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Removal of Methanethiol, Dimethyl Sulfide, Dimethyl Disulfide, and Hydrogen Sulfide from Contaminated Air by *Thiobacillus thioparus* TK-m

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Methanethiol, dimethyl sulfide, dimethyl disulfide, and hydrogen sulfide were efficiently removed from contaminated air by *Thiobacillus thioparus* TK-m and oxidized to sulfate stoichiometrically. More than 99.99% of dimethyl sulfide was removed when the load was less than 4.0 g of dimethyl sulfide per g (dry cell weight) per day.

Methanethiol (methylmercaptan [MM]), dimethyl sulfide (DMS), dimethyl disulfide (DMDS), and hydrogen sulfide (H_2S) are malodorous compounds which exceed the odor threshold at low concentrations (5). They are produced by the wood-pulping industry, in oil refineries, and in manure and sewer systems and cause malodorous air pollution. Various methods have been used for the removal of malodorous compounds from contaminated air. The biological deodorizing method is now attracting attention because of its low operating cost. However, there is little knowledge available on the microorganisms which cause the degradation of methyl sulfides. MM was degraded with a biofilter made of pine bark (7), but there was no direct evidence of any degradation by isolated microorganisms. DMS was degraded by *Thiobacillus* sp. strain MS1 (6), *Thiobacillus thioparus* TK-m (4), *Hyphomicrobium* sp. strain S (1), and *Hyphomicrobium* sp. strain EG (8, 9). DMDS mixed with DMS was degraded by *Thiobacillus* sp. strain MS1 (7). Recently, Suylen and Kuenen (8) have reported that available cultures of *Thiobacillus* sp. strain MS1 could no longer metabolize DMS. There are no reports on the removal of methyl sulfides from contaminated air by identifiable bacteria.

In order to develop efficient methods for biological deodorization, the deodorizing potential of *T. thioparus* TK-m was examined. Results of this study indicate that MM, DMS, DMDS, and H_2S can be efficiently removed from contaminated air by use of this strain.

MATERIALS AND METHODS

Organism, media, and preculture conditions. The isolation of *T. thioparus* TK-m has been described previously (4). Basal medium C (4) was used in precultures and deodorization tests. A preparation of medium CS (2.5 g of $Na_2S_2O_3 \cdot 5H_2O$ and 15 g of agar added to 1 liter of medium C) was used to maintain *T. thioparus* TK-m and to measure the number of *T. thioparus* TK-m cells in the cultures. Medium N, which contained 2 g of nutrient broth (Difco Laboratories, Detroit, Mich.) and 15 g of agar in 1 liter of distilled water, was used to measure the number of heterotrophic cells in the cultures. Precultures (50 ml) were grown on DMS in 300-ml flasks that were sealed with rubber stoppers, as described previously (4), and shaken at 25°C (unless indicated otherwise).

Deodorizing tests. A schematic diagram of the deodorizing apparatus used in this study is shown (Fig. 1). The acrylic tubes (55 mm by 1 m) were filled with 700 ml of precultures made up of *T. thioparus* TK-m and 1,300 ml of sterilized medium C. Malodorous gases (1 to 5 ml/liter of nitrogen) were diluted with air and supplied to the bottom of the tubes through fritted glass diffusers. The flow rate of the gases and the air was controlled by mass flow controllers (Teledyne Hastings-Raydist) or was regulated and monitored with flow meters. The bacterial cultures in the tubes were maintained at 25°C and at pH 6.8 ± 0.4 by titration with 1 M K_2CO_3.

Analytical methods. Growth of the cultures in deodorizing tubes was monitored by either measuring the A_{660} in a spectrophotometer (101; Hitachi) or measuring the total organic carbon (TOC) in the cells in a TOC analyzer (TOC-500; Shimadzu). TOC in cells represented TOC in cultures after the deduction of TOC from culture filtered through membrane filters (pore size, 0.45 μm; Millipore Corp., Bedford, Mass.). Dry cell weights were calculated from their relationship to the A_{660}, in which an A_{660} value of 0.1 was equal to 75.5 mg (dry cell weight) per liter. Cell yields were calculated on the basis of increased A_{660} values and increased sulfate concentrations. In order to measure the number of cells, 1 ml of culture was diluted with sterilized water and 0.1 ml of the diluted liquid was applied to a medium in a plate. This medium in the plate was incubated for between 9 and 11 days at 25°C, and the number of visible colonies was counted. The number of round colonies with precipitated elemental sulfur appearing on medium CS were determined as the number of *T. thioparus*; the number of colonies on medium N was determined as the number of heterotrophs. Sulfate was determined with an ion chromatographic analyzer (IC500; Yokogawa Hokushin Electric, Tokyo, Japan). MM, DMS, DMDS, and H_2S were measured by gas chromatography on a gas chromatograph (GC-5A; Shimadzu) equipped with a flame photometric detector and a glass column (length, 3 m) packed with 25% β,β'-oxydipropionitrile on 60-80 mesh Chromosorb W. The lower limit for detection was 0.1 μl/liter. Human volunteers were used to detect malodorous compounds and proved to be much more effective than the gas chromatographic technique. It has been reported that human volunteers recognize 2.1 nl of MM per liter, 1.0 nl of DMS per liter, and 0.47 nl of H_2S per liter (5).

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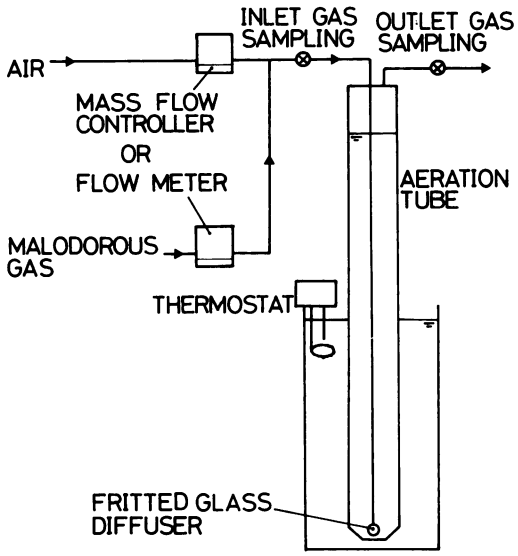

FIG. 1. Schematic diagram of the deodorizing apparatus used in this study.

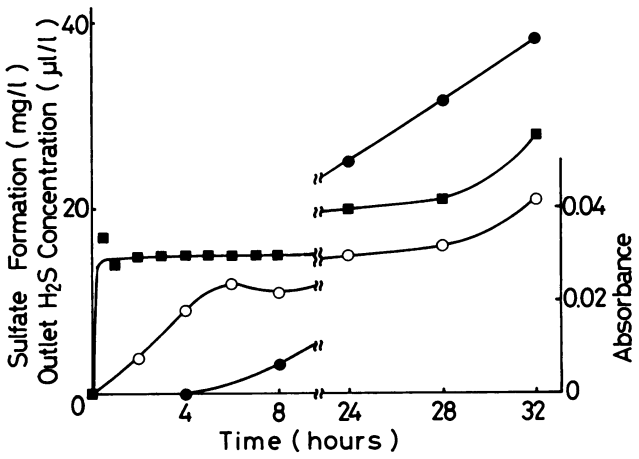

FIG. 2. Removal of H_2S by a sterilized medium. H_2S gas (140 μl/liter) was supplied at 250 Ncm^3 per min. Symbols: ■, outlet H_2S concentration; ○, A_{660}; ●, sulfate.

Gases. MM, DMS, DMDS, and H_2S gases (1 to 5 ml/liter of nitrogen) in 10- or 40-liter gas tanks were obtained from Seitetsu Chemical Industries Ltd. (Osaka, Japan) and Takachiho Chemical Industries Ltd. (Tokyo, Japan).

RESULTS

Removal of malodorous compounds by a sterilized medium. The aeration tube was filled with 2 liters of sterilized medium C. DMS gas (60 μl/liter) was supplied at 250 Ncm^3 (Ncm^3, volume when the gas is at 0°C and 1 atm) per min. The DMS concentration of the outlet gas after 5 h was as high as that of the inlet gas. There was no increase in the sulfate concentration in the medium over a 24-h period. Thus, DMS was not removed by the sterilized medium. MM and DMDS gas showed the same behavior pattern in the sterilized medium.

H_2S gas (140 μg/liter) showed a different behavior pattern (Fig. 2). The H_2S concentration of the outlet gas after 24 h was only 20 μl/liter. The absorbance of the medium increased, probably because of the reaction between H_2S and the metal ion in the medium, to form insoluble sulfides. Yellowish white precipitates that were thought to be elemental sulfur appeared in the bottom of the tube. No sulfate was formed during the first 4 h of gassing in the reactor. However, 3.2 mg of sulfate per liter formed after 8 h and 25.0 mg of sulfate per liter formed after 24 h, indicating that 9 and 24% of supplied H_2S were oxidized to sulfate, respectively. Thus, a fairly large amount of H_2S was removed by the sterilized medium, but complete removal was impossible.

Removal of DMS by *T. thioparus* TK-m. DMS gas (100 μl/liter) was supplied at 250 Ncm^3 per min to the aeration tube that was inoculated with *T. thioparus* TK-m (Fig. 3). Gas bubbles reached the surface within 3 s. The initial cell concentration was an A_{660} value of 0.008. The outlet gas contained 25 μl of DMS per liter after 2 h, but DMS was not detected after 24 h (A_{660}, 0.032) or subsequently in the outlet gas by either gas chromatography or the human volunteers. The culture was uniformly turbid at all times. No bacterial flocks appeared in the culture. Almost all the cells that were observed through a microscope were small bacilli. Plate counts showed relative numbers of the organisms in the 10-day culture to be 10 TK-m to 1 heterotroph. Cell yield in the initial 10 days was 21 g (dry cell weight) per mol of DMS. Sulfate was formed stoichiometrically. Growth of the cells terminated when the A_{660} value reached nearly 1.0, probably because of the lack of a nitrogen source.

Next, the maximum degradation rate of DMS by the cells was measured. A total of 20 ml of a 40-day culture was transferred to another aeration tube containing 1,980 ml of medium C. DMS gas (50 μl/liter) was supplied for 4 days at 250 Ncm^3 per min; then, the DMS concentration was increased stepwise (Fig. 4). When the DMS load was not over 4.0 g/g (dry cell weight) per day, no DMS was detected in the outlet gas by either gas chromatography or the human volunteers. This shows that the maximum degradation rate was 4.0 g/g per day.

In order to estimate the maximum capacity of the apparatus to remove DMS, the DMS load to the aeration tube was increased stepwise according to cell growth. A medium that was prepared by adding 1.8 g of NH_4Cl and 4 ml of trace metal solution to 1 liter of medium C was used in this experiment. DMS gas (100 to 760 μl/liter) was supplied at 500 to 1,610 Ncm^3 per min. The maximum DMS load in this experiment was 2.42 g/liter per day. When the load reached a maximum, the cell concentration was 1.02 g/liter, and DMS gas (760 μl/liter) was supplied at 1,610 Ncm^3 per min. More DMS did not flow into the aeration tube, probably because of the narrow pipe lines. DMS was not detected in the outlet gas in this experiment.

Removal of MM by *T. thioparus* TK-m. MM gas (106 μl/liter) was supplied to the aeration tube at 250 Ncm^3 per min (Fig. 5). The initial cell concentration was an A_{660} value of 0.028. The initial MM load to the cells was 2.0 g/g (dry cell weight) per day. MM was not detected in the outlet gas at any time throughout the experiment by either gas chromatography or the human volunteers. Sulfate was formed stoichiometrically. After 11 days, plate counts showed the relative number of organisms to be 25 TK-m to 1 heterotroph. The cell yield on MM was 20 g/mol of MM.

Removal of H_2S by *T. thioparus* TK-m. H_2S gas (220 μl/liter) was supplied at 250 Ncm^3 per min. The initial cell concentration was an A_{660} value of 0.028. The initial H_2S load to the cells was 2.9 g/g (dry cell weight) per day. H_2S was not detected in the outlet gas at any time. Sulfate was

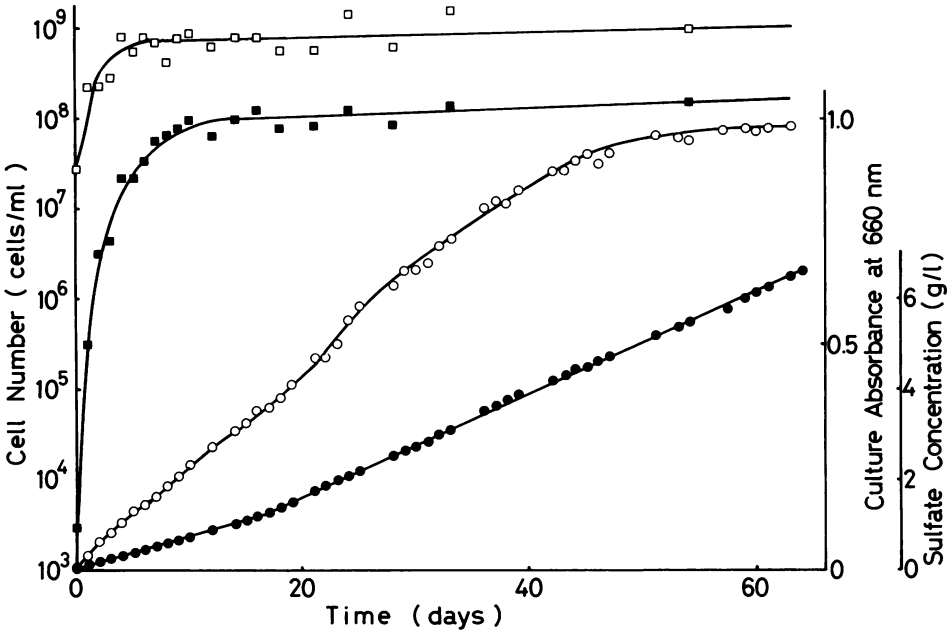

FIG. 3. Removal of DMS by *T. thioparus* TK-m. DMS gas (100 μl/liter) was supplied at 250 Ncm^3 per min. After 16 days, the DMS concentration of the inlet gas was increased to 150 μl/liter. The outlet gas contained 25 μl of DMS per liter after 2 h, but DMS was not detected after 1 day or subsequently in the outlet gas by either gas chromatography or the human volunteers. Symbols: ○, A_{660}; ●, sulfate; □, cell number of *T. thioparus* TK-m; ■, number of heterotrophic cells.

formed stoichiometrically. As described above, H_2S oxidation occurred in sterilized medium C, but the rate was very low. Therefore, in this experiment, H_2S oxidation was thought to be carried out mainly by the microorganisms. After 11 days, the relative number of organisms was 10 TK-m to 1 heterotroph. The cell yield on H_2S was 9.2 g/mol of H_2S.

Removal of DMDS by *T. thioparus* TK-m. Precultures grown on DMDS were inoculated into the aeration tube. The initial cell concentration was an A_{660} value of 0.018. DMDS gas (65 μl/liter) was supplied at 250 Ncm^3 per min. The initial DMDS load to the cells was 3.6 g/g (dry cell weight) per day. Outlet gas contained 3 μl of DMDS per liter after 2 and 5 h, but no DMDS was detected in the outlet gas after 24 h (A_{660}, 0.042). Sulfate was formed stoichiometrically. The cell yield on DMDS was 33 g/mol of DMDS. Precultures grown on DMS did not degrade DMDS in the aeration tube over 3 days.

DISCUSSION

This is the first study in which it has been demonstrated that methyl sulfides are removed from contaminated air by an identified microorganism and are oxidized to sulfate stoichiometrically. More than 99.99% of the DMS was removed by *T. thioparus* TK-m, when the DMS load to the cells was 4.0 g/g (dry cell weight) per day. Fukuyama et al. (2) have reported that the maximum DMS load for acclimated activated sludge to obtain a 99.9% removal is 9.0 mg/g of mixed liquor suspended solids (MLSS) per day. The maximum load for removing more than 90% of reduced sulfur contained in kraft pulp wastewater, i.e., MM, DMS, DMDS, and H_2S, by activated sludge has been reported to be 15 mg of S per g of MLSS per day (3). Therefore, the cultures of *T. thioparus* TK-m removed DMS 100-fold more rapidly than did the acclimated activated sludges. The cul-

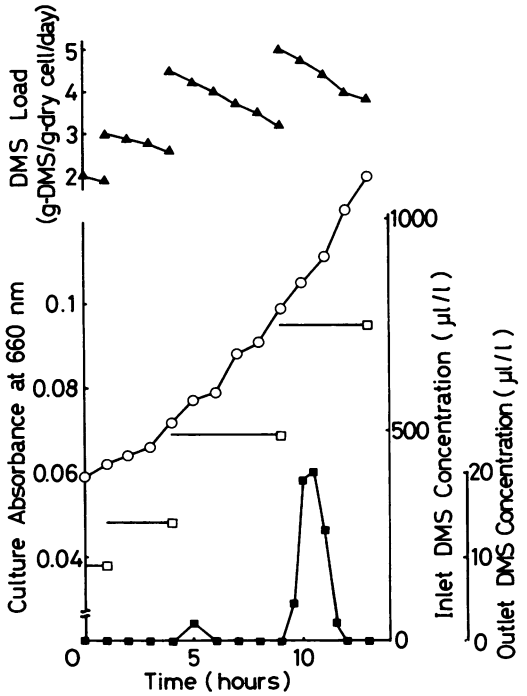

FIG. 4. Removal efficiency of DMS for the different loadings. DMS gas (180 to 750 μl/liter) was supplied at 250 Ncm^3 per min. The DMS load was calculated from the relationship of the A_{660} to the dry cell weight, in which an A_{660} value of 0.1 = 75.5 mg/liter. Symbols: ○, A_{660}; □, inlet DMS concentration; ■, outlet DMS concentration; ▲, DMS load.

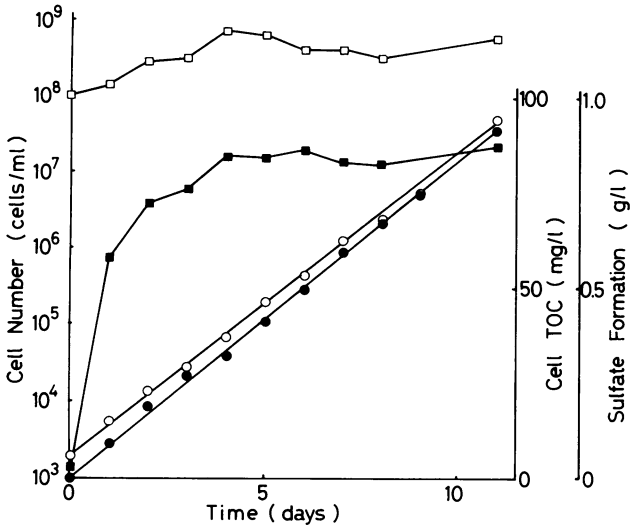

FIG. 5. Removal of MM by *T. thioparus* TK-m. MM gas (106 μl/liter) was supplied at 250 Ncm^3 per min. MM was not detected in outlet gas at any time throughout the experiment by either gas chromatography or the human volunteers. Symbols: ○, cell TOC; ●, sulfate; □, cell number of *T. thioparus* TK-m; ■, number of heterotrophic cells.

tures in the aeration tube degraded DMS completely for more than 2 months. Moreover, MM, DMDS, and H_2S were also removed by *T. thioparus* TK-m as efficiently as DMS was. Thus, *T. thioparus* TK-m proved very useful for removing malodorous methyl sulfides and hydrogen sulfide.

After the inoculation of pure cultures of *T. thioparus* TK-m in the aeration tube, heterotrophs grew very rapidly, but the plate counts always showed that the relative number of organisms was more than 10 TK-m to 1 heterotroph. *T. thioparus* TK-m has been reported to utilize not only reduced sulfur but also the carbons in the DMS molecule (4); consequently, there was very little of the energy source remaining in the culture broth after the growth of *T. thioparus* TK-m. Thus, the mixed cultures in the aeration tube consisted of a large amount of strain TK-m but little of the other organisms.

The cell yields on H_2S and DMS were 9.2 and 21 g/mol, respectively. These values are very similar to those for *Hyphomicrobium* sp. strain EG, the yields of which were reported to be 8 to 10 and 19.1 g/mol of consumed H_2S and DMS, respectively (9). The cell yields on MM and DMDS were 20 and 33 g/mol, respectively. It was very interesting to observe from the point of view of a biochemist that there was very little difference between the cell yield on MM and that on DMS.

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