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Thermophilic biofiltration of H_2S and isolation of a thermophilic and heterotrophic H_2S-degrading bacterium, *Bacillus* sp. TSO3

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ABSTRACT

Thermophilic biofiltration of H_2S-containing gas was studied at 60 °C using polyurethane (PU) cubes and as a packing material and compost as a source of thermophilic microorganisms. The performance of biofilter was enhanced by pH control and addition of yeast extract (YE). With YE supplement and pH control, H_2S removal efficiency remained above 95% up to an inlet concentration of 950 ppmv at a space velocity (SV) of 50 h^{-1} (residence time = 1.2 min). H_2S removal efficiency strongly correlated with the inverse of H_2S inlet concentrations and gas flow rates. Thermophilic, sulfur-oxidizing bacteria, TSO3, were isolated from the biofilter and identified as *Bacillus* sp., which had high similarity value (99%) with *Bacillus thermoleovorans*. The isolate TSO3 was able to degrade H_2S without a lag period at 60 °C in liquid cultures as well as in the biofilter. High H_2S removal efficiencies were sustained with a periodic addition of YE. This study demonstrated that an application of thermophilic microorganism for a treatment of hot gases may be an economically attractive option since expensive pre-cooling of gases to accommodate mesophilic processes is not required.

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1. Introduction

Malodorous gases are emitted from many industrial and environmental facilities under hot as well as moderate temperature conditions [1]. For most gases, the Henry's law coefficient increases with temperature, resulting in decreased solubility of the compound in water [2–4]. Therefore, in order to treat hot odor waste gases by conventional scrubbing methods, the gas stream needs to be cool to applicable temperatures. However, cooling the air to ambient temperatures would be expensive [5,6]. One alternative treatment method that does not require pre-cooling of emission gas is the thermophilic biofiltration, which utilizes the metabolic activity of thermophilic organisms [5]. Thermophilic biofilters take advantages of increased rate of microbial metabolism and more economical treatment processes [1–3,5]. Although microbial metabolic activities increase at higher temperatures, most investigations on the biofiltration of odorous gas have focused on mesophilic biofilters that operate at ambient temperature. On the other hand, successful application of thermophilic biofilters and biotrickling filters have been reported for treatment of hot waste gases containing volatile organic compounds [1–5]. A thermophilic fungi biofilter achieved a high removal rate of 80 $g\,m^{-3}\,h^{-1}$ ethanol [5], and other thermophilic biofilters achieved removal rates greater than 100 $g\,m^{-3}\,h^{-1}$ of ethyl acetate at 45 to 50 °C [5]. Removal rates of methanol and α-pinene in a thermophilic biotrickling filter were reported to be 100 and 60 $g\,m^{-3}\,h^{-1}$, respectively [3]. Thermophilic biodegradation of BTEX (benzene, toluene, ethylbenzene, xylene) by both pure culture and mixed culture has been reported by various researchers [7–9]. A successful degradation of a mixture of benzene and toluene was reported with a polyurethane (PU) biofilter at 60 °C [1]. However, little information is available on thermophilic biofiltration for the treatment of sulfur-containing malodorous gases such as H_2S, CH_3SH, $(CH_3)_2S$, and $(CH_3)_2S_2$, which are the major constituents in hot waste gases emitted from many industries and environmental treatment facilities [10].

A variety of sulfur-oxidizing bacteria for the removal of sulfur-containing malodorous gases have been reported. Most of them are mesophilic and chemolithoautotrophic bacteria such as *Acidithiobacillus thiooxidans* [11,12], and *Thiobacillus thioparus* [13]. Some mesophilic and heterotrophic bacteria including *Xanthomanas* sp. [14], *Rhodococcus* sp. [15], *Streptomyces* sp. [16], *Bacillus* sp. [17], and *Pseudomonas* sp. [18] have been described as sulfide oxidizers. There is little report on thermophilic, heterotrophic sulfur-oxidizing bacteria in the literature even though thermophilic, autotrophic sulfur-oxidizing bacteria have been well documented [19]. *Sulfolobus* spp. are representative thermophilic and autotrophic sulfur oxidizers [20,21] that grow in volcanic springs with optimal growth occurring at pH 2–3 and

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temperatures of 75–80 °C. These bacteria have been applied for thermophilic bioleaching processes [21]. Thermophilic purple sulfur bacteria, *Thermochromatium tepidum*, have been shown to anaerobically oxidize hydrogen sulfide to elemental sulfur [22].

To develop a thermophilic biofilter for sulfur-containing malodorous gases, effective microbial resources, which can be applied in the thermophilic biofilter is necessary. Therefore, in this paper, the feasibility of thermophilic biofiltration of H_2S was investigated using a lab-scale polyurethane biofilter at 60 °C. Moreover, a novel thermophilic and heterotrophic H_2S-degrading bacterium was isolated from the thermophilic PU biofilter, and its ability to oxidize H_2S was verified in a liquid culture and in a laboratory-scale biofilter system.

2. Materials and methods

2.1. Packing material and inoculums for biofilter

The experimental biofilter was packed with 1-cm PU foam cubes (Seilsponge, Seoul, Korea). The bulk density, water holding capacity, porosity, average pore size, and surface area of the material were 0.015 g cm^{-3}, 57 g-H_2O g^{-1}, 98.8%, 0.8 mm, and 76.81 m^2 g^{-1}, respectively. Seed microorganisms for the biofilter were obtained from high-temperature compost. The large particles were first removed from the compost by sieving (200 mesh) and then the sieved materials was suspended in mineral salts solution (MS solution; 1.5 g l^{-1} of KH_2PO_4, 9.0 g l^{-1} of $Na_2HPO_4 \cdot 12H_2O$, 3 g l^{-1} of $(NH_4)_2SO_4$, 0.01 g l^{-1} of $CaCl_2 \cdot 12H_2O$, and 0.15 g l^{-1} of $MgSO_4$).

2.2. Lab-scale biofilter and operation conditions

A laboratory-scale biofilter was constructed from an acryl column with a diameter of 10 cm (ID) and a height of 40 cm. The column was packed with 30 g (dry weight) of PU cubes to a packing height of 160 mm (packing volume: 1256 ml). Prior to packing, the PU cubes were pre-absorbed with the compost suspension. The column was then placed in a water bath at 60 °C to maintain a thermophilic condition in the biofilter. Desired inlet concentrations of H_2S gas was obtained by diluting H_2S gas from a cylinder (146,000 ppmv) with air from a compressor in a mixing chamber, and the humidified H_2S gas was supplied to the biofilter at a constant flow rate to maintain the desired space velocity (SV, gas flow rate per unit packing volume) [11].

The biofilter was first acclimatized for 15 days with an inlet H_2S concentration (100–200 ppmv) at a SV of 50 h^{-1}. After steady state conditions were achieved, the effect of inlet H_2S concentrations and gas flow rates on the biofilter performance was examined at the following inlet concentrations and SVs: 25–950 ppmv at 50 h^{-1}, 50–400 ppmv at 100 h^{-1}, 30–100 ppmv at 200 h^{-1}, and 10–70 ppmv at 300 h^{-1}. After each SV condition, the biofilter was re-acclimated to the original condition by supplying 100–200 ppmv H_2S at 50 h^{-1} for 3 and 4 days before changing the gas flow rate.

During the biofilter operation, 50 ml of sterile MS solution supplemented with 5 g l^{-1} of yeast extract (YE) was poured from the top of the biofilter every day to maintain moisture content of more than 70%. The drainage from the biofilter was sampled every day, and its pH was monitored. If its pH decreased below 5.5, the biofilter was neutralized by adding $CaCO_3$ solution. The removal efficiency and removal capacity of H_2S by the biofilter were calculated using the inlet and outlet concentrations of H_2S and SVs [11].

2.3. Effect of YE addition and pH adjustment on biofilter performance

The effect of YE addition and pH adjustment on the performance of biofilter for H_2S removal was investigated at a SV of 50 h^{-1} and at H_2S concentrations of 25–950 ppmv. During these experiments, in addition to providing both YE and pH adjustment, the biofilter was also operated (1) without $CaCO_3$ neutralization only and (2) without both YE addition and $CaCO_3$ neutralization to evaluate the effects of YE addition and pH adjustment.

2.4. Isolation of thermophilic sulfur-oxidizing bacterium

At the termination of the biofiltration experiment, a thermophilic sulfur-oxidizing bacterium (SOB) was isolated from the PU-packed biofilter. Approximately 5 g (wet weight) of PU cubes were sampled from the middle of the biofilter bed, suspended in 50 ml of sterile distilled water, and shaken vigorously. The resulting cell suspension enriched in four different SOB mediums: (1) SOB medium at pH 7, (2) SOB medium at pH 7 supplemented with 5 g l^{-1} of YE, (3) SOB medium at pH 4, (2) SOB medium at pH 4 supplemented with 5 g l^{-1} of YE. SOB contained the following constituents: 2.0 g l^{-1} of K_2HPO_4, 2.0 g l^{-1} of KH_2PO_4, 0.4 g l^{-1} of NH_4Cl, 0.2 g l^{-1} of $MgCl_2 \cdot 7H_2O$, 0.01 g l^{-1} of $FeSO_4 \cdot 7H_2O$, 10 g l^{-1} of elemental sulfur (S^0). The pH of the media was adjusted to 7 and 4 using 2N NaOH and 2N HCl solution, respectively. The enrichment cultures were started by seeding 5 ml of PU cell suspension into 95 ml of each SOB medium.

All enrichment culture flasks were incubated at 60 °C on a rotary shaker at 180 rpm for 15 days. Following the incubation period, the enrichment cultures with decreasing pH and increasing sulfate concentration were selected and spread on the SOB + YE agar plates (pH 7) for incubation at 60 °C for 4 days. Ten colonies, which had different shapes and color, were selected and cultivated in fresh SOB + YE liquid medium (pH 7) in the same manner as described above. One colony having the highest sulfur oxidation ability was chosen and named TSO3. The isolate TSO3 was identified through a partial 16S rDNA sequence analysis as previously described [23].

Growth and sulfur oxidation activity of the isolate was examined in the SOB + YE medium (pH 7) with yeast extract concentrations ranging from 0 to 10 g l^{-1}. To evaluate the effect of different carbon sources on the sulfur oxidation rate, 5 g l^{-1} of glucose, sucrose, and peptone supplementation to the SOB medium (pH 7) was compared using a 500 ml flask containing 100 ml of culture broth, The liquid cultures were incubated at 60 °C on a rotary shaker (180 rpm) and culture samples (10 ml) were obtained every 1 or 2 days during incubation to measure optical density (at 600 nm) and sulfate concentration. Specific growth rate and sulfur oxidation rate in each culture condition was calculated using the same method as previously described [11,24]. Sulfur oxidation rate was determined from the consumed sulfur concentration (S^0) during culture time. Consumed sulfur concentration was calculated based on sulfate produced from sulfur oxidation ($S^0 + 3/2O_2 + H_2O \rightarrow 2H^+ + SO_4^{2-}$). All experiments were conducted in duplicate.

2.5. H_2S degradation by isolate

Liquid cultures of the isolate were established by inoculating the cell suspension into a 500 ml glass bottle containing 300 ml of fresh SOB + YE medium (pH 7). An identical bottle without the isolate was prepared as a control. Experimental and control bottles were placed in a water bath (60 °C) and H_2S gas (100–200 ppmv) was supplied into the bottles with a gas bubble diffuser at a SV of 70 h^{-1} via a humidifier. Inlet and outlet concentrations of H_2S were intermittently measured to determine the H_2S removal by the isolate.

To examine H_2S removal by the isolate in a laboratory-scale biofilter, cells were first harvested by centrifuging 800 ml of culture broth at 8000 rpm and the pellet was then resuspended in 200 ml of SOB + YE medium (pH 7). The resulting cell suspension was absorbed to 25 g (dry weight) polyurethane cubes

(1 cm × 1 cm × 1 cm). A glass column (10 cm Ø × 25 cm *H*) was packed with the seeded polyurethane cubes (a packing height of 14 cm) and the column was placed in a water bath (60 °C) and H_2S gas (50–200 ppmv) was supplied to the column via a humidifier at 70 h^{-1} of SV. The PU cubes were manually sprayed with 50 ml of sterile SOB + YE medium every 4 days to maintain moisture levels.

2.6. Analysis

Inlet and outlet H_2S gas concentrations were analyzed by gas chromatography (5890 plus II, HP, USA) equipped with flame photometric detector and HP-1 capillary column (0.25 mm Ø × 3000 mm *L*, HP, USA). The temperature of the oven, injector and detector were fixed at 35, 100, and 200 °C, respectively. A standard curve of H_2S gas was prepared by injecting H_2S permeation tube (P-tube) through a permeator (PD-13, Gastec, Japan) with the P-4 permeation tube held at 30 °C. Sulfate concentrations were determined by Ion Chromatography (Waters 510, USA; conductivity detector, Waters 432; IC-Pak-TM Anion column: 4.6 mm Ø × 50 mm *L*) with sodium borate/gluconate solution as the mobile phase.

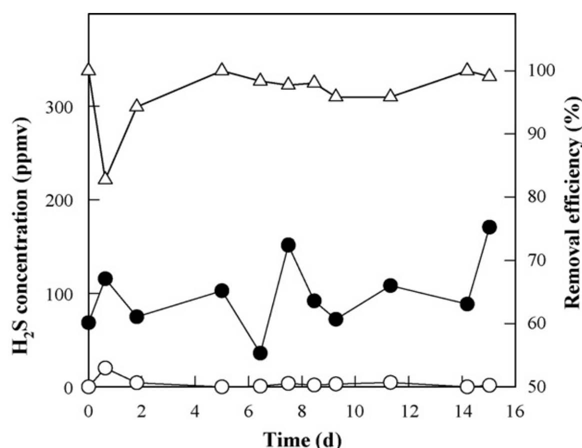

Fig. 1. Removal of hot H_2S gas (60 °C) in thermophilic PU biofilter during acclimation period (SV = 50 h^{-1}). Symbols: (●) inlet concentration; (○) outlet concentration; (△) removal efficiency.

3. Results and discussion

3.1. H_2S removal by thermophilic biofilter

The feasibility of thermophilic biofiltration of H_2S at 60 °C was examined using PU cubes as a packing material and high-temperature compost as an inoculum source. For inlet H_2S concentrations ranging from 50 to 950 ppmv, H_2S removal in the PU biofilter operated at SV of 50 h^{-1} is shown in Fig. 1. After 2 days, less than 5 ppmv of H_2S gas was detected in the outlet, and removal efficiency of greater than 95% was maintained throughout the 15 day-acclimation period. On the other hand, when the similar concentration of H_2S gas was supplied to an abiotic PU filter (no inoculation), the outlet concentration of H_2S reached 98% of the inlet concentration in 5–10 min, suggesting that adsorption of H_2S to PU packing materials was negligible (data not shown). These

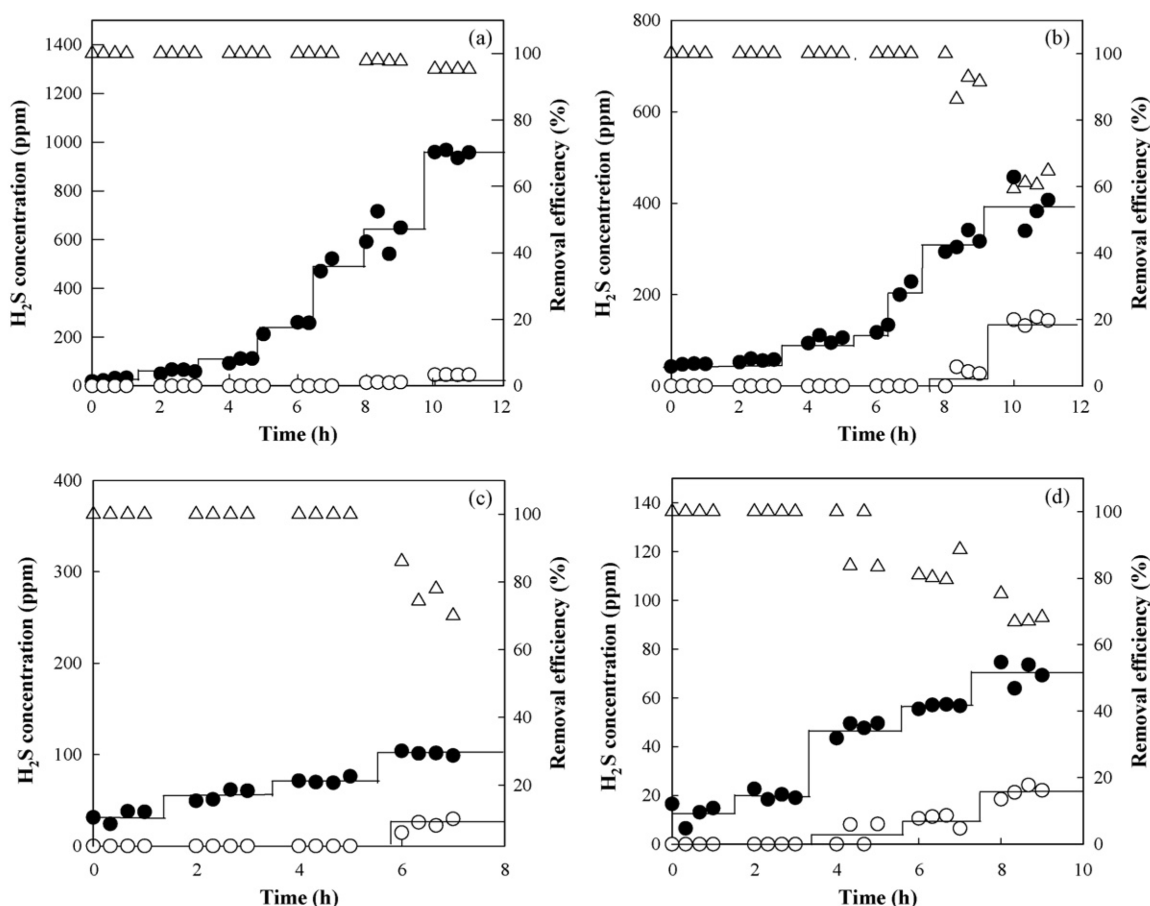

Fig. 2. Removal of hot H_2S gas (60 °C) in thermophilic PU biofilter at different inlet concentrations and SVs. (a) SV = 50 h^{-1}, (b) SV = 100 h^{-1}, (c) SV = 200 h^{-1}, (d) SV = 300 h^{-1}. Symbols: (●) inlet concentration; (○) inlet concentration; (△) removal efficiency.

results indicate that H_2S removal in the PU biofilter is associated with microbial activities of thermophilic seed microorganisms.

Presence of acclimation period has been commonly observed during the initial start-up of biofilters seeded with microbial mixed cultures such as soil, activated sludge, digested sludge, and earthworm [25,26]. However, in this study, acclimation period for H_2S oxidation was not observed as over 83% of inlet H_2S was removed during the first 2 days of operation. This result suggests that the compost materials contained substantial amount of thermophilic H_2S-degrading microorganisms. Cho et al. (2007) also reported that the mixed gases of benzene and toluene were readily removed in a PU biofilter inoculated with compost materials without any acclimation period [1]. Compost is microbial resource consisted of a variety of microorganisms including thermophiles as well as mesophiles [27]. Therefore, compost is one of the promising sources of seed microorganisms for the biofiltration of hot waste gases.

Packing materials is also one of key factors influencing biofilter performance [5]. An ideal packing material must have a high porosity to reduce head loss and a high specific surface area for the attachment of microorganisms [1,28,29]. For thermophilic biofiltration, water holding capacity of packing material is also important because of higher evaporation of water at elevated temperatures. PU cubes have a high moisture holding capacity and therefore may be suitable for thermophilic applications [1].

3.2. *Effect of flow rate and inlet concentration on biofilter performance*

Fig. 2 shows H_2S removal in the thermophilic PU biofilter at SVs of 50–300 h^{-1} under the conditions of YE addition and pH control. At a SV of 50 h^{-1}, the removal efficiencies remained above 95% up to an inlet concentration of 950 ppmv. At a SV of 100 h^{-1}, the removal efficiencies of 99% were achieved at inlet H_2S concentration up to around 200 ppmv; the removal efficiency of the biofilter decreased sharply to 61% at 400 ppmv. Similar results were observed with SVs of 200 and 300 h^{-1} as the removal efficiencies significantly decreased with increasing inlet concentrations (Fig. 2).

Removal capacity of H_2S as a function of influent H_2S loading at four different gas flow rates (SV = 50–400 h^{-1}) is shown in Fig. 3a. For a SV of 50 h^{-1}, the removal capacity increased linearly with the range of tested loading rates. The maximum H_2S removal capacity was 56.6 g m^{-3} h^{-1} at a SV 50 h^{-1}. For SVs of 100, 200 and 300 h^{-1}, the maximum removal capacities for over 90% H_2S removal were 39.1, 17.8, and 16.2 g m^{-3} h^{-1}, respectively. Fig. 3b shows the removal efficiencies of H_2S as a function of the inlet concentrations at each SV. High removal efficiency of H_2S (>95%) at a SV 50 h^{-1} could be obtained when inlet concentration was increased to 950 ppmv. However, H_2S removal efficiencies strongly correlated with the inverse of the inlet concentration at SVs of 100, 200 and 300 h^{-1}. The maximum inlet concentrations of H_2S guaranteed the removal efficiency of >90% were 340, 70, and 47 ppmv, respectively.

3.3. *Effect of YE addition and pH control on biofilter performance*

Fig. 4 shows the effect of YE addition and pH control on the biofilter performance for H_2S removal at a SV of 50 h^{-1} and H_2S concentration of 25–950 ppmv. Under conditions of YE addition and pH control, the maximum H_2S removal capacity was 56.6 g m^{-3} h^{-1}. When only YE was supplied the maximum removal capacity of H_2S without pH control (37.1 g m^{-3} h^{-1}) was 66% of that with pH control (56.6 g m^{-3} h^{-1}). On the other hand, when the thermophilic biofilter was operated without pH control and YE addition, the biofilter performance was significantly deteriorated. The maximum removal capacity of H_2S was only 8.9 g m^{-3} h^{-1}. The pH of drain water from the biofilter decreased from 7.2 to 5.5 during the operation without pH control. The H_2S removal efficiency of biofilter decreased

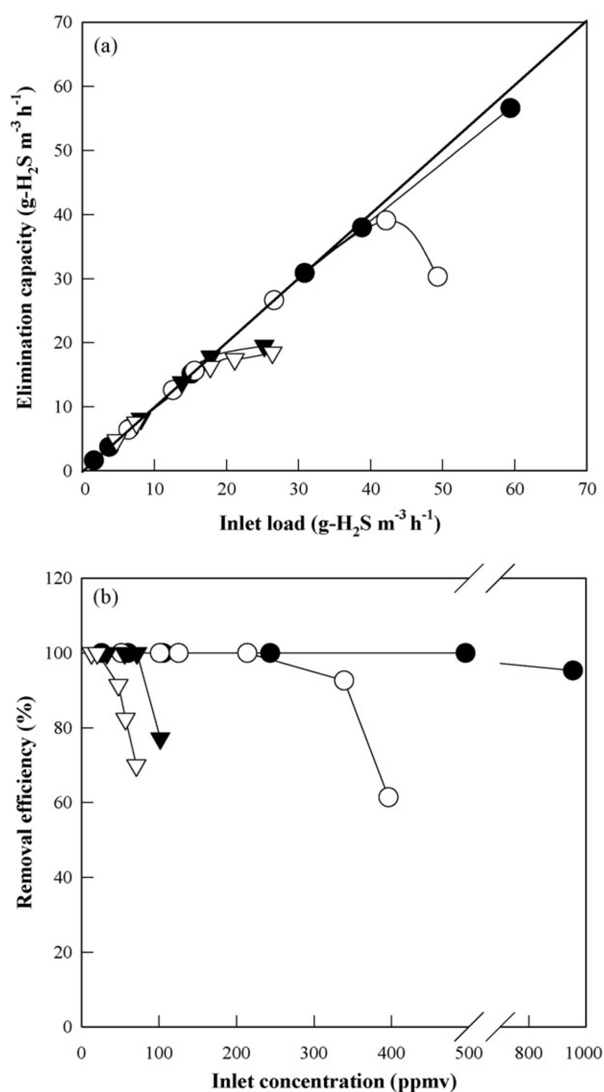

Fig. 3. Relationships between inlet load and elimination capacity (a), and inlet concentration and removal efficiency (b). Symbols: (●) SV = 50 h^{-1}; (○) SV = 100 h^{-1}; (▼) SV = 200 h^{-1}; (▽) SV = 300 h^{-1}.

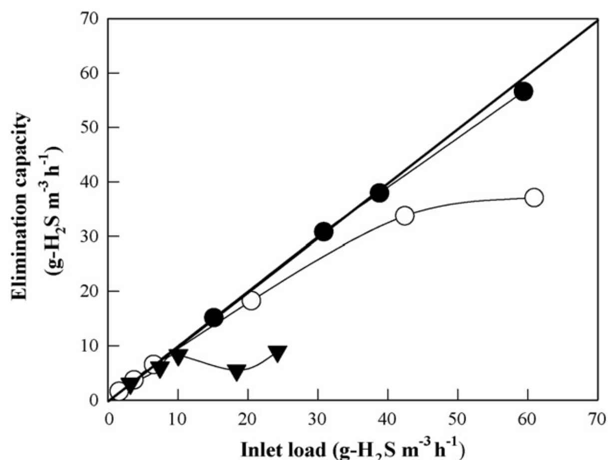

Fig. 4. Effect of YE addition and pH control on thermophilic biofilter performance. Symbols: (●) w/YE addition and w/pH control; (○) w/YE addition and w/o pH control; (▼) w/o YE addition and w/o pH control.

sharply when its pH value decreased to <5.5 (data not shown). These results suggest that the addition of YE is essential for improving the performance of biofilter. Similar results were also observed in the biofilter for the removal of hot benzene gas [1].

3.4. Isolation and characterization of thermophilic H_2S-oxidizing bacteria

In order to isolate a H_2S-degrading bacterium from the biofilter, the bacterial suspension obtained from PU cubes was enriched in SOB medium under four different conditions at 60 °C: (1) SOB medium at pH 7, (2) SOB medium at pH 7 supplemented with 5 g l^{-1} of YE, (3) SOB medium at pH 4, (2) SOB medium at pH 4 supplemented with 5 g l^{-1} of YE. Sulfate accumulation was only detected in pH 7 SOB + YE medium (data not shown). Thermophilic, sulfur-oxidizing bacteria, TSO3, isolated from pH 7 SOB + YE culture broth were identified as *Bacillus* sp., which had high similarity value (99%) with *Bacillus thermoleovorans* (Accession no. AF385083) (Fig. 5).

The effect of YE on the specific growth rate and sulfur-oxidizing activity of TSO3 is shown in Fig. 6. TSO3 could not grow and oxidize sulfur under the absence of YE. However, both the sulfur-oxidizing rate and specific growth rate of TSO3 increased with increasing YE concentrations. This fact suggests that TSO3 can heterotrophically oxidize sulfur. The addition of peptone, glucose, and sucrose as well as YE could enhance the growth as well as the sulfur-oxidizing rates for the strain OST3 as shown in Fig. 7. The N-containing organic compounds such as YE and peptone stimulate cell activities more than sugar such as glucose and sucrose.

Fig. 8a shows H_2S removal by *Bacillus* sp. TSO3 cultures containing SOB-YE medium. In contrast, minimal H_2S removal was observed in the abiotic control reactor. H_2S removal occurred immediately without any lag period, indicating that intracellular enzymes responsible for H_2S degradation may be constitutive. Addition of YE resulted in longer-lasting and more-efficient H_2S degradation.

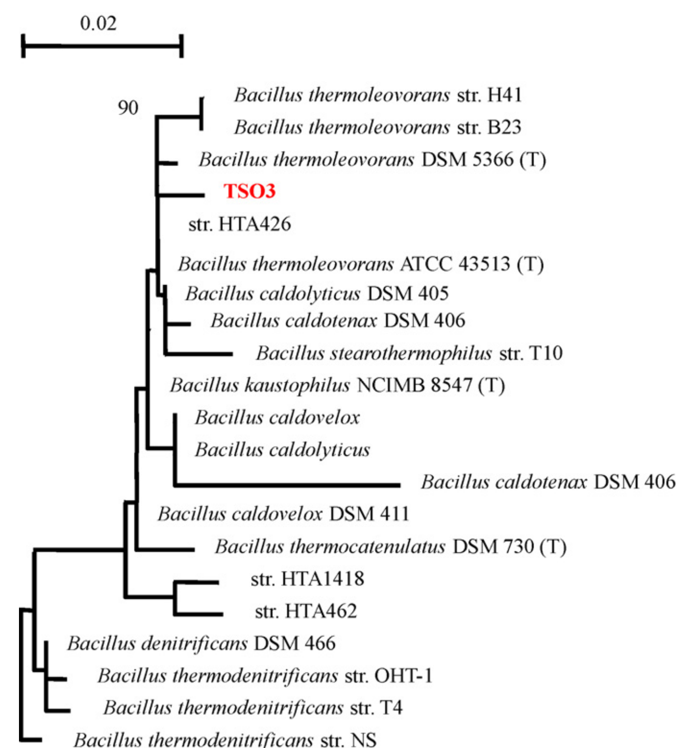

Fig. 5. Phylogenetic relationship among isolate TSO3 and other bacteria closely related with TSO3.

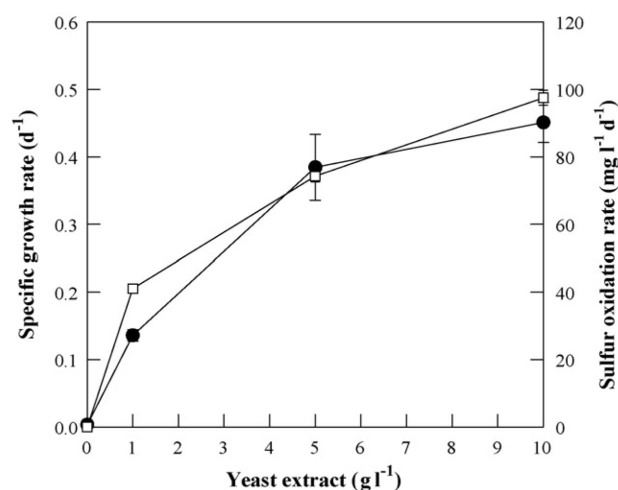

Fig. 6. Effect of YE concentration on specific growth rate and sulfur oxidation rate of the isolate TSO3. Symbols: (●) specific growth rate; (□) sulfur oxidation rate.

The TSO3-inoculated biofilter efficiently removed H_2S without a lag period (Fig. 8b). The biofilter performance deteriorated over time and periodic addition of YE was needed to maintain high removal efficiencies. Sulfate accumulation was detected in the biofilter drainage as well as the liquid medium (data not shown). H_2S removal by *Bacillus* sp. TSO3 (Fig. 8) corresponded to that by thermophilic biofilter (Fig. 3), suggesting that *Bacillus* species including TSO3 may play an important role for the removal of H_2S in thermophilic biofilters.

Microbial removal of H_2S has been associated with a wide range of sulfur-oxidizing bacteria [11–19,30]. In this study, the removal of H_2S in hot gas streams was quantitatively demonstrated using a PU-packed biofilter with compost as a source of microorganisms. Thermophilic heterotrophic bacteria, *Bacillus* sp. TSO3, were isolated from the biofilter that treated hot H_2S-containing gas stream. These bacteria were closely related to *B. thermoleovorans*. Feitkenhauer et al. (2003) reported that *B. thermoleovorans* A2 degraded phenol at 65 °C [31], and Kato et al. (2001) described alkane-degradation by two strains of *B. thermoleovorans* at temperatures ranging 50–80 °C [32]. However, degradation of H_2S by *Bacillus* sp. TSO3 under a heterotrophic, thermophilic and aerobic condition has not been reported. Further study is required on the degradation pathway of H_2S by this bacterium.

Application of thermophilic microorganism for a treatment of hot gases may be an economically attractive option since expen-

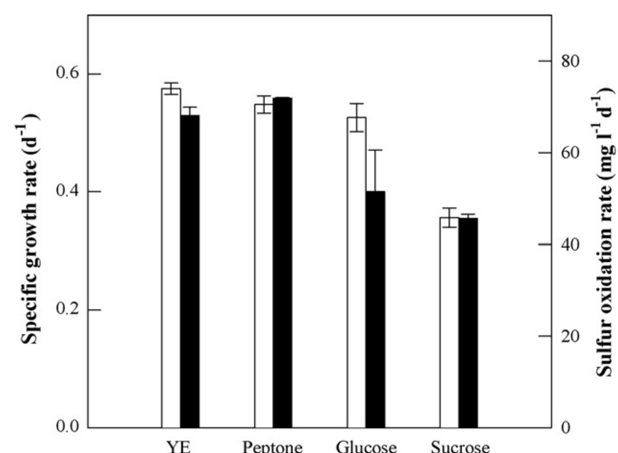

Fig. 7. Effect of organic compounds on specific growth rate and sulfur oxidation rate of the isolate TSO3. Symbols: (■) specific growth rate; (□) sulfur oxidation rate.

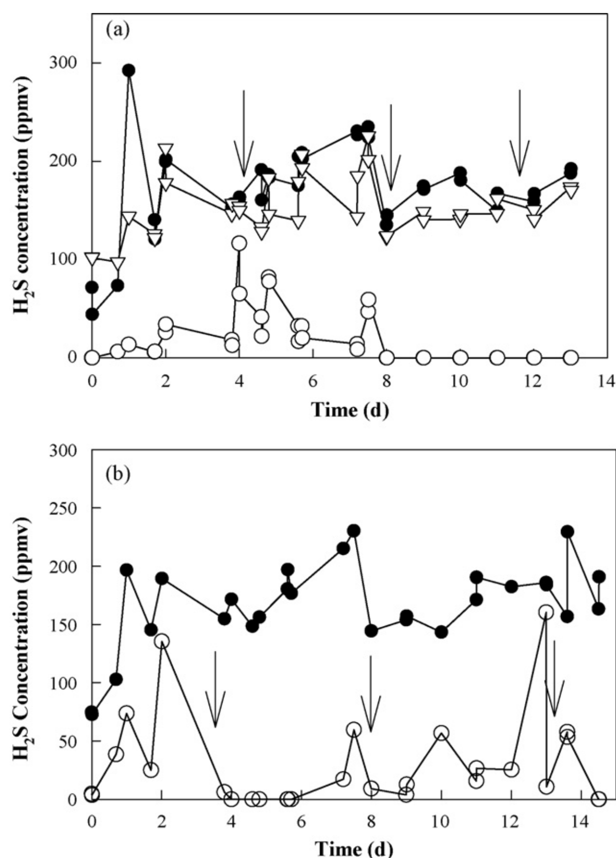

Fig. 8. H_2S degradation in liquid cultures and PU biofilter inoculated with the isolate TSO3. (a) Liquid culture system, (b) PU biofilter. Symbols: (●) inlet concentration; (○) outlet concentration (with TSO3); (▽) outlet concentration (without TSO3).

sive pre-cooling of gases to accommodate mesophilic processes is not required. Moreover, mass production of *Bacillus* sp. TSO3 may be more practical than autotrophic thermophiles due to its heterotrophic growth characteristics.

4. Conclusions

Hot H_2S-containing gas was successfully treated in a thermophilic biofilter containing polyurethane cubes as a packing material and compost as a source of seed organisms. At a space velocity of 50 h^{-1} (residence time = 1.2 min), removal efficiencies of greater than 95% were obtained up to an inlet concentration of 950 ppmv. Maximum elimination capacity was calculated to be 56.6 $g\,m^{-3}\,h^{-1}$. Thermophilic, sulfur-oxidizing bacteria, TSO3, isolated from the biofilter were identified as *Bacillus* sp., which had high similarity value (99%) with *B. thermoleovorans*.

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