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Short Communication

# Efficient regulation of elemental sulfur recovery through optimizing working height of upflow anaerobic sludge blanket reactor during denitrifying sulfide removal process

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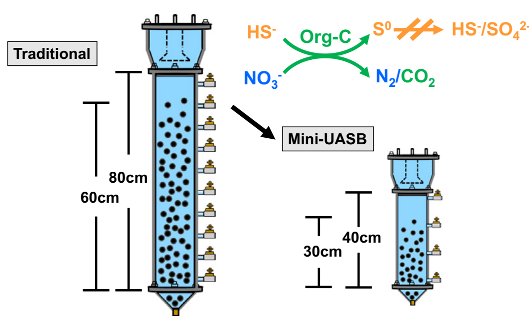
## HIGHLIGHTS

- Efficient regulation of $S^0$ recovery by optimizing working height of UASB reactor.
- Sulfide over oxidization to sulfate was observed in the traditional reactor.
- After working height improved, $S^0$ recovery rate increased from 7.4% to 78.8%.
- Bacterial community was remarkably modified as working height/volume altered.
- Desulfurization and denitrification genera were predominant in the improved reactor.

## GRAPHICAL ABSTRACT

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## ABSTRACT

In this study, two lab-scale UASB reactors were established to testify $S^0$ recovery efficiency, and one of which (M-UASB) was improved from the previous T-UASB by shortening reactor height once $S^{2-}$ over oxidation was observed. After the height was shortened from 60 to 30 cm, $S^0$ recovery rate was improved from 7.4% to 78.8%, and while, complete removal of acetate, nitrate and $S^{2-}$ was simultaneously maintained. Meanwhile, bacterial community distribution was homogenous throughout the reactor, with denitrifying sulfide oxidization bacteria predominant, such as *Thauera* and *Azoarcus* spp., indicating the optimized condition for $S^0$ recovery. The effective control of working height/volume in reactors plays important roles for the efficient regulation of $S^0$ recovery during DSR process.


## 1. Introduction

High amount of sulfate ($SO_4^{2-}$) and nitrate ($NO_3^-$) are generated from manufacturing industries, which cause erosion and destruction of water body and the potential carcinogenicity to human

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beings (Krayzelova et al., 2014). Sulfide ($S^{2-}$), produced from $SO_4^{2-}$ reduction, is toxic, effluvial and one of the most commonly detected forms in $SO_4^{2-}$ polluted wastewater (Jiang et al., 2009). The recent developed denitrifying sulfide removal (DSR) process, which transfers $S^{2-}$ to elemental sulfur ($S^0$) by utilizing $NO_3^-$ as electron acceptors, supplies an effective biological means for $NO_3^-$ removal and simultaneous $S^0$ recovery from wastewater (Chen et al., 2008a; Wang et al., 2005).

Several studies were conducted to regulate $S^0$ recovery in various bioreactors (Chen et al., 2008a; Jing et al., 2009), such as the upflow anaerobic sludge blanker (UASB) reactor, expanded granular sludge blanket (EGSB) reactor, etc. According to standard Gibbs free energy change, $S^{2-}$ oxidization to sulfate takes place easily than $S^0$, with the hypothetical biochemical Eqs. (1) and (2) listed as follows (Chen et al., 2008b):

$$\begin{aligned} & S^{2-} + 1.25CH_3COOH + 3.6NO_3^- + 1.6H^+ \\ & \rightarrow SO_4^{2-} + 2.5CO_2 + 1.8N_2 + 2.3H_2O + 2OH^- \Delta G^{0,} \\ & = -2100.1\ \text{kJ/reaction} \end{aligned} \tag{1}$$

$$\begin{aligned} & S^{2-} + 1.25CH_3COOH + 2.4NO_3^- + 2.4H^+ \\ & \rightarrow S^0 + 2.5CO_2 + 1.2N_2 + 2.7H_2O + 2OH^- \Delta G^{0,} \\ & = -1463.1\ \text{kJ/reaction} \end{aligned} \tag{2}$$

This made $S^{2-}$ over oxidization to $SO_4^{2-}$ as a big obstacle for the efficient $S^0$ recovery. To solve this problem, several studies have attempted to seek for the high $S^0$ recovery strategies through optimizing $NO_3^-$ supplement by applying the different carbon/nitrate/sulfide loading ratios. Chen et al. (2008a,b) found the selective $S^0$ recovery was improved when S/N molar ratio set at 5/2 rather than 5/5 or 5/8 in an up flow reactor. Huang et al. (2015a) examined the influence of $S^{2-}/NO_3^-$ molar ratio ranged from 5/2 to 5/9 and found the optimized $S^{2-}/NO_3^-$ ratio was evaluated as 5/6 for the high $S^0$ reclaiming rate.

Besides loading ratios, reactor configuration or working area would be other factors that largely impact the recovery efficiency. Previously, Kubota et al. (2014) and Lu et al. (2015) reported that the UASB height would affect both the inner microbial community and biochemical reaction efficiency. However, so far, reaction mechanism involved functional bacteria and migration and transformation roles of $S^{2-}$, $SO_4^{2-}$ and $S^0$ inside UASB reactor are rarely concerned. Identification of spatial information of the responsible bacteria and chemical transformation are vital for better regulation of $S^0$ recovery through reactor structural improvement.

Therefore, in this study, two lab-scale UASB reactors were established to testify $S^0$ recovery efficiency during the simultaneous removal of organic carbon (acetate), $S^{2-}$ and nitrate, one of which (M-UASB) was improved from the traditional reactor (T-UASB) by shorten reactor height after $S^{2-}$ over oxidation to $SO_4^{2-}$ inside T-UASB was observed. Migration of $S^{2-}$, $SO_4^{2-}$ and $S^0$ and functional bacteria distribution were investigated along with the height of T-UASB and M-UASB reactors to gain a deeper insight of the interplays between bacteria and substance transformation. Objectives of the study were to demonstrate the important role of working volume/height for regulation of DSR process and supply a novel thought to improve $S^0$ recovery and avoid $S^{2-}$ over oxidization by reactor working height/volume improvement.

## 2. Methods

### 2.1. Experimental set up

Two kinds of reactors were operated in this study: traditional UASB reactor (T-UASB) with working depth of 60 cm and working volume of 1.7 L and improved UASB reactor from T-UASB with a shorter length (M-UASB), harboring the working depth of 30 cm and working volume of 0.85 L. The two reactors were wrapped with the resistance wire around to maintain the inside temperature of 30 °C. Both of the reactors were fed with synthetic wastewater with the loading volume of 8 L. Other running parameters were as shown in details in Table 1. An equal volume of sludge (16 g TSS/L) from the anaerobic sludge thickener of WenChang Wastewater Treatment Plant (Harbin, China) was chosen as inoculum of the two reactors. The influent acetate/$NO_3^-$/$S^{2-}$ ratio was optimized by Huang et al. (2015a,b), which contained 113.4 mg $L^{-1}$ acetate–C, 105 mg $L^{-1}$ $NO_3^-$–N and 200 mg $L^{-1}$ $S^{2-}$–S, bicarbonate (1 g $L^{-1}$) as well as the trace element solution with the composition and detailed concentration described by Chen et al. (2008a). Bicarbonate was employed to maintain the influent pH of 8.0 ± 0.3. Both of the reactors were operated at a fixed inflow volume of 5 L per day. Concentration of acetate, $NO_3^-$ and $S^{2-}$ were monitored at intervals until the reactors treatment efficiency achieved at the steady state after more than 60 days.

### 2.2. Sampling and chemical analytical methods

After achieved at the steady state, samples of influent, effluent and sample ports along with reactor height were collected every two days and ten days, respectively, and concentration of acetate, $NO_3^-$, $S^{2-}$, $SO_4^{2-}$ and $S^0$ in samples were continuously monitored. Acetate was determined with HPLC (Waters 2996, Waters Incorporation, USA) and a C18 column (5 mm, 4.6 × 250 mm) through authentic standard UV–visible light analysis, as described in detail by Chen et al. (2008a). Concentration of $NO_3^-$, $S^{2-}$, $SO_4^{2-}$, and thiosulfate ($S_2O_3^{2-}$), were determined by an ion chromatography (ICS-90A; Dionex, USA) with column (Ion-Pac AG4A AS4A-SC 4 mm, Dionex, USA) after diluted five times and filtrated with 0.45 μm of millipore filter. Production of elemental sulfur in effluent was calculated according to the following equation (De Graaff et al., 2012): $[S^0]$ = [Influent $S^{2-}$] + [Influent $SO_4^{2-}$] + [Influent $S_2O_3^{2-}$] − [Effluent $S^{2-}$] − [Effluent $SO_4^{2-}$] − [Effluent $S_2O_3^{2-}$].

### 2.3. DNA extraction and Illumina sequencing

After the reactors reached the steady state, samples in T-UASB reactor with the height of 20, 40 and 60 cm and M-UASB reactor with the height of 10, 20 and 30 cm were collected and stored in 5 ml freezing tubes at −80 °C before went for DNA analysis. DNA was extracted using the PowerSoil DNA Isolation kit (MoBio Laboratories Inc, USA) according to the manufacturer's instructions. Concentration and purity of the extracted DNA were measured with Nanophotometer (P-class, Implen, Germany). Bacterial V1–V3 region of 16S rRNA gene was amplified using the forward primer 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse primer 533R (5′-TTACCGCG GCTGCTGGCAC-3′). PCR products were purified using GeneJET™ PCR purification kit (Fermentas, USA) and then went for Illumina sequencing platform. The sequences obtained from Illumina sequencing were analyzed following the pipelines of Quantitative Insights into Microbial Ecology (QIIME) software (www.microbio.me/qiime) as described by the previous studies (Huang et al., 2015b). Taxonomic classification of each phylotype was determined using the SILVA rRNA database project with over 97% of sequence similarity. The 16S rRNA gene sequence data was deposited in NCBI Sequence Read Archive under the accession number of SRP064180.

**Table 1**
Average results for removals of acetate, nitrate and sulfite at steady state in T-UASB and M-UASB reactors.

| Reactor | Acetate–C | | Nitrate–N | | $S^{2-}$ | | $S_2O_3^{2-}$–S | $SO_4^{2-}$–S | $S^0$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Influent (mg $L^{-1}$) | Removal rate (%)[a] | Influent (mg $L^{-1}$) | Removal rate (%) | Influent (mg $L^{-1}$) | Removal rate (%) | Effluent (mg $L^{-1}$) | Effluent (mg $L^{-1}$) | Effluent (mg $L^{-1}$) | Generation rate (%)[b] |
| T-UASB | 225 ± 9.1[c] | 100.0 | 107 ± 6.4 | 100.0 | 210.5 ± 8.5 | 100.0 | 40.5 ± 5.2 | 155.1 ± 6.2 | 14.9 ± 6.1 | 7.4 ± 3.0 |
| M-UASB | 225 ± 9.1 | 100 | 105 ± 5.9 | 100.0 | 204.5 ± 9.5 | 100.0 | 37.5 ± 5.7 | 8.9 ± 5.4 | 158.1 ± 8.4 | 78.8 ± 2.8 |

[a] Removal rate (%) was calculated by dividing the effluent concentration with the influent concentration.
[b] $S^0$ generation rate (%) was calculated by dividing the effluent concentration of $S^0$ with the concentration of $SO_4^{2-}$ in influent.
[c] The data was the average results from triplicate samples with the standard deviation shown on the right side of " ± ".

## 3. Results and discussion

### 3.1. Performance in T-UASB reactor

At steady state, the removal of acetate, $NO_3^-$ and $S^{2-}$ in T-UASB reactor approached the optimal conditions with the removal efficiency achieved at around 100% (Table 1), however, the generated $S^0$ in effluent was much lower, compared with the introduced $S^{2-}$ in influent during continuous running for 60 days (Fig. 1A). $S^0$ recovery rate was calculated as 7.4% in average, accompanied with large amount of $SO_4^{2-}$ ($SO_4^{2-}$–S, 155.1 mg $L^{-1}$) and $S_2O_3^{2-}$ ($S_2O_3^{2-}$–S, 40.5 mg $L^{-1}$) generated (Table 1), which indicated the occurrence of $S^{2-}$ over oxidize to $SO_4^{2-}$ (Huang et al., 2015a).

Concentration of $S^{2-}$, $SO_4^{2-}$ and $S_2O_3^{2-}$ on 20, 40 and 60 cm of height in T-UASB reactor were determined (Fig. 1B). Large amount of $S^0$ generated at the first 20 cm height. However, the produced $S^0$ gradually decreased after 20 cm, and instead of that, concentration of $SO_4^{2-}$ dramatically increased and approached to the highest at 60 cm. Concentration of $S^0$ decreased from 171 mg $L^{-1}$ (85.5% of recovery rate) to 5 mg $L^{-1}$ (2.5% of recovery rate) with the height increased from 20 to 60 cm. The results indicated that the oxidization of $S^{2-}$ to $S^0$ were occurred majorly at the initial area of reactor (at around 20 cm of height) and then $S^0$ was over oxidized to $SO_4^{2-}$ by utilizing $NO_3^-$ as electron acceptor between 40 and 60 cm.

Batch experiments were conducted by sampling sludge at 20, 30, 40, 50 and 60 cm of the height in reactor, and the highest $S^0$ recovery rates were appeared between 20 and 30 cm (date not shown), indicated the appropriate bacterial community for $S^0$ recovery. Combing with the result that $S^{2-}$ was over oxidized to $SO_4^{2-}$ between 40 and 60 cm (Fig. 1), the effective length of T-UASB was selectively modified as 30 cm to optimize the reactor performance.

### 3.2. Performance in M-UASB reactor

Under the same loading ratio, the removal of acetate, $NO_3^-$ and $S^{2-}$ and recovery of $S^0$ were monitored in M-UASB with the functional height shortened from 60 to 30 cm (Table 1 and Fig. 2). Removal efficiency of acetate, $NO_3^-$ and $S^{2-}$ approached to 100%, $S^0$ recovery rate was improved to 78.8% and only a few $SO_4^{2-}$ ($SO_4^{2-}$–S, 8.9 mg $L^{-1}$) and $S_2O_3^{2-}$ ($S_2O_3^{2-}$–S, 37 mg $L^{-1}$) were detected in effluent (Table 1; Fig. 2A). Sulfur distribution along with the reactor height was described in detail in Fig. 2B. As concentration of $S^{2-}$ decreased, generated $S^0$ was increased gradually along with the reactor, and while, concentration of $SO_4^{2-}$ and $S_2O_3^{2-}$ were not dramatically increased. The high $S^0$ recovery rate and low $SO_4^{2-}$ concentration indicated $S^{2-}$ oxidization to $S^0$ was the major sulfur cycling process, and $S^0$ over oxidization to $SO_4^{2-}$ was effectively inhibited because of the improvement of reactor height.

### 3.3. The bacterial community structure and diversity inside T-UASB and M-UASB reactors

To better understand the correlation between performance and bacterial structure variations in T-UASB and M-UASB reactors,

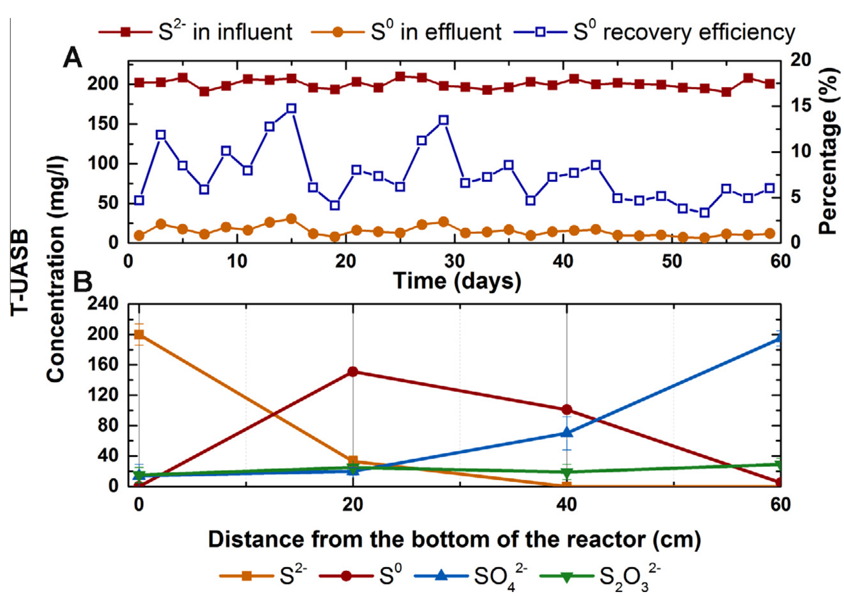


**Fig. 1.** Performance of T-UASB reactor with the working height of 60 cm. (A) Performance of $S^0$ recovery in T-UASB reactor during the continuous running for 60 days. (B) Concentration of sulfide, elemental sulfur, sulfate and thiosulfate at the different height within T-UASB reactor.

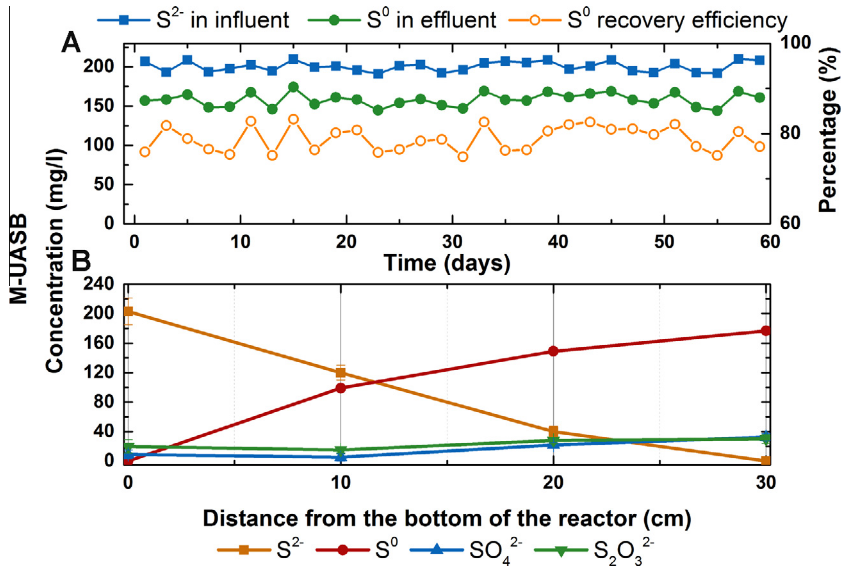

**Fig. 2.** Performance of M-UASB reactor with the working height of 30 cm. (A) Performance of $S^0$ recovery in M-UASB reactor during the continuous running for 60 days. (B) Concentration of sulfide, elemental sulfur, sulfate and thiosulfate at the different height within M-UASB reactor.

bacterial communities were analyzed through Illumina sequencing at different sample ports along with reactor height, respectively (Fig. 3). More than 33 types of bacterial genera (relative abundance $\geqslant$ 1%) were generated in total, with 23 types and 15 types in T-UASB and M-UASB reactors, respectively. Among of them, *Azoarcus* gen. were the most dominant in T-UASB (at 20 cm), and while, *Thauera* gen. were the most dominant in T-UASB (at 10, 20 and 30 cm).

The diverse bacterial composition was observed at the different height of T-UASB (Fig. 3A). At 20 cm, three genus in charge of denitrifying sulfur oxidization were predominant, including *Azoarcus* (34.9%), *Thauera* (28.4%), and *Arcobacter* (4.8%). Of which, *Azoarcus* sp. and *Thauera* sp. were two ubiquitous denitrifying sulfur oxidizing bacteria, which were able to covert $S^{2-}$ to $S^0$ and simultaneous reduce $NO_3^-$ to $N_2$ (Liu et al., 2006). *Arcobacter* sp. could oxidize sulfide into filamentous sulfur and simultaneously fix carbon dioxide to organic compound (Wirsen et al., 2002). While, at 40 and 60 cm, the percentage of denitrifying sulfur oxidizing genera was much lower (about 20%) (Fig. 3B); instead, *Halothiobacillaceae* gen. (48.3%) and *Anaerolineaceae* gen. (37.1%) were respectively dominant, which were widely existed in many sulfur oxidizing bioreactor and whereas, the function of them was not defined yet (Vannini et al., 2008).

In comparison, bacterial diversity in M-UASB was lower and bacterial distribution along with the reactor was relative homogeneous (Fig. 3A). About 55–70% of community was composed of denitrifying sulfur oxidizing bacteria (Fig. 3B), that *Thauera* (54.8%, 10 cm; 26.8%, 20 cm; 51.6%, 30 cm) were the most

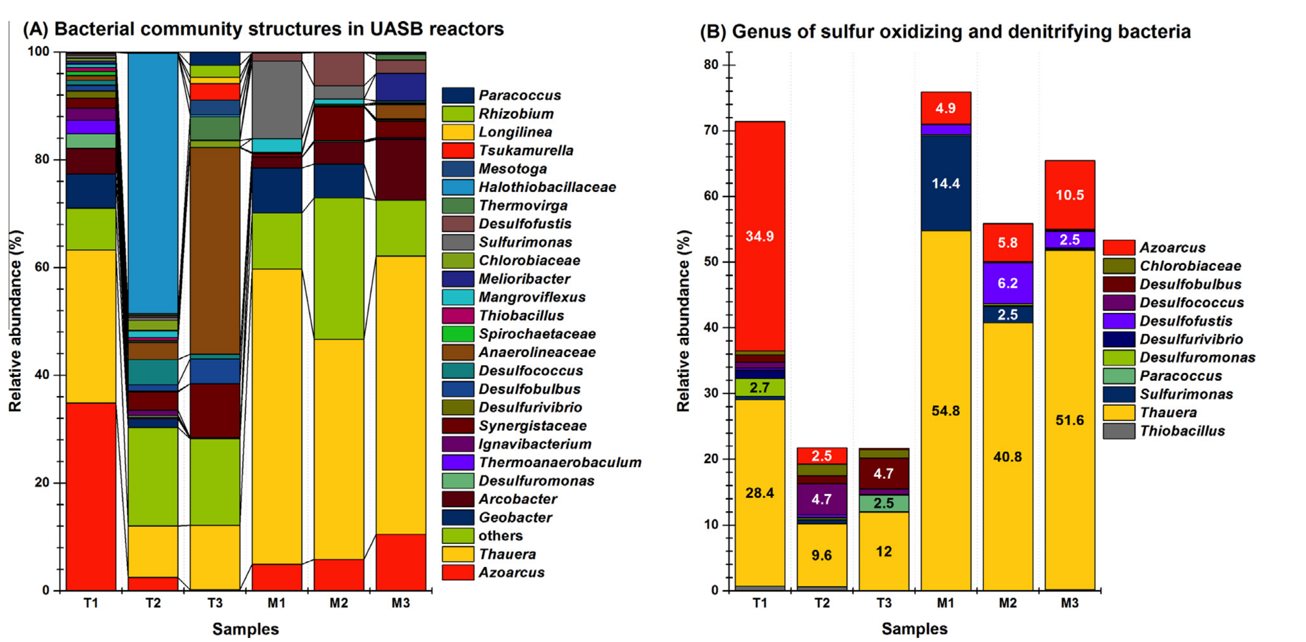

**Fig. 3.** Bacterial community structures in lever of genus in UASB reactors corresponding to the different sample height. T1: 20 cm height of T-UASB, T2: 40 cm height of T-UASB, T3: 60 cm height of T-UASB, M1: 10 cm height of M-UASB, M2: 20 cm height of M-UASB, M3: 30 cm height of M-UASB.

dominant. Besides, *Azoarcus* sp. and *Desulfurivibrio* sp., the sulfur oxidizing bacteria in charge of sulfide to sulfate (Sorokin et al., 2008), were also abundant. The bacterial structure in M-UASB was similar with that at 20 cm of T-UASB, except that bacterial diversity decreased, and meanwhile, the involved functional genera were more abundant.

### 3.4. Interrelations between reactor performance and bacterial communities

Focusing on DSR process, many studies have addressed to improve $S^0$ recovery rate by either optimizing organic compound/$NO_3^-$/$S^{2-}$ loading ratios (Cai et al., 2008; Cardoso et al., 2006) or regulating the microbial communities (Huang et al., 2015a). However, little study has concerned on the effects of reactor configuration/working volume to system performance (Chen et al., 2008a). The study presented here, clearly demonstrated that reactor height/volume significantly affected both the $S^0$ recovery performance and bacterial structure; after UASB reactor height was optimized, $S^0$ recovery was dramatically improved and bacterial community correspondingly altered.

Most of $S^{2-}$ was oxidized to $S^0$ before 30 cm height of UASB reactor (Figs. 1 and 2), with sulfide oxidizing and denitrifying bacteria highly enriched, such as *Azoarcus* and *Thauera* spp. After 30 cm, sulfur oxidizer and denitrifier were less abundant, and replaced with some uninvolved bacteria, such as *Halothiobacillaceae* and *Anaerolineaceae* sp., which resulted in $S^0$ over oxidization to $SO_4^{2-}$ (Figs. 1–3). Therefore, it is inferred when carbon, nitrate and sulfide were simultaneously supplied, denitrification and sulfide oxidization processes were dominant at initial; after most of $S^{2-}$ was converted to $S^0$, $S^0$ over oxidization was occurred as nitrate was sufficient. Therefore, the precise control of working height/area was essential for both $S^0$ reclamation and regulation of the end product of sulfide oxidization. Also, bacterial communities varied as sulfur forms changed and bacterial structure corresponded well with the sulfide oxidization product. Optimizing the working volume/height effectively regulated both the $S^{2-}$ oxidization process and bacterial community. Further studies on the effects of the other impacting factors, such as loading ratio, are warranted under the optimized working length/volume in UASB reactor.

## 4. Conclusion

Briefly, the study reported the effective regulation of $S^0$ recovery during simultaneous recovery of $S^{2-}$, $NO_3^-$ and acetate through improvement of working height/volume in UASB reactor. After the working height of UASB reactor shortened from 60 cm to 30 cm, the $S^0$ recovery efficiency was improved from 7.4% to 78.8%. Meanwhile, the bacterial community was effectively regulated with a more homogenous distribution and predominant with the denitrifying sulfide oxidization genera, such as *Thauera* and *Azoarcus*, indicated the effective regulation of DSR process for the effective recovery of $S^0$.

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