J Ind Microbiol Biotechnol (2009) 36:95–104
DOI 10.1007/s10295-008-0476-9

ORIGINAL PAPER

Enhanced removal of dimethyl sulfide from a synthetic waste gas stream using a bioreactor inoculated with *Microbacterium* sp. NTUT26 and *Pseudomonas putida*

Chin-Hang Shu · Ching-Kuo Chen

Received: 21 June 2008 / Accepted: 16 September 2008 / Published online: 25 September 2008

Abstract The removal of dimethyl sulfide (DMS) from industrial gas streams has received a high priority due to its very low odorous threshold value and relatively low biodegradability compared to other reduced sulfur compounds. A variety of bacteria that utilize DMS as a carbon/energy source have been studied and the degradation pathway elucidated. However, to date, there have been few reports on the industrial application of such bacteria inoculated into a bioreactor for DMS treatment. An additional problem of such systems is the accumulation of intermediate metabolites that strongly impact on DMS removal by the microbe. The results reported here were obtained using a bioreactor inoculated with the H_2S-degrader *Pseudomonas putida* and the DMS-degrader *Microbacterium* sp. NTUT26 to facilitate removal of metabolic intermediates and DMS. This bioreactor performed well (1.71 g-S/day/kg-dry packing material) in terms of DMS gas removal, based on an evaluation of the apparent kinetics and maximal removal capacity of the system. Under varying conditions (changes in start-up, inlet loading, shutdown, and re-start), the bioreactor inoculated with *Microbacterium* sp. NTUT26 and *P. putida* enhanced removal of high concentrations of DMS. Our results suggest that this type of bioreactor system has significant potential applications in treating (industrial) DMS gas streams.

C.-H. Shu · C.-K. Chen
Department of Chemical and Materials Engineering,
National Central University, Jhongli 320, Taiwan, ROC

C.-K. Chen (✉)
Institute of Biotechnology,
National Taipei University of Technology,
Taipei 106, Taiwan, ROC
e-mail: f10460@ntut.edu.tw; stonewave@pchome.com.tw

Keywords Dimethyl sulfide · Bioreactor · Inoculation · Biofiltration

Introduction

Dimethyl sulfide (DMS) is the most abundant biological sulfur compound emitted into the atmosphere and a major contributor to total sulfur emission in nature through biogenic processes [1]. Its characteristic slightly unpleasant smell becomes highly odorous at higher concentrations, often when the source of the compound is the off-gases from pulp mills, oil refineries, manure and sewer systems, and wastewater treatment plants [6, 7]. Dimethyl sulfide produced by phytoplankton in the oceans is often oxidized to other sulfur-containing compounds, such as dimethyl sulfoxide (DMSO), methanesulfonate, and sulfate in the atmosphere, and these compounds have been regarded as precursors of tropospheric aerosols, resulting in the reflection of solar radiation and modulation of rain. Therefore, it has been suggested that DMS has both a significant impact on the Earth's climate and a central role in the sulfur cycle [36].

The olfactory detection threshold (0.001 ppm) of DMS is lower than that of other sulfur compounds, such as methyl mercaptan (MM), dimethyl disulfide (DMDS), hydrogen sulfide (H_2S), carbon disulfide (CS_2), DMSO, and sulfur dioxide (SO_2) [32], and although its odor does not necessarily pose a health hazard at ambient levels, it can also seriously lower real estate property values and result in lower worker productivity [35]. Hence, the deodorization or biodegradation of DMS gas from an industrial gas stream is preferred.

The traditional approaches to the removal of odorous compounds from a gas stream include incineration, catalytic oxidation, adsorption, radio-frequency plasma, and chemical

scrubbing [32]. Such chemical and/or physical methods are often effective in their primary objective—the removal of the target compound—but they are associated with both high energy demands and high costs and easily result in secondary pollutants. In comparison, biological waste gas treatments, such as those consisting of a biofilter, are ecologically and economically more favorable, especially for the removal of odorous gases at low concentrations [4]. Dimethyl sulfide is the least biodegradable compound among the odorous sulfur-containing gases [5, 27]; consequently, improved systems need to be designed that enhance DMS degradation.

A variety of bacteria can utilize DMS as a carbon and energy and/or sulfur source [26]. Methylotrophs (*Hyphomicrobium* sp., *Methylophaga sulfidovorans*) can degrade DMS via MM [12, 37], but *Methylomicrobium* sp. transforms DMS to DMSO [13]. Some strains of sulfur oxidizers (*Thiobacillus thioparus, Thiobacillus* sp.) also degrade DMS via MM [8, 33]. Partially phototropic strains (*Rhodobacter sulfidophilus*, *Thiocapsa roseopersicina*) transform DMS to DMSO and utilize it as an electron donor [16, 34], and some heterotrophic strains (*Pseudomonas acidovorans*, *P. fluorescens*, *Acinetobacter* sp.) have also been found to able to transform DMS to DMSO [19, 20, 38]. Marine isolates, such as *Marinobacterium* sp., have recently been found to be able to utilize DMS [14, 18]. The identification of these species and their ability to utilize/catalyze DMS suggest that a microbial-based DMS conversion system is feasible.

To date, only limited attempts have been made to use bacteria inoculated into a bioreactor for the treatment/break-down of DMS gas. Documents clearly demonstrated that the competitive effects between the mixtures (including intermediate) strongly affect the removal of each pollutant either because of the preferential biodegradation of one substrate over another or toxic interactions [21, 35]. Thus, further study of DMS removal from a gas steam by systems using the biofiltration principle is necessary. To this end we isolated *Microbacterium* sp. NTUT26, a DMS degrader, from the wastewater sludge of a wood-pulp factory and determined the characteristics of the isolated strain to degrade DMS under batch and continuous conditions. We were also able to enhance DMS removal by inoculating the H_2S-degrader *Pseudomonas putida*, into the biofilter system. During this study, we carried out an apparent kinetics analysis and established the most suitable operating conditions and maximal removal capacity of the bioreactor.

Material and methods

Microorganisms and cultivation

A 10-g sample of wet sludge taken from the wastewater treatment plant of a wood-pulp factory was mixed with 100-ml sterile mineral medium in a 250-ml flask. The mineral medium contained KH_2PO_4 2 g/l, K_2HPO_4 2 g/l, NH_4Cl 0.4 g/l, $MgCl_2{\cdot}6H_2O$ 0.2 g/l, and $FeSO_4.7H_2O$ 0.01 g/l. The initial pH of the medium was 7.09. Dimethyl sulfide gas was supplied to the flask continuously at a rate of 10 ml/min. The inlet DMS concentration was step-wise increased from 10 to 100 ppm at 5-day intervals. Once a concentration of 100 ppm was reached, the sludge solution was drawn out and vortexed with the same volume of sterile 0.95% saline solution. The mixture was centrifuged at 6,000*g* for 20 min and the pellet removed and, fully mixed with 100 ml mineral medium in a 250-ml flask. Dimethyl sulfide gas was gradually introduced to the flask from 100 to 200 ppm at 14-day intervals. Following a 56-day acclimation period, the dominant colony was isolated in solid mineral medium containing 0.1 g/l DMS by the plate-counting method. The cells of the dominant isolate were lysed and the DNA extracted. Subsequent PCR amplification and sequencing procedures were according to Sandaa et al. [28] and resulted in the isolate being identified as *Microbacterium* sp. NTUT26.

Batch experiments

The isolate was cultured in nutrient broth at 35°C for 1 day. One milliliter of the cell solution (10^8 cfu/ml) was added to a flask containing 100-ml mineral medium, and the flask was sealed with a butyl rubber stopper. Sterile DMS liquid was then injected into the flask by a syringe to give a headspace gas concentration of 200 ppm, and the flask was then shaken at 180 rpm on a rotary shaker at 35°C. The headspace DMS concentration was measured at hourly intervals by gas chromatography. The liquid phase concentration was estimated by Henry's Law, and the total DMS concentration was calculated by adding up the concentration of DMS in the gas and liquid phases. After 4 h, the specific degradation rate of DMS in the mineral medium by *Microbacterium* sp. NTUT26 was 8.4×10^{-13} g-S/cell h.

To evaluate the effect of pH on the capability of the isolate strain to degrade DMS, we added 1 ml of the cell solution (10^8 cfu/ml), after a 1-day cultivation, to flasks each containing 100-ml mineral medium at a different pH (4–9) (pH was adjusted with 0.1 N NaOH or HCl solution). The flasks were then sealed with butyl rubber stoppers, and sterile DMS liquid was injected into the flasks to give a headspace gas concentration of 100 ppm. The flasks were then shaken at 180 rpm on the rotary shaker at 35°C for 4 h, at which time the specific degradation rates of DMS under different pH conditions by *Microbacterium* sp. NTUT26 were determined. To evaluate the effect of temperature on the capability of the isolate strain to degrade DMS, we followed the same procedure as that described

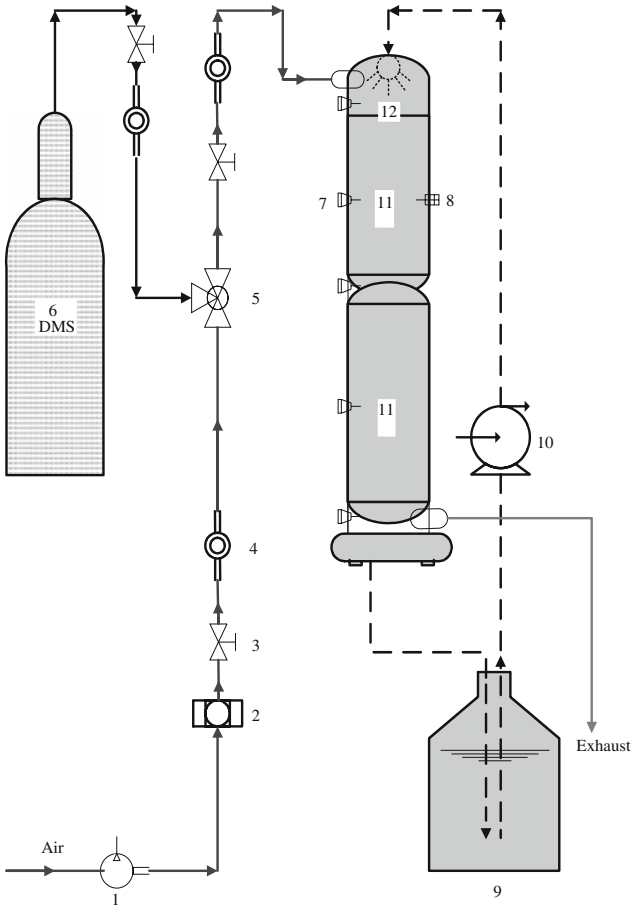

Fig. 1 The set-up and design of the bioreactor inoculated with *Microbacterium* sp. NTUT26 alone or *Microbacterium* sp. NTUT26 and *Pseudomonas putida*: *1* air compressor; *2* air filter; *3* regulator; *4* flowmeter; *5* three-way connector; *6* DMS gas cylinder; *7* gas sampler port; *8* sampler port of packing material; *9* nutrient tank; *10* peristaltic pump; *11* bioreactor; *12* sprinkling system

for the optimum pH determination, but varied the cultivation temperature from 25 to 45°C while maintaining the pH at 8.5.

Apparatus and gas removal in the continuous operation

To investigate the capacity of the packing material to adsorb DMS gas, we carried out a the Bed Depth Service Time (BDST) experiment described by Chung et al. [9] using a series of three inter-connected glass columns (10 cm ϕ × 5 cm working height) that were packed with a GAC and peat mixture (1:1, v/v) without microbes.

The set-up and design of the bioreactor inoculated with *Microbacterium* sp. NTUT26 alone or *Microbacterium* sp. NTUT26 and *P. putida* is shown in Fig. 1. A series of two inter-connected transparent PVC columns, each 10 cm (i.d.) × 35 cm (working height), were packed with sterile GAC and peat mixture (1:1, v/v), and a perforated sieve plate of PVC material was fitted at the bottom of the columns to allow the circulating liquid to flow out. The densities of the GAC and peat packing material were 0.48 and 0.67 g/cm^3, respectively. The total packed volume and dry weight of the packing material in the bioreactor were 5.5 l and 3.2 kg, respectively. The pure DMS gases, supplied from a gas cylinder, were first diluted with compressed air, then passed through an air filter (pore size 0.2 μm, model LIDA 3000-06; USA), and then flowed downward through the top of the bioreactor. The mineral medium containing 8×10^8 cfu/ml *Microbacterium* sp. NTUT26 stored in the nutrient tanks was continuously recirculated by a peristaltic pump and sprayed onto the medium with a spray nozzle at 3 l/min for 25-day immobilization process. Following the immobilization, the sterile mineral medium stored in the nutrient tanks was recirculated by peristaltic pumps at 6 l/min for 5 min, six times a day, to maintain the moisture of the bioreactor and supply nutrients to the attached cells. Unless specifically stated otherwise, fresh medium was generally added to the mineral medium in the tanks at 50-day intervals to replace that lost due to evaporation.

To examine the operating performance of the bioreactor, we defined operation periods for five phases (Table 1). A cooling circulator bath (BL-40, Tungtec Co., Taiwan) was used to maintain the operating temperature at a constant 35°C. In the first phase, 60 ppm DMS was introduced into the bioreactor with the aim of identifying the possible DMS removal mechanism. In the second phase, we introduced increasing concentrations of DMS into the bioreactor at a gradual rate in order to avoid any possible negative effects of a high DMS concentration. In the third phase, we introduced 120 ppm DMS into the bioreactor at different gas retention times (GRTs) to evaluate the effect of gas retention time on DMS removal. In the fourth phase, different DMS concentrations were introduced into the bioreactor at different GRTs to evaluate the effect of inlet loadings and shock loading on DMS removal. In the last phase, only air was introduced into the bioreactor for the first 16 days and re-introduced DMS was introduced in the remaining days to evaluate the effect of the shutdown operation. To gain an understanding of the possible mechanism of DMS biodegradation by *Microbacterium* sp. NTUT26, we collected and analyzed the possible intermediates in the outlet and sulfate in the leachate during the period 130–184 days.

To enhance DMS removal, the sterile mineral medium containing H_2S-degrading bacterium *P. putida* (2×10^8 cfu/ml) and *Microbacterium* sp. NTUT26 (6×10^8 cfu/ml) was continuously recirculated by a peristaltic pump to another bioreactor with a similar structure at 3 l/min for a 25-day immobilization process. In the first phase (1–30 days), 60 ppm DMS was introduced into the

Table 1 The operating conditions of the bioreactor inoculated with *Microbacterium* sp. NTUT26

	First phase (1–30 days)	Second phase (30–100 days)	Third phase (100–198 days)	Fourth phase (198–298 days)	Fifth phase (298–330 days)
DMS concentration (ppm)	60	10–200	120	5–200	0–120
Flow rate (l/h)	660	990	330–1980	330–990	330–990
Gas retention time (s)	30	20	10–60	20–60	20–60
Loading (g-S/m^3/h)	9.4	2.4–47.0	9.4–56.5	0.392–47.02	0–28.2

bioreactor at 30 s GRT. In the second phase (30–86 days), 120 ppm DMS was introduced into the bioreactor at different GRTs (20–50 s). In the last phase (86–120 day), only air was introduced into the bioreactor for the first 14 days and DMS was re-introduced at 120 ppm at 20 s GRT in the remaining days.

Apparent kinetic analysis

The DMS removal rate in the bioreactor was calculated using the following equation derived from the Michaelis–Menten equation [17]:

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

where R (g-S/day/kg-dry packing material) = apparent removal rate; C_{ln} (ppm) = $(C_o - C_e)/\ln(C_o/C_e)$, the logarithmic mean concentration of DMS at the inlet and outlet of the bioreactor; V_m (g-S/day/kg-dry packing material) = maximum apparent removal rate; K_s (ppm) = apparent half-saturation constant. From the linear relationship between $1/C_{ln}$ and $1/R$, V_m and K_s were calculated from the slope and intercept. In this experiment, the GRT was kept at longer than 20 s to minimize the mass-transfer limitation.

Criteria for designing a scale-up of bioreactor

Different DMS concentrations at different flow rates were introduced into the bioreactor to evaluate the DMS removal capacity of the bioreactor. These data were collected to establish the relationship between inlet loading and the removal capacity of the bioreactor. Additionally, the maximum removal capacity (g-S/m^3/h) or design guideline can be obtained from the upper limit of this linear relationship. The inlet loading and removal capacity are generally defined as follows:

$$\text{Inlet loading} = \frac{Q \times C}{V} \quad (2)$$

$$\text{Removal capacity} = \frac{Q \times C}{V} \times R \quad (3)$$

where Q (l/h) = gas flow rate; C (g-S/l) = inlet DMS concentration; V (m^3) = packing material volume; R (%) = removal efficiency.

Analytical methods

Gas concentrations of sulfur-containing compounds (DMS, MM, H_2S) were analyzed in a Fisons-8000 gas chromatograph (Fisons, UK) equipped with a GS-Q column (0.53 mm × 30 m) and a flame photometric detector (FPD). The concentration of sulfate in the leachate was analyzed on an ion chromatograph (Shimadzu HIC6A) equipped with CDD-6A conductivity detector. The oxygen concentration was measured by gas detector (Gastec). Formaldehyde concentration in leachate was quantified by a colorimetric method [2]. The moisture content of the packing material was determined by first removing a 0.8-g sample of packing material from the sampling port of the bioreactor, then weighing and drying it for 24 h at 103 ± 0.5°C. To measure the pH in the bioreactor, we removed 0.4 g of packing material from a similar location and mixed it with 4 ml distilled water. The sample was vortexed for 5 min, and the pH value was then determined using a pH meter. To estimate cell number, we removed 0.5 g of packing material from the sampling port of the bioreactor and mixed it with 5 ml sterile saline solution (0.95% w/v NaCl). The samples were then vortexed for 5 min, and the microbes were cultivated in nutrient agar; cell numbers were determined by plate-counting methods. The stability of the inoculated strains during the experiments was verified by denaturing gradient gel electrophoresis (DGGE) on a Bio-Rad (Hercules, CA, USA) apparatus. The relative amounts of inoculated strains in the bioreactor were analyzed with Quantity One 4.5.0 software (Bio-Rad). The steps used in the analysis are described in detail in Chung [10]. All measurements were conducted after sprinkling for 2 h, and the tests were carried out at least in duplicate.

Results and discussion

Basic characteristic of DMS removal in batch reaction

Figure 2a shows the effect of pH on the capability of *Microbacterium* sp. NTUT26 to degrade DMS in the

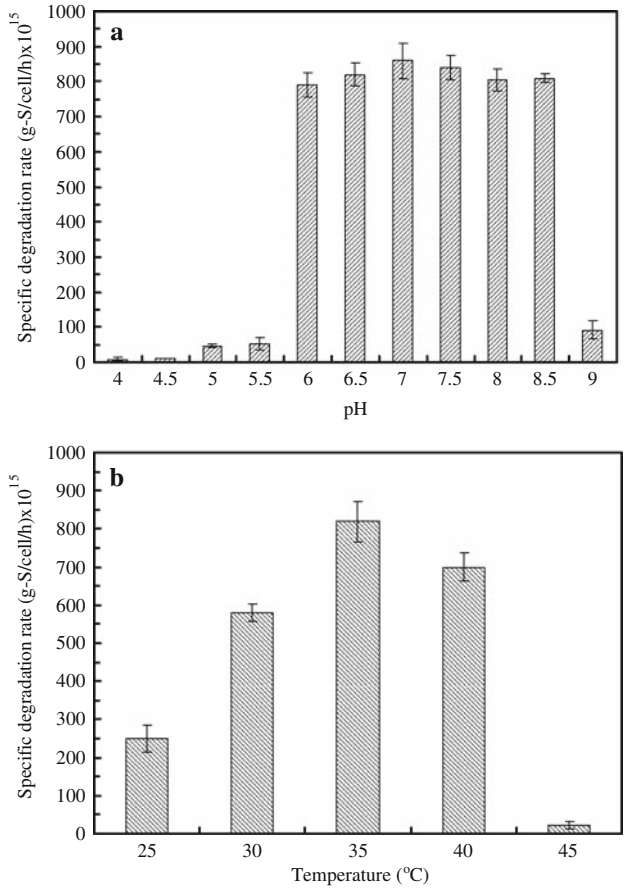

Fig. 2 pH effect on the specific degradation rate of DMS in the mineral medium at 35°C by *Microbacterium* sp. NTUT26 (**a**) and temperature effect on the specific degradation rate of DMS in the mineral medium at pH 8.5 by *Microbacterium* sp. NTUT26 (**b**)

mineral medium at 35°C. A control group consisting of the mineral medium containing dead microbial cells was also included in the study in order to determine the absorption effect of the mineral medium in total DMS removal. During the experimental period, the change in cell number was insignificant. The results indicate that the effect of pH on the specific degradation rate of DMS by *Microbacterium* sp. NTUT26 was statistically insignificant between pH 6 and 8.5 (7.9×10^{-13}–8.6×10^{-13} g-S/cell/h). When the pH in the medium was higher than 8.5 or lower than 6.0, the specific degradation rate of DMS by *Microbacterium* sp. NTUT26 decreased by at least tenfold. The decrease in the specific degradation rate may be due to the activity of *Microbacterium* sp. NTUT26 being inhibited under inappropriate pH conditions or DMS solubility decreasing under the acid conditions. We considered a medium pH of 8.5 to be the optimal operating condition based on DMS degradation rate and absorption capacity of the solution to DMS.

Figure 2b shows the effect of temperature on the capability of *Microbacterium* sp. NTUT26 to degrade DMS in the mineral medium at pH 8.5. The results indicate that the optimal temperature for DMS degradation by *Microbacterium* sp. NTUT26 is 35°C; the specific degradation rate of DMS at 35°C was 75 and 29% higher than at 40 and 30°C, respectively. The higher temperature would result in low DMS solubility in the medium and/or an inhibition of microbial activity, while microbial DMS biodegradation activity would not be activated at the lower temperature. The highest specific degradation rate of DMS under the optimal temperature condition was 8.6×10^{-13} g-S/cell/h, which was far higher than the results of 6.36×10^{-15}, 1.01×10^{-15}, and 2.7×10^{-14} g-S/cell/h presented by Tiwaree et al. [31], Cha et al. [5] and Geng et al. [15], respectively.

DMS removal in a continuous operation by a bioreactor inoculated with *Microbacterium* sp. NTUT26

Dimethyl sulfide is often emitted as a waste gas from (industrial) processing activities, such as those carried out at pulp mills, oil refineries, manure and sewer treatment plants, and wastewater treatment plants. An additional complicating factor in treating DMS is that it is relatively difficult to biodegrade compared to other reduced sulfur-containing compounds [27]. We therefore evaluated DMS removal by the bioreactor under a number of different conditions (different loading, shocking loading, and shut-down operations) that would be present in a continuous operational system. Figure 3a presents the DMS removal efficiencies of the bioreactor under different inlet loadings at 35°C during a 330-day operating period. During the experimental period, the oxygen concentration in the bioreactor and the pH, moisture content and cell number in the packing material were $19.8 \pm 0.6\%$, 7.3 ± 0.5, $35.7 \pm 4.5\%$, and $3.5 \pm 1.5 \times 10^9$ cfu/g-packing material, respectively. These values indicate that the mainly chemical and biological conditions in the bioreactor which would impact on DMS removal during the operating period were acceptably stable. The theoretical saturated adsorption capacity of DMS, based on the BDST experiment, for the packing material was 26 days; however, 100% DMS removal was observed for 32 days. This indicates that not only pure physical and chemical adsorption occurred in the bioreactor, but that biological oxidation of DMS occurred after 26 days. During the period 32–198 days, DMS removal efficiency showed a tendency towards an inverse relationship with inlet DMS loading: when inlet loading was at its highest, 56.5 g-S/m^3/h (0.097 g-S/kg/h) during the period 102–144 days, DMS removal efficiency dropped to $58.4 \pm 0.6\%$. To simulate the general emission behavior of different emission sources, we tested three levels (3×, 120×, and 20×) of shock loadings on days 198–204, 218–220, and 226–228, respectively. The results indicated that

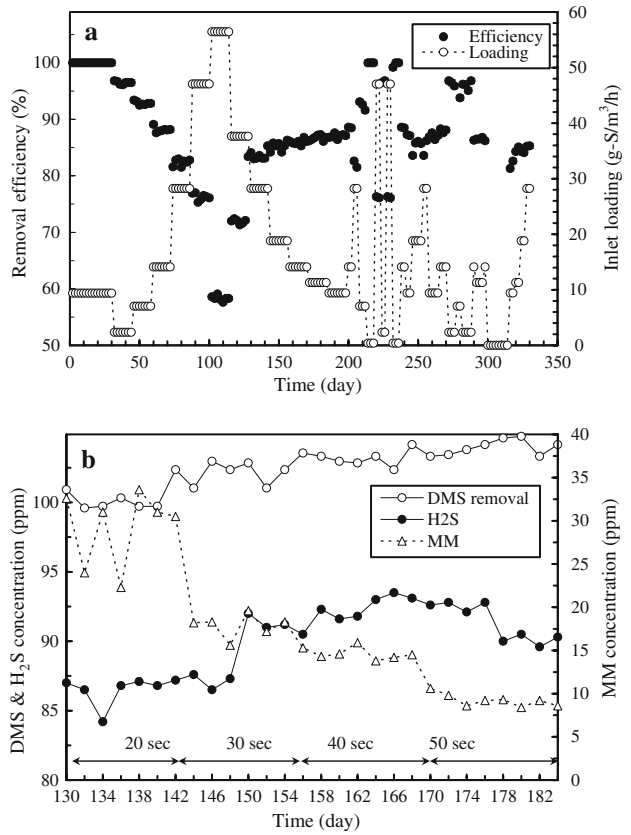

Fig. 3 Profiles of removal efficiency and inlet loading for DMS by the bioreactor inoculated with *Microbacterium* sp. NTUT26 at 35°C (**a**) and profile of concentrations of DMS removed and H_2S and MM produced by the bioreactor inoculated with *Microbacterium* sp. NTUT26 at 35°C (**b**)

shock loading occurring within a short period of time did not significantly affect DMS removal by the bioreactor in comparison with a non-shock-loading operation condition. The operational system was shut down occurred for 16 days during days 298–330. When the gas stream containing DMS was re-introduced into the bioreactor, the DMS removal efficiency was 3–5% lower than that under normal operating conditions for several days after start-up, reaching normal operation levels after 8 days.

Figure 3b shows the variations in the concentrations of DMS removed and metabolites produced (H_2S and MM) by the bioreactor during days 130–184. The results indicate that the amount of DMS removed and the amount of H_2S produced increased with increasing GRT under almost all conditions. The amount of intermediate MM accumulated decreased with increasing GRTs, which may be due to the rapid transformation of MM to H_2S gas. The concentrations of the other metabolite sulfate (1.8–3.2 mg/l) and formaldehyde (HCHO) (2.6–4.2 mg/l) in the leachate did not show a statistically significant trend. The generally aerobic metabolic pathway for DMS by bacteria has been documented [33]. Dimethyl sulfide is oxidized to MM and HCHO, and MM further is oxidized to HCHO and H_2S. Hydrogen sulfide is following oxidized to sulfate. Dimethyl sulfide has also been found to be oxidized to the intermediate DMSO by *Pseudomonas acidovorans*, *Basidiomycete* sp., *Marinobacterium* sp., and *Acinetobacter* sp. [18, 19, 25, 38]. In our study, the concentration of DMSO in the leachate was lower than the detection limit of the instrument; consequently, we assumed that the biodegradation metabolic pathway for DMS by *Microbacterium* sp. NTUT26 in the bioreactor was that reported by Visscher and Taylor [33] and Bentley and Chasteen [3]. This is first report of DMS degradation and its metabolism pathway by *Microbacterium* sp.

Effect of the important operating parameters on DMS removal efficiency

After a 30-day acclimation period, 10–200 ppm DMS was introduced in a gradual stepwise manner, starting with low concentrations and working towards high concentrations, into the bioreactor at 20 s GRT to evaluate the effect of inlet DMS concentration on DMS removal efficiency. Figure 4a shows that the higher concentration resulted in a lower DMS removal efficiency, with the relationship being a well-fitting linear negative one. When 30 ppm DMS was introduced into the system, the removal efficiency was 93%; however, a 76% removal efficiency was still obtained even when the relatively high concentration of 200 ppm DMS was fed into the system. Compared to the results of DMS biodegradation by *Thiobacillus novellus* SRM (30 ppm at GRT = 36 s, $R\% = 73\%$), *Hyphomicrobium* sp. I55 (60 ppm at GRT = 120 s, $R\% = 83\%$), activated sludge obtained from pulp mill (10.8 ppm, GRT = 38 s, $R\% = 35\%$), a mixed population of heterotrophs and *Hypomicrobium* sp. (120 ppm, GRT = 45 s, $R\% < 20\%$), *Thiobacillus thioparus* TK-m (55 ppm, GRT = 10.4 min, $R\% = 99.9\%$) and activated sludge obtained from municipal wastewater treatment plant (22 ppm, GRT = 40 sec, $R\% = 44\%$), the removal efficiency of the bioreactor inoculated with *Microbacterium* sp. NTUT26 was clearly superior [5, 6, 22, 35, 37, 39].

To evaluate the effect of gas retention time on DMS removal, we introduced 120 ppm DMS into the system with the various GRTs. The operational period was equal to the third phase of Table 1. Figure 4b indicates an insignificant effect on DMS removal when the GRT is longer than 20 s, with the removal efficiency being higher than 83%. To the best of our knowledge, this is the best removal efficiency reported to date under similar operating conditions. When the GRT was shorter than 20 s, the curve of removal efficiency showed a statistically significant change, and the efficiency decreased by at least 10%. It

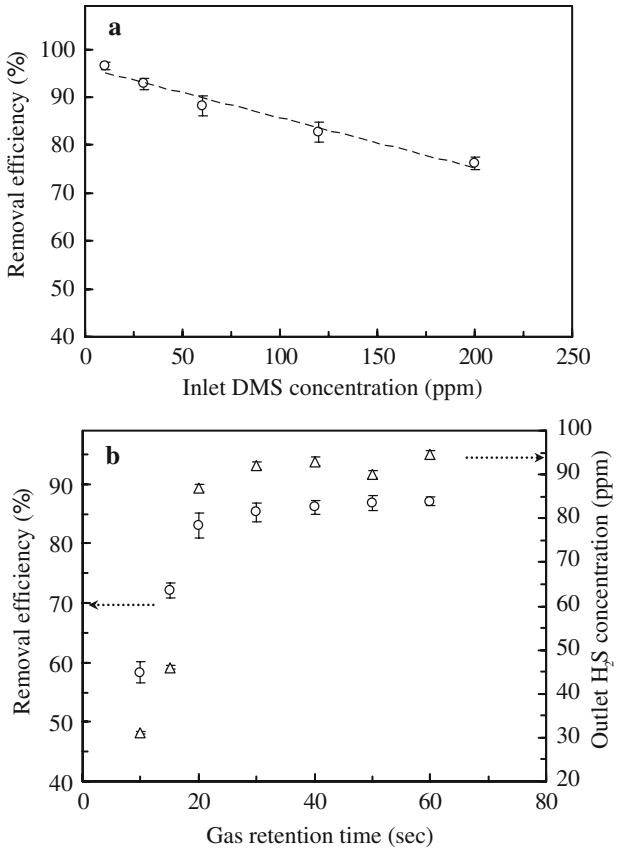

Fig. 4 Effect of inlet concentration (**a**) and gas retention time (**b**) on DMS removal of the inoculated with *Microbacterium* sp. NTUT26 bioreactor

would appear that short GRTs result in a mass-transfer limitation on gas removal [11]. To understand the underlying factors causing this insignificant DMS removal when the GRT was longer than 20 s, we determined the pH and bacterial number in the packing material. The changes in pH (7.2 ± 0.4) and bacterial number ($3.2 \pm 0.8 \times 10^9$ cfu/g-packing material) were statistically insignificant during this experimental stage and, consequently, we were able to exclude the effect of acidification or alkalization on DMS removal in the system. However, we did find a relatively high concentration (87–95 ppm) of H_2S when the GRT was longer than 20 s. Thus, H_2S derived from DMS oxidation may further inhibit the activity of the DMS oxidizer even when the GRT is adequate.

Enhancement of DMS removal by a bioreactor inoculated with *Microbacterium* sp. NTUT26 and *P. putida*

To enhance DMS removal, it is essential to effectively remove H_2S by seeding with H_2S-oxidizing bacteria. The inoculated H_2S degrader-*P. putida* was obtained from the

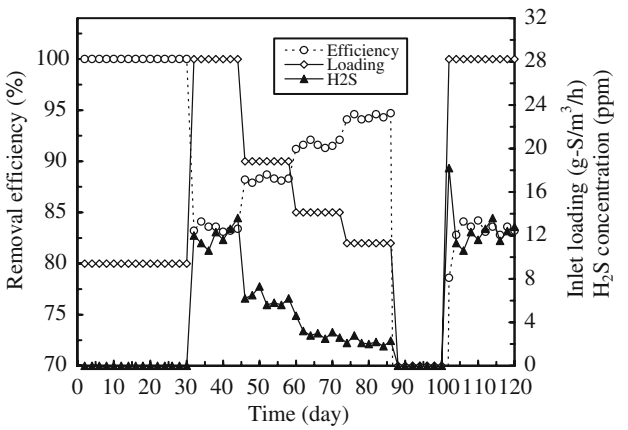

Fig. 5 Profiles of DMS removal efficiencies, inlet DMS loading, and H_2S concentration in the system conducted by the bioreactor inoculated with *Microbacterium* sp. NTUT26 and *Pseudomonas putida*

American Type Culture Collection (ATCC) and has been shown to be capable of degrading H_2S but not DMS. After the immobilization period, the average pH, moisture content, and total cell number in the packing material of the bioreactor inoculated with *Microbacterium* sp. NTUT26 and *P. putida* during the operating period were 6.7 ± 0.5, $35.9 \pm 4.2\%$, and $7.6 \pm 2.8 \times 10^9$ cfu/g-packing material, respectively. Because H_2S was mainly adsorbed by the packing material—and not by bio-oxidation during the immobilization period—no H_2S emission in the outlet (Fig. 5) and a low sulfate concentration (0.6–2.1 mg/l) in the leachate were measured. In the second phase (30–86 days), DMS removal efficiency increased with decreasing inlet loading (increasing GRT). When the GRT was longer than 30 s, efficiency of DMS removals increased by 3–8% compared to the system in which only *Microbacterium* sp. NTUT26 had been inoculated. For example, DMS removal efficiency reached 94.3% at a GRT of 50 s by the co-immobilization system while only 86.1% removal was achieved by the inoculated *Microbacterium* sp. system shown in Fig. 4b. Additionally, H_2S emission was lower than 6.2 ppm, when the GRT was longer than 30 s, and it was successfully transformed to sulfate or sulfur. In this operating period, sulfate concentrations ranged from 15.6 to 20.5 mg/l. In comparison with the inoculated *Microbacterium* sp. system (Fig. 3b), effective H_2S removal demonstrated the advantage of co-immobilization with *Microbacterium* sp. NTUT26 and *P. putida* in removing DMS. Sulfate accumulation was still found during the first 14 days of the last phase (days 86–100) even though the shutdown operation had been carried out, apparently due to continuing biodegradation that possibly resulted from the microbes oxidizing DMS/H_2S adsorbed on the packing material. Thereafter, 120 ppm DMS was re-introduced and only 2 days of recovery time were

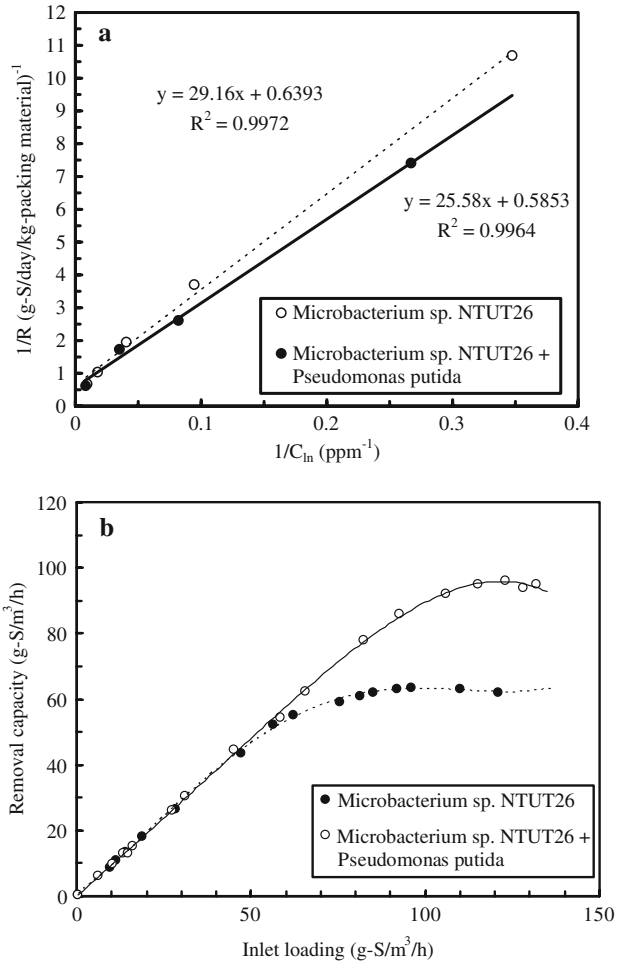

Fig. 6 Relationship between DMS degradation $1/R$ and $1/C_{ln}$ in the bioreactor inoculated with *Microbacterium* sp. NTUT26 alone, and *Microbacterium* sp. NTUT26 and *Pseudomonas putida* (**a**) and relationship between inlet loading and removal capacity for DMS gas (**b**)

required to reach normal levels under standard operating conditions (days 32–44).

To gain an understanding of the stability of the inoculated *Microbacterium* sp. NTUT26 and *P. putida* populations during the experiments, we analyzed the bacterial community in the bioreactor by DGGE on days 1, 30, 86, 100 and 120 of the experiment. The results showed that the total bacterial community comprised approximately 50.5–52.1% *Microbacterium* sp. NTUT26 and 46.6–48.6% *P. putida*, with other bacterial strains accounting for 0.2–2.6%. The observed variations were closely related to operating conditions, but under all conditions *Microbacterium* sp. NTUT26 and *P. putida* remained the dominant species present. Thus, the co-immobilization bioreactor system with *Microbacterium* sp. NTUT26 and *P. putida* exhibits characteristics of excellent DMS and H_2S removal as well as the capability to adapt to changing operating conditions.

Apparent kinetic analysis and criteria for designing a scale-up bioreactor

Our results indicate that H_2S accumulation in the bioreactor would result in the decreased activity of DMS-oxidizing bacteria to degrade DMS gas. Confirmation of this result can be demonstrated by the apparent kinetic analysis. The apparent kinetic parameters of the maximum apparent removal rate and the apparent half-saturation constant for DMS degradation under different conditions were calculated using the Lineweaver–Burk method [23] and are shown in Fig. 6a. The regression equation expressed as $y = 29.16\,x + 0.6393$ is for the bioreactor system inoculated only with *Microbacterium* sp. NTUT26 and $y = 25.58\,x + 0.5853$ is for the system inoculated with both *Microbacterium* sp. NTUT26 and *P. putida* system. The maximum apparent removal rate and the apparent half-saturation constant of DMS by the two systems were calculated to be $V_m = 1.56$ and 1.71 g-S/day/kg-dry packing material and $K_s = 45.62$ and 43.37 ppm, respectively, from the slope and intercept of the regression equation. If the physical meaning of K_s is analogous to that in enzymatic kinetics, a decrease in K_s suggests an enhancement in biomass affinity for the target substrate. Certainly, the high maximum apparent removal rate shows relatively high removal rate between the two biosystems. It would therefore appear that the bioreactor inoculated with both *Microbacterium* sp. NTUT26 and *P. putida* exhibits a higher efficiency in removing DMS than the bioreactor alone inoculated with *Microbacterium* sp. NTUT26. Its maximum apparent removal rate (1.71 g-S/day/kg-dry packing material) in this study was superior to the 0.38 g-S/day/kg-peat reported by Hirai et al. [17], 0.59 g-S/day/kg-peat reported by Zhang et al. [37], and 0.03 g-S/day/kg-peat reported by Park et al. [24].

Practical experience with such systems have shown that inlet gas flow rate and concentrations often play important roles in the design of a scale-up bioreactor if the packing volume is constant. The maximal removal capacity is often dependent on the design of the structure of the bioreactor, the microbe used as inoculum, and the operating conditions. To establish design criteria for the scale-up of the bioreactor and identify the difference between the two biosystems, the relationship between inlet loading and removal capacity needs to be studied. As shown in Fig. 6b, the relationship curve first rose and then leveled off to a maximum level. The extrapolated correlation line suggests that the maximum inlet loading was 63.5 and 96.1 g-S/m^3/h for the bioreactor inoculated with *Microbacterium* sp. NTUT26 and with *Microbacterium* sp. NTUT26 and *Pseudomonas putida*, respectively. The value of 96.1 g-S/m^3/h is much higher than those reported for other biosystems (7.1, 36.6, 10.8, 51.8, and 29.9 g-S/m^3/h) [4, 26, 27, 29, 30]. Thus, the bioreactor inoculated with *Microbacterium* sp. NTUT26

and *P. putida* can reduce its working volume or treat high inlet DMS loading better than other biosystems.

Conclusions

The results of this study indicate the possible DMS degradation pathway by *Microbacterium* sp. NTUT26. They also demonstrate that the bioreactor inoculated with *Microbacterium* sp. NTUT26 and *P. putida* can indeed improve the removal of high concentrations of DMS from a synthetic waste gas stream. We have shown that the strategy of a bioreactor that is simultaneously inoculated with DMS-oxidizing bacteria and H_2S-oxidizing bacteria is effective, based on an evaluation of the apparent kinetics analysis and its maximal removal capacity. As such, this type of bioreactor provides a potential new approach to purifying a gas that is difficult to degrade in those cases in which the bioreactor's performance is hindered due to incomplete oxidation or intermediate accumulation. During changes in the operational set-up of the bioreactor, the bioreactor inoculated with *Microbacterium* sp. NTUT26 and *P. putida* achieved excellent performance regardless of the changes in shutdown, re-start, or inlet loading. Therefore, based on our results, we suggest that this bioreactor has significant potential for treating DMS from real waste gas streams containing high concentrations of DMS.

References

1. Andreae MO (1990) Ocean–atmosphere interactions in the global biogeochemical sulfur cycle. Mar Chem 30:1–29. doi:10.1016/0304-4203(90)90059-L
2. Avigad G (1983) A simple spectrophotometric determination of formaldehyde and other aldehydes: application to periodate-oxidized glycol system. Anal Biochem 134:499–504. doi:10.1016/0003-2697(83)90330-5
3. Bentley R, Chasteen TG (2004) Environmental VOSCs-formation and degradation of dimethyl sulfide, methanethiol and related materials. Chemosphere 55:291–317. doi:10.1016/j.chemosphere.2003.12.017
4. Bo ID, Heyman J, Vincke J, Verstraete W, Van Langenhove H (2003) Dimethyl sulfide removal from synthetic waste gas using a flat poly(dimethylsiloxane)-coated composite membrane bioreactor. Environ Sci Technol 37:4228–4234. doi:10.1021/es020168f
5. Cha JM, Cha JS, Lee JH (1999) Removal of organo-sulphur odour compounds by *Thiobacillus novellus* SRM, sulphur-oxidizing microorganisms. Process Biochem 34:659–665. doi:10.1016/S0032-9592(98)00139-3
6. Chan AA (2006) Attempted biofiltration of reduced sulphur compounds from a pulp and paper mill in Northern Sweden. Environ Prog 25:152–160. doi:10.1002/ep.10131
7. Cheng X, Peterkin E, Burlingame GA (2005) A study on volatile organic sulfide causes of odors at Philadelphia's Northeast Water Pollution Control Plant. Water Res 39:3781–3790. doi:10.1016/j.watres.2005.07.009
8. Cho KS, Hirai M, Shoda M (1991) Degradation characteristics of hydrogen sulfide, methanethiol, dimethyl sulfide and dimethyl disulfide by *Thiobacillus thioparus* DW44 isolated from peat biofilter. J Ferment Bioeng 71:384–389. doi:10.1016/0922-338X(91)90248-F
9. Chung YC, Huang C, Tseng CP (1997) Removal of hydrogen sulphide by immobilized *Thiobacillus* sp. strain CH11 in a biofilter. J Chem Technol Biotechnol 68:58-62. doi:10.1002/(SICI)1097-4660(199705)69:1 < 58::AID-JCTB660 > 3.0.CO;2-H
10. Chung YC (2007) Evaluation of gas removal and bacterial community diversity in a biofilter developed to treat composting exhaust gases. J Hazard Mater 144:377–385. doi:10.1016/j.jhazmat.2006.10.045
11. Chung YC, Ho KK, Tseng CP (2007) Two-stage biofilter for effective NH_3 removal from waste gases containing high H_2S concentration. J Air Waste Manage Assoc 57:337–347
12. De Zwart JMM, Nelisse PN, Kuenen JG (1996) Isolation and characterization of *Methylophaga sulfidovorans* sp. nov.: an obligately methylotrophic, aerobic, dimethylsulfide oxidizing bacterium from a microbial mat. FEMS Microbiol Lett 20:261–270
13. Fuse H, Ohta M, Takimura O, Murakami K, Inoue H, Yamaoka Y et al (1998) Oxidation of trichloroethylene and dimethyl sulfide by a marine *Methylomicrobium* strain containing soluble methane monooxygenase. Biosci Biotechnol Biochem 62:1925–1931. doi:10.1271/bbb.62.1925
14. Fuse H, Takimura O, Murakami K, Yamaoka Y, Omori T (2000) Utilization of dimethyl sulfide as a sulfur source with the aid of light by *Marinobacterium* sp. strain DMS-S1. Appl Environ Microbiol 66:5527–5532. doi:10.1128/AEM.66.12.5527-5532.2000
15. Geng AL, Chen XG, Gould WD, Ng YL, Yan R, Lee CC et al (2004) Removal of odorous sulphur-containing gases by a new isolate from activated sludge. Water Sci Technol 50:291–297
16. Hanlon SP, Holt RA, Moore GR, McEwan AG (1994) Isolation and characterization of a strain of *Rhodobacter sulfidophilus*: a bacterium which grows autotrophically with dimethylsulphide as electron donor. Microbiol 140:1953–1958
17. Hirai M, Ohtake M, Shoda M (1990) Removal kinetic of hydrogen sulphide, methanethiol and dimethyl sulphide by peat biofilters. J Ferment Bioeng 70:334–339. doi:10.1016/0922-338X(90)90145-M
18. Hirano H, Yoshida T, Fuse H, Endo T, Habe H, Nojiri H et al (2003) *Marinobacterium* sp. strain DMS-S1 uses dimethylsulphide as a sulphur source after light-dependent transformation by excreted flavins. Environ Microbiol 5:503–509. doi:10.1046/j.1462-2920.2003.00444.x
19. Horinouchi M, Kasuga K, Nojiri H, Yamane H, Omori T (1997) Cloning and characterization of genes encoding an enzyme which oxidizes dimethyl sulfide in *Acinetobacter* sp. strain 20B. FEMS Microbiol Lett 155:99–105. doi:10.1111/j.1574-6968.1997.tb12692.x
20. Ito T, Miyaji T, Nakagawa T, Tomizuka N (2007) Degradation of dimethyl disulfide by *Pseudomonas fluorescens* strain 76. Biosci Biotechnol Biochem 71:366–370. doi:10.1271/bbb.60295
21. Juliette LY, Hyman MR, Arp DJ (1993) Inhibition of ammonia oxidation in *Nitrosomonas europaea* by sulfur compounds: thioethers are oxidized to sulfoxides by ammonia monooxygenase. Appl Environ Microbiol 59:3718–3727
22. Kim JY, Kim BW (2003) Removal of dimethyl sulfide in ceramic biofilters immobilized with *Thiobacillus thioparus* TK-m. J Microbiol Biotechnol 13:866–871
23. Laidler KJ (1958) The chemical kinetics of enzyme action. Oxford University Press, Oxford

24. 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. J Ferment Bioeng 76:55–59. doi:10.1016/0922-338X(93)90053-B
25. Phae CG, Shoda M (1991) A new fungus which degrades hydrogen sulfide, methanethiol, dimethyl sulfide and dimethyl disulfide. Biotechnol Lett 13:375–380. doi:10.1007/BF01027686
26. Pol A, Op den Camp HJ, Mees SG, Kersten MA, van der Drift C (1994) Isolation of a dimethylsulfide-utilizing *Hyphomicrobium* species and its application in biofiltration of polluted air. Biodegradation 5:105–112. doi:10.1007/BF00700635
27. 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. J Air Waste Manage Assoc 51:11–16
28. Sandaa RA, Enger O, Torsvik V (1999) Abundance and diversity of Archaea in heavy-metal-contaminated soils. Appl Environ Microbiol 65:3293–3297
29. Sercu B, Nunez D, Van-Langenhove H, Aroca G, Verstraete W (2005) Operational and microbiological aspects of a bioaugmented two-stage biotrickling filter removing hydrogen sulfide and dimethyl sulfide. Biotechnol Bioeng 90:259–269. doi:10.1002/bit.20443
30. Smet E, Chasaya G, Van Langenhove H, Verstraete W (1996) The effect of inoculation and the type of carrier material used on the biofiltration of methyl sulphides. Appl Microbiol Biotechnol 45:293–298. doi:10.1007/s002530050686
31. Tiwaree RS, Cho KS, Hirai M, Shoda M (1992) Biological deodorization of dimethyl sulfide using different fabrics as the carriers of microorganisms. Appl Biochem Biotechnol 32:135–148. doi:10.1007/BF02922154
32. Tsai CH, Huang YJ, Chen JC, Liao WT (2003) Deodorization of dimethyl sulfide using a discharge approach at room temperature. J Air Waste Manage Assoc 53:1225–1232
33. Visscher PT, Taylor BF (1993) A new mechanism for the aerobic catabolism of dimethyl sulfide. Appl Environ Microbiol 59:3784–3789
34. Visscher PT, van Gemerden H (1991) Photo-autotrophic growth of *Thiocapsa roseopersicina* on dimethyl sulfide. FEMS Microbiol Lett 81:247–250. doi:10.1111/j.1574-6968.1991.tb04766.x
35. Wani AH, Lau AK, Branion MR (1999) Biofiltration control of pulping odors-hydrogen sulfide: performance, microkinetics and coexistence effects of organo-sulfur species. J Chem Technol Biotechnol 74:9–16. doi:10.1002/(SICI)1097-4660(199901)74:1<9::AID-JCTB981>3.0.CO;2-B
36. Welsh DT (2000) Ecological significance of compatible solute accumulation by micro-organisms: from single cells to global climate. FEMS Microbiol Rev 24:263–290. doi:10.1111/j.1574-6976.2000.tb00542.x
37. Zhang L, Hirai M, Shoda M (1991) Removal characteristics of dimethyl sulfide, methanethiol and hydrogen sulfide by *Hyphomicrobium* sp. I55 isolated from peat biofilter. J Ferment Bioeng 72:392–396. doi:10.1016/0922-338X(91)90093-V
38. Zhang L, Kuniyoshi I, Hirai M, Shoda M (1991) Oxidation of dimethyl sulfide by *Pseudomonas acidovorans* DMR-11 isolated from peat biofilter. Biotechnol Lett 13:223–228. doi:10.1007/BF01025822
39. Zhang YF, Liss SN, Allen DG (2006) The effects of methanol on the biofiltration of dimethyl sulfide in inorganic biofilters. Biotechnol Bioeng 95:734–743. doi:10.1002/bit.21033