [12] J. Wang, H. Lu, G.H. Chen, G.N. Lau, W.L. Tsang, M. van Loosdrecht, A novel sulfate reduction, autotrophic denitrification, nitrification integrated (SANI) process for saline wastewater treatment, *Water Res.* 43 (2009) 2363–2372.
- [13] F. Carmen, M.C. Anuska, C.J. Luis, M. Ramón, Post-treatment of fish canning effluents by sequential nitrification and autotrophic denitrification processes, *Process Biochem.* 9 (2013) 1368–1374.
- [14] J. Reyes-Avila, E. Razo-Flores, J. Gomez, Simultaneous biological removal of nitrogen, carbon and sulfur by denitrification, *Water Res.* 38 (2004) 3313–3321.
- [15] I. Manconi, A. Carucci, P. Lens, S. Rossetti, Simultaneous biological removal of sulphide and nitrate by autotrophic denitrification in an activated sludge system, *Water Sci. Technol.* 53 (2006) 91–99.
- [16] C. Chen, A.J. Wang, N.Q. Ren, X. Zhou, D.J. Lee, Simultaneous biological removal of sulfur, nitrogen and carbon using EGSB reactor, *Appl. Microbiol. Biotechnol.* 78 (2008) 1057–1063.
- [17] A.J.H. Janssen, A. De Keizer, G. Lettinga, Colloidal properties of a microbiologically produced sulphur suspension in comparison to a LaMer sulphur sol, *Colloids Surf. B* 3 (1994) 111–117.
- [18] A.J.H. Janssen, A. De Keizer, A. Van Aelst, R. Fokkink, H. Yangling, G. Lettinga, Surface characteristics and aggregation of microbiologically produced sulphur particles in relation to the process conditions, *Colloids Surf. B* 6 (1996) 115–129.
- [19] R. Kleerbezem, R. Mendez, Autotrophic denitrification for combined hydrogen sulfide removal from biogas and post-denitrification, *Water Sci. Technol.* 45 (2002) 349–356.
- [20] P.L.F. van den Bosch, O.C. van Beusekom, C.J.N. Buisman, A.J.H. Janssen, Sulfide oxidation at halo-alkaline conditions in a fed-batch bioreactor, *Biotechnol. Bioeng.* 97 (2007) 1053–1063.
- [21] C. Chen, N.Q. Ren, A.J. Wang, Z.G. Yu, D.J. Lee, Microbial community of granules in expanded granular sludge bed reactor for simultaneous biological removal of sulfate, nitrate, and lactate, *Appl. Microbiol. Biotechnol.* 78 (2008) 1071–1077.
- [22] APHA, AWWA, WPCF, Standard Methods for Water and Wastewater Examination, 21st ed., APHA, Washington, DC, USA, 2005.
- [23] R.E. Moosbrugger, M.C. Wentzel, G.A. Ekama, G.V.R. Marais, Simple titration procedures to determine H₂CO₃ alkalinity and short-chain fatty acids in aqueous solutions containing known concentrations of ammonium, phosphate and sulfide weak acid/bases, Water Research Commission, Pretoria, 1992, Report No. TT 57/92.
- [24] F.G. Neytzell-DeWilde, G.R. Nurse, J. Groves, Treatment of effluents from ammonia plants. Part IV. Denitrification of an inorganic effluent from a nitrogen-chemicals complex using methanol as a carbon source, *Water SA* 3 (1977) 142–154.
- [25] G.M. Jiang, K.R. Sharma, A. Guisasaola, J. Keller, Z.G. Yuan, Sulfur transformation in rising main sewers receiving nitrate dosage, *Water Res.* 43 (2009) 4430–4440.
- [26] B. Ravishankar, J. Blais, H. Benmoussa, R. Tyagi, Bioleaching of metals from sewage sludge: elemental sulfur recovery, *J. Environ. Eng.* 120 (1994) 462–470.
- [27] M. de Graaff, J.B.M. Klok, M.F.M. Bijmans, G. Muyzer, A.J.H. Janssen, Application of a 2-step process for the biological treatment of sulfidic spent caustics, *Water Res.* 46 (2011) 723–730.
- [28] M. Yoda, M. Kitagawa, Y. Miyaji, Long term competition between sulfate-reducing and methane-producing bacteria for acetate in anaerobic biofilm, *Water Res.* 21 (1987) 1547–1556.
- [29] P.L.W. Hulshoff, P.N.L. Lens, A.J.M. Stams, G. Lettinga, Anaerobic treatment of sulphate-rich wastewaters, *Biodegradation* 9 (1998) 213–224.
- [30] P. Fox, V. Venkatasubbiah, Coupled anaerobic/aerobic treatment of high-sulfate wastewater with sulfate reduction and biological sulfide oxidation, *Water Sci. Technol.* 34 (1996) 359–366.
- [31] R.E. Speece, Anaerobic biotechnology for industrial wastewater treatment, *Environ. Sci. Technol.* 17 (1983) 416–427.
- [32] C. Chen, A.J. Wang, N.Q. Ren, D.J. Lee, J.Y. Lai, High-rate denitrifying sulfide removal process in expanded granular sludge bed reactor, *Bioresour. Technol.* 100 (2009) 2316–2319.
- [33] L.L. Barton, G.D. Fauque, Biochemistry, physiology and biotechnology of sulfate-reducing bacteria, *Adv. Appl. Microbiol.* 68 (2009) 41–98.
- [34] D. Prato-Garcia, F.J. Cervantes, G. Buitrón, Azo dye decolorization assisted by chemical and biogenic sulfide, *J. Hazard. Mater.* 250 (2013) 462–468.
- [35] P.H. Smith, R.E. Hungate, Isolation and characterization of *Methanobacterium ruminantium* n. sp., *J. Bacteriol.* 75 (1958) 713–718.
- [36] F. Inagaki, K. Takai, K.H. Nealson, K. Horikoshi, *Sulfurovum lithotrophicum* gen. nov., sp. nov., a novel sulfur-oxidizing chemolithoautotroph within the epsilon-Proteobacteria isolated from Okinawa Torugh hydrothermal sediments, *Int. J. Syst. Evol. Microbiol.* 54 (2004) 1477–1482.
- [37] E.V. Odintsova, H.W. Jannasch, J.A. Mamone, T.A. Langworthy, *Thermothrix azorensis* sp. nov., an obligately chemolithoautotrophic, sulfur-oxidizing, thermophilic bacterium, *Int. J. Syst. Bacteriol.* 46 (1996) 422–428.
- [38] B. Song, M.M. Haggblom, J. Zhou, J.M. Tiedje, N.J. Palleroni, Taxonomic characterization of denitrifying bacteria that degrade aromatic compounds and description of *Azoarcus toluvorans* sp. nov. and *Azoarcus toluclasticus* sp. nov., *Int. J. Syst. Bacteriol.* 49 (1999) 1129–1140.
- [39] H. Uemoto, H. Saiki, Nitrogen removal by tubular gel containing *Nitrosomonas europaea* and *Paracoccus denitrificans*, *App. Environ. Microbiol.* 62 (1996) 4224–4228.