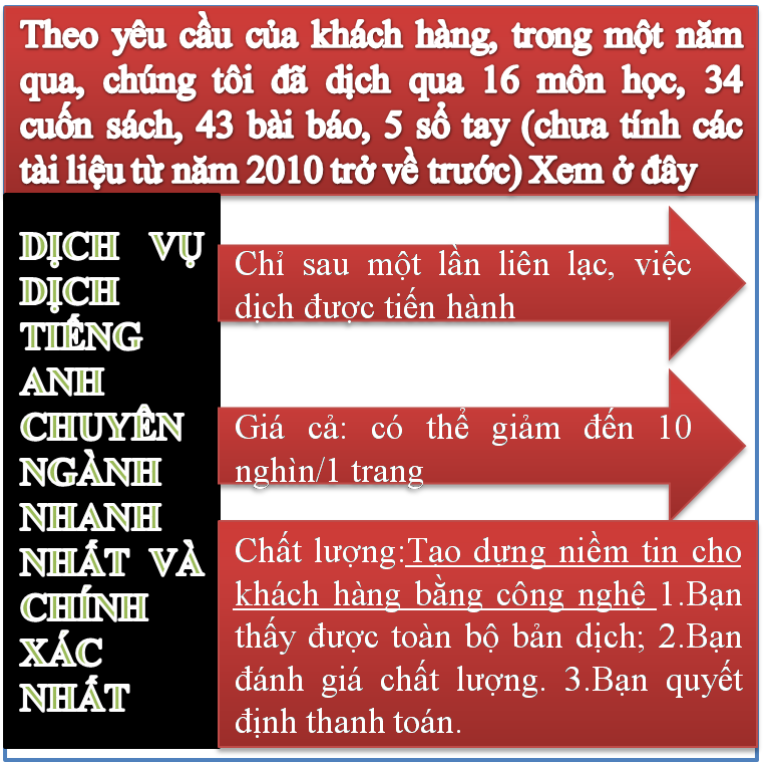

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Removal of H2S in down-flow GAC biofiltration using sulfide oxidizing bacteria from concentrated latex wastewater	Loại bỏ H2S trong quá trình lọc sinh học GAC chảy xuôi bằng vi khuẩn oxy hóa sunfua lấy từ nước thải mũ cao su đậm đặc chảy xuôi: chảy từ trên xuống dưới	
A biofiltration system with sulfur oxidizing bacteria immobilized on granular activated carbon (GAC) as packing materials had a good potential when used to eliminate H2S. The sulfur oxidizing bacteria were stimulated from concentrated latex wastewater with sulfur supplement under aerobic condition. Afterward, it was immobilized on GAC to test the performance of cell-immobilized GAC biofilter. In this study, the effect of inlet H2S concentration, H2S gas flow rate, air gas flow rate and long-term operation on the H2S removal efficiency was investigated. In addition, the comparative performance of sulfide oxidizing bacterium immobilized on GAC (biofilter A) and GAC without cell immobilization (biofilter B) systems was studied. It was found that the efficiency of the H2S removal was more than 98% even at high concentrations (200-4000 ppm) and the maximum elimination capacity was	Một hệ thống lọc sinh học bằng vi khuẩn oxy hóa lưu huỳnh cố định trên than hoạt tính dạng hạt (GAC) đóng vai trò như vật liệu đóng gói có tiềm năng to lớn khi sử dụng để loại bỏ H2S. Vi khuẩn oxy hóa lưu huỳnh được lấy từ nước thải cao su đậm đặc có bổ sung lưu huỳnh trong điều kiện hiếu khí. Sau đó, nó được cố định trên GAC để kiểm tra hiệu suất của bộ lọc sinh học GAC cố định tế bào. Trong nghiên cứu này, chúng tôi khảo sát ảnh hưởng của nồng độ H2S nạp vào, tốc độ dòng khí H2S, lưu lượng không khí và hoạt động thời gian dài đến hiệu suất loại bỏ H2S. Thêm vào đó, chúng tôi cũng so sánh hiệu suất của các hệ dựa trên vi khuẩn oxy hóa sunfua cố định trên GAC (bộ lọc sinh học A) và GAC không cố định tế bào (bộ lọc sinh học B). Chúng tôi thấy rằng hiệu suất loại bỏ H2S lớn hơn 98% ngay cả ở các nồng độ cao (200-4000 ppm) và khả	

about 125 g H2S/m of GAC/h in the biofilter A. However, the H2S flow rate of 15-35 l/h into both biofilters had little influence on the efficiency of H2S removal. Moreover, an air flow rate of 5.86 l/h gave complete removal of H2S (100%) in biofilter A. During the long-term operation, the complete H2S removal was achieved after 3-days operation in biofilter A and remained stable up to 60-days.	năng loại bỏ tối đa khoảng 125 g H2S / m GAC / h trong bộ lọc sinh học A. Tuy nhiên, lưu lượng H2S 15-35 l / h vào cả hai bộ lọc sinh học có ảnh hưởng rất ít đến hiệu suất loại bỏ H2S. Hơn nữa, lưu lượng khí 5.86 l / h đã loại bỏ hoàn toàn H2S (100%) trong bộ lọc sinh học A. Trong quá trình hoạt động lâu dài, H2S bị loại bỏ hoàn toàn sau 3 ngày hoạt động trong bộ lọc sinh học A và duy trì ổn định lên đến 60 ngày.	
1. Introduction Anaerobic treatment of concentrated latex wastewater, which is sulfate-rich wastewater, can generate hydrogen sulfide (H2S) as a by-product in biogas. H2S is produced naturally during the reduction of sulfate and sulfur-containing organic compounds by anaerobic bacteria. H2S is a colorless, toxic, flammable gas that is responsible for the foul odor of rotten eggs, an odor that is a major nuisance in municipal, industrial and biological wastewater treatment systems. H2S is extremely toxic to living organisms and plants. At a level of 0-5 ppm in the air, it can be detected easily. At levels greater than 10 ppm it	[redacted]	

can affect human health, while levels of more than 600 ppm can cause death (Droste, 1997). Biofiltration has recently been recognized as one of the most popular and efficient technologies for odor treatment. A typical biofiltration process consists of two steps. Firstly, the pollutant is transferred from the air stream into liquid film and adsorbed on a solid medium; then the pollutant is biodegraded by microbes living in the liquid phase or on the packing material. Therefore, the operating conditions of the biofilter, supporting material, and inoculated microbes are important parameters to consider (Duan et al., 2007). Recently, cell-immobilized biofiltration has become one of the most important biological processes for treating H2S gases. This process has low capital and operating costs for its regeneration and recirculation. Moreover, it requires less energy and no additional chemicals or fuels. Above all, it was public acceptance as an envi-ronment-friendly process for reducing secondary pollution (Ma et al., 2006a).	[redacted]	

Several different packing materials have been used in biofiltration for the removal of H2S. However activated carbon has been recognized as the most extensively used material, due to its capacity for adsorbing substrates quickly and then slowly releasing them for microbial degradation (Duan et al., 2005b). The major function of activated carbon is to support the microorganisms and act as a buffer for fluctuating loading (Duan et al., 2005a). The immobilization of microorganisms to activated carbon in biofiltration is the self-attachment of the microorganisms to the filter, which is defined as attached growth system. The advantages of attached microbial film compared to suspended microorganisms are higher biomass concentrations, higher metabolic activity and greater resistance of toxicity (Cohen, 2001).

It has been reported that various sulfide oxidizing bacterium, including Thiobacillus, Xanthomonas and Pseudomonas have great

potential to metabolize H2S effectively for its low acid production and fast oxidation rate in activated carbon (Ma et al., 2006a, 2006b; OyarzUn et al., 2003; Chung et al., 1996b). Most researches studied H2S removal using one microorganism immobilized on carrier (Ma et al., 2006a, 2006b; Son and Lee, 2005; OyarzUn et al., 2003; Chung et al., 1996b).

However, there are limitations on employing pure cultures for concentrated latex industrial application to remove H2S using biofiltration. Although some researches used mixed culture from compost (Morgan-Sagastume and Noyola, 2006) and wastewater treatment plant sludge (Duan et al., 2006; Kim et al., 2008), there are still a lot of microbial diversity in different sources.

In addition, the microorganisms from sulfate reduction tank of concentrated latex industry for H2S removal have never been reported.
Therefore, this research used

sulfide oxidizing bacteria obtained from concentrated latex wastewater. The performance of biofiltration system using cell-immobilized granular activated carbon (GAC) was investigated. The objectives of this research were to determine the optimum operating parameters, including inlet H2S concentration, H2S gas flow rate, air flow rate and long-term operation.

2. Methods

2.1. Microorganisms and cell immobilization on GAC

Sulfide oxidizing bacteria were stimulated from concentrated latex wastewater with 2.21 g sulfur supplement under aerobic condition for 3 days. For cell immobilization, commercial granular activated carbon (GAC) was selected as the support material. GAC was sieved to obtain the particle size of 6-7 mesh (2.83-3.36 mm). After microbial stimulation process, sulfide oxidizing bacteria were harvested by centrifugation (8000 rpm for 10 min). Then, the pallet was put into a 5 l plastic tank containing 3 l sterile concentrated latex wastewater. At the same time, 500 g GAC was added into for

microbial attachment.

The cultivation was conducted under aerobic condition. During the cultivation period, the cultured liquid in plastic tank was removed and then new sterile concentrated latex wastewater was replaced every 3 days. The cell numbers of microbial immobilization on GAC were estimated everyday by the traditional plate-counting method using a thiosulfate mineral medium. After 15 days, the microorganisms immobilized on GAC reached 4.0 x 108cfu/g dry GAC. Then the cell-immobilized GAC was transferred into biofilters.

2.2. Thiosulfate mineral medium

Thiosulfate mineral medium, which is a selective medium for sulfide oxidizing bacteria, contained the following (g/l): 2.0 KH_2PO_4, 2.0 K_2HPO_4, 0.4 NH_4Cl, 0.2 $MgCl_2 \cdot 6H_2O$, 0.01 $FeSO_4 \cdot 7H_2O$ and 8.0 $Na_2S_2O_3 \cdot 5H_2O$ (Jin et al., 2005a). It was used for cell number estimation and isolation of sulfide oxidizing bacterium. Also, it was employed as the solution for humidification in biofilters.

2.3. Experiment setup and operation

The experiment was performed in two laboratory-scale down- flow biofilters (Fig. 1). Each biofilter was made of stainless steel, with 0.055 m inner diameter and 0.6 m height (a working volume of 1 l). One biofilter was packed with 40 cm of cell-immobilized GAC (biofilter A) and another was packed with 40 cm of the GAC without cell immobilization (biofilter B). A packed bed volume in biofilters was 0.67 l (about 400 g wet weight of GAC). Air was supplied with an air compressor, with flow rate controlled by flow meter. The air was passed through a column of the thiosulfate mineral medium for humidification. Humid air was then mixed with H2S gas, which was generated from a PVC pipe (0.05 m ID and 0.6 m length), by an equimolar reaction of Na2S and HCl solution. Biofilters were operated in a down flow mode. The inlet mixture gas

was fed to the top of the biofilters at constant flow rate. The sampling points were provided at the inlet and outlet of the biofilters. A series of tests were conducted to investigate the performance of bio-filters. In the first set of tests, the effect of inlet H2S concentration, H2S gas flow rate and air flow rate on H2S removal was studied (Table 1). Inlet and outlet H2S gas concentrations of biofilters were periodically measured. At the end of the first test, the 0.5 g of GAC was taken from biofilters. Then it was mixed with 10 ml distilled water and was vortexed for 2 min. The solution was then determined for pH, total sulfur and sulfate concentrations. Moreover, sulfide oxidizing bacteria from GAC in biofilter A was isolated and identified. In the second set of tests, the performance of biofilter A at the optimum condition was examined in long-term operation (Table 1). The efficiency of H2S removal was evaluated. The biofiltration system was	[redacted]	

operated at room temperature (2732 °C) throughout all the experiments.

2.4. Analytical method

H2S gas concentrations were determined by the Cadmium sulfide method (Jacob, 1996). The concentrations of sulfur in biofilters were measured by the gravimetric method (AOAC, 1990) and sulfate concentrations were measured by turbidimetric method (APHA, 1998).

For the estimation of cell numbers, 0.5 g of GAC was mixed with 5 ml 0.85% NaCl and then was shaken for 3 min. The cell number in the sample solution was enumerated by traditional plate-counting method. Microorganisms that appeared on the thiosulfate mineral medium were isolated. A 16S rRNA gene sequence (500 base pairs) was also carried out to identify the isolated strains. Genomic DNA was extracted using the standard method and then amplified by GeneAmpPCR system 96000 (Altschul et al., 1997) followed by a homology

search using the BLASTn program from the NCBI database.

Time (day)

Fig. 2. Effect of inlet H2S concentrations on H2S removal efficiencies.

of the H2S removal remained higher than 98% at high inlet concentration in biofilter A (Fig. 2).

The H2S capacity to remove through biofiltration was carried out by elimination capacity (EC) (g/m /h). EC is amount of contaminant being removed in the biofiltration. This parameter was calculated according to the following equations:

Elimination capacity (EC) = Q (Cin - Cout) (1)

where Q is the flow rate (m3/h), V is the medium volume (m3), and Cin and Cout are the inlet and outlet concentration of H2S, respectively (g/m3).

H2S removal and elimination capacity as a function of the inlet H2S loading was estimated and presented in Fig. 3. The elimination

capacity is defined as the amount of pollutant degraded per unit of time, normalized to the volume of the packed bed. The inlet H2S loading is defined as the amount of inlet gas per unit time and volume of packed bed (g/m3/h) (Chung et al., 1996b). It was found that H2S removal was not affected by the shock loading when the inlet

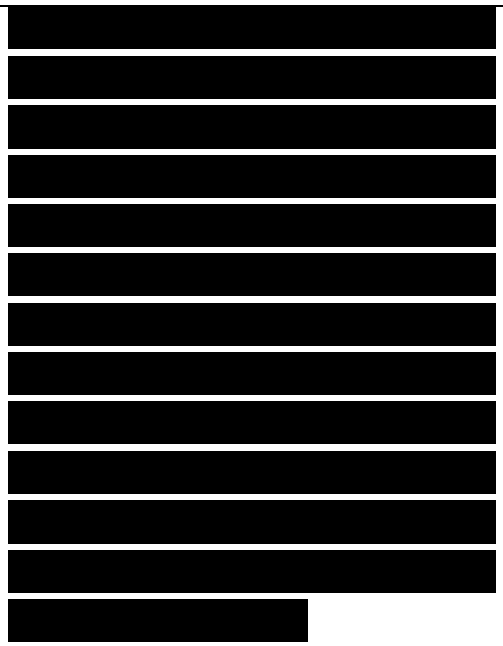


Fig. 3. H2S removal and elimination capacity (EC) as a function of the inlet H2S loading in boifilters A and B.

H2S loading was suddenly increase from 19 g/m3/h (500 ppm) to 149g/m3/h (4000 ppm). Moreover, the maximum elimination capacity was determined as 125 g H2S/m3/h in biofilter A and 112gH2S/m3/h in biofilter B at inlet H2S loading of 149g/m3/h. In addition, it was found that elimination capacity of the H2S in this study was higher than that from other reports presented in Table 2. However, it depends on microbial strains and type of packing materials (Potivichayanoin et al., 2006; Cho et al., 2000).

3.2. Effect of H2S gas and air flow rate

The effect of the H2S gas flow rate on H2S removal efficiency was investigated during 21 days. The inlet H2S concentration and air flow rate were set at 200 ppm and 0.75 l/h, respectively. The experiment was carried out at the H2S gas flow rate of 15, 25 and 35 l/h. The results are showed in Fig. 4. It was found that the H2S removal efficiency was not significantly different at various H2S gas flow rates. The average H2S removal efficiency of biofilters A and B reached 99% and 84%, respectively. Chung et al. (1996b) reported that H2S removal efficiencies at gas flow rates between 36 and 76 l/h showed little variation but it was significantly different at a gas flow rate of 150 l/h. The stable removal efficiency H2S flow rate (15-35 l/h) can be explained by the adaptation of microorganisms to the new environment when the slow diffusion of H2S gas took place (Tuuguu et al., 2006). Furthermore, the effect of the air flow rate on H2S removal efficiency was investigated for

21 days. The inlet H2S concentration and H2S gas flow rate were kept constantly at 200 ppm and 35 l/h, respectively. The varied air flow rates of 0.75, 1.34 and 5.83 l/h were examined. The results show that complete H2S removal (100%) was found in biofilter A at 5.83 l/h of air flow rate (Fig. 5).

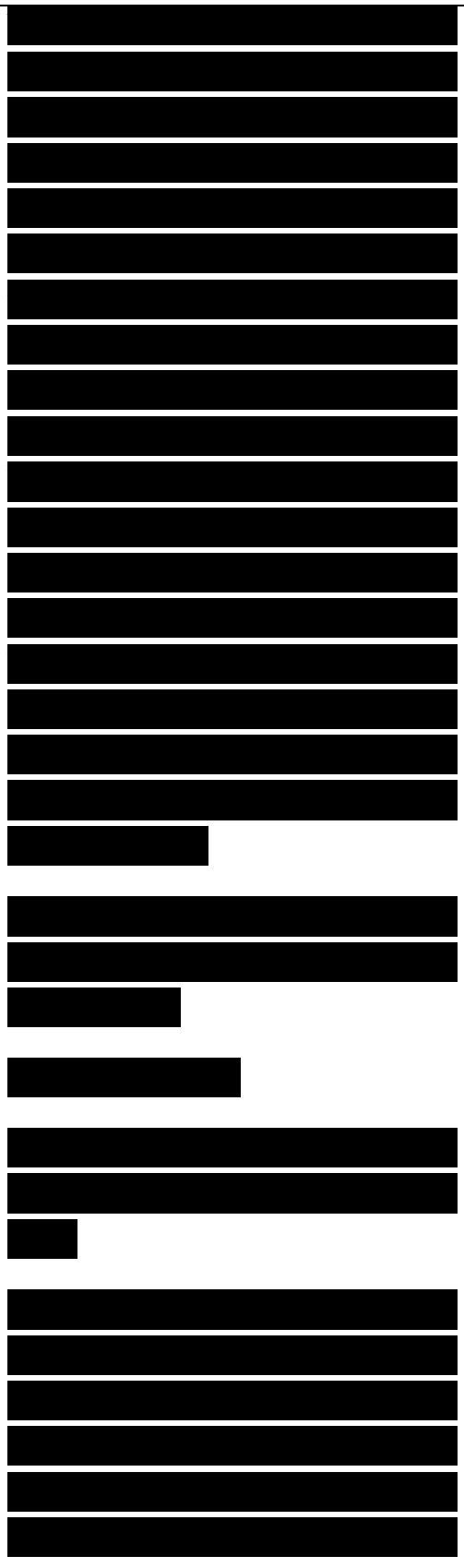
This may cause from adequate oxygen supplement in bifiltration system. Oxygen is the key parameter that controls the level of oxidation (Alcantara et al., 2004). Insuffi-

Fig. 4. Effect of H2S gas flow rate on H2S removal efficiencies.

Time (day)

Fig. 5. Effect of air flow rate on H2S removal efficiencies.

cient oxygen may cause a shift in the proportion of the end-products and prevent complete removal of H2S. Generally, sulfuric acid and H2O is the end-product of H2S oxidation when the oxygen is adequate.



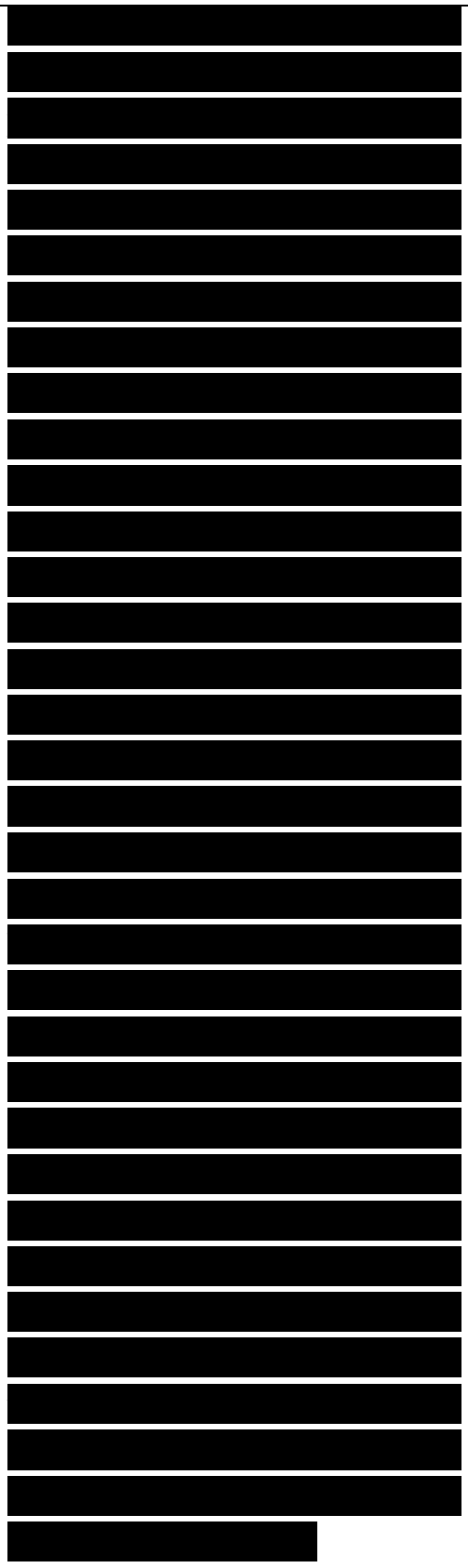
However, elemental sulfur may become the end-product when oxygen is consumed and apparently decreased (Potivicha- yanoin et al., 2006).

3.3. Metabolic products and microorganism on GAC

Acidification and metabolic products are significant parameters for acid gas treatment. Chung et al. (2001) claimed that the major metabolic products of heterotrophic Pesudomonas putida CH11 for H2S treatment were sulfur and sulfate. In this present study, the levels of pH, sulfur and sulfate in the biofiltration system for H2S removal were considered as parts of the functions of biofiltration. During the 82-days operation in the first set of tests, the pH, sulfur and sulfate values in biofiltration were determined before and after the experiment. It was found that pH decreased from 8.52 to 2.04, sulfur concentration increased from 0.73 to 55.3 g/l of packing bed and sulfate concentration increased from 0.07 to 38.7 g/l of packing bed

in biofilter A (Table 1). Whereas the pH decreased from 7.52 to 1.99, sulfur concentration increased from 0.60 to 14.0 g/l of packing bed and sulfate concentration increased from 0 to 24.5 g/l of packing bed in biofilter B (Table 1). However, the variations of pH, sulfur and sulfate were not influenced on the efficiency of H_2S removal (Figs. 4 and 5). Corresponding to Jin et al. (2005b), they reported that the H_2S removal was slightly changed between pH 2.00 and 7.00. This may be due to different microbial groups dominating and having activities at different pH.

At the end of the experiment, GAC in biofilter A was withdrawn for isolation and identification of sulfide oxidizing bacteria. It was found that only two isolates were grown on thiosulfate mineral medium agar plate. Afterwards, isolates were identified by 16S rDNA sequencing. One isolated strain was accession number, CP 000304, for the 16S rDNA gene sequence, which was 100% similar to that Pseudomonas stutzeri A1501.

Another isolated strain was accession number, AY 509223, for the 16S rDNA gene sequence, which was 100% similar to Microbacterium oxydans strain S28n. P. stutzeri is a heterotrophic, gram-negative, and rod-shaped bacte-rium. Its colonies were round, convex, regular and green with the average diameter of 2 mm on thiosulfate mineral medium agar plate. The US Patent issued on December 5, 2000 showed that P. stutzeri oxidized H2S to S4o6~ without gaining energy from the reaction process for the purification of gases containing hydrogen sulfide in bioscrubber (US patent, 2000). M. oxydans is a heterotro-phic, gram-positive, and rod-shaped bacterium. This strain was yellow colonies on thiosulfate mineral medium agar plate. Li et al. (2005) reported that Microbacterium strain ZD-M2 can be able to remove dibenzothiophene and other organic sulfur compound. However, M. oxydans has never been reported as microorganisms for H2S removal.		

3.4. Comparison of biological and conventional oxidation processes H2S removal by biofilter A (biological oxidation) could be maintained at the high level of over 98%. In comparison with biofilter B (conventional oxidation), it was found that biofilter A can remove H2S more effectively than biofilter B by about 15%. It indicated that H2S mainly oxidized auto-catalytically when it deposited on GAC (about 80-85% H2S removal) (Figs. 2-5). However, biodegradation of H2S by sulfide oxidizing bacteria enhanced the efficiency of H2S removal in GAC biofilter. Furthermore, the maximum elimination capacity of biofilter B (112 g H2S/m~3/h) was lower than biofilter A (125 gH2S/m~3/h) due to a higher oxygen requirement. Generally, the conventional oxidation by GAC as an adsorption process requires a much air/oxygen being supplied to the waste gas but biological aerobic oxidation by microbial H2S degradation to elemental sulfur and sulfates is not required (Zicari,	[redacted]	

2003).

Therefore, these preliminary results indicate that the combination of conventional oxidation and biological oxidation was suitable for H2S removal, with its lower air/oxygen requirement, and has potential for industrial application.

3.5. The long-term performance of biofiltration for H2S removal

H2S at high concentrations is often emitted from various industries or manufacturing processes. It is necessary to test the H2S removal and characteristics by sulfide oxidizing bacteria GAC reactor (biofilter A) over a long-term operation. The selected inlet concentrations of H2S gas was 200 ppm. The H2S and air gas flow rates were 35 l/h and 5.83 l/h, respectively. The H2S removal was studied for 60 days operation. It was found that the H2S removal efficiency achieved a value of 100% after 3 days of operation and it remained stable during 4-60 days.

During the 60 days of operation, pH decreased from 8.35 to 2.10 resulting from sulfate formation. Sulfate concentration increased from 0.04 to 33.9 g/l of GAC. Sulfur also generated as one of metabolic products. Sulfur concentration increased from 0.45 to 11.2 g/l of GAC (Table 1).

5. Conclusions

The sulfide oxidizing bacteria were stimulated from concentrated wastewater with sulfur supplement and then were immobilized on GAC biofilter to study the performance of biofilter for H2S removal. In comparison of GAC biofilter without cell immobilization, it was found that sulfur oxidizing bacteria enhanced to re-moval H2S in GAC biofilter. The efficiency of the H2S removal reached more than 98% even at high concentrations (2004000 ppm) in sulfide oxidizing bacteria immobilization on GAC biofilter. In addition, a high elimination capacity (125 g H2S/m3 of GAC/h) was achieved. Furthermore, it was found that H2S flow rates of 15-35 l/h and air flow rates of 0.75-5.83 l/h into biofilters had little variation on the

efficiency of the H2S removal. However, the complete H2S removal (100%) was found at the inlet H2S concentrations of 200 ppm, the H2S gas flow rate of 35 l/h and air flow rate of 5.83 l/h by sulfide oxidizing bacteria immobilization on GAC biofilter even though biofilter was operated for long term (up to 60-day operation period). An acidification phenomenon occurred in this system during H2S removal. Sulfur and sulfate were found to be the metabolic products of biofilters. Moreover, the sulfur oxidizing bacteria isolated from cell-immobilization GAC biofilter were P. stutzeri and M. oxydans. From the results, it can be concluded that the conventional H2S oxidation of the GAC biofilter combined with biodegradation of H2S by the sulfide oxidizing bacteria showed a good performance in treating H2S gas.	[redacted] Từ những kết quả này, chúng ta có thể kết luận rằng sự oxy hóa H2S thông thường của bộ lọc sinh học GAC kết hợp với sự phân hủy sinh học H2S do vi khuẩn oxy hóa sunfua có hiệu suất xử lý khí H2S tốt. checked	
