academicJournals

Vol. 13(16), pp. 1739-1744, 16 April, 2014
DOI: 10.5897/AJB12.1436
Article Number: DB6946143986
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Inhibition of carbon disulfide on bio-desulfurization in the process of gases purification

Ziyu Song[1, 2], Qiang Li[1], Dan Wang[3] Hang Tang[1, 2], Maohua Yang[1] and Jianmin Xing[1]*

[1]National key Laboratory of Biochemical Engineering, Institute of process Engineering, Chinese Academy of Sciences, Beijing 100190, People's Republic of China.
[2]Graduate University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China.
[3]College of Chemistry and Chemical Engineering, Chongqing University, Chongqing 400044, People's Republic of China.

Received 5 April, 2012; Accepted 26 November, 2012

Biological desulfurization is a novel technology for the removal of hydrogen sulfide from some biogas or sour gas, in which there are always a certain amounts of carbon disulfide together with much hydrogen sulfide. Nowadays, carbon disulfide is found to have negative effect on the biological desulfurization, but seldom research is afforded to investigate how carbon disulfide inhibits the process of biological desulfurization. In this paper, we investigated the effect of carbon disulfide both on the growth of *Thiobacillus thioparus* and the resting cells under various concentrations, including 0.01, 0.05, 0.10, 0.15 and 0.20%. In this process, the rate of the cell growth was characterized by the rate of nitrogen consumption in order to solve the problem that the adsorption of cells to sulfur granules have on the accuracy of biomass test. Under the cell density of 23.92 mg N/L, which is lower than the maximum of cell density 36.13 mg N/L, the average rate of thiosulfate oxidation reached the maximum (26.50 $S_2O_3^{2-}$ mg/L-h). Carbon disulfide at titers of 0.01% significantly inhibited the growth of cells, but hardly affected the biological desulfurization of resting cells. Although carbon disulfide at titers of 0.05% had negative effect on the biological desulfurization of resting cells, the effect of inhibition could be relieved by the increased density of resting cells. For the resting cells, the parameters of Michaelis-Menten equation were calculated by the method of Lineweaver-Burk. The V_{max} of biological desulfurization was decreased from 27.93 to 14.0 $S_2O_3^{2-}$ mg/L-h, and the K_m was increased from 0.264 to 0.884 mM, with the concentration of carbon disulfide rising up from 0.0 to 0.1%. These results show that the growth of cells was sensitive to carbon disulfide, and the resting cells had resistance to the low level of carbon disulfide (0.05%). Thus, the inhibition of carbon disulfide to biological desulfurization should be attributed to *T. thioparus* growth suppression function.

Key words: Carbon disulfide, bio-desulfurization, inhibition, *Thiobacillus thioparus*, resting cell, gases purification.

INTRODUCTION

Hydrogen sulfide (H_2S) is a highly toxic, corroded and malodor gas, which is a common ingredient in natural gas and biogas (Zhang et al., 2008; Kim et al., 2005). It was reported that the concentration of H_2S could get to as high as 17 000 ppm, when sulfate-rich wastewater was converted to biogas by anaerobic digestion (Chaiprapat

et al., 2011). Biological desulfurization is a novel method for the removal of H_2S from gases stream by sulfide oxidizing bacteria (SOBs) that have capacity of oxidizing low state sulfur compounds. In the process of biological desulfurization, H_2S is firstly adsorbed by alkaline adsorbent, and then is oxidized to element sulfur by SOBs under oxygen limitation (Equations 1 to 2). Profiting from the regeneration of hydroxide, the alkaline adsorbents could be recycled (Equation 2). It has been considered as the best alternative of the classic chemical methods for desulfurization, and has perspective application in the fields of the desulfurization of gases, such as natural gas and biogas (Abatzoglou and Boivin, 2009).

$$H_2S + OH^- \rightarrow HS^- + H_2O \quad (1)$$

$$2HS^- + O_2 \xrightarrow{SOBs} 2S + 2OH^- \quad (2)$$

In the raw natural gas or biogas, there are also some volatile organic sulfur compounds (VOSCs), such as methanethiol, dimethyl disulfide, dimethyl sulfide, carbon disulfide and carbonyl sulfide (Mata-Alvarez and Llabrés, 2000; Böresson, 2001; Sheng et al., 2008). These VOSCs could also be absorbed by the alkaline adsorbents, and make significant effect on the activity of SOBs (Lobo et al., 1999). It was reported that *Thiobacillus thioparus* DW44 had the closed specific uptake rates ($g \cdot S \cdot Cell^{-1} \cdot h^{-1}$) of H_2S (7.49×10^{-14}), methanethiol (3.45×10^{-14}), dimethyl disulfide (1.24×10^{-14}) and dimethyl sulfide (4.14×10^{-15}) (Kyeoung et al., 1991).

However, it had been reported that most of *Thiobacilli* were not only incapable of oxidizing carbon disulfide, but were inhibited by VOSCs, including *T. thiooxidans* ATCC 19377, *T. ferrooxidans* ATCC 23270, *T. neapolitanus* DSM 581, *T. versutus* DSM 582, *T. thioparus* DSM 505, *T. acidophilus* DSM 700, *T. thiooxidans* ATCC 8085, *T. aquaesulis* DSM 4255 and *T. tepidarius* DSM 3134 (Neil et al., 1988). Seldom acidic *Thiobacillus* strains were able to use carbon disulfide as sole energy source. For example, *Thiobacillus* strain TJ330 DSM 8985 that is similar to *T. thiooxidans* ATCC 19377 and *T. ferrooxidans* ATCC 23270 could take carbon disulfide as one of the substrates for growth (Hartikainen et al., 2000). Few literatures have reported the inhibitory mechanism of carbon disulfide on the biological desulfurization process.

One approach to enhance biological desulfurization focused on the carbon disulfide inhibition. Although the carbon disulfide slightly dissolved in the water, little carbon disulfide could obviously inhibit the growth of cells. In this paper, the effect of carbon disulfide on the biological desulfurization was investigated, and the inhibitory kinetics was proposed for the first time.

MATERIALS AND METHODS

Microorganism

In this study, *T. thioparus* CGMCC 4826 isolated from the effluent of sulfate reducing bioreactor in our laboratory was used. It had been proved that this strain was not able to grow on CS_2. This strain could oxidize thiosulfate to elemental sulfur and sulfate (Equation 3). Compared with sulfide, thiosulfate was not sensitive to chemical oxidation and non-toxic to cells. These benefits would make it easy to estimate the effect of carbon disulfide on desulfurization.

$$S\text{-}SO_3^{2-} + 1/2O_2 \xrightarrow{SOBs} S + SO_4^{2-} \quad (3)$$

Medium and Culture

The culture medium contained (g/L): KNO_3, 0.50; K_2HPO_4, 4.00; KH_2PO_4, 4.00, $MgSO4 \cdot 7H_2O$ 0.10, $CaCl_2$, 0.10; $FeCl_3 \cdot 6H_2O$, 0.02; $MnSO_4 \cdot H_2O$, 0.02; $Na_2S_2O_3 \cdot 5H_2O$, 15.00 g. The pH was adjusted to 7.0 with 1.0 M NaOH or 1.0 M HCl. *T. thioparus* was pre-cultured at 30°C and180 rpm for 36 h. And then, it was inoculated in medium volume of 1.0 L in a 3.0 L bioreactor (Bioflo 110 fermenter, New Brunswick Scientific, Edison, NJ) at the ratio of 5% (v/v). With the pH value being controlled at 6.8 to 7.2, the concentration of dissolved oxygen was maintained at 4 to 6 mg/L for the adequate supply of oxygen.

Biomass assay

The biomass concentration was measured by the amount of total N and N consumption (van den Bosch et al., 2006). Thiosulfate was oxidized to elemental sulfur and sulfate by *T. thioparus*, so there were many sulfur granules suspended in the medium. Approximately, 90% of biomass was absorbed by the sulfur particles. As a result, it was difficult to accurately measure the concentration of cell by the method of turbidimetry and protein assay. On basis of the mass balance, the growth rate of cell was determined by the consumption rate of nitrate (Visser et al., 1997).

Desulfurization by resting cell

The resting cells were prepared by the method of N source limitation. The bacteria could not grow without N source. *T. thioparus* was pre-cultured at 30°C and 180 rpm for 36 h. The culture was centrifuged at 500 rpm for 10 min to remove elemental sulfur particles. The supernatant was centrifuged at 6 000 rpm for 10 min. The precipitation was washed twice by physiological saline (0.9% NaCl), and was resuspended by the medium without potassium nitrate and thiosulfate. After stirred at 30°C, 180 rpm for 30 min, the thiosulfate solution (10×) was added. If carbon disulfide was needed, it was added into medium before being stirred.

Analytical method

Samples were filtered over 0.22 μm membrane. Sulfate, thiosulfate

*Corresponding author. E-mail: jmxing@home.ipe.ac.cn. Tel: +86 010 62550913. Fax: +86 010 62550913.

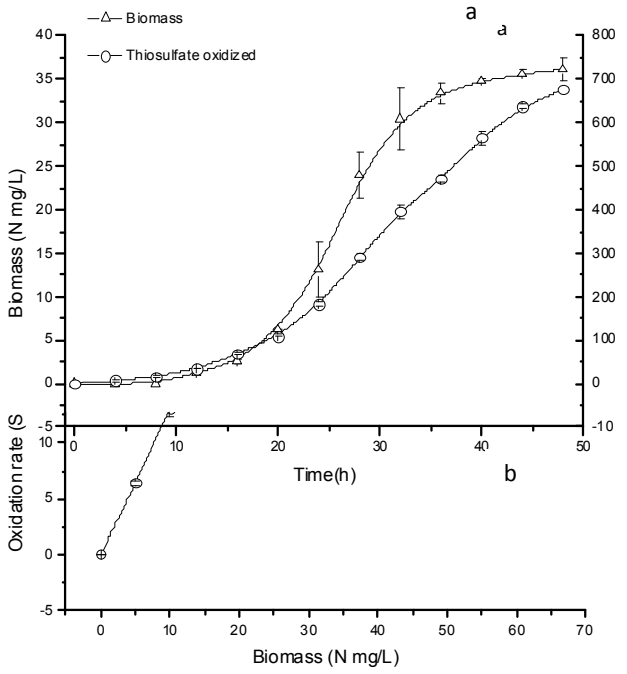

Figure 1. (a) The relation between cell growth and thiosulfate oxidation without carbon disulfide. **(b)** The desulfurization activity of the resting cell of *T. thioparus* under different cell density. The resting cell could be regarded as enzyme. The optimal density of resting cell was 20 to 25 N mg/L, and the corresponding maximum oxidation rate was about 25 $S_2O_3^{2-}$ mg/L-h.

and nitrate were analyzed by ion chromatography (Dionex model ICS 900, Dionex, Sunnyvale, CA), which was equipped with an electrical conductivity detector (Dionex Sunnyvale, CA). An ionpac AS14 column (Dionex Sunnyvale, CA) was operated at 25°C; the mobile phase was 8.0 mM Na_2CO_3/1.0 mM $NaHCO_3$ at a flow rate of 1.0 ml/min. The injection volume was 10 µL. The desulfurization activity of resting cells was determined by the method of termination reaction. After 60 min, the samples were taken from the reaction system, and immediately centrifuged at 10 000 rpm for 10 min to terminate the reaction. The samples were immediately analyzed by ion chromatography method. The unit of desulfurization activity was $S_2O_3^{2-}$ mg/L-h. The cells were cracked by sodium dodecyl sulfate (SDS) lysis buffer (Catalogue Number: 20 to 163, Millipore, USA), and the protein was stained with coomassie brilliant blue G-250 and the absorption value was measured at 595 nm (Hu et al., 2009). The average percentage of N in protein was 16% and the cell density was calculated by the below equation:

Cell density (mg-N/L) = 16% Protein content (mg-protein/L)

RESULTS

The growth of cells without carbon disulfide

As a control, *T. thioparus* was firstly cultured without carbon disulfide. The cell growth rate and the thiosulfate oxidation rate were investigated (Figure 1a). The delay phase of the cell growth was 20 h, and the platform phase appeared at the 40 h. The maximum of cell density was 36.13 mg-N/L. During the logarithmic phase, the average rate of the cell growth was 1.70 mg-N/L-h. It was found that the thiosulfate oxidation rate was not synchronously increased with the cell growth. After 28 h, there was a clear acceleration in the rate of thiosulfate oxidation. It was 12 h later than the logarithmic phase of the cell growth. When the concentration of cell was above 23.92 mg-N/L, the average rate of thiosulfate oxidation achieved the highest value, 26.50 $S_2O_3^{2-}$ mg/L-h. After the growth of cell had stopped, the thiosulfate oxidation rate was still kept at the constant level. These results suggest that the oxidation of thiosulfate was not completely attributed to the cell growth. The cells of *T. thioparus* mainly played a role of enzyme in the process of sulfur compounds oxidation, so that *T. thioparus* could be immobilized in the same way of enzyme (Qiu et al., 2006).

Effect of various concentrations of carbon disulfide on the cell growth

During the adsorption of hydrogen sulfide, carbon disulfide is absorbed into the absorbent. Carbon disulfide slightly dissolves in water, and its solubility in water is 0.20% at standard conditions. So, the maximum concentration of carbon disulfide in this experiment was set as 0.20%. To determine the effect of the carbon disulfide on the growth of *T. thioparus*, 0.01, 0.05, 0.10, 0.15, and 0.20% was added respectively to the medium with the other parameters unchanged. The results obviously showed that carbon disulfide had substantially negative effect on the growth of *T. thioparus* (Figure 2a).

When the concentration was 0.01%, the carbon disulfide extended the delay phase from 20 to 24 h. After that, *T. thioparus* was adapted to the carbon disulfide and presented similar curve of logarithmic growth to the control. The maximum cell density and the average growth rate of logarithmic phase were 35.62 and 1.59 mg-N/L-h, respectively (Figure 2b) both of them were closed to the bank control. However, under the condition of 0.05% carbon disulfide, the growth of cell was significantly inhibited by carbon disulfide. The maximum cell density and the average growth rate of logarithmic phase respectively decreased to 19.75 and 1.04 mg-N/L-h, which were only the 54.67 and 61.18% of the control group. The delay phase reached almost 32 h later. As the concentration of carbon disulfide increased to 0.10 and

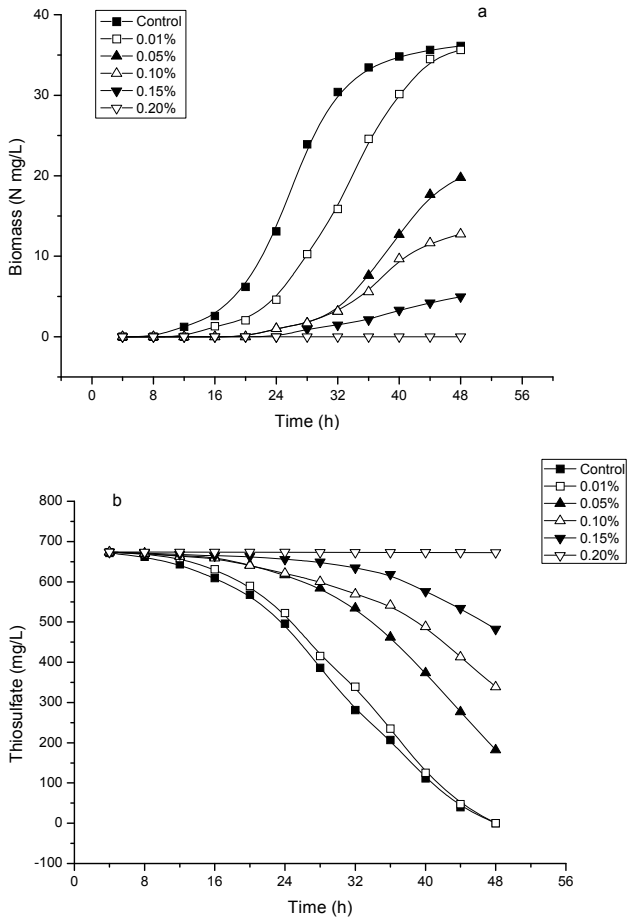

Figure 2. The effects of carbon disulfide on the thiosulfate oxidation and cell proliferation during cell growth. Various concentrations of carbon disulfide were added into the medium at the beginning of culture. It was obvious that carbon disulfide could negatively affect the growth of *T. thioparus* at the low concentration (a). However, there were no significant differences between control and 0.01% in the thiosulfate oxidized by the resting cell (b). It demonstrated that the thiosulfate oxidation was not coupled with the cell growth.

0.15%, the logarithmic phases of growth disappeared, the curves of growth cell were similar to the line. When the concentration of carbon disulfide reached 0.20%, the growth of cell was completely inhibited. The 0.05% carbon disulfide outstandingly inhibited the growth of cell, but the line of thiosulfate oxidation mostly paralleled the one of the control group. It suggested that carbon disulfide firstly affected the growth of cell, and then declined the desulfurization activity of cell.

Desulfurization activity of resting cell

On basis of the above results, it was deducted that the cell of *T. thioparus* could catalyze the thiosulfate oxidation in similar way as the enzyme. In this experiment, the growth of cell was limited by the absence of N resource, and the resting cell was taken as enzyme and the enzyme activity was estimated. In the middle of logarithmic phase, the cell was gathered by centrifu-gation, washed twice with physiological saline, and then resuspended by the medium without N resource. The cell density was measured by protein assay, and then was adjusted to 5, 10, 15, 20, 25, 30, 40 and 60 mg-N/L, respectively. The initial rate of thiosulfate oxidation was determined (Figure 1b). The initial rate reached the maximum value (25.19 $S_2O_3^{2-}$ mg/L-h) at the cell density of 20 mg-N/L.

Under the density of resting cell was set the optimal 20 mg-N/L, the concentration of substrate thiosulfate was changed to 0.5, 1, 2, 5, 10 and 20 g/L, and the initial rate of desulfurization was determined. The Michaelis-Menten equation was used for the process modeling. The K_m and V_{max} of the resting cell were calculated by the Lineweaver-Burk method (Kim et al., 2004; Li et al., 2008). The K_m and V_{max} were 0.264 mM and 27.93 $S_2O_3^{2-}$ mg/L-h, respectively (Figure 3a).

Inhibition of resting cell by carbon disulfide

In order to investigate the effect of carbon disulfide on resting cell, 0.05, 0.01 and 0.20% carbon disulfide was added to the solutions containing 10, 20, 30, 40, 50 mg-N/L resting cell. The initial rate of thiosulfate oxidation was detected after 60 min. In comparison with the control group, 0.05% carbon disulfide hardly impacted the rate of thiosulfate oxidation (Figure 4). When the concentration of carbon disulfide got to 0.10%, the rate of thiosulfate oxidation obviously decreased, but it could partially be restored with the increasing of biomass. In batch culture, the maximum cell density was about 40 mg-N/L. Under this cell density and 0.1% carbon disulfide, the rate of thiosulfate oxidation was only 14.0 $S_2O_3^{2-}$ mg/L-h (54.28% of the control group).

After that, the concentration of carbon disulfide was respectively set as 0.5 and 0.10%; the density of resting cell was 20 mg-N/L. The parameters of Michaelis-Menten equation was calculated by the method of Lineweaver-Burk (Figure 3b). When the concentration of carbon disulfide was 0.05%, the V_{max} was 26.77 mg $S_2O_3^{2-}$ /L-h, which was closed to the 27.93 $S_2O_3^{2-}$ mg/L-h of the control group. However, the K_m increased from 0.264 to 0.35 mM, and it continued to increase to 0.884 mM under 0.10% carbon disulfide.

DISCUSSION

In our experiments, the maximum cell density of *T. thioparus* CGMCC 4826 reached 36.13 mg-N/L, and the maximum growth rate was 10.9 mg-N/L-h. It had been reported that the *haloalkaliphilic Thioalkalivibiro* and *Thioalkalimicrbium* consortium used for hydrogen sulfide

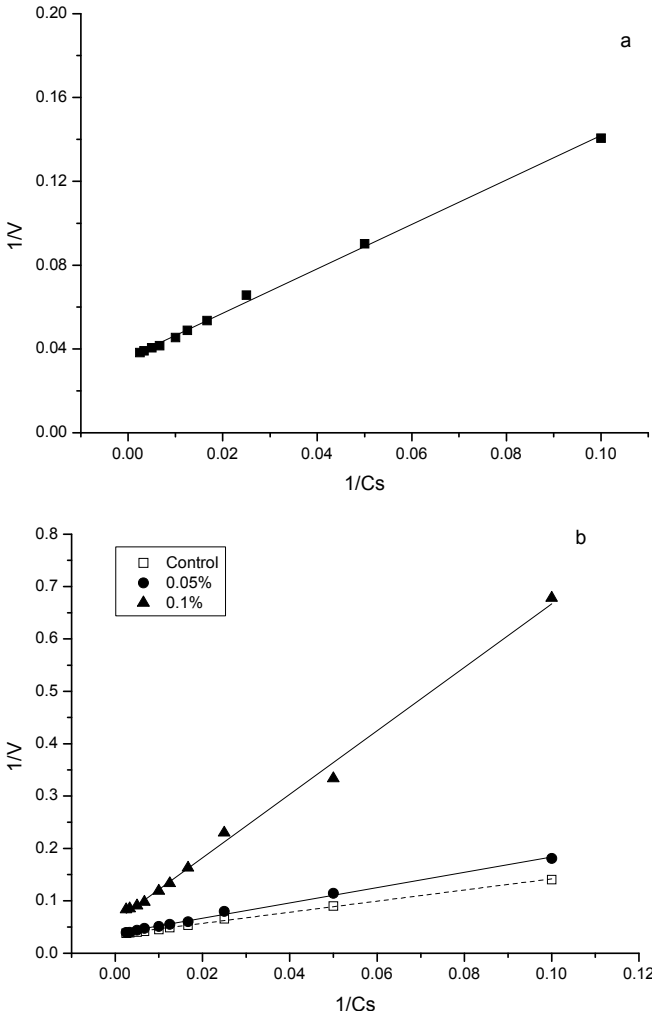

Figure 3. (a) The Michaelis-Menten parameters calculated by the method of Linewaver-Burk. K_m=29.61 mM, V_{max}= 27.93 $S_2O_3^{2-}$ mg/L-h (R^2=0.9979). **(b)** The effect of carbon disulfide on the Michaelis-Menten parameters of resting cell. When the concentration of carbon disulfide was 0.05%, the K_m and V_{max} were respectively, 39.21 mM and 26.77 $S_2O_3^{2-}$ mg /L-h. When it was 0.10%, the K_m and V_{max} were, respectively, 99.07 mM and 16.35 $S_2O_3^{2-}$ mg /L-h.

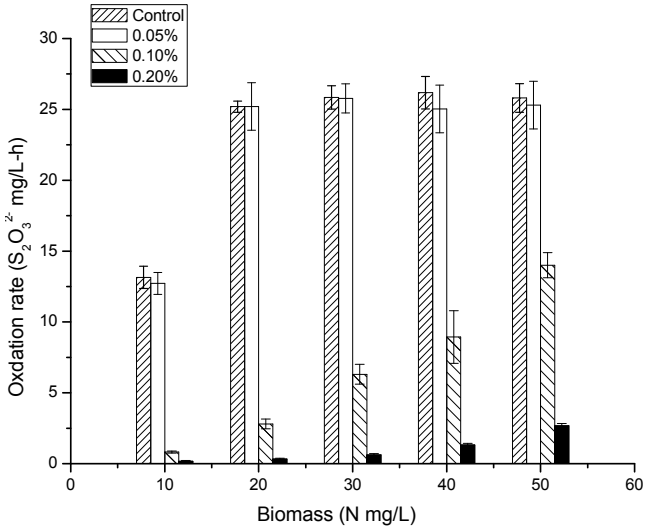

Figure 4. The effect of carbon disulfide on the desulfurization activity of resting cell. The 0.05% carbon disulfide hardly had effect on desulfurization activity of the resting cell.

oxidation had the maximum growth rate of 7.8 mg-N/L-h under sulfate producing condition (van Den et al., 2009). The biomass concentration of *T. denitrificans* could increase from 350 to 600 mg-protein/L (56 to 96 mg-N/L) with 4.7 mM (65.8 mg-N/L) NH_4^+ utilized in batch cultivation, and it was about 180 mg-protein/L (28.8 mg-N/L) in a continuous stirred-tank reactor under aerobic conditions (Sublette, 1987). It could be deduced that *T. thioparus* CGMCC 4826 could grow very well on the thiosulfate medium with nitrate as N source under the strict aerobic condition.

According to the results, biomass growth rate was not consistent with the thiosulfate oxidation rate, which indicated that thiosulfate oxidation was not completely coupled with biomass growth. Under the lower concentrations, the carbon disulfide violently inhibited the cell growth, but the thiosulfate oxidation was still running very well. The cells action in desulfurization was more like that of an enzyme.

This presumption could be proved by the immobilized SOBs, which were good for the enhancement of microbiological desulfurization. *Thiobacillus* sp. had been successfully immobilized into different material like Ca-alginate, K-carrageenan, agar and polyurethane by entrapment methods. After being pre-cultured 4 to 5 days, the immobilized *Thiobacillus* sp. could be reused for 10 cycles with the sulfide oxidation of 94 to 96% (Ravichandra et al., 2009). Thus, the resting cell of *T. thioparus* can also be investigated for the activity of desulfurization and the effect of carbon disulfide. The thiosulfate oxidation rate was rose up with the increase of biomass concentration, and its maximum was gained at the cell density of 20 mg-N/L.

Carbon disulfide was common ingredient in sour natural gas and biogas, some tail gases of plants. The concentrations of carbon disulfide in landfill gas from four landfill sites were in the range of from 25 to 5352 ppb, and the highest ratio of H_2S to CS_2 was about 6:1 (Kim et al., 2005), which was highly toxic to the strain *T. thioparus*.

The bad ability of removing carbon disulfide must lead to the accumulation of carbon disulfide in the recycle of adsorbents, which would negatively affect the biological desulfurization. When its concentration was 0.01%, *Thiobacillus* sp. could oxidize the carbon disulfide at pH 7.0 and 30°C, but the degradation activity was caused above levels of 0.015% (Plas et al., 1993). In this study, when its concentration was as low as 0.01%, carbon

disulfide could obviously decrease the cell growth rate. As the level was increased to 0.05%, the maximum of biomass was only 19.75 mg-N/L; 54.68% of the control group.

In contrast, carbon disulfide had less effect on the desulfurization activity of the resting cell. As its concentration was up to 0.05%, there were no obvious differences to the control batches. Under 0.05 and 0.1% carbon sulfide, the K_m of resting cell increased to 0.35 and 0.884 mM, respectively, which indicated that the affinity of resting cell to the substrate might decline. The thiosulfate was oxidized by the enzymes of Sox pathway located in bacterial periplasm, including a series of reactions (Bamford et al., 2002; Kelly et al., 1997). The possible reason was that carbon disulfides can affect the selective permeability of cell membrane, which leads to losing the ion balance of cell membrane. The $S_2O_3^{2-}$ or electron could not be correctly transferred to the sits of oxidase on the membrane.

Conclusion

The sulfur compounds oxidation was not coupled with the growth of *T. thioparus,* the cells could be considered as enzyme, and the optimal density of resting cell was 20 mg-N/L. Although the carbon disulfide slightly dissolved in the water, little carbon disulfide could obviously inhibit the growth of cells. Moreover, it would significantly inhibit the desulfurization activity of resting cell for its cell toxicity, when its concentration was above 0.15%. In the process of biological desulfurization, carbon disulfide could not be oxidized by the SOBs; otherwise it may accumulate to a higher amount in the adsorbent. It would cause significant inhibition of desulfurization by restraining the cells density to an optimal value. If its concentration was above 0.05%, it would directly inhibit the desulfurization activity of cells. Therefore, 0.05% was the dangerous value of carbon disulfide for the biological desulfurization. Thus, under the optimized bio-desulfurization process, it was necessary to control the concentration of carbon disulfide in the absorbent below 0.05% by renewing the absorbent.

Conflict of interests

The author(s) have not declared any conflict of interests.

REFERENCES

Abatzoglou N, Boivin S (2009). A review of biogas purification processes. Biofuels Bioprod. Biorefining 3:42-71.

Bamford VA, Bruno S, Rasmussen T, Ayme CA, Cheesman MR, Berks BC, Hemmings AM (2002). Structural basis for the oxidation of thiosulfate by a sulfur cycle enzyme. EMBO J. 21:5599-5610.

Böresson G (2001). Inhibition of methane oxidation by volatile sulfur compounds (CH_3SH and CS_2) in landfill cover soils. Waste Manage Res. 19:314-319.

Chaiprapat S, Mardthing R, Kantachote D, Karnchanawong S (2011). Removal of hydrogen sulfide by complete aerobic oxidation in acidic biofiltration. Process Biochem. 46:344-352.

Hartikainen T, Ruuskanen J, Räty K, Von WA, Martikainen PJ (2000). Physiology and taxonomy of *Thiobacillus* strain TJ330, which oxidizes carbon disulphide (CS_2). J. Appl. Microbiol. 89:580-586.

Hu Z, Lin BK, Xu Y, Zhong MQ, Liu GM (2009). Production and purification of agarase from a marine agarolytic bacterium *Agarivorans* sp. HZ 105. J. Appl. Microbiol. 106:181-190.

Kelly DP, Shergill JK, Lu WP, Wood AP (1997). Oxidative metabolism of inorganic sulfur compounds by bacteria. Antonie Van Leeuwenhoek 71:95-107.

Kim CS, Ji ES, Oh DK (2004). Characterization of a thermostable recombinant β-galactosidase from *Thermotoga maritima*. J. Appl. Microbiol. 97:1006-1014.

Kim KH, Choi YJ, Jeon EC, Sunwoo Y (2005). Characterization of malodorous sulfur compounds in landfill gas. Atmos. Environ. 39:1103-1112.

Kyeoung SC, Hirai M, Shoda M (1991). Degradation characteristics of hydrogen sulfide, methanethiol, dimethyl sulfide and dimethyl disulfide by *Thiobacillus thioparus* DW44 isolated from peat biofilter. J. Biosci. Bioeng. 71:384-389.

Li N, Meng K, Wang YR, Shi PJ, Luo HY, Bai, YG, Yang, PL, Yao B (2008). Cloning, expression, and characterization of a new xylanase with broad temperature adaptability for *Streptomyces* sp. S9. J. Appl. Microbiol. 80:231-240.

Lobo R, Revah S, Viveros-Garcia T (1999). An analysis of a trickle-bed bioreactor: Carbon disulfide removal. Biotechnol. Bioeng. 63:98-109.

Mata-Alvarez J, Llabrés SM (2000). Anaerobic digestion of organic solid wastes: An overview of research achievements and perspectives. Bioresour. Technol. 74:3-16.

Neil AS, Kelly DP (1988). Oxidation of carbon disulfide as the sole source of energy for the autotrophic growth of *Thiobacillus thioparus* strain TK-m. J. Gen. Microbiol. 134:3041-3048.

Plas C, Winner K, Holubar P, Mattanovich D, Danner H, Jelinek E, Harant H, Braun R (1993). Degradation of carbon disulfide by a *Thiobacillus* isolate. Appl. Microbiol. Biotechnol. 38:820-823.

Qiu GL, Li YL, Zhao K (2006). *Thiobacillus thioparus* immobilized by magnetic porous beads: Preparation and characteristic. Enzyme Microb Technol 39:770-777.

Ravichandra P, Gopal M, Annapurna J (2009). Biological sulfide oxidation using autotrophic *Thiobacillus* sp.: Evaluation of different immobilization methods and bioreactors. J. Appl. Microbiol. 106:1280-1291.

Sheng YQ, Chen FZ, Wang XM, Sheng GY, Fu JM (2008). Odorous volatile organic sulfide in wastewater treatment plants in Guangzhou, China. Water Environ. Res. 80:324-330.

Sublette KL (1987). Aerobic oxidation of hydrogen sulfide by *Thiobacillus denitrificans*. Biotechnol. Bioeng. 29:690-695.

Van-den BPLF, De Graaff M, Fortuny-Picornell M, Van Leerdam RC, Janssen AJH (2009). Inhibition of microbiological sulfide oxidation by methanethiol and dimethyl polysulfides at natron-alkaline conditions. Appl. Microbiol. Biotechnol. 83:579-587.

Van-den BPLF, Van BOC, Buisman CJN, Janssen AJH (2006). Sulfide oxidation at halo-alkaline conditions in a fed-batch bioreactor. Biotechnol. Bioeng. 97:1053-1063.

Visser JM, Robertson LA, Van VHW, Kuenen JG (1997). Sulfur production by obligately chemolithoautotrophic *Thiobacillus* species. Appl. Environ. Microbiol. 63:2300-2305.

Zhang LH, De Schryver P, De Gusseme B, De Muynck W, Boo N, Verstrate W (2008). Chemical and biological technologies for hydrogen sulfide emission control in sewer systems: A review. Water Res. 42:1-12.