

Removal of Hydrogen Sulphide by Immobilized *Thiobacillus* sp. strain CH11 in a Biofilter

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(Received 20 June 1996; revised version received 27 September 1996; accepted 30 September 1996)

Abstract: An autotrophic *Thiobacillus* sp. CH11 was isolated from piggery wastewater containing hydrogen sulphide. The removal characteristics of hydrogen sulphide by *Thiobacillus* sp. CH11 were examined in the continuous system. The hydrogen sulphide removal capacity was elevated by the BDST (Bed Depth Service Time) method (physical adsorption) and an immobilized cell biofilter (biological conversion). The optimum pH to remove hydrogen sulphide ranged from 6 to 8. The average specific uptake rate of hydrogen sulphide was as 1.02×10^{-13} mol-S cell⁻¹ h⁻¹ in continuous systems. The maximum removal rate and saturation constant for hydrogen sulphide were calculated to be $V_m = 30.1$ mmol-S day⁻¹ (kg-dry bead)⁻¹ and $K_s = 1.28$ μ mol dm⁻³, respectively. A criterion to design a scale-up biofilter was also studied. The maximum inlet loading in the linear region (95% removal) was 47 mmol-S day⁻¹ (kg-dry bead)⁻¹. Additionally, the biofilter exhibited high efficiency (>98.5%) in the removal of hydrogen sulphide at both low (<0.026 mg dm⁻³) and high (0.078 mg dm⁻³) concentrations. The results suggested that the *Thiobacillus* sp. CH11 immobilized with Ca-alginate is a potential method for the removal of hydrogen sulphide.

Key words: biofilter, hydrogen sulphide, immobilized cell, *Thiobacillus* sp.

INTRODUCTION

Hydrogen sulphide (H₂S) is an extremely toxic gas and has the potential for causing injury to central nervous systems at low-dose exposures.¹ A considerable amount of H₂S is produced in association with industrial processes, such as petroleum refining, wastewater treatment, food processing, paper and pulp manufacturing, and also in the treatment of fuels.^{2,3} Physical and chemical processes, including activated carbon adsorption, ozone oxidation and incineration,^{4–6} have been used to purify H₂S from waste gas and wastewater. However, biological processing is increasingly being considered as a method for the removal of H₂S because it is less expensive, has a higher removal efficiency and gives rise to less secondary pollution than physical and chemical means for decomposing toxic H₂S.⁷

A number of microbial processes for H₂S removal have been studied using some specific packing materials as carriers, including fibrous peat, compost and activated carbon.^{8,9} However, these processes either have an unsatisfactory removal efficiency or an undesirable removal capacity, especially at a relatively low concentration (0.026 mg dm⁻³).¹⁰ For example, the compost or peat which is packed into a biofilter causes aging phenomena, including compaction and lumping of material, that decrease the effectiveness of the biofilter.¹¹ In addition to the appropriate packing material, it is important to select an appropriate microorganism to optimize the removal efficiency of H₂S. Various microorganisms, such as *Xanthomonas* sp., *Hyphomicrobium* sp., *Pseudomonas* sp. and *Thiobacillus* spp. have been reported to be capable of H₂S oxidation in biological reactors.^{12–16} The products of H₂S oxidation are dependent on the strain of *Thiobacillus* sp.^{17,18} *Hyphomicrobium* genera can oxidize H₂S to elemental

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sulphur which is stored in the cell. The elemental sulphur will be further oxidized to sulphate when the concentration of H₂S in the environment is low. The resulting pH reduction will significantly retard the microbial activity and inhibit H₂S oxidation.¹³

In this study, we developed an innovative biofilter using immobilized *Thiobacillus* sp. CH11, commonly used in wastewater treatment, to elevate the efficiency of removing H₂S gas especially at low concentration conditions.

2 MATERIALS AND METHODS

2.1 Microorganism and cultivation

An autotrophic *Thiobacillus* sp. CH11 was isolated from piggery wastewater containing H₂S. The bacterium was purified by repeated transfer of the bacteria to fresh medium. The thiosulphate medium was prepared for cultivation of the isolate containing (dm⁻³): KH₂PO₄ 2 g, K₂HPO₄ 2 g, NH₄Cl 0.4 g, MgCl₂·6H₂O 0.2 g, FeSO₄·7H₂O 0.01 g and Na₂S₂O₃·5H₂O 8 g. For the continuous experiment, a basal mineral medium was prepared; this was the same as the thiosulphate medium except for the absence of sodium thiosulphate. The final pH was adjusted to 7 by using 2 mol dm⁻³ NaOH for both the thiosulphate and basal mineral media.

The growth pattern of the bacteria as well as the change in pH value, and sulphate and thiosulphate concentrations in the thiosulphate medium was monitored during the incubation (reciprocal shaking: 200 rpm, at 30°C). Bacterial numbers on the thiosulphate medium were determined after dilution by the plating count technique. Both sulphate (SO₄²⁻) and thiosulphate (S₂O₃²⁻) concentrations were measured by ion chromatography (Dionex 4500i). The separating column was an inorganic negative-ion column of IonPac AS4A-SC. The mobile phase was composed of 1.8 mmol dm⁻³ Na₂CO₃ and 1.7 mmol dm⁻³ NaHCO₃. The eluent flow rate and operational temperature were 2.0 cm³ min⁻¹ and 25°C, respectively.

2.2 Preparation of immobilized microbes

Bacteria grown in 100 cm³ thiosulphate medium were harvested by centrifugation (7500 × g, 10 min) and washed aseptically three times with distilled water. The organisms (10⁵ organisms cm⁻³) were immersed in a sterilized 4% (w/v) Na-alginate solution, and the Na-alginate solution dropped into 4% (w/v) CaCl₂ solution by syringe. The immobilized beads, with a diameter of 3.0 mm, formed immediately. The beads were activated by flushing aseptically with buffer solution for 5 h. The activated beads exhibited excellent mechanical strength for continuous experiments.

2.3 H₂S removal rate in continuous system

Three glass columns (60 mm φ × 18 cm working length) connected in series were packed with Ca-alginate beads without microbes to investigate the adsorption capacity of H₂S by alginate beads. H₂S gas (0.0065 mg dm⁻³) was supplied to those glass columns at 725 cm³ min⁻¹. The desired concentration of H₂S at the breakthrough was 0.0026 mg dm⁻³ (i.e.: C_e/C₀ = 0.4).

The beads of immobilized organisms were packed into a glass column (60 mm φ × 25 cm working length). The packing volume, bead dry weight and initial number of immobilized bacteria in each column were 0.7 dm³, 0.25 kg and 10⁵ cfu (g dry bead)⁻¹, respectively. H₂S gases with different concentrations were supplied to the column at a flow rate of 36 dm³ h⁻¹ or 45 dm³ h⁻¹. The inlet and outlet H₂S concentrations were continuously measured by Single Point Monitor (MDA Scientific, Inc. USA). The basal mineral medium which was contained in an air-bubbling bottle was supplied to the immobilized microbes using an air compressor. The change in pH value and sulphate concentration in the upper and middle layers of the column were measured.

2.4 Kinetic analysis

The rate of H₂S removal in the biofilter was carried out using the following equation which was derived from the Michaelis–Menten equation:¹⁹

$$\frac{1}{R} = \frac{K_s}{V_m} \times \frac{1}{C_{1n}} + \frac{1}{V_m} \quad (1)$$

where R (mol-S day⁻¹ kg-dry bead⁻¹) = removal rate, C_{1n} (mol dm⁻³) = logarithmic mean concentration of H₂S at the inlet and outlet of the biofilter, V_m (mol-S day⁻¹ kg-dry bead⁻¹) = maximum removal rate and K_s (mol dm⁻³) = saturation constant. From the linear equation between $1/C_{1n}$ and $1/R$, the V_m and K_s values were calculated from the slope and intercept, respectively.

2.5 Criteria for designing a scale-up of biofilter

The continuous study was also conducted at different concentrations and flow rates of H₂S into the biofilter with various packing heights. This study was to investigate the relationship between inlet loading and the removal capacity of the biofilter. The maximum removal capacity (mmol-S day⁻¹ kg-dry bead⁻¹) can be obtained from the upper limit of this linear relationship.

3 RESULTS AND DISCUSSION

3.1 Growth characteristics of the isolated microorganism

Thiobacillus sp. CH11 was classified as a chemolitho-autotroph according to its physiological and biochemical characteristics; it was motile, Gram-negative and short-rod. The colony (diameter of 0.5–1 mm) on the thiosulphate agar was whitish-yellow because of extracellular deposition of sulphur. *Thiobacillus* sp. CH11 grew autotrophically with elemental sulphur, thiosulphate and sulphide, but hardly grew in facultative autotrophic and heterotrophic conditions. The optimum pH range for the growth of *Thiobacillus* sp. CH11 was 6–8. The growth pattern of *Thiobacillus* sp. CH11 in the thiosulphate medium is shown in Fig. 1. The cell numbers reached their maximum level, 10^8 cfu cm^{-3} , on the fifth day and then rapidly decreased on the sixth day. During the initial 6 days of cultivation, the decrease in thiosulphate concentration was accompanied by an increase in the cell number and sulphate concentration. Visible elemental sulphur accumulated in the medium. These results hinted that *Thiobacillus* sp. CH11 could oxidize thiosulphate to sulphate as the final product via the accumulation of intermediate sulphur. After the sixth day of cultivation, the significant decrease in the cell number was accompanied by an increase in sulphate concentration. It suggested that the accumulation of sulphate was over the buffer capability of medium which caused the pH value and cell number to decrease significantly. These results confirmed that *Thiobacillus* sp. CH11 was sensitive to low pH.

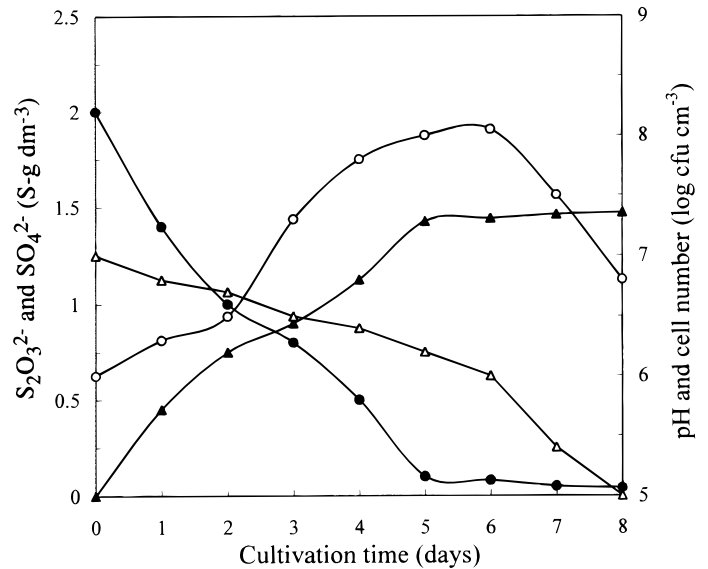


Fig. 1. Growth pattern, pH value and $\text{S}_2\text{O}_3^{2-}$ changes of *Thiobacillus* sp. CH11 grown in the thiosulphate medium. ●, $\text{S}_2\text{O}_3^{2-}$; ▲, SO_4^{2-} ; ○, cell number; △, pH value.

3.2 H_2S removal in continuous system

The physical adsorption of Ca-alginate beads to remove H_2S is shown in Fig. 2. The adsorption process is described by eqn (2):²⁰

$$t = \left(\frac{N_0}{C_0 V} \right) X \frac{1}{k C_0} \ln \left(\frac{C_0}{C_e} - 1 \right) \quad (2)$$

where C_0 and C_e are the inlet concentration and the desired concentration of gas at breakthrough (mg dm^{-3}), respectively; V , t and X are hydraulic

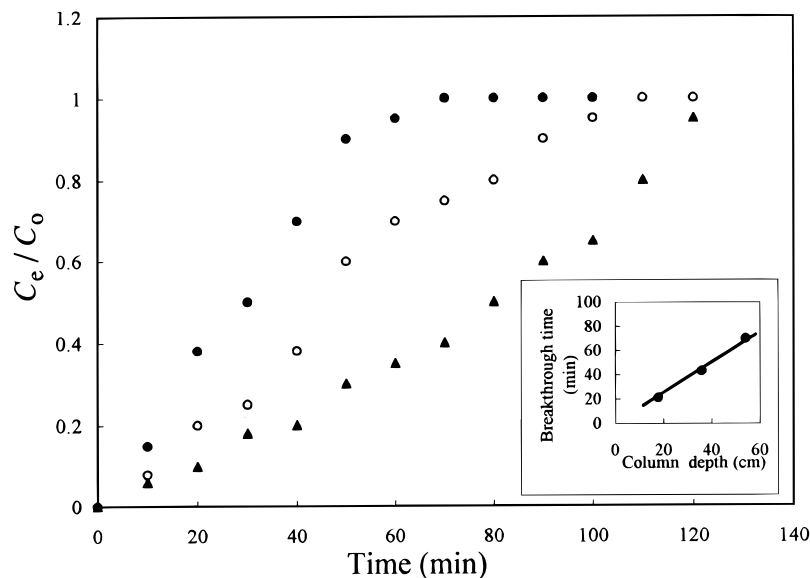


Fig. 2. Breakthrough of hydrogen sulphide adsorption by the Ca-alginate bead column. The inset diagram shows the linear correlation between packing height and breakthrough time according to the calculation of Eqn (2) by using breakthrough data. ●, Column I; ○, column II; ▲, column III.

loading (cm min⁻¹), service time (min) and bed depth (cm); N_0 and k are adsorptive capacity (mg dm⁻³) and adsorption rate constant (mg⁻¹ dm³ min⁻¹).

The constants of eqn (2) were obtained from the slope and interception of Fig. 2 by the regression method. The values of slope and interception were 1.36 and 4.33. The adsorptive capacity (N_0) and the adsorption rate constant (k) were 7.1 $\mu\text{mol dm}^{-3}$ and 26.5 $\mu\text{mol}^{-1} \text{dm}^3 \text{h}^{-1}$, respectively. The H₂S adsorption capacity by pure Ca-alginate beads calculated from this equation can provide a reference value of the removal capacity by cell-laden Ca-alginate beads.

The fluctuation of the inlet H₂S concentration was examined in the range of 0.0013 to 0.078 mg dm⁻³ at flow rates of 36 dm³ h⁻¹ and 45 dm³ h⁻¹. At a flow rate of 36 dm³ h⁻¹, the average removal efficiency of H₂S was 99.5% on the seventh day even when the inlet H₂S concentration was as high as 0.078 mg dm⁻³. When the flow rate was increased to 45 dm³ h⁻¹, the average removal efficiency was still maintained at 98.6%. The changes in the pH value and sulphate concentration in the upper and middle layers of the biofilter are shown in Fig. 3. During this period, the pH value decreased with the accumulation of sulphate ions. The inlet H₂S from the bottom of the biofilter caused the sulphate concentration to be higher in the middle layer than in the upper layer. Similarly, the decline of pH in the middle layer was also greater than in the upper layer. The results also suggested that *Thiobacillus* sp. CH11 partially oxidized H₂S to sulphate according to sulphur mass balance. The residual H₂S might be converted to sulphur or organic sulphur. Hence, the pH value was maintained between 7 and 6.5 during the operation period. An experiment was also conducted

without controlling the temperature (23–30°C) for 90 days to understand the stability of the biofilter. H₂S removal efficiencies of 99% or greater were consistently achieved when H₂S concentrations were varied between 0.00065 and 0.0780 mg dm⁻³ at a flow rate of 36 dm³ h⁻¹. Hence, the biofilter has a potential for application in real situations.

3.3 Kinetic analysis

The kinetic parameters of the maximum removal rate and saturation constant to degrade H₂S under different conditions are calculated by the Lineweaver–Burk method. The specific uptake rate (1.02×10^{-13} mol-S cell⁻¹ h⁻¹) in the continuous system was about one hundred orders of magnitude higher than the previous reports (9.68×10^{-15} , 3.38×10^{-15} , 2.43×10^{-15} mol-S cell⁻¹ h⁻¹).^{14,21,22} The maximum removal rate and saturation constant of H₂S were calculated to be $V_{\max} = 30.1$ mmol-S day⁻¹ (kg-dry bead)⁻¹ and $K_s = 1.28$ $\mu\text{mol dm}^{-3}$, respectively. The saturation constant in this study was smaller than that in previous reports (1.59 μM , 2.25 μM).^{19,21} If we suppose a physical meaning for K_s analogous to that in enzymatic kinetics, a decrease in the K_s value suggests the enhancement of biomass affinity for the H₂S.

3.4 Criteria for designing a scale-up of biofilter

The H₂S can be removed completely below a certain inlet loading. Above this certain inlet loading, the H₂S would be constantly detected at the outlet of the biofilter. Hence, to find an optimum inlet loading of H₂S

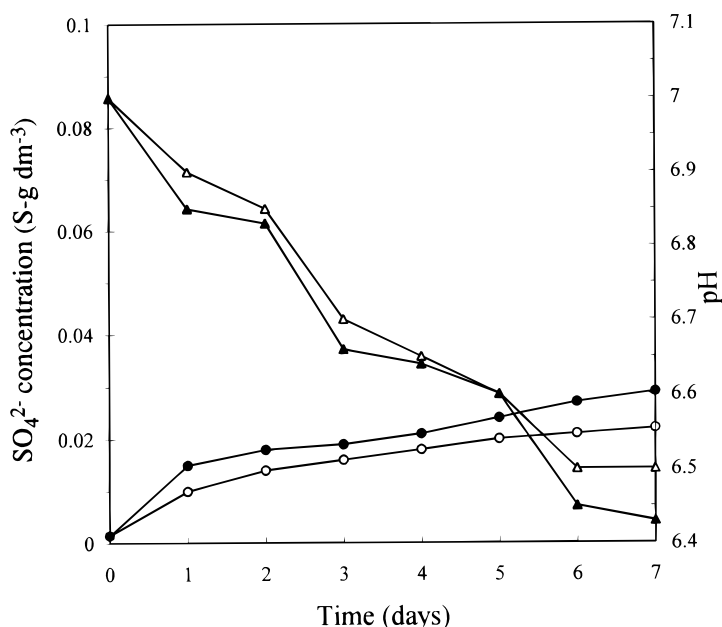


Fig. 3. pH value and SO_4^{2-} concentration in the upper and middle layers of biofilter. Δ , pH change in the upper layer of biofilter; \blacktriangle , pH change in the middle layer of biofilter; \circ , SO_4^{2-} concentration in the upper layer of biofilter; \bullet , SO_4^{2-} concentration in the middle layer of biofilter.

during the operation is very important. The inlet loading is defined as the amount of inlet gas per unit time and the weight of packing material ($\text{g-S day}^{-1} \text{kg-dry bead}^{-1}$). Hence, gas flow rate, inlet gas concentration and the weight of packing material play important roles in designing a scale-up biofilter. In this study, the maximum inlet loading of H_2S was $47 \text{ mmol-S day}^{-1} (\text{kg-dry bead})^{-1}$ in the linear region (95% removal) and was much larger than the $14 \text{ mmol-S day}^{-1} (\text{kg-dry bead})^{-1}$ reported previously. This maximum inlet loading can be used as a criterion for designing a real scale-up of the immobilized *Thiobacillus* sp. CH11 biofilter. In practice, it was found favourable to put a constant weight of packing beads in the biofilter. When the theoretical loading was larger than $47 \text{ mmol-S day}^{-1} (\text{kg-dry bead})^{-1}$, the flow rate could be reduced to maintain greater than 95% removal efficiency. When the theoretical loading was much less than $47 \text{ mmol-S day}^{-1} (\text{kg-dry bead})^{-1}$, the flow rate could be elevated to achieve the maximum inlet loading.

4 CONCLUSIONS

This study demonstrates that the immobilized *Thiobacillus* sp. CH11 biofilter is a potential remediation system to purify H_2S emissions. Feed concentrations ranging from 0.013 to 0.078 mg dm^{-3} were removed with more than 98.5% removal. Removal capacity as high as $47 \text{ mmol-S day}^{-1} (\text{kg-dry bead})^{-1}$ was achieved. Both the high average specific uptake rate and low saturation constant infers that *Thiobacillus* sp. CH11 has high removal ability for H_2S . Therefore, the immobilized *Thiobacillus* sp. CH11 biofilter becomes a potential process for treatment of small volumes of effluents and low concentrations of pollutants.

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