Degradation of Hydrogen Sulfide by Xanthomonas sp. Strain DY44 Isolated from Peat

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Xanthomonas sp. strain DY44, capable of degrading H2S, was isolated from dimethyl disulfide-acclimated peat. This bacterium removed H2S either as a single gas or in the presence of the sulfur-containing compounds methanethiol, dimethyl sulfide, and dimethyl disulfide. The maximum specific H2S removal rate, obtained in the late stationary phase, was 3.92 mmol g of dry cells\(^{-1}\) h\(^{-1}\) \(7 \times 10^{-16}\) mol cell\(^{-1}\) h\(^{-1}\) at pH 7 and 30°C through a batch experiment in a basal mineral medium. Since Xanthomonas sp. strain DY44 exhibited no autotrophic growth with H2S, the H2S removal was judged not to be a consequence of chemolithotrophic activity. By using X-ray photoelectron spectroscopy, the metabolic product of H2S oxidation was determined to be polysulfide, which has properties very similar to those of elemental sulfur. Autoclaved cells (120°C, 20 min) did not show H2S degradation, but cells killed by γ-irradiation and cell extracts both oxidized H2S, suggesting the existence of a heat-labile intracellular enzymatic system for H2S oxidation. When Xanthomonas sp. strain DY44 was inoculated into fibrous peat, this strain degraded H2S without lag time, suggesting that it will be a good candidate for maintaining high H2S removability in the treatment of exhaust gases.

Hydrogen sulfide (H2S) is one of the main malodorous compounds occurring in exhaust gases. This compound is often evolved in the highest concentration together with sulfur-containing volatile compounds such as methanethiol (MT), dimethyl sulfide (DMS), and dimethyl disulfide (DMDS) from wastewater and night soil treatment plants. To remove H2S, physicochemical methods are generally used. However, biological methods, using microbial activity for H2S removal, have drawn attention since they are more efficient and more economical than physicochemical methods if proper operational conditions are maintained. A number of microbial processes for H2S removal have been proposed that are based on oxidation by thiobacilli and other sulfur microorganisms. Thiobacillus spp. have been used to oxidize H2S to sulfate in liquid (19) and in packed tower systems such as peat biofilters (4, 5, 21, 23). Mixed cultures of bacteria from the genera Thiobacillus (14) and the photosynthetic bacterium Chlorobium thiosulfatophilum have been proposed for use in the oxidation of H2S to elemental sulfur from the gas stream (6). Most of them are autotrophic bacteria, but these bacteria are often difficult to handle, mainly because their growth rates are significantly lower than those of heterotrophic bacteria. In applying phototrophic microorganisms, the supply of solar or artificial energy is a limiting factor for growth.

This paper describes some characteristics of a new H2S-degrading heterotrophic bacterium, a Xanthomonas sp., isolated from DMDS-acclimated peat; the advantageous features of this bacterium over autotrophic bacteria as an H2S remover are also discussed.

MATERIALS AND METHODS

Media and cultivation conditions. A nutrient medium was used for cultivation of the isolate and contained the following (in grams per liter): meat extract, 3.0; peptone, 15.0; yeast extract, 3.0; Na₂HPO₄·12H₂O, 2.0; and NaCl, 3.0. For batch- and continuous-supply H2S experiments, a basal mineral medium, containing 2.0 g of K₂HPO₄, 2.0 g of KH₂PO₄, 2.0 g of KH₂PO₄, 0.4 g of NH₄Cl, 0.2 g of MgCl₂·6H₂O, and 0.01 g of FeSO₄·7H₂O (each per liter), was used. The pH was adjusted to 7 by using 2 N NaOH in both the nutrient and basal media.

Isolation and identification of the bacterium. A heterotrophic bacterium was isolated by chance in the process of isolating the autotrophic bacterium Thiobacillus thioparus DW44 on thiosulfate-agar medium from DMDS-acclimated peat (3, 4). The heterotrophic bacterium was purified by repeated transfer of the cells to fresh nutrient medium and was labeled DY44. Identification of strain DY44 was carried out by the National Collections of Industrial and Marine Bacteria Limited, Tottori Research Station, Aberdeen, United Kingdom.

H2S removal in batch system. Cells grown in 100 ml of the nutrient medium for 4 days at 30°C were harvested by centrifugation (7,500 × g, 10 min), washed in sterilized, distilled water, and resuspended in 100 ml of sterilized water. One milliliter of the cell suspension (about 10¹⁵ cells) was added to 99 ml of the basal mineral medium in a 500-ml flask. One milliliter of an autoclaved cell suspension (120°C for 20 min) and 1 ml of γ-irradiated cells (50°C for 1 h at 1 megarad h⁻¹) were added to similar flasks. As a control, 1 ml of sterilized, distilled water was added to a fourth flask. The flasks were tightly sealed with butyl rubber stoppers, H₂S gas from a cylinder containing 20,000 μl of H₂S liter⁻¹ in N₂ (Takachiho Co., Inc., Tokyo, Japan) was injected into each flask through the stopper to give a total H₂S amount of 10 μmol per flask (the aqueous H₂S concentration at 10 μmol was 44.9 μM), and the flasks were shaken at 120 rpm at 30°C. The headspace of each flask was sampled periodically by using a gastight syringe, and the H₂S concentration was determined by gas chromatography (model GC-14 with a flame photometric detector; Shimadzu Co. Ltd., Kyoto, Japan). The aqueous H₂S concentration was calculated from the gas concentration by using Henry’s constant (13). Details

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of experimental and analytical methods were described previously (5, 9).

\( \text{H}_2\text{S} \) removal by cell extract. Cells harvested in the manner described above were suspended in 10 mM Tris-HCl buffer (pH 8) containing 0.2 M NaCl, 100 \( \mu \)M EDTA, and 10 \( \mu \)M phenylmethylsulfonyl fluoride and passed twice through a chilled French pressure cell at 1,000 kg cm\(^{-2}\). After DNA digestion was digested by the addition of DNase, intact cells were removed by centrifugation at 10,000 \( \times \) g for 10 min. The supernatant was centrifuged at 30,000 \( \times \) g for 1 h at 4°C and used as the crude cell extract. One milliliter of the cell extract and 9 ml of 10 mM Tris-HCl buffer (pH 7.4; final protein concentration, 0.87 mg ml\(^{-1}\)) were put into a 123-ml vial bottle which was then sealed with a butyl rubber stopper. A second 123-ml vial bottle containing 10 ml of 10 mM Tris-HCl buffer without cell extract was used as a control. \( \text{H}_2\text{S} \) gas was injected to give total \( \text{H}_2\text{S} \) amounts of 4 \( \mu \)mol per bottle (aqueous \( \text{H}_2\text{S} \) concentration at 4 \( \mu \)mol, 166.9 \( \mu \)M), and the \( \text{H}_2\text{S} \) concentration was measured periodically as described above.

Growth pattern and \( \text{H}_2\text{S} \) removal rates in different growth phases. Cells grown in nutrient-agar medium were suspended in 10 ml of sterilized water, and 1 ml of the suspension (about \( 10^{10} \) cells) was inoculated into 99 ml of fresh nutrient medium in each of eight 500-ml flasks. The flasks were incubated at 30°C, and the growth of strain DY44 was monitored by measuring dry weight after the whole cells in one flask were dried at 110°C for 4 h at each cultivation time. The pH of the culture broth was also measured. \( \text{H}_2\text{S} \) removal rates after 1 and 4 days of cultivation time were determined as follows. The cells were harvested by centrifugation at each time and were washed and resuspended in 10 ml of distilled water. The cell suspension was diluted with distilled water at different dilution ratios, with 1 ml of each differently diluted suspension being added to 99 ml of the basal mineral medium (pH 7) in a 500-ml flask. \( \text{H}_2\text{S} \) removal by the bacterial suspension was determined for each flask by the same procedures as described above. The specific removal rate (millimoles of \( \text{H}_2\text{S} \) per gram of dry cells per hour) was calculated from the total amount of \( \text{H}_2\text{S} \) removed in both the gas and liquid phases divided by the dry cell mass.

Experiment with a continuous supply of \( \text{H}_2\text{S} \) in the basal mineral medium. Figure 1a shows the bubbling system for the experiment using a continuous supply of \( \text{H}_2\text{S} \). Ten milliliters of cell suspension (about \( 10^{11} \) cells; dry weight of cells, 0.2 g) was added to a 300-ml glass bubbling bottle containing 240 ml of the basal mineral medium. About 200 to 250 \( \mu \)l of \( \text{H}_2\text{S} \) gas liter\(^{-1}\), which was generated by the dilution of \( \text{H}_2\text{S} \) gas from a cylinder (20,000 \( \mu \)l liter\(^{-1}\)) with air from a compressor, was supplied into the bottle at a flow rate of 100 ml min\(^{-1}\) at 30°C for 16 days. During the supply of \( \text{H}_2\text{S} \) gas, the inlet and outlet concentrations of \( \text{H}_2\text{S} \) were intermittently measured; the culture broth was also sampled, and the pH and the viable cell count on the nutrient-agar medium were determined.

\( \text{H}_2\text{S} \) removal in the presence of organic compounds. \( \text{H}_2\text{S} \) removal by the isolate in the presence of organic compounds was studied in batch and continuous systems. In the batch experiments, glucose, maltose, and acetate were added to the basal mineral medium to give a final concentration of 2 g liter\(^{-1}\), and \( \text{H}_2\text{S} \) removal was monitored in the same manner as described above. In the continuous experiment, 1 ml of cell suspension (about \( 10^{10} \) cells) was added to a 300-ml bottle containing 199 ml of the basal mineral medium supplemented with 2 g of yeast extract liter\(^{-1}\), and about 20 \( \mu \)l of \( \text{H}_2\text{S} \) liter\(^{-1}\) was supplied into the bottle at a flow rate of 200 ml min\(^{-1}\) at 30°C.

Identification of the product of removed \( \text{H}_2\text{S} \). One milliliter of culture broth obtained from the continuous-supply experiment described above was filtered through a 0.45-\( \mu \)m-pore-size membrane. Anionic sulfur compounds in the filtrate were analyzed by ion chromatography (HIC-6A; Shimadzu). The rest of the culture broth was lyophilized (FD-1; Tokyo Rikakikai Co. Ltd., Tokyo, Japan) and used for analysis of sulfur compounds by X-ray photoelectron spectroscopy (XPS) (ESCA750; Shimadzu). Non-\( \text{H}_2\text{S} \)-supplied culture broth was used as a reference. To observe the difference between the two, a mixture of the lyophilized culture broth supplied with \( \text{H}_2\text{S} \) (1.25 g) and elemental sulfur (1 g) was also subjected to analysis by XPS.

\( \text{H}_2\text{S} \) removal in the presence of other sulfur-containing gases. Two milliliters of cell suspension prepared as described above was added to 98 ml of the basal mineral medium in each of four 500-ml flasks. The flasks were tightly sealed with butyl rubber stoppers, and \( \text{H}_2\text{S} \) gas was injected into each flask through the stopper (total \( \text{H}_2\text{S} \) amount, about 8 \( \mu \)mol per flask). The following volumes of MT, DMS, or DMDS were then injected to give total amounts of 2, 1, and 2 \( \mu \)mol per flask, respectively: 10 ml of MT gas from a cylinder containing 8,200 \( \mu \)l of MT liter\(^{-1}\) in \( \text{N}_2 \) (Takachiho); 0.1 ml of DMS gas from the headspace of a glass vessel containing a 98% DMS solution (Kanto Chemical Co. Inc., Tokyo, Japan) at 25°C; and 2 ml of DMDS gas from the headspace of a 99% DMDS solution (Tokyo Kasei Co. Inc., Tokyo, Japan) at 25°C. The fourth flask contained \( \text{H}_2\text{S} \) gas only as a control. During shaking at 120 rpm at 30°C, the headspace of each flask was sampled, and gas concentra-
tions were determined periodically by gas chromatography.

\( \text{H}_2\text{S} \) removal experiment using a peat biofilter inoculated with strain DY44. An experiment on the removal of \( \text{H}_2\text{S} \) by strain DY44 inoculated on peat was carried out in a labora-
tory-scale peat column, which is referred to as a peat biofilter (4, 5) (Fig. 1b). Forty-five grams of dry fibrous peat,
which had been neutralized previously with 0.3 M Ca(OH)₂.
kg of dry peat⁻¹ and sterilized by γ-irradiation with ⁶⁰Co for
3 h at 1 megarad h⁻¹, was sprayed with 100 ml of cell
suspension of strain DY44. The peat was then packed into a
glass column (50 mm in inner diameter by 500 mm in height),
and the packing volume, density, height, initial moisture
content, initial pH of the peat, and initial cell concentration
were adjusted to 0.4 liter, 115 g of dry peat liter⁻¹, 20 cm,
70%, 7, and 5 × 10¹⁰ CFU g of dry peat⁻¹, respectively. H₂S
gas (150 to 250 µl liter⁻¹) was supplied to the column at a
flow rate of 200 ml min⁻¹ (space velocity [i.e., aerated
volume at standard state/packed volume of peat], 36 h⁻¹),
and the inlet and outlet concentrations were intermittently
measured. Sterilized water (50 ml) was sprayed manually
every 2 days to adjust the moisture content to around 70%.
The pH of the drainage water was also monitored.

RESULTS

Characteristics of strain DY44. DY44 was isolated as a
strain commensal with the DMDS-degrading bacterium T.
thioparus DW44 in thiosulfate-agar medium from DMDS-
acclimated peat (3, 4).

Its characteristics are as follows: colony morphology on
nutrient-agar plate, lemon yellow, semitranslucent, round,
regular, entire, shiny and low convex, old colony turns
brown; cell morphology, rod shaped; mobile; gram negative;
non-spore forming; growth, positive at 25 and 37°C and
negative at 41 and 45°C; catalase positive; cytochrome
oxidase positive; acid from glucose, negative; arginine de-
hydrolase, negative; urease negative; β-galactosidase posi-
tive; phosphatase positive; NO₃ reduction, negative; assim-
ilation positive for glucose, N-acetylglucosamine, maltose,
and acetate; assimilation negative for arabinose, mannose,
mannitol, gluconate, caprate, adipate, malate, citrate, and
phenylacetate; hydrolyzes Tween 80, gelatin, and casein;
weakly hydrolyzes esculin and urea; hydrolysis of starch is
restricted; does not produce hydrogen sulfide, indole, levan,
DNase, arginine dihydrolase, or lysine decarboxylase; neg-

ative for pigment production in King’s B medium; negative
for alkalization of tartrate; negative for egg yolk agar opac-
ity; no change in test of reaction in litmus milk. Its strong
proteolytic capabilities preclude it from being identified
among the yellow-pigmented Pseudomonas spp. On the
basis of its characteristics, strain DY44 was determined to be
a Xanthomonas sp.

H₂S removal in batch system. Figure 2 shows H₂S removal
by strain DY44 and by cell extract in the basal mineral medium.
No change of H₂S concentration was observed in the
cell-free basal mineral medium. However, 10 µmol of
H₂S was removed within 1.5 h by the viable cells. Although
no viable cells appeared on the nutrient-agar medium after
treatment by γ-irradiation, the γ-irradiated cell suspension
showed nearly the same ability of H₂S degradation as that of
viable cells. No H₂S degradation was observed for autoclaved
cells. H₂S degradation by the cell extract was obvious in
comparison with that in 10 mM Tris-HCl buffer. H₂S
removal by strain DY44 was therefore considered to be
biological. The optimum pH and temperature for H₂S de-
gradation by strain DY44 were determined to be 7 and 30°C,
respectively (data not shown).

Growth pattern and specific H₂S removal rates in different
growth phases. Figure 3a shows the growth pattern of strain
DY44 in the nutrient medium. Its growth was accompanied
by an increase of pH in the culture broth, and maximum
growth was attained after 1.5 days. The H₂S removal rate by
strain DW44 was apparently greater after 4 days than after 1
day (Fig. 3b). After 5 days, the rate fell to about one half of the 4-day value, mainly because of autolysis of the cells (data not shown), reflecting the fact that H$_2$S-removing activity is at a maximum during cultivation time. From the slope of Fig. 3b, the specific H$_2$S removal rates of cells cultured for 1 and 4 days in the nutrient medium were determined as 1.42 and 3.92 mmol g of dry cells$^{-1}$ h$^{-1}$, respectively.

**Removal of H$_2$S in continuous supply in basal mineral medium.** The removal pattern of H$_2$S which was continuously supplied at 100 ml min$^{-1}$ in the basal mineral medium is shown in Fig. 4b. *Xanthomonas* sp. strain DY44 removed H$_2$S gas without lag time, indicating that the intracellular enzymes responsible for H$_2$S degradation are constitutive. Viable cell counts gradually decreased, mainly because of cell autolysis. No change in the pH of the culture broth was found (Fig. 4a). The total amount of H$_2$S removed by strain DY44 in the basal mineral medium over 16 days was estimated to be 0.1 mol g of dry cells$^{-1}$, which was nearly the same as that for $\gamma$-irradiated cells, 0.11 mol g of dry cells$^{-1}$.

**H$_2$S removal in the presence of organic compounds.** *Xanthomonas* sp. strain DY44 assimilates glucose, acetate, and maltose, and its growth in the basal mineral medium supplemented with these carbon sources (2 g liter$^{-1}$) was confirmed (data not shown). In the presence of these compounds, H$_2$S removal by this strain was studied with a batch system (Fig. 5a). The cell-free basal mineral medium supplemented with these compounds showed no decline in H$_2$S concentration (data not shown). However, H$_2$S degradation was observed in all of the flasks inoculated with the cells. The presence of organic compounds had no significantly adverse effects on H$_2$S removal rates, although the rate in the presence of glucose was relatively slower.

Figure 5b shows the removal of continuously supplied H$_2$S in the basal mineral medium with or without yeast extract. H$_2$S degradation with only strain DY44 in the basal mineral medium finally deteriorated in a way similar to that seen in Fig. 4. This reflects a decrease in the viable cell number, as shown in Table 1. The addition of yeast extract to the basal mineral medium resulted in longer-lasting and more-efficient H$_2$S degradation. This is mainly because the growth of strain DY44 was significant, as can be seen in Table 1. It is obvious that cell-free yeast extract in the medium reacted with H$_2$S chemically, and an apparent decrease in H$_2$S concentration.

**TABLE 1.** Changes of cell number in strain DY44 during continuous supply of H$_2$S in the basal mineral medium with or without 2 g of yeast extract liter$^{-1}$

<table>
<thead>
<tr>
<th>Medium</th>
<th>No. of cells (CFU ml$^{-1}$)</th>
<th>Initial</th>
<th>After supply of H$_2$S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 days</td>
</tr>
<tr>
<td>Without yeast extract</td>
<td>1.75 x 10$^9$</td>
<td>4.61 x 10$^8$</td>
<td>2.38 x 10$^7$</td>
</tr>
<tr>
<td>With yeast extract</td>
<td>1.75 x 10$^9$</td>
<td>2.45 x 10$^{10}$</td>
<td>1.11 x 10$^{10}$</td>
</tr>
</tbody>
</table>
was observed. This fact indicates that the selection of the medium is of primary importance in verifying the purely biological degradation of H₂S.

**Identification of product of removed H₂S.** During continuous supply of H₂S in the basal mineral medium (Fig. 4), whitish-yellow precipitates appeared in the culture broth. No anionic sulfur compounds were detected in the filtrate of the culture broth by ion chromatography. Figure 6 shows the spectra by XPS analysis for lyophilized samples of culture broth without (Fig. 6a) and with (Fig. 6b) H₂S supply. Sulfur peaks, S₂s and S₂p, appeared in the H₂S-supplied culture at 227.4 and 163.1 eV, respectively. The binding energy value of S₂p was very close to that of elemental sulfur, 164.05 eV (24), but this difference of 1 eV is significant. Two peaks at S₂s and S₂p were clearly observed when a mixture of lyophilized culture supplied with H₂S and elemental sulfur was analyzed (Fig. 6c). When the lyophilized sample of H₂S-supplied culture broth was extracted by CS₂, elemental sulfur was obtained. On the basis of these results, the metabolite of H₂S oxidized by strain DY44 was judged to be polysulfide, which has properties very similar to elemental sulfur.

**H₂S removal in the presence of sulfur-containing gases.** The H₂S removal pattern in the presence of the sulfur-containing gases MT, DMS, and DMDS is shown in Fig. 7. Strain DY44 removed H₂S in the presence of MT, DMS, or DMDS, although the H₂S removal rate in the presence of MT was 40% of that in the presence either DMS or DMDS. The strain had the ability to remove MT, but the removal rate was only 1/100 that of H₂S removal (data not shown). Neither DMS nor DMDS was removed in a single gas or in mixed gases with H₂S by this strain (data not shown).

**H₂S removal in a peat biofilter.** Strain DY44 was inoculated into peat, and its H₂S degradation ability on the peat column shown in Fig. 1 was tested. Figure 8 shows the H₂S removal pattern in the peat biofilter. Clearly, strain DY44 exhibited
the ability to degrade H$_2$S on the peat without a lag time. No decline of pH in drainage water was detected during the experimental period.

**DISCUSSION**

We have demonstrated the characteristics of H$_2$S removal by Xanthomonas sp. strain DY44 isolated from DMDS-acclimated peat. Diverse microorganisms capable of H$_2$S oxidation have previously been reported: colorless sulfur bacteria such as Thiobacillus spp. (4, 5, 12, 18, 21, 23), Thiothrix sp. (1), and Beggiatoa sp. (2); methylotrophs such as Hyphomicrobium sp. (20); photosynthetic such as Chlorobium spp. (11, 17), Chromatium sp. (7), Rhodobacter sp. (8), and Ectothiorhodospira sp. (22); and cyanobacteria (15). However, there have been few reports on H$_2$S oxidation by aerobic heterotrophic microorganisms (16), and this is the first extensive report on H$_2$S removal by a heterotrophic bacterium.

H$_2$S removal by Xanthomonas sp. strain DY44 seems to be specific to this strain because four strains of Xanthomonas campestris (03-01420, 03-01424, 03-01426, and 03-01428) supplied by the National Institute of Agrobiological Resources (Tsukuba, Japan) were subjected to H$_2$S removal tests, but no H$_2$S removal was detected (data not shown). The specific H$_2$S removal rate by 4-day-old cells in the nutrient medium was maximum at 3.92 mmol g of dry cells$^{-1}$ h$^{-1}$ (6.7 x 10$^{-19}$ mol cell$^{-1}$ h$^{-1}$) (Fig. 3). As strain DY44 was isolated as a strain commensal with T. thioparus DW44, we speculate that T. thioparus DW44 and Xanthomonas sp. strain DY44 are major bacteria able to degrade H$_2$S in the neutral pH region and in environmental conditions where the concentration of organic matter is high. We have already reported another autotrophic bacteria isolated from a peat biofilter as H$_2$S-oxidizing bacteria, namely Thiobacillus intermedius (23) and Thiobacillus sp. strain HA43 (5), in which optimal activity was expressed in the acidic pH region.

The autoclaved cells (120°C, 20 min) did not degrade H$_2$S, but the cells treated by γ-irradiation showed nearly the same H$_2$S-degrading ability as that of viable cells, and the cell extract degraded H$_2$S, suggesting the existence of a heat-stable intracellular enzymatic system for H$_2$S oxidation (Fig. 2). The fact that strain DY44 cultivated in the nutrient medium degraded H$_2$S immediately after the supply of H$_2$S started (Fig. 4) suggests that the intracellular enzymatic system for H$_2$S degradation formed in the nutrient medium was constitutive. After 16 days of H$_2$S degradation, H$_2$S removal stopped and the cell number decreased, indicating that H$_2$S removal was not a consequence of chemolithotrophic activity. Since this strain oxidized neither thiosulfate nor elemental sulfur in the basal mineral medium, and no growth of the strain was detected (data not shown), it is obvious that it does not grow chemolithotrophically with only these sulfur compounds. On the other hand, this strain degraded H$_2$S in the presence of organic compounds such as glucose, acetate, maltose, and yeast extract (Fig. 5), and its growth in these compounds was not affected by the presence of H$_2$S. These results suggest that H$_2$S degradation is not closely linked to the utilization of organic compounds which are associated with energy production. The oxidation of H$_2$S by Xanthomonas sp. strain DY44 is speculated to be physiologically an H$_2$S detoxification process.

The facts that whitish-yellow precipitates were generated in the culture broth during the supply of H$_2$S, no decline of pH was observed (Fig. 4), and no anionic sulfur compounds were detected in the culture filtrate indicate that H$_2$S removal mechanism of this strain is different from that of the autotrophic bacteria reported previously (4, 5, 12, 18, 21, 23). Actually, the product of H$_2$S removal by Xanthomonas sp. strain DY44 was identified as polysulfide by analysis with XPS (Fig. 6). During H$_2$S oxidation by strain DY44, O$_2$ consumption was confirmed with a dissolved-oxygen electrode, suggesting that O$_2$ was required for H$_2$S oxidation (data not shown). A detailed biochemical study on the mechanism of H$_2$S oxidation in Xanthomonas sp. strain DY44 is in progress.

There are several advantages of strain DY44 for practical application in removing H$_2$S in a peat biofilter. Although the specific H$_2$S removal rate of strain DY44 is lower than those of purified Thiobacillus spp. (4, 5, 23), harvesting of the cell mass for inoculation is easy because of its rapid growth in the nutrient medium. No pH change was detected in the peat biofilter inoculated with strain DY44 mainly because the final product was polysulfide (Fig. 8). Sulfate is a final product of most autotrophic H$_2$S-oxidizing bacteria, and pH decline is significant. Polysulfide as a final product is preferable to sulfate, chiefly because a deterioration of microbial activity due to a decline in pH will not occur. We have also already demonstrated that the removability of MT, DMS, and DMS, as well as of H$_2$S, was sensitive to a decline in the pH of the peat (3, 10, 25). Considering the result shown in Fig. 8b, higher efficiency in H$_2$S removal can be obtained by supplying organic compounds such as treated wastewaters to a peat biofilter inoculated with Xanthomonas sp. strain DY44. The fact that removal of H$_2$S was not influenced by the coexistence of MT, DMS, or DMD (Fig. 7) suggests that this bacterium can be applied to the treatment of exhaust gases in wastewater or night soil treatment plants where these gases are the main malodorous components. Xanthomonas sp. strain DY44 can be also used to improve the removability of MT, DMS, and DMD in mixed cultures with MT-, DMS-, and DMD-degrading microorganisms, where the removability of these compounds is inhibited by the coexistence of H$_2$S.

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**REFERENCES**