CHEMICAL ENGINEERING TRANSACTIONS, Volume 15, 2008
Edited by Renato Del Rosso
 ISBN 978-88-95608-09-9

Removal of hydrogen sulphide by immobilized *Acidithiobacillus thiooxidans* in a Biotrickling filter packed with polyurethane foam.

M. Ramirez[1,*], K. Sedmikova[2], J.M Gómez[1], J. Paca[2] and D. Cantero[1]
[1]Department of Chemical Engineering, Food Technology and Environmental Technologies. Faculty of Sciences. University of Cádiz. 11510 Puerto Real (Cádiz)
[2]Institute of Chemical Technology, Department of Fermentation Chemistry and Bioengineering, Prague, Czech Republic.
*Corresponding author: Tel:+34956016554; Fax:+34956016411. E-mail. martin.ramirez@uca.es

In this work, a laboratory scale biotrickling filter was applied for removal of hydrogen sulphide packed with *Acidithiobacillus thiooxidans* (DSM11478) immobilized in polyurethane foam particles (PUF). The biotrickling filter has removal efficiency greater than 99% for inlet hydrogen sulphide concentration of 65.9 ppmv and EBRT of 24.4 seconds. The system removed over 98% of the hydrogen sulphide in the pH range of 0.44-7.29. A concentration of 58 $g.l^{-1}$ of biological sulphate concentration was reached without inhibition effect.

1 Introduction

Malodorous gases emitted from many industrial activities are not only a nuisance, also cause significant health problems for workers and nearby residents. It is well known that more than three hundred substances can cause this problem, but hydrogen sulphide is the one that often exists in our surroundings. Large amounts of hydrogen sulphide are generated and released from industrial processes, such as wastewater treatment, petrochemical refining and Kraft pulping processes. Usually, the low concentration of these odorous compounds in the gas emissions needs other technologies apart from the traditional ones as incineration, adsorption or chemical scrubbing. So, based on the cost for the equipment and operation, biological treatment is believed to be the most economical option. The biofiltration process has shown to have a very high efficiency to treat diluted and easily biodegradable waste gases. Thus, hydrogen sulphide is an excellent candidate for removal by biofiltration.
Many microorganisms has been used for removal of hydrogen sulphide, principally *Acidithiobacillu*s and *Thiobacillus*. In this group we found acidophilic bacteria as *Acidithiobacillus thiooxidan*s (Aroca et al. 2007; Cho et al. 2000; Lee et al. 2006; Sercu et al. 2005; Shinabe et al. 1995; Wu et al. 2001), neutrophilic bacteria as *Thiobacillus novellus* (Chung et al. 1998), *Thiobacillus thioparus* (Cho et al. 1992; Cho et al. 1991; Chung et al. 2000; Chung et al. 1996; Cox and Deshusses 2002; Oyarzun et al. 2003; Park et al. 1993; Tanji et al. 1989) and *Thiobacillus denitrificans* (Ma et al. 2006; Ma et al. 2006). Heterophilic bacterias as *Xantomonas sp.* (Cho et al. 1992), *Pseudomonas putida* CH11 and *Arthrobacter oxidanas* CH8 (Chung et al. 1996; Chung et al. 2004; Chung et al. 2001) has been used in biofilter for hydrogen sulphide removal. Other bacteria as *Hyphomicrobioum* (Cho et al. 1992; Hirai et al. 2001; Sercu et al. 2005; Zhang et al. 1991) have been used for H_2S removal with very good results.

For H_2S removal, the packing material media used in conventional biofilter beds consist mostly of peat and compost, however many authors add other materials as wood chips or perlite to avoid the bed compaction (Jones et al. 2004; Li et al. 2003; Wani et al. 1999). Also other carrier materials such as polypropylene pall rings (Jin et al. 2005; Jin et al. 2005; Potivichayanon et al. 2006; Tanji et al. 1989), porous lava (Chitwood et al. 1999; Morton and Caballero 1997) ceramics (Hirai et al. 2001; Lee et al. 2005; Ruokojärvi et al. 2001; Shinabe et al. 2000), active carbon (Duan et al. 2007), and polyurethane foam (Gabriel et al. 2004; Gabriel and Deshusses 2003) have been used in biotrickling filters. Literature contains few accounts of the use of polyurethane foam for removal of malodorous gases with biotrickling filters.

2 Materials And Methods

2.1 Organism cultivation and medium preparation

The original pure-culture strain of autotrophic *Acidithiobacillus thiooxidans* (DSM11478) was obtained from Minas Gerais (Brazil). This stock culture was grown using a rotary shaker at optimal temperature (30ºC) and pH 2.5. The microorganism was cultivated in iron-free 9K medium (Silverman and Lundgren 1959). Composition in grams per liter: 3.0 g of $(NH_4)_2SO_4$; 0.5 g of $MgSO_4$; 0.5 g of K_2HPO_4; 0.1 g of KCl; 0.1 g of $Ca(NO_3)_2$ and with powdered sulphur as the energy source (10 grams per liter).

2.2 Packing material

Polyurethane foam particles (PUF) of 1 cm^3 were used as a filter packed bed. This packing material has a surface area of approximately 600 $m^2.m^{-3}$ and a density of 20 $kg.m^{-3}$ (Devinny et al. 1999). It is an inert material with low density, large porosity (near 96%), good scaling-up possibilities and very low commercial cost (McNevin and Barford 2000; Moe and Irvine 2000). Low density provides advantage in a construction and minimizes problems of compaction of the packing material. High porosity permits uniform gas flow distribution needed for maximum contact between the gas stream and biofilm biomass. The total weigh of PUF in the biofilter was 10 grams.

2.3 Immobilization method

Three Erlenmeyer flask of 1L were used. Each flask contains 500 ml of medium, 6 g of powdered sulphur, 100 ml of inoculum and 3.5 g of PUF. The culture was incubate at 150 rpm and 30ºC. The evolution of pH was followed and before it was of 1.0, the medium was drained and replaced with 600 mL of fresh medium without inoculation. Several consecutive batches were run on a “drawn and fill” basis until steady-state biomass levels had been achieved.

2.4 Experimental set-up

The experimental set up is shown in Figure 1. A PVC column (63 mm of diameter) was used to build-up the biofilter with two stages of the bed height 0.205 m each (total working volume of 1.278 L).

The air supply used was compressed air available in the building. Pressure regulation and filtering were achieved by having four filters: silica gel, active carbon, wool glass and Millipore Filter SLG05010 (0.45 μm). Air was humidified using fine bubble diffusion. Flow rates were controlled with Flow controller (Bronkhorst, Model F-201C).

A solution with a composition similar to the liquid culture medium without powdered sulphur, the energy source, was added to supplement nutrients (19.04 $l.h^{-1}$ flow rate). The pH of medium was controlled between 2.0-2.1 with controller CRISON PH28 and the temperature of experiment was maintained at 30ºC (Heidolph EKT3001).

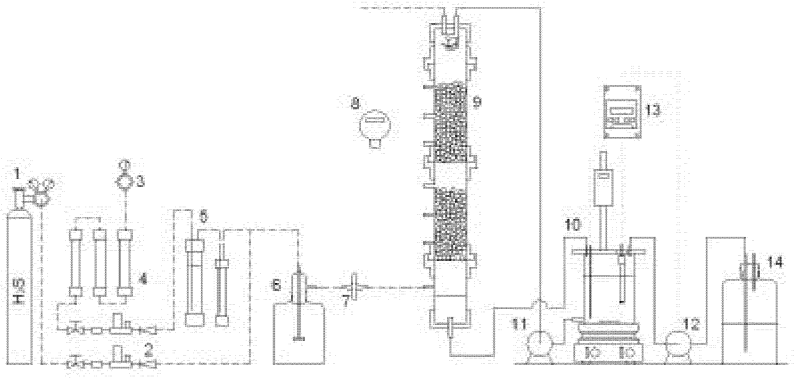

Figure 1. Diagram of the experimental setup

1. Hydrogen sulphide gas cylinder (H_2S/synthetic air); 2. Mass flow controller; 3. Pressure air regulator; 4. Air prefilter; 5. Humidification system; 6. Expansion deposit; 7. Air filter; 8. H_2S sensor; 9. Biotrickling filter; 10. Recirculation tank; 11. Recirculation pump; 12. pH control pump; 13. pH controller; 14. NaOH deposit.

2.5 Analytical techniques

A CRISON (5202) pH meter with an Ag electrode was used to follow the pH. The sulphuric acid obtained by bacterial oxidation of sulphur was analysed by titration with a 0.02 N sodium hydroxide solution. The sulphate concentration in the medium was measure using a modification of Classic Turbidimetric Method (Clesceri and Eaton 2007) and free bacterial population was determined by counting in a Neubauer chamber in conjunction with an optical microscope (Olympus BH-2). The immobilized biomass concentration was measured by counting of total biomass in a Neubauer chamber. A unit of carrier is removed from the reactor and squeezed lightly in order to remove the interstitial liquid. Then, it was submerged in an erlenmeyer flask containing 25 ml of iron-free 9K medium (pH 2.5). In a second step, the flask was placed in an ultrasonic bath at room temperature for 15 min. These conditions led to the total desorption of adhered cells. In the last stage, the Neubauer chamber re-count method for the submerged cells was carried out in the liquid phase. The carrier was subsequently removed from the flask and dried in an oven at 80 °C during 24 h. It was then possible to calculate the number of immobilized cells per grams of carrier (de Ory et al. 2004; Gómez et al. 2000). This technique has been previously validated by developing experiments concerned with cellular resistance to ultrasonic treatment and studying the desorption efficiency.

Hydrogen sulphide was analysed using a specific sensor of Crowcon (Model GASFLAG, TXGARD-IS).

3 Results And Discussion

3.1 Immobilized biomass

The total immobilized biomass was of $1.58 \pm 0.44 \cdot 10^{10}$ cells.g^{-1} at the end of 4th cycle. The duration of the experiment was 30 days. When the immobilization was finished, the carrier was put into the biotrickling filter (5 grams in each stage-dry carrier). Previously the polyurethane foam has been used by immobilization of microbial consortium (Gabriel and Deshusses 2003). Similar bacteria as *Acidithiobacillus ferrooxidans* with concentration of $8.0 \cdot 10^{10}$ and $1.2 \cdot 10^{12}$ cells.g^{-1} (Mesa et al. 2002) have been immobilized in PUF .

3.2 Removal of hydrogen sulphide

3.2.1 Effect of sulphate concentration

In the biological treatment of H_2S, sulphur and sulphate are produced and accumulated in the biotrickling filter. Sulphate is an inhibitory compound (Jin et al. 2005; Koe and Yang 2000; Ruokojärvi et al. 2001; Sercu et al. 2005; Tanji et al. 1989; Yang and Allen 1994). Therefore, the influence of sulphate concentration on *Acidithiobacillus thiooxidans* was examined. The EBRT was 49 seconds, the pH was controlled between 2.0-2.1 and the load of H_2S was of 12.7 $gS.m^{-3}.h^{-1}$ and after 311 hours it increased to 26.2 $gS.m^{-3}.h^{-1}$. The sulphate concentration was increased by biooxidation of H_2S from 28.9 to 56.7 $g.l^{-1}$ (biological sulphate concentration) and the removal efficiency was above of 98%. At 450 h the sulphate concentration was increased through the addition of Na_2SO_4 to 97.3 $g.l^{-1}$ (chemical sulphate concentration) and the removal efficiency didn't decrease (Figure 2). Lee et al. (2005) study the effect of $(NH_4)SO_4$ on the growth an oxidation rate o the *Acidithiobacillus thiooxidans* TAS found that the accumulation to 60 $g.l^{-1}$ has no lethal effect on the growth and sulphur oxidation reaction. This high resistance to sulphate concentration reduces the frequency of change of the medium in the biotrickling filter. In others studies, authors need to change the medium when the sulphate concentration was great than 5 $g.l^{-1}$ (Tanji et al. 1989) or 15 $g.l^{-1}$ (Ruokojärvi et al. 2001; Sercu et al. 2005).

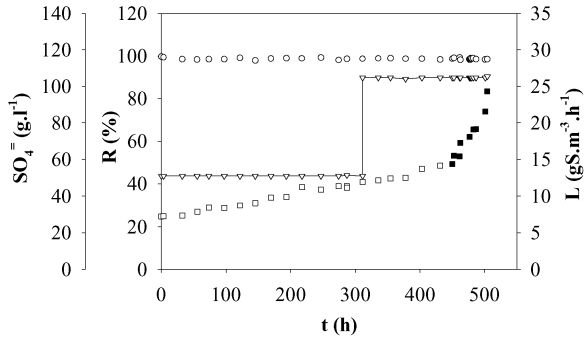

Fig 2. Study of the sulphate effect. Removal efficiency R (?), inlet load L(▽), biological sulphate concentration (?), and chemical sulphate concentration (■) versus time.

3.2.2 Effect of pH

The effect of pH on H_2S removal was studied in the range form 0.44 to 7.30 (Figure (3a)). Each pH was maintained constant for a period over 20h.

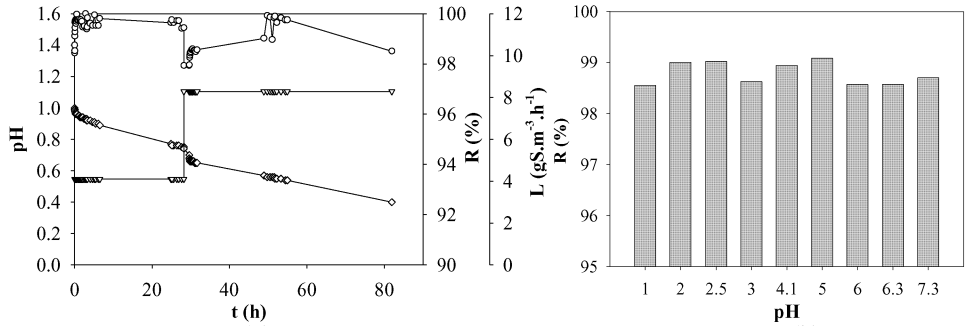

Fig3. Study of the effect of the pH. (a) Removal efficiency R (?), inlet load L (▽), and pH (◇) versus time. (b)Removal efficiency versus pH.

The pH was controlled from 1.0 to 7.30. In order to know the low limit of pH at pH 1.00 the controller was stopped, and the pH decreased quickly by H_2SO_4 formation.
The EBRT was 49 seconds, the sulphate concentration was maintained below 30 $g.l^{-1}$ and the inlet load of H_2S was of 4.10 g $gS.m^{-3}.h^{-1}$ and after 28.2 hours was increased to 8.28 $gS.m^{-3}.h^{-1}$. The H_2S was removal successfully in whole range with removal efficiency greater than 98% (Figure 3b). The optimal pH for H_2S removal found by Shinabe et al. (1995) was 2.5, although the elimination was similar from pH 1 to 6.

3.2.3 Effect of load

The inlet load was increased every two hours from 4.10 to 58.49 $gS.m^{-3}.h^{-1}$ at EBRT of 24.4 seconds, pH was controlled between 2.0-2.1 and the sulphate concentration was smaller than 30 $g.l^{-1}$. The removal efficiency was great of 90% in the first stage (from 0 to 0.205 meter). For the second stage (from 0.205 to 0.410 meter) the removal efficiency was greater than 98% (Figure 4). In the literature is reporter a maximum elimination capacity of 810 $gS.m^{-3}.h^{-1}$ in a biotrickling filter by Lee et al. (2005) using a ceramic carrier.

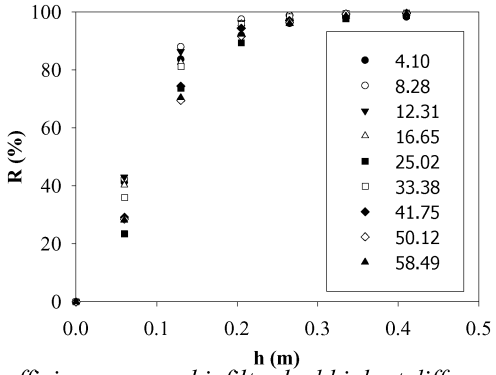

Fig 4. Removal efficiency versus biofilter bed high at different inlet loads

3.2.4 Effect of empty bed resident time

The effect of EBRT was studied at constant load of 12.47 $gS.m^{-3}.h^{-1}$ and a constant inlet hydrogen sulphide concentration of 65.9 ppmv. The EBRT was change each two hours. The pH was controlled between 2.0-2.1, the sulphate concentration was smaller than 30 $g.l^{-1}$.

In the study for constant load the removal efficiency was greater than 98% for EBRT of 25 seconds (Figure 5a). For constant inlet concentration (65.9 ppmv) the removal efficiency was greater than 98% for EBRT of 16 seconds (Figure 5b).

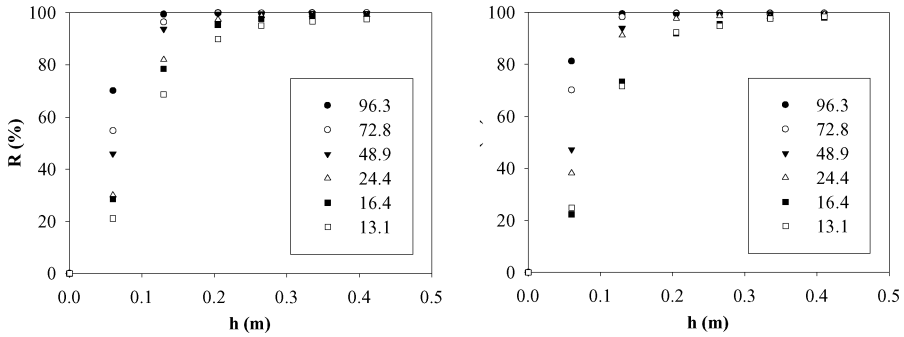

Fig 5. Removal efficiency versus biofilter bed high at different EBRT. (a) constant load of 12.47 $gS.m^{-3}.h^{-1}$. (b) constant inlet hydrogen sulphide concentration of 65.9 ppmv

4 Conclusions

The maximum biomass immobilized in PUF was $1.58\pm0.44\cdot10^{10}$ cells.g^{-1} at the end of 4th cycle. The biotrickling filter has removal efficiency greater than 99% for inlet hydrogen sulphide concentration of 65 ppmv and EBRT of 24 seconds. The system removed over 98% of the hydrogen sulphide in the pH range of 0.44-7.29. A concentration of 58 g.l^{-1} of biological sulphate concentration was reached without inhibition effect.

These results had shown a high removal efficiency and very high resistance of the immobilized microorganism to the pH and sulphate concentration.

5 Acknowledgements

Authors wish express sincere gratitude to Spanish Government for financial support of this work through Project CTM2006-05497.

6 References

Aroca G, Urrutia H, Nuñez D, Oyarzún P, Arancibia A, Guerrero K. 2007. Comparison on the removal of hydrogen sulfide in biotrickling filters inoculated with *Thiobacillus thioparus* and *Acidithiobacillus thiooxidans*. Electronic Journal of Biotechnology 10(4):514.

Chitwood DE, Devinny JS, Reynolds FE, Jr. 1999. Evaluation of a two-stage biofilter for treatment of POTW waste air. Environmental Progress 18(3):212-221.

Cho KS, Hirai M, Shoda M. 1992. Enhanced removal efficiency of malodorous gases in a pilot-scale peat biofilter inoculated with *Thiobacillus thioparus* DW44. Journal of Fermentation and Bioengineering 73(1):46-50.

Cho KS, Ryu HW, Lee NY. 2000. Biological deodorization of hydrogen sulfide using porous lava as a carrier of *Thiobacillus thiooxidans*. Journal of Bioscience and Bioengineering 90(1):25-31.

Cho KS, Zhang L, Hirai M, Shoda M. 1991. Removal characteristics of hydrogen sulphide and methanediol by *Thiobacillus sp.* isolated from peat in biological deodorization. Journal of Fermentation and Bioengineering 71(1):44-49.

Chung YC, Huang C, Pan JR, Tseng CP. 1998. Comparison of autotrophic and mixotrophic biofilters for H_2S removal. Journal of Environmental Engineering (Reston, Virginia) 124(4):362-367.

Chung YC, Huang C, Rushing Pan J, Tseng CP. 2000. Biotreatment of H_2S- and NH_3-containing waste gases by co-immobilized cells biofilter. Chemosphere 41(3):329-336.

Chung YC, Huang C, Tseng CP. 1996. Operation optimization of *Thiobacillus thioparus* CH11 biofilter for hydrogen sulfide removal. Journal of Biotechnology 52(1):31-38.

Chung YC, Lin YY, Tseng CP. 2004. Control of H_2S waste gas emissions with a biological activated carbon filter. Journal of Chemical Technology and Biotechnology 79(6):570-577.

Chung YC, Huang C, Tseng CP. 2001. Biological elimination of H_2S and NH_3 from waste gases by biofilter packed with immobilized heterotrophic bacteria. Chemosphere 43(8):1043-1050.

Clesceri LS, Eaton AD. 2007. ALPHA, AWWA, WEF, Standard Methods for the Examination of Water and Wastewater.

Cox HHJ, Deshusses MA. 2002. Co-treatment of H_2S and toluene in a biotrickling filter. Chemical Engineering Journal 87(1):101-110.

de Ory I, Romero LE, Cantero D. 2004. Optimization of immobilization conditions for vinegar production. Siran, wood chips and polyurethane foam as carriers for *Acetobacter aceti*. Process Biochemistry 39(5):547-555.

Devinny JS, Deshusses MA, Webster TS. 1999. Biofiltration for Air Pollution Control, CRC, Press, Lewis, Boca Raton, Fla.

Duan H, Yan R, Koe LCC, Wang X. 2007. Combined effect of adsorption and biodegradation of biological activated carbon on H_2S biotrickling filtration. Chemosphere 66(9):1684.

Gabriel D, Cox HHJ, Deshusses MA. 2004. Conversion of full-scale wet scrubbers to biotrickling filters for H_2S control at publicly owned treatment works. Journal of Environmental Engineering 130(10):1110-1117.

Gabriel D, Deshusses MA. 2003. Retrofitting existing chemical scrubbers to biotrickling filters for H_2S emission control. Proceedings of the National Academy of Sciences of the United States of America 100(11):6308-6312.

Gómez JM, Cantero D, Webb C. 2000. Immobilisation of *Thiobacillus ferrooxidans* cells on nickel alloy fiber for ferrous sulfate oxidation. Applied Microbiology and Biotechnology 54(3):335-340.

Hirai M, Kamamoto M, Yani M, Shoda M. 2001. Comparison of the biological H_2S removal characteristics among four inorganic packing materials. Journal of Bioscience and Bioengineering 91(4):396-402.

Jin Y, Veiga MC, Kennes C. 2005. Autotrophic deodorization of hydrogen sulfide in a biotrickling filter. Journal of Chemical Technology and Biotechnology 80(9):998-1004.

Jin Y, Veiga MC, Kennes C. 2005. Effects of pH, CO_2, and flow pattern on the autotrophic degradation of hydrogen sulfide in a biotrickling filter. Biotechnology and Bioengineering 92(4):462-471.

Jones KD, Martinez A, Maroo K, Deshpande S, Boswell J. 2004. Kinetic Evaluation of H_2S and NH_3 Biofiltration for Two Media Used for Wastewater Lift Station Emissions. Journal of the Air and Waste Management Association 54(1):24-35.

Koe LCC, Yang F. 2000. A bioscrubber for hydrogen sulfide removal. Water Science and Technology 41(6):141-145.

Lee EY, Cho K-S, Ryu Hee W. 2005. Simultaneous removal of H_2S and NH_3 in biofilter inoculated with *Acidithiobacillus thiooxidans* TAS. Journal of bioscience and bioengineering 99(6):611-5.

Lee EY, Lee NY, Cho KS, Ryu HW. 2006. Removal of hydrogen sulfide by sulfate-resistant *Acidithiobacillus thiooxidans* AZ11. Journal of Bioscience and Bioengineering 101(4):309.

Li H, Mihelcic JR, Crittenden JC, Anderson KA. 2003. Field Measurements and Modeling of Two-Stage Biofilter that Treats Odorous Sulfur Air Emissions. Journal of Environmental Engineering (Reston, VA, United States) 129(8):684-692.

Ma YL, Yang BL, Zhao JL. 2006. Removal of H_2S by *Thiobacillus denitrificans* immobilized on different matrices. Bioresource technology 97(16):2041-6.

Ma YL, Zhao JL, Yang BL. 2006. Removal of H_2S in waste gases by an activated carbon bioreactor. International Biodeterioration and Biodegradation 57(2):93.

McNevin D, Barford J. 2000. Biofiltration as an odour abatement strategy. Biochemical Engineering Journal 5(3):231-242.

Mesa MM, Macias M, Cantero D. 2002. Mathematical Model of the Oxidation of Ferrous Iron by a Biofilm of *Thiobacillus ferrooxidans*. Biotechnology Progress 18(4):679-685.

Moe WM, Irvine RL. 2000. Polyurethane foam medium for biofiltration. I: Characterization. Journal of Environmental Engineering 126(9):815-825.

Morton RL, Caballero RC. Removing hydrogen sulfide from wastewater treatment facilities' air process streams with a biotrickling filter; 1997.

Oyarzun P, Arancibia F, Canales C, Aroca GE. 2003. Biofiltration of high concentration of hydrogen sulphide using *Thiobacillus thioparus*. Process Biochemistry (Oxford, United Kingdom) 39(2):165-170.

Park SJ, Cho KS, Hirai M, Shoda M. 1993. Removability of malodorous gases from a night soil treatment plant by a pilot-scale peat biofilter inoculated with *Thiobacillus thioparus* DW44. Journal of Fermentation and Bioengineering 76(1):55-59.

Potivichayanon S, Pokethitiyook P, Kruatrachue M. 2006. Hydrogen sulfide removal by a novel fixed-film bioscrubber system. Process Biochemistry 41(3):708-715.

Ruokojärvi A, Ruuskanen J, Martikainen PJ, Olkkonen M. 2001. Oxidation of gas mixtures containing dimethyl sulfide, hydrogen sulfide, and methanethiol using a two-stage biotrickling filter. Journal of the Air and Waste Management Association 51(1):11-16.

Sercu B, Van Langenhove H, Nuñez D, Aroca G, Verstraete W. 2005. Operational and microbiological aspects of a bioaugmented two-stage biotrickling filter removing hydrogen sulfide and dimethyl sulfide. Biotechnology and Bioengineering 90(2):259-269.

Shinabe K, Oketani S, Ochi T, Kanchanatawee S, Matsumura M. 2000. Characteristics of hydrogen sulfide removal in a carrier-packed biological deodorization system. Biochemical Engineering Journal 5(3):209-217.

Shinabe K, Oketani S, Ochi T, Matsumura M. 1995. Characteristics of hydrogen sulfide removal by *Thiobacillus thiooxidans* KS1 isolated from a carrier-packed biological deodorization system. Journal of Fermentation and Bioengineering 80(6):592-598.

Silverman MP, Lundgren DG. 1959. Studies on the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans*. I. An improved medium and a harvesting procedure for securing high cell yields. J. Bacteriol. 77:642.

Tanji Y, Kanagawa T, Mikami E. 1989. Removal of dimethyl sulfide, methyl mercaptan, and hydrogen sulfide by immobilized *Thiobacillus thioparus* TK-m. Journal of Fermentation and Bioengineering 67(4):280-285.

Wani AH, Lau AK, Branion RMR. 1999. Biofiltration control of pulping odors - hydrogen sulfide: performance, macrokinetics and coexistence effects of organo-sulfur species. Journal of Chemical Technology & Biotechnology 74(1):9-16.

Wu L, Loo YY, Koe LCC. 2001. A pilot study of a biotrickling filter for the treatment of odorous sewage air. Water Science and Technology 44(9):295-299.

Yang Y, Allen ER. 1994. Biofiltration control of hydrogen sulfide. 1. Design and operational parameters. Air & Waste 44(7):863-8.

Zhang L, Hirai M, Shoda M. 1991. Removal characteristics of dimethyl sulfide, methanethiol and hydrogen sulfide by *Hyphomicrobrium sp.* I55 isolated from peat biofilter. Journal of Fermentation and Bioengineering 72(5):392-396.