JOURNAL OF
Biotechnology

ELSEVIER

Journal of Biotechnology 52 (1996) 31-38

Operation optimization of *Thiobacillus thioparus* CH11 biofilter for hydrogen sulfide removal

Ying-Chien Chung[a], Chihpin Huang[a,*], Ching-Ping Tseng[b]

[a]*Institute of Environmental Engineering, National Chiao Tung University, Hsinchu, Taiwan 30039, ROC*
[b]*Institute of Biological Science and Technology, National Chiao Tung University, Hsinchu, Taiwan 30039, ROC*

Received 19 June 1996; revised 25 September 1996; accepted 26 September 1996

Abstract

Members of the autotrophic species, *Thiobacillus thioparus* CH11, were isolated from swine wastewater and immobilized with Ca-alginate to produce pellet packing materials for a novel biofilter system that controls hydrogen sulfide emission. The effect of operating parameters, including retention time, temperature, and inlet gas concentration, on the removal efficiency and capacity was evaluated. Criteria necessary for a scale-up design of the biofilter were established and the sulfur balances at various loadings were tabulated. High and satisfactory H_2S removal efficiency levels were maintained during operation and the optimal retention time was found to be 28 s corresponding to a H_2S removal efficiency greater than 98%. The pH drop was insignificant in this biofilter. The optimal inlet S-loading can be noted as 25 g m^{-3} h^{-1} that is at the upper end of linear correlation between inlet loading and removal capacity. We suggest that the *Thiobacillus thioparus* CH11 immobilized with Ca-alginate is a potent method to control hydrogen sulfide emissions. Copyright © 1996 Elsevier Science B.V.

Keywords: Biofilter; Hydrogen sulfide; Immobilized cell; *Thiobacillus thioparus*

1. Introduction

Considerable amounts of hydrogen sulfide are produced in association with industrial processes such as petroleum refining, wastewater treatment, food processing, paper and pulp manufacturing and in the treatment of fuels (EiKum and Storhang, 1986; Yang and Allen, 1994). Hydrogen sulfide concentrations of 5–60 ppm were generally emitted from those processes (Langenhove and Schamp, 1986; Cho et al., 1992b; Chung et al., 1996a). Physical and chemical processes, including activated carbon adsorption, ozone oxidation and incineration have been used to purify H_2S from waste gas and wastewater (Day, 1966; Eby and Wilson, 1969; Barth et al., 1984; Mannebeck, 1986). However, recently the focus has shifted

* Corresponding author.

0168-1656/96/$15.00 Copyright © 1996 Elsevier Science B.V. All rights reserved
PII S0168-1656(96)01622-7

toward using biofiltration (Bohn, 1992). Biofiltration has advantages over physical and chemical methods by virtue of its low maintenance and operating costs, as well as high removal efficiency (Bohn, 1992). Reduced secondary pollution is a special benefit (Bohn, 1992). In view of these advantages, biofilters have been researched by using different packing materials as carriers and diverse microorganisms to enhance removal efficiency (Richard, 1982). Several biofilter packing materials have been studied (Carlson and Leiser, 1966; Rands et al., 1981; Lee and Shoda, 1989). Soil bed was the first attempt on H_2S removal (15 ppm). Although high removal efficiency was achieved in this study, the low volumetric load (4.8 m^3 m^{-2} h^{-1}) was found to be caused by the low air permeability of the soil (Carlson and Leiser, 1966). Compost performed well for removing H_2S over short-term periods, however, for long-term performance, compost filter entailed a short circuiting problem because of aging effects (Langenhove et al., 1992). Activated carbons performed well, but they are so expensive not as to justify the efficiency difference (Medina et al., 1995). Fibrous peat packing material has been demonstrated to be preferable to soil, compost, or activated carbon (Leson and Winer, 1991). However, peat biofilters require a relatively large installation space to install because of their high air permeability (Hodge and Devinny, 1994).

In addition to a suitable packing material, choosing an appropriate microorganism to optimize H_2S removal efficiency is important. Various microorganisms capable of H_2S oxidation have previously been reported (Nelson, 1990). However, not many bioreactors for suitable H_2S removal from gas emissions have been developed. The autotrophic *Thiobacillus* species have been seeded into different packing materials to metabolize H_2S, the products of which are elemental sulfur or sulfate dependent on the strain of *Thiobacillus sp.* used (Moriarty and Nicholas, 1970; Wada et al., 1986; Sublette and Sylvester, 1987). As for heterotrophic bacteria, only *Pseudomonas putida*, and *Xanthomonas sp.* have been used in biofilters for H_2S oxidation because of their low affinity for H_2S (Cho et al., 1992a; Chung et al., 1996b). A biofilter being packed with microorganism-laden peat has also been reported to remove hydrogen sulfide at concentrations ranging between 25 and 45 ppm. However, it failed to remove hydrogen sulfide at relatively low concentrations (< 20 ppm; Cho et al., 1992b). Although Tanji et al. (1989) had designed a packed reactor (50 mm $\phi \times 1250$ mm) to improve the removal efficiency at low H_2S concentrations, they found a significant pressure loss during operation.

In this study, we present an innovative biological reactor with immobilized *Thiobacillus thioparus* CH11, commonly used in wastewater treatment, that reduces the required biofilter working volume and enhances H_2S removal efficiency especially under low-concentration conditions. We also include operational and design standards for the biofilter.

2. Materials and methods

2.1. Microorganisms and cultivation

Autotrophic *Thiobacillus thioparus* CH11 was isolated from swine wastewater. The thiosulfate medium was prepared for cultivation of the isolate containing (in grams per liter): KH_2PO_4 1.2 g, K_2HPO_4 1.2 g, NH_4Cl 0.4 g, $MgCl_2 \cdot 6H_2O$ 0.2 g, $FeSO_4 \cdot 7H_2O$ 0.01 g and $Na_2S_2O_3 \cdot 5H_2O$ 8 g. The *Thiobacillus thioparus* CH11 was motile, gram-negative and short-rod. It grew autotrophically with elemental sulfur, thiosulfate and sulfide, but hardly grew in facultative autotroph and heterotroph conditions. The optimum pH for the growth of *Thiobacillus thioparus* CH11 was 6–8. For all continuous experiments, the same basal mineral medium (in grams per liter), including KH_2PO_4 1.2 g, K_2HPO_4 1.2 g, NH_4Cl 0.4 g, $MgCl_2 \cdot 6H_2O$ 0.2 g, and $FeSO_4 \cdot 7H_2O$ 0.01 g, was used. The final pH was adjusted to 7 by using 2 N NaOH for both the thiosulfate and basal mineral media.

2.2. Preparation of immobilized cells

Bacteria cells grown in 100 ml thiosulfate medium were harvested by centrifugation (7500 ×

g, 10 min) and then washed with aseptically distilled water three times. The organisms (10^5 cells per ml) were immersed in a sterilized 4% Na-alginate solution and the Na-alginate solution containing cells was dropped into a 4% $CaCl_2$ solution by syringe. The immobilized beads were formed immediately. The immobilized 3 mm-diameter beads were activated by flushing with aseptically distilled water. The activated beads exhibited excellent mechanical strength in continuous experiments.

2.3. Apparatus and H_2S removal in continuous system

A laboratory-scale experimental biofilter was shown in Fig. 1 and described previously (Chung et al., 1996b). The cell-laden beads were packed into a glass column (60 mm $\phi \times 25$ cm of working length). The packing volume, bead dry weight and initial cell number in one column were 0.7 l, 0.25 kg and 10^5 cfu per g-dry bead, respectively. The basal mineral medium contained in a humidification bottle was supplied to the immobilized cells by purging air through the humidification bottle. Relative humidities between 95 and 100% were routinely and continuously achieved during the operation. H_2S gas at different concentrations (5–100 ppm) was supplied to the column at varied flow rates (18–185 l h^{-1}). The experimental temperature was held at 28 ± 2°C and the effect of temperature on H_2S removal was studied over the range of 15–50°C at 72 l h^{-1}.

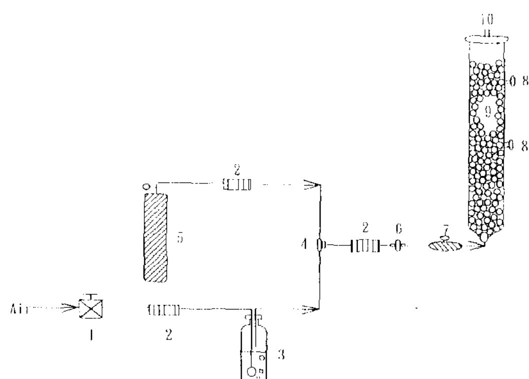


Fig. 1. Laboratory-scale experimental biofilter system. (1) Air compressor. (2) Flow meter. (3) Humidification bottle. (4) Three way valve. (5) H_2S gas cylinder. (6) Air filter. (7) Inlet chamber (8) Sampling port. (9) Glass column. (10) Gas outlet.

2.4. Analytical methods

Inlet and outlet H_2S gas concentrations in the column were continuously measured using a Single Point Monitor (MDA Scientific, USA) in the range of 50–1500 ppb or periodically measured by gas detector tubes (GASTEC, Japan) in the range of 1–60 ppm. Cell-laden beads (5 g wet-weight) were dissolved in 95 ml of 0.1 M sodium citrate solution and the pH value of the solution containing free cells was measured. Solutions with different dilution ratios were spread on the thiosulfate solid medium. After incubation for 5 days at 30°C, the numbers of colonies on the plates were counted. Sulfate ion concentrations in the solutions were measured by ion chromatography (Dionex 4500i). Sulfite was determined by titration using a standard potassium iodide-iodate titrant and a starch indicator (APHA, 1992). Sulfide was determined using an ion-specific electrode. Elemental sulfur was determined by reaction with cyanide to produce thiocyanate, which was quantitated as $Fe(SCN)_6^{3-}$ (Schedel and Truper, 1980).

3. Results

3.1. H_2S removal in continuous operation

A long-term investigation into removal of different H_2S concentrations (5, 20, 60 ppm) was conducted at a flow rate of 36 l h^{-1} for 3 months. To evaluate the adaptability *Thiobacillus thioparus* CH11 biofilters to upset conditions, biofilters were supplied with 5 ppm H_2S for the first month, and then a 60 ppm H_2S 'shock-load' was fed for the remaining 2 months. The H_2S removal efficiency of the biofilter and the change in pH in the middle portion of the biofilter are shown in Fig. 2. After 7 days of start-up were required, after which better than 98.5% removal efficiency was achieved at inlet concentration of 60 or 20 ppm. However, when the H_2S gas supplied was increased from 5

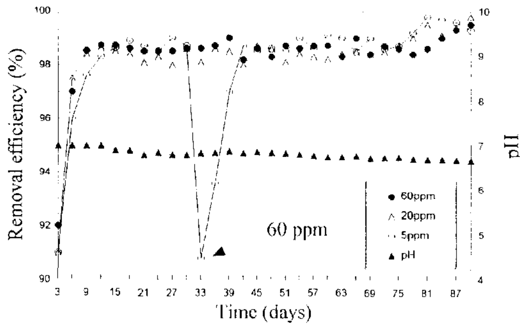


Fig. 2. H_2S removal efficiency of the biofilter at a flow rate of 36 l h^{-1}, and changes in pH in the middle portion of the biofilter.

to 60 ppm on the 30th day, a short-term decline (8%) in performance was observed. The biofilter required about 5 days to recovery its microorganism oxidation abilities, after the 12-fold shock loading, this result in accordance with the theory presented by Peters, 1993. On the 39th day, the removal efficiency returned to 98%. When the biofilter was supplied with 60 ppm H_2S, the pH in the middle portion of the biofilter is shown in Fig. 2. Clearly, pH variation was insignificant. To determine the metabolized products of H_2S removal by *Thiobacillus thioparus* CH11, the sulfate, sulfide, sulfite, and elemental sulfur concentrations in the middle portion of the biofilter were assessed after 7 days of operation at a flow rate of 36 l h^{-1}. The biofilter was continually supplied with 60 or 5 ppm H_2S at a flow rate of 36 l h^{-1} for 7 days, and the sulfur mass balance in the biofilter is as show in Table 1. The equation for H_2S removal is given below:

$$E = \frac{R\% \times C_1 \times F \times t \times \alpha}{W1} \quad (1)$$

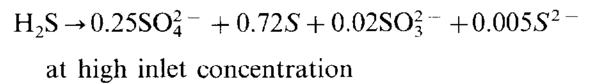
where E is the H_2S removal (S per bead (g kg^{-1})); $R\%$, the average removal efficiency; C_1, the inlet H_2S concentration (ppm); F, the flow rate (l h^{-1}); t, operation time (h); α,: conversion coefficient (1.3×10^{-12} g l^{-1} ppm^{-1}); W_1, packing bead weight (kg).

The amount of produced S compound is calculated by the following equation:

$$P = \frac{C_2 \times D}{W_2} \quad (2)$$

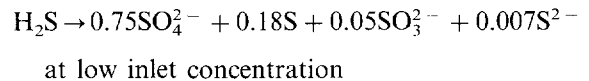
where P is the S compound accumulation (S per bead (g kg^{-1})); C_2, the concentration of S compound in sodium citrate solution (g l^{-1}); D, dilution volume (l); W_2, the sampling bead weight (kg).

When 60 ppm was fed to the biofilter, the total amount of H_2S consumption was 1.860 S per bead (g kg^{-1}) and the sulfate, elemental sulfur, sulfite, and sulfide accumulations were 0.460, 1.340, 0.037 and 0.009 S per bead (g kg^{-1}), respectively. The major product was elemental sulfur and the ratio of H_2S conversion was 72.0%. Note that an adverse tendency was found at low inlet concentrations. When 5 ppm was introduced to the biofilter, the major product became sulfate and 74.5% of the H_2S was converted to sulfate. Hence, the sulfur balance in Table 1 can be described as follows:



$$H_2S \rightarrow 0.25SO_4^{2-} + 0.72S + 0.02SO_3^{2-} + 0.005S^{2-}$$

at high inlet concentration



$$H_2S \rightarrow 0.75SO_4^{2-} + 0.18S + 0.05SO_3^{2-} + 0.007S^{2-}$$

at low inlet concentration

3.2. Effect of retention time and temperature on H_2S removal

The effect of retention time in the biofilter on H_2S removal was studied by introducing 60 ppm H_2S to the biofilter at various flow rates, and the results are shown in Fig. 3. The retention time of H_2S gas in the biofilter was calculated by the following equation:

$$RT = \frac{V}{F} \quad (3)$$

where RT is the retention time in seconds; V, the volume of packing beads (l); F, the flow rate (l s^{-1}).

High removal efficiency ($>98\%$) was achieved when the retention time was in the range of 28–140 s. When the retention time was shortened to 14 s, the removal efficiency decreased significantly (by 18%). Compared to a *Pseudomonas putida* biofilter, this biofilter is less sensitive to

Table 1
Sulfur mass balances in the biofilter at 60 and 5 ppm H_2S, fed at a flow rate of 36 l h^{-1} for 7 days

Inlet concentration (ppm)	H_2S removed (*S* per bead (g kg^{-1}))	SO_4^{2-} produced (*S* per bead (g kg^{-1}))	*S* produced (*S* per bead (g kg^{-1}))	SO_3^{2-} produced (*S* per bead (g kg^{-1}))	S^{2-} produced (*S* per bead (g kg^{-1}))
60	1.860	0.460 (24.7%)	1.340 (72.0%)	0.037 (2.0%)	0.009 (0.50%)
5	0.153	0.114 (74.5%)	0.028 (18.3%)	0.008 (5.2%)	0.001 (0.65%)

changes in retention time (Chung et al., 1996b). The effect of temperature on H_2S removal efficiency was conducted in a range from 15 to 50°C, see Fig. 4. The results shows that high H_2S removal efficiencies were consistently observed with little variation (97.5–98.0%) in the range from 20 to 37°C, regardless of whether inlet concentrations were high or low. However, the removal efficiencies dropped significantly at low (15°C) temperature and high (50°C) temperature and a great reduction was seen at 50°C. These can be attributed to the sensitivity of the microorganism to high temperature. Based on removal efficiency, the optimum temperature for our system operation was 30°C. This is near the optimum temperature for *Thiobacillus thioparus* CH11 cultivation in batch culture (data not shown).

3.3. Effect of flow rate and inlet concentration on H_2S removal

The H_2S removal efficiency as functions of gas flow rates and H_2S concentrations is shown in Fig. 5A. Removal efficiency decreased progressively with increasing gas flow rates and H_2S concentrations. H_2S removal efficiencies between 36 and 72 l h^{-1} showed little variation. However, significant difference was noticed when the flow rate was increased to 150 l h^{-1}. The higher inlet concentration also produced a large effect on removal efficiency. The elimination capacity versus the inlet H_2S concentrations at different flow rates (36, 72, 150 l h^{-1}) is shown in Fig. 5B. Note that an increase in gas flow rate from 36 to 72 l h^{-1} yielded almost a doubled removal capacity, while an increase from 72 to 150 l h^{-1} resulted in an advanced improvement of removal capacity. Clearly, the gas flow rate strongly influenced both the removal efficiency (Fig. 5A) and the removal capacity (Fig. 5B) in the range of inlet H_2S concentrations from 10 to 100 ppm.

3.4. Criteria for designing a scale-up of biofilter

Complete H_2S removal can be achieved only less than a critical inlet loading. If this critical inlet loading is exceeded, hydrogen sulfide will be

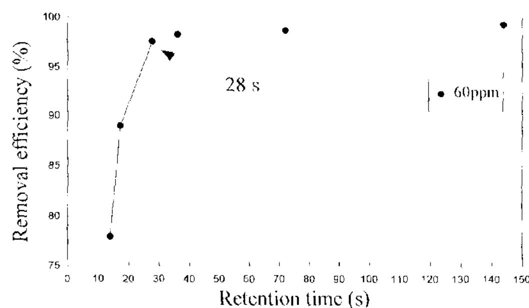


Fig. 3. Effect of gas retention time on H_2S removal efficiency at 60 ppm of inlet gas. The removal efficiency required 15 days of operation to reach a steady state.

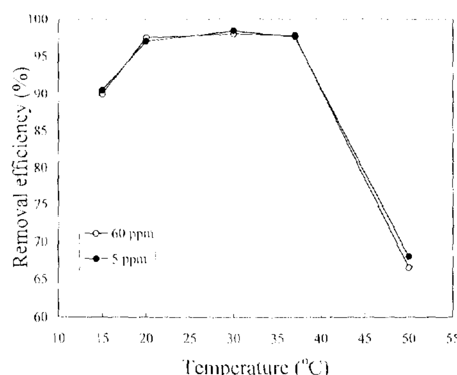


Fig. 4. Effect of temperature on H_2S removal efficiency at a flow rate of 72 l h^{-1}.

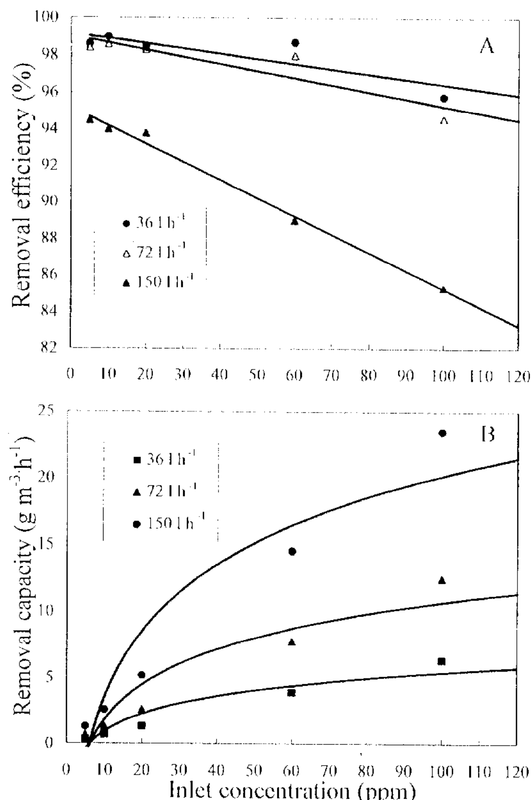


Fig. 5. (A) H_2S removal efficiency versus inlet H_2S concentration at different flow rates (36, 72, 150 l h^{-1}). (B) Elimination capacity versus inlet H_2S concentration at different flow rates (36, 72, 150 l h^{-1}).

detected at the outlet of the biofilter. Finding optimal inlet loading is therefore important for the operation of the biofilter. The inlet loading is defined as the amount of inlet gas per unit time and volume of packing material (g m^{-3} h^{-1}) and the equation was expressed as follows:

$$\text{Load} = \frac{F \times C}{V} \tag{4}$$

where F is the flow rate (l h^{-1}); C, the inlet H_2S concentration (g l^{-1}) and V, the volume of packing beads (m^3).

Thus, both gas flow rate and inlet gas concentration play important roles in designing a real scale-up biofilter if the volume of packing material is constant. The relationship between the inlet loading and the removal capacity for hydrogen sulfide is shown in Fig. 6. It is obvious that the maximum inlet S-loading of hydrogen sulfide was approximately 33 g m^{-3} h^{-1} and only 70% H_2S removal was achieved. If we considered the linear region between inlet S-loading and removal capacity, the optimal inlet loading is 25 g m^{-3} h^{-1}. For example, when high H_2S concentration is introduced into the biofilter, we need lower the flow rate of H_2S gas or increase the volume of packing beads to achieve optimal removal capacity (25 g m^{-3} h^{-1}). Conversely, we can elevate the gas flow rate or decrease the volume of packing beads in treating diluted concentration of inlet gas by using the scale-up criteria.

4. Discussion

Our results show that the *Thiobacillus thioparus* CH11 biofilter has excellent adaptability to upset condition even at a 12-fold shock loading. The major metabolic products, identified as elemental sulfur or sulfate, are dependent on inlet concentrations. To gain more energy, *Thiobacillus thioparus* CH11 oxidizes hydrogen sulfide to sulfate at low inlet loading. A similar result can be found in the work of Buisman et al. (1990) and the chemical oxidation equations to metabolize H_2S were also presented as follows (Buisman et al., 1990):

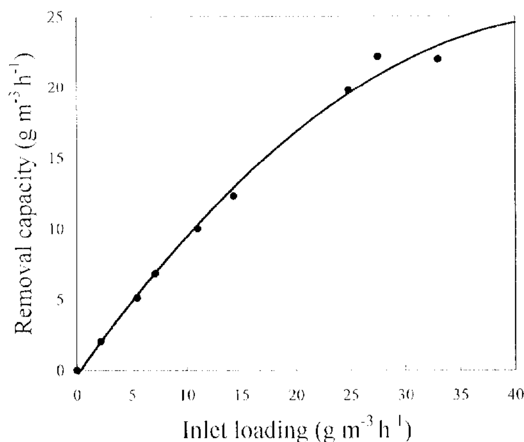
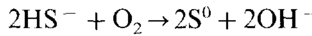
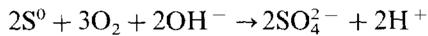


Fig. 6. Relationship between the inlet loading and H_2S removal capacity.



$$2HS^- + O_2 \rightarrow 2S^0 + 2OH^-$$

$$\Delta G^0 = -169.35 \text{ kJ mol}^{-1}$$



$$2S^0 + 3O_2 + 2OH^- \rightarrow 2SO_4^{2-} + 2H^+$$

$$\Delta G^0 = -563.23 \text{ kJ mol}^{-1}$$

Fortunately, no acidification occurs in the biofilter judging by the pH change in the biofilter during three months of operation. High removal efficiency (> 98%) can be achieved as long as the retention time exceeds 28 s. A related study indicated that the microorganism could metabolize hydrogen sulfide within 1–2 s (Sublette and Sylvester, 1987). Hence, the reduction in removal efficiency caused by short retention time results from slow diffusion from the gas phase into the liquid phase. The more rapid decline of removal efficiency at high temperature (50°C) than at low temperature (15°C) is probably due to either the repression of microbial enzymes or the death of microorganisms. A reduction in microorganism numbers at 15°C appears less than that at 50°C (data not shown), inferring that the decline in removal efficiency is due to repression of microbial enzymes at 15°C, but also that a large quantity of microorganism are killed at 50°C. This result can be confirmed by comparing the specific uptake rate at different temperatures (data not shown). The removal efficiency and removal capacity exhibit adverse tendencies at higher flow rates. An analogous result was reported in both the work of Sublette et al. (1994) and Chung et al. (1996b). It may be concluded that this phenomenon is due to diffusion limitation, but limitation in the activity of microbial enzymes. Finally, the results show that the immobilized *Thiobacillus thioparus* CH11 biofilter was successful in eliminating H_2S over an extended period of time. Greater than 98.5% removal efficiency and excellent adaptability to upset condition have been demonstrated even at an inlet concentration of 5 ppm. The optimal inlet *S*-loading for this biofilter is suggested to be 25 g m^{-3} h^{-1}.

References

American Public Health Association. (1992) Standard Method: Examination of Water and Wastewater, 18th edn. APHA, New York, 1992.

Barth, C.L., Elliott, F.L. and Melvin, S.W. (1984) Using odour control technology to support animal agriculture. T. ASAE 27, 859–864.

Bohn, H. (1992) Consider biofiltration for decontaminating gases. Chem. Eng. Prog. 88, 35–40.

Buisman, C.J.N., Geraats, B.G., Ijspeert, P. and Lettinga, G. (1990) Optimization of sulphur production in a biotechnological sulphide-removing reactor. Biotechnol. Bioeng. 35, 50–56.

Carlson, K.Y. and Leiser, C.P. (1966) Soil beds for the control of sewage odors. J. Wat. Pollut. Control Fed. 38, 829–834.

Cho, K.S., Hirai, M. and Shoda, M. (1992a) Degradation of hydrogen sulfide by Xanthomonas sp. Strain DY44 isolated from peat. Appl. Environ. Microbiol. 58, 1183–1189.

Cho, K.S., Hirai, M. and Shoda, M. (1992b) Enhanced removal efficiency of malodorous gases in a pilot-scale peat biofilter inoculated with *Thiobacillus thioparus* DW44. J. Ferment. Bioeng. 73, 46–50.

Chung, Y.C., Huang, C. and Tseng, C.P. (1996a) Reduction of H_2S/NH_3 production from pig feces by controlling environmental conditions. J. Environ. Sci. Health 31 (A), 139–155.

Chung, Y.C., Huang, C. and Tseng, C.P. (1996b) Microbial oxidation of hydrogen sulfide with biofilter. J. Environ. Sci. Health 31 (A), 1263–1278.

Day, D.L. (1966) Liquid hog manure can be deodourized by treatment with chlorine or lime. Illinois Res. 127, 16–18.

Eby, H.J. and Wilson, G.B. (1969) Poultry house dust, odour and their mechanical removal. In: Agricultural Waste Management Proceedings, Cornell University, Conference on Agricultural Waste Management, Syracuse, NY, pp. 303–308.

EiKum, A.S. and Storhang, R. (1986) Odour problems related to waste water and sludge treatment. In: Neilsen, V.C., Voorburg, J.H. and Hermite, P.L. (Eds.), Odour Prevention and Control of Organic Sludge and Livestock Farming. Elsevier, London, pp. 12–18.

Hodge, D.S. and Devinny, J.S. (1994) Biofilter treatment of ethanol vapors. Environ. Prog. 13, 167–173.

Langenhove, H.V., Wuyts E. and Schamp, N. (1986) Elimination of hydrogen sulphide from odorous air by wood bark biofilter. Water. Res. 20, 1471–1476.

Langenhove, H.V., Bendinger, B., Oberthur, R. and Schamp, N. (1992) Organic sulfur compounds: persistent odorants in biological treatment of complex waste gases. In: Dragt, A.J. and Ham, J.V. (Eds.), Biotechniques for Air Pollution Abatement and Odour Control Policies. Elsevier, Amsterdam, pp. 177–182.

Lee, S.K. and Shoda, M. (1989) Biological deodorization using activated carbon fabric as a carrier of microorganisms. J. Ferment. Bioeng. 68, 437–442.

Leson, G. and Winer, A.M. (1991) Biofiltration: an innovative air pollution control technology for VOC emission. J. Air Waste Manage. Assoc. 41, 1045–1054.

Mannebeck, H. (1986) Covering manure storing tanks to control odour. In: Neilsen, V.C., Voorburg J.H. and Hermite, P.L. (Eds.), Odour prevention and control of organic sludge and livestock farming. Elsevier, London, pp. 188–192.

Medina, V.F., Webster, T. and Devinny, J.S. (1995) Treatment of gasoline residuals by granular activated carbon based biological filtration. J. Environ. Sci. Health 30 (A), 407–422.

Moriarty, D.J.W. and Nicholas, D.J.D. (1970) Products of sulphide oxidation in extracts of *Thiobacillus concretivorus*. Biochim. Biophys. Acta 197, 143–151.

Nelson, D.C. (1990) Physiology and biochemistry of filamentous sulfur bacteria. In: Schegel, H.G. and Bowien, B. (Eds.), Autotrophic Bacteria. Springer-Verlag, Berlin, pp. 219–238.

Peters, D.A. (1993) Laboratory assessment of biofiltration for fuel-derived VOC emmission control. In: Proc. 86th Ann. Meet. Air and Waste Management Association, Denver, Colorado.

Rands, M.B., Cooper, D.E., Woo, C.P., Fletcher, G.C. and Rolfe, K.A. (1981) Compost filters for H_2S removal from anaerobic digestion and rendering exhausts. J. Wat. Pollut. Control Fed. 53, 185–189.

Richard, D.P. (1982) Biological treatment of odorous air. J. Wat. Pollut. Control Fed. 54, 1541–1545.

Schedel, M. and Truper, H.G. (1980) Anaerobic oxidation of thiosulfate and elemental sulfur in Thiobacillus denitrifans. Arch. Microbiol. 2–3, 205–210.

Sublette, K.L. and Sylvester, N.D. (1987) Oxidation of hydrogen sulfide by continuous cultures of *Thiobacillus denitrificans*. Biotechnol. Bioeng. 29, 753–758.

Sublette, K.L., Hesketh, R.P. and Hasan, S. (1994) Microbial oxidation of hydrogen sulfide in a pilot-scale bubble column. Biotechnol. Prog. 10, 611–614.

Tanji, Y., Kanagawa, T. and Mikami, E. (1989) Removal of dimethyl sulfide, methyl mercaptan, and hydrogen sulfide by immobilized *Thiobacillus thioparus* TK-m. J. Ferment. Bioeng. 67, 280–285.

Wada, A., Shoda, M., Kubota, H., Kobayashi, T., Katayama-Fujimura, Y. and Kuraishi, H. (1986). Characteristics of H_2S oxidizing bacteria inhabiting a peat biofilter. J. Ferment. Technol. 64, 161–167.

Yang, Y. and Allen, E.R. (1994) Biofiltration control of hydrogen sulfide 1. Design and operational parameters. J. Air Waste Manage. Assoc. 44, 863–868.