Removal of odorous sulphur-containing gases by a new isolate from activated sludge


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Abstract Biological treatment of odorous sulphur-containing compounds is attracting attention due to its benign eco-friendliness, energy-savings and low operating costs. As the biological treatment efficiency of dimethylsulphide (DMS) reported was often low and variable, selection of useful DMS-degrading microorganisms is of importance for the enhancement of the biological deodorizing process. This paper reports the successful isolation of a DMS-degrading bacterium from activated sludge, using the enrichment isolation technique. The isolate was identified by 16S rRNA gene sequencing, and found to belong to the alpha group of Proteobacteria, with an identity of 99.4% and 99.1% to the 16S rRNA gene sequences of Afipia felis and Pseudomonas carboxydohydrogena, respectively. The isolate was able to metabolize DMS as well as hydrogen sulphide (H2S). A batch experiment was performed to assess the removal characteristics of DMS by the isolate. The results showed that over half of DMS could be removed by the isolate in 3 hours when the initial DMS amount was approximately 10 µmol and 25 µmol. Removal of H2S by the isolate was evaluated by a continuous test in a 2-L gas-bubbling bottle. Although part of the H2S removal by the mineral medium itself was observed in the control test, the majority of H2S removal was believed to be attributed to the metabolic activity of the isolate. In conclusion, the isolate might be potentially useful for the enhancement of the biological deodorizing processes.

Keywords Bacteria; dimethylsulphide; hydrogen sulphide; isolation; odour; sulphur-containing compounds

Introduction Formation of odorous sulphur-containing compounds, e.g. hydrogen sulphide (H2S) and dimethylsulphide (DMS), has been observed in wastewater treatment plants as well as in several industrial processes such as brewing and paper manufacturing (Kelly and Smith, 1990). These compounds are causing environmental problems due to their high odour index and toxicity. Furthermore, acid rain can be formed when these compounds are oxidized to SO2 by the OH– radical in the troposphere (Charlson et al., 1987).

There are several existing approaches to treat foul gases using on-site odour control processes, i.e. incineration, chemical scrubbing, activated carbon adsorption and some chemical masking or neutralization. Although these techniques can effectively remove the odorants from foul air under certain conditions, the requirement for daily chemical addition and adsorbent replacement gives rise to a relatively high operating cost (Pomeroy, 1982; Williams and Miller, 1992). The biological treatment method, nevertheless, is attracting attention as a viable alternative, due to its benign eco-friendliness, energy-savings and low operating costs. At present, there are two basic biological treatment processes for odour abatement. One is bioscrubbing, which uses scrubbing and biological oxidation, and the other is biofiltration, which uses adsorption and biological oxidation. In both processes, odour-removing microorganisms play a key role in the system performance.

During recent decades, there has been abundant research on DMS and H2S removal using isolated microbial species as well as microbial consortia obtained from biofilters.
In general, the removal rate of DMS is rather low – one-tenth of that of H₂S and one-third of that of methanethiol (Hirai et al., 1990). Meanwhile, the diversity of DMS-utilizing microorganisms is fairly limited. Up to now, only a few bacterial strains within the genera *Hyphomicrobium* (Zhang et al., 1991a), *Thiobacillus* (Kanagawa and Mikami, 1989), *Pseudomonas* (Zhang et al., 1991b) and *Alcaligenes* (Bendinger, 1992), and several fungal strains (Phae and Shoda, 1991), were reported to be able to remove DMS by various pathways. As a result, the biofiltration efficiency of methyl sulphides in waste gases was reported to be often low and variable (0–81%), whereas carbonyl compounds such as isovaleraldehyde were removed efficiently (above 98%) (Van Langenhouve et al., 1991). This indicates the necessity of improving DMS removability by exploring useful microorganisms. This paper reports the successful isolation of a DMS-degrading bacterium from activated sludge by using the enrichment isolation technique. The removal characteristics of DMS and H₂S using the new isolate are also presented.

**Materials and methods**

**Media**

The mineral medium used for cultivation and experiments contained (in g L⁻¹) K₂HPO₄ 1.5, KH₂PO₄ 1.5, NH₄Cl 0.3, MgCl₂·7H₂O 0.5, CaCl₂·2H₂O 0.2; pH 7.0. Bacto-agar was added at 15 g L⁻¹, to achieve a solid medium. The media were sterilized by autoclaving at 121°C for 15 min. Sodium thiosulphate (Na₂S₂O₃·5H₂O) was filter sterilized and added to the mineral medium to form the thiosulphate medium.

**Enrichment and isolation procedure**

Activated sludge was collected from Jurong Industrial Water Works, Singapore, and stored at −20°C before use. Ten millilitres of the sludge were aseptically added to a stoppered shaking flask containing 100 ml of mineral medium. DMS was dosed by adding 20 µl of DMS solution to the flask every other day. The flask was incubated in an orbital incubator (120 rpm) at 25°C. Two weeks later, 10 ml of the culture broth were transferred to a new shaking flask containing 100 ml mineral medium dosed with DMS. After another two weeks of incubation, the culture broth was series-diluted using 1 × PBS buffer, and spread on the mineral medium agar plates supplemented with DMS. Supplementation of DMS was carried out by adding 20 µl of DMS solution to a piece of sterilized glass-slip placed on the plate lid. The plates were immediately sealed with parafilm after DMS was added, and incubated at 25°C for 10 days. Individual bacterial colonies were picked up and subcultured on the DMS agar plates and the thiosulphate agar plates for several times. Strain SH was obtained after several such subcultures. Purity was confirmed by microscopic examination. The purified cultures were then preserved in a thiosulphate medium supplemented with 20% sterilized glycerol at −80°C.

**Identification of the isolate with 16S rRNA gene sequencing**

Partial sequences of the 16S rRNA gene were determined by direct sequencing of the polymerase-chain-reaction (PCR)-amplified 16S rDNA. A whole-cell direct lysis PCR amplification of the 16S rDNA and purification of the PCR products were carried out as described previously (Zhuang et al., 2002). After purification, a DNA region corresponding to the nucleotides 530 to 907 of *Escherichia coli* 16S rDNA was sequenced using the ABI model 310A DNA sequencer (Applied Biosystems, Perkin-Elmer) and the ABI PRISM® BigDye™ Terminator Cycle Sequencing ready-reaction kit (Applied Biosystems, Perkin-Elmer). The partial 16S rDNA sequences were analysed using a BLAST search of the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST/).
Growth pattern of the isolate
Strain SH was inoculated into 400 ml of the thiosulphate medium in a 2-L shaking flask, and incubated at 30°C on an orbital shaker (150 rpm). The growth of the strain was monitored by measuring the concentrations of thiosulphate and sulphate, pH and cell number, during the incubation. Concentration of thiosulphate was measured by using the standard iodometric titration method (APHA–AWWA–WEF, 2000). Concentration of sulphate was measured according to the turbidimetric method, with the aid of flow inject analyser (FIA, Model QUICKCHEM 8000, LACHAT). Cell number determination was carried out by DAPI (4′,6-diamidino-2-phenylindole) stain, as previously described (Kuwae and Hosokawa, 1999).

Removal of DMS by the isolate in a batch test
A batch experiment was conducted to assess the removal characteristics of DMS by the isolate. Cells of strain SH were harvested by centrifugation at 4,000 rpm for eight minutes, washed twice with mineral medium and resuspended in mineral medium to form the culture solution. The pH of the culture solution was maintained at 7.0. After that, 20 ml of the culture solution with the cell density at 10^8 cell/ml were added into a 60-ml culturing tube. Twenty millilitres of the mineral medium without the bacterium were added into another of the same type of culturing tube as a negative control. Each tube was then sealed with a butyl rubber stopper. A proper amount of DMS was injected into the tubes, using a gas-tight syringe. The culturing tubes were shaken at 120 strokes at 25°C. DMS concentration in the gas phase of the tubes was analysed periodically on a gas chromatograph (Agilent 6890N) equipped with a flame photometric detector. The total amount of DMS was calculated from the DMS concentration in the gas phase of the culturing tube, according to Henry’s law (Murakami et al., 1987).

Removal of H_2S by the isolate in a continuous test
Continuous H_2S removal study was performed in a 2-L gas-bubbling bottle. Culture solution was prepared similarly as described in the previous section. The 2-L bottle was then filled with 500 ml of the culture solution with a cell concentration of 10^6 cell/ml. Some 100 ppm of H_2S gas was supplied to the bottom of the bottle through a fritted glass diffuser. The gas flowrate was controlled at 150 ml/min by using a mass flowmeter controller (Brooks 5850E). A negative control test was performed at the same time, using 500 ml of mineral medium without adding the bacterium. In this control test, the inlet H_2S concentration was 70 ppm and the gas flow rate was 150 ml/min. Concentration of H_2S was determined by using a Draeger tube (Dräger, Germany).

Results and discussion
Enrichment, isolation and identification of the DMS-utilizing bacterium
A steady decrease of DMS in the gas phase of the enrichment culture was observed. After four weeks of enrichment, a dominant bacterium, strain SH, was successfully isolated from the enrichment culture, using the DMS supplemented mineral medium agar plate. Strain SH was also found to be able to grow on the thiosulphate agar plates without any organic carbon source, indicating that the strain could grow autotrophically. A partial 16S rDNA sequence of 445 nucleotide bases was obtained for the isolate, and compared with the currently available sequences of organisms belonging to the domain bacteria. The result showed that SH belonged to the alpha group of Proteobacteria, with an identity of 99.4% and 99.1% to the 16S rRNA gene sequences of Afipia felis and Pseudomonas carboxydohydrogena, respectively.

During the last decade, several DMS-converting microbial species have been isolated...
from different microbial environments (Kelly and Smith, 1990). However, the number of DMS-utilizing microorganisms reported so far is quite limited. Most strains belong to the methylotrophic Hyphomicrobium genus or the autotrophic Thiobacillus genus, utilizing DMS as an energy and/or carbon source. In addition, Zhang and his colleagues (1991b) reported the isolation of a Pseudomonas acidovorans strain that can stoichiometrically convert DMS to dimethyl sulphoxide (DMSO); and Bendinger (1992) obtained an Alcaligenes faecalis strain using dimethyl disulphide (DMDS) and possibly also DMS as a sulphur source when supplemented with acetate as a carbon source. So far, removal of DMS by the species Afipia felis or Pseudomonas carboxydohydrogena has not been reported in the literature. From the findings presented in this study, the DMS-utilizing bacterial family could be extended.

**Growth pattern of the isolate**
The growth pattern of the isolate SH in the thiosulphate medium is shown in Figure 1. It is interesting to find that thiosulphate in the culture medium was not stoichiometrically oxidized to sulphate, even at the end of the test (day 9). Only 41.87% of the thiosulphate was converted to sulphate. The rest, however, could be oxidized to other products such as elemental sulphur. In fact, after six days of incubation of strain SH in the thiosulphate medium, a thin film could be observed on the surface of the medium. At the same time, the growth medium became cloudy white. Microscopic observation also confirmed the presence of small crystals, which might be elemental sulphur in the medium.

The growth of the isolate was also indicated by the increase in total cell number and the decrease in pH due to the accumulation of sulphate. The total cell number reached a maximum of $8.54 \times 10^7$ cell/ml on day 5, and then gradually decreased to $3.44 \times 10^7$ cell/ml on day 9. The decrease in the cell number during the last phase could be due to product inhibition or the resultant acidity.

For the control test, a slight decrease in thiosulphate and a slight increase in sulphate were observed, whereas no change of pH was found in the course of the experiment. The minor changes in thiosulphate and sulphate concentration could be due to the auto-oxidation of thiosulphate.

**Removal of DMS by the isolate in a batch test**
The time profiles of DMS removal by the isolate SH are given in Figure 2. Some 72.07% and 67.59% of DMS was removed by the isolate when the initial DMS amount was approximately 10 µmol and 25 µmol, respectively. In both experiments, DMS was virtually removed in 15 hours. The removal of DMS by the isolate could be divided into two stages. The first stage took about three hours, and removed 57.95% and 56.36% of DMS, respectively. The second stage took about 12 hours, in which only 14.12% and 11.23% of DMS were removed respectively. Loss of DMS (approximately 13.50%) was observed in the control tube, probably due to the imperfect nature of the testing system or the measuring method used. Nevertheless, the loss was not significant when compared with the decrease of DMS in the tubes with the presence of the bacterium. After the systematic loss was deducted, over half of the DMS removal was attributed to the bacterial metabolic activity.

Zhang and his co-workers reported 90% DMS removal, in a sealed-flask study using their DMS-degrading isolate, Hyphomicrobium sp. I55 (Zhang et al., 1991a). Although the DMS removal efficiency of strain SH was lower than that of Hyphomicrobium sp. I55, it is encouraging to find that strain SH could survive and degrade DMS at a high concentration (six times higher than that in Zhang’s study). It has been proved that a high concentration of DMS is growth inhibiting (Pol et al., 1994). Therefore, strain SH might be particularly useful when applied in a high DMS-loading bio-deodorization process.
Removal of H$_2$S by the isolate in a continuous test

Figure 3 shows the removal of H$_2$S by the isolate in a chemostat test. The mineral medium itself was found to be able to partially remove H$_2$S. This is reflected by the H$_2$S removal curve in the control test, where no bacterial culture was adopted and the inlet H$_2$S concentration was 70 ppm. The H$_2$S removal rate levelled off at around 50% after one day of the test. The removal is most likely due to the auto-oxidation of H$_2$S or the reactions between H$_2$S and the metal ions in the mineral medium. However, no significant decrease in the pH of the medium was observed.

Figure 1 Growth pattern of the isolate SH in the thiosulphate medium. (a) Time courses of the concentrations of thiosulphate (squares) and sulphate (triangles) of the culture medium in the control test (solid shapes) and the SH inoculated test (hollow shapes); (b) Time courses of pH (squares) of the culture medium in the control test (solid shapes) and the SH inoculated test (hollow shapes); and time course of total cell number (triangles) in the SH inoculated test.

Figure 2 Time profiles of DMS amounts in the culturing tubes.
In the H$_2$S removal test using the isolate SH, where the inlet H$_2$S concentration was 100 ppm, the outlet H$_2$S concentration increased from 20 ppm at half an hour to 40 ppm at three hours, due to the saturation of H$_2$S in the medium. After one day of the test, the outlet H$_2$S concentration dropped to 25 ppm, corresponding to a removal efficiency of 75%. The removal efficiency levelled off at 70–80% afterwards. As shown by the control test, some H$_2$S removal was achieved by the mineral medium itself (around 50% at the inlet concentration of 70 ppm, or 35 ppm removal); nevertheless, a majority of the H$_2$S removal in this test (35–43 ppm) is believed to be attributable to the metabolic activity of the isolate. This is also supported by the obvious decrease in pH during the test, whereas no significant change of pH was observed in the control (Figure 3(b)).

Although the H$_2$S removal by the isolate SH in the present study was not very high, the versatility of SH in removing both DMS and H$_2$S is affirmable. In fact, this is important in a practical industrial application, since most of the foul gases discharged by industries are polluted with H$_2$S and DMS simultaneously.

Conclusions

1. A DMS-degrading bacterium, strain SH, was successfully isolated from activated sludge by using the enrichment isolation technique. The isolate was identified by 16S rRNA gene sequencing and found to belong to the alpha group of Proteobacteria, with an identity of 99.4% and 99.1% to the 16S rRNA gene sequences of Afipia felis and Pseudomonas carboxydohydrogena, respectively.
2. The isolate could grow on the DMS-supplemented mineral medium as well as the thiosulphate-supplemented mineral medium. Its growth on the thiosulphate medium was indicated by a decrease in thiosulphate concentration and pH; and an increase in total cell numbers.
3. A batch experiment was performed to assess the removal characteristics of DMS by the
isolate. The results showed that the isolate could survive and degrade DMS at a high concentration. Over half of DMS could be removed by the isolate in three hours, when the initial DMS amount was approximately 10 µmol and 25 µmol.

4. Removal of H\textsubscript{2}S by the isolate was evaluated by a continuous test in a 2-L gas-bubbling bottle. Although part of H\textsubscript{2}S removal by the mineral medium itself was observed in the control test, a majority of H\textsubscript{2}S removal (35–43 ppm) is believed to be attributable to the metabolic activity of the isolate. This affirmed the versatility of the isolate in removing both DMS and H\textsubscript{2}S.

5. The findings showed that the isolate SH is potentially useful for the enhancement of the biological deodorizing processes.

**References**


