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Isolation of a dimethylsulfide-utilizing *Hyphomicrobium* species and its application in biofiltration of polluted air

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Abstract

The methylotrophic bacterium *Hyphomicrobium* VS was enriched and isolated, using activated sewage sludge as inoculum in mineral medium containing dimethylsulfide (DMS) at a low concentration to prevent toxicity. DMS concentrations above 1 mM proved to be growth inhibiting. *Hyphomicrobium* VS could use DMS, dimethylsulfoxide (DMSO), methanol, formaldehyde, formate, and methylated amines as carbon and energy source. Carbon was assimilated via the serine pathway. DMS-grown cells respired sulfide, thiosulfate, methanethiol, dimethyldisulfide and dimethyltrisulfide.

To test *Hyphomicrobium* VS for application in biofiltration of air polluted with volatile sulfur compounds two laboratory scale trickling biofilters with polyurethane and lava stone as carrier material were started up by inoculation with this bacterium. Both methanol- and DMS-grown cells could be used. Only a short adaptation period was needed. Short term experiments showed that high concentrations of DMS (1–2 μmol l^{-1}) were removed very efficiently by the biofilters at space velocities up to 100 h^{-1}.

Abbreviations: VSC – volatile sulfur compounds, DMS – dimethylsulfide, DMDS-dimethyldisulfide, DMTS – dimethyltrisulfide, MT – methanethiol, DMSO – dimethylsulfoxide

Introduction

Formation of volatile sulfur compounds (VSC), in particular dimethylsulfide (DMS) has been observed in various microbial ecosystems such as soils, algal mats, compost stacks and sewage treatment systems, as well as in several industrial processes such as brewing and paper manufacture (Kelly & Smith 1990). Especially in marine environments large amounts of DMS are formed as a result of degradation of dimethylsulfoniopropionate, an osmoprotectant of algae (Kiene 1988). The production of VSC causes environmental problems due to their high odor index and toxicity. Moreover it has been shown that sulfur-containing trace gases are oxidized by the OH· radical in the troposphere to SO_2, which causes acid rain and contributes to cloud formation (Charlson et al. 1987). As a result of more stringent rules on the emission of harmful gases to the atmosphere the need was felt for a DMS-utilizing microorganism, which could be used in the construction of a biological filter for DMS removal. Such a filter should be applicable for treatment of waste gases originating from for instance indoor composting facilities (Derikx et al. 1991) and sewage treatment plants (Buisman et al. 1990). Biological deodorization of VSC is now attracting attention because of the low operating cost and high ability as compared with chemical or physical processes (Kanagawa & Kelly 1986; Shoda 1991). The isolation and characterization of a *Hyphomicrobium* species able to utilize DMS as a carbon and energy source, and initial attempts to use this

strain for purification of DMS-containing air streams are presented in this study.

Materials and methods

Media

A mineral medium was used for enrichment and cultivation. The medium contained (in g l^{-1}): K_2HPO_4, 0.4; KH_2PO_4, 1.0; NH_4Cl, 0.4; $MgCl_2 \cdot 6H_2O$, 0.1; $CaCl_2 \cdot 2H_2O$, 0.01; Na_2CO_3, 0.5; and 2 ml l^{-1} trace element solution. Trace element solution was prepared according to Suylen & Kuenen (1986). The final pH was adjusted to 7.1. Substrates tested for growth experiments were added to a final concentration of 10 mM except for formaldehyde and sulfur compounds, which were tested at 1 mM.

Enrichment and isolation

Enrichment of DMS-utilizing bacteria, using activated sewage sludge as inoculum, was achieved at room temperature in mineral medium containing DMS at a low concentration (0.015 mM liquid concentration during the first transfers and 0.4 mM for the following ones) as the only source of carbon and energy. Enrichment was performed in 120 ml stoppered bottles containing 20 ml medium, and DMS was added by syringe; after depletion of DMS new amounts were added. After 6 transfers agar plates, composed of the same mineral medium plus 1.5% (w/v) agar, were inoculated with the enrichment culture and incubated at 30 °C in a jar containing 15 mmol l^{-1} DMS in the gas phase. Purity of cultures was checked microscopically after growth in defined medium. Additional purity tests were performed with mineral medium enriched with glucose, yeast extract and tryptone, each 2 g l^{-1}. The latter medium does not allow growth of methylotrophic hyphomicrobia. Growth tests were performed at least in duplicate at 30 °C.

Continuous cultivation

Continuous cultivation was performed at 30 °C in a 350 ml glass fermentor (New Brunswick Scientific, Nutley, NJ, USA). Methanol was added to the medium after sterilization to a final concentration of 20 mM. DMS as growth substrate was added by leading an air stream containing DMS through the culture liquid. The pH was maintained at 7.0 by automatic titration with 0.2 M NaOH. Sulfide was added to a methanol-limited culture to study the effect on the yield. Sulfide was sterilized under nitrogen and separately introduced under the liquid level of the culture.

Respiration measurements

Respiration rates of whole cells were measured polarographically in a Biological Oxygen Monitor, with a Clark-type oxygen electrode at 30 °C. Rates were expressed as nmol O_2 min^{-1} mg $protein^{-1}$ and were corrected for endogenous respiration.

Preparation of cell-free extracts

Cell-free extracts were prepared by suspending cells (0.5 g wet weight per ml) in 50 mM Tris-HCl buffer (pH 7.0) and breaking them by passage through a French pressure cell at 110 MPa. After centrifugation at 40,000 × *g* at 4 °C for 20 min the supernatant (crude extract) was directly used or stored at - 20 °C.

Enzyme assays

Methanol dehydrogenase was assayed at pH 9.0 according to Duine et al. (1978). Methanethiol (MT) oxidase activity was determined gaschromatographically by measuring the disappearance of MT in the headspace of an incubation mixture (total volume 1.2 ml) in a stoppered 100 ml bottle. The mixture contained 50 mM Tris-HCl buffer, pH 8.2, 1 μmol MT and about 0.15 mg of protein. DMS mono-oxygenase activity was assayed according to De Bont et al. (1981). NAD^+- and DCPIP-dependent formaldehyde dehydrogenase and formate dehydrogenase were assayed according to Suylen et al. (1986). NAD^+-dependent malate dehydrogenase was assayed by following the oxaloacetate-dependent oxidation of NADH at 340 nm. Reaction mixtures contained 25 mM glycylglycine buffer (pH 7.4), 0.24 mM NADH, 0.25 mM oxaloacetate and an appropriate amount of cell extract (about 0.15 mg protein per ml). Hydroxypyruvate reductase was determined according to Blackmore & Quayle (1970) except that 50 mM phosphate buffer (pH 7.0) was used as buffer. Catalase was assayed according to Haywood & Large (1981).

Air biofiltration

A lab-scale biofilter (trickling filter principle) was constructed of a glass-cylinder (90 mm Ø × 450 mm)

packed either with oblong pieces (15 cm in length) of polyurethane, having a T-shaped cross-section (10 × 10 × 3 mm), or with lava stone (8–16 mm Ø). DMS-containing air was saturated with water and supplied at the bottom of the cylinder at room temperature. The air flow is expressed as space velocity, i.e., the flow rate divided by the biofilter volume. From a 1-liter container growth medium was recirculated intermittantly in order to remove the sulfuric acid produced in the biofilter. Every 30 min 0.5 liter of medium was sprayed on top of the packing material via a perforated plate. This took about 10 s. The medium was pH regulated between 6.7 and 7.0 by simultaneous addition of fresh medium and a NaOH solution (0.2 M). In this way the concentration of Na_2SO_4 never exceeded 20 mM. The filter was inoculated by replacing 400 ml of the recirculation fluid with a culture of *Hyphomicrobium* VS with an optical density at 600 nm (OD_{600}) of about 1.0.

Analytical procedures

DMS and MT were determined gaschromatographically as described earlier (Derikx et al. 1990). For cell-free extracts protein was determined with the Bio-Rad (Bio-Rad Laboratories, Richmond, CA, USA) protein reagent with bovine serum albumin as a standard. For protein analysis of whole cells the Lowry method was used. Methanol was determined gaschromatographically according to Teunissen et al. (1989). Sulfate and thiosulfate were determined using the HPLC-system described by Van Alebeek et al. (1992).

Results

Isolation and characterization of a DMS-utilizing bacterium

A DMS-utilizing bacterium was successfully enriched using activated sludge as inoculum in a mineral medium in which DMS was present as carbon and energy source. Low concentrations of DMS were a prerequisite, since DMS concentrations above 1 mM proved to be growth inhibiting. After streaking on agar plates and incubation for about 14 days in a jar, containing DMS and air in the gas phase, small colonies were visible. Upon microscopical examination all colonies appeared to consist of *Hyphomicrobium*-like cells. A number of colonies were selected and all were able to grow on methanol. One colony was subcultured further and designated *Hyphomicrobium* strain VS. Cells were ovoid (0.5–1 × 1.2–2.3 μm) with a single polar hypha. Hyphae were up to three times the length of the cell and produced buds. Motility was observed for small cells and maturing buds. *Hyphomicrobium* VS was unable to grow on a complex medium containing glucose, yeast extract and tryptone. Its methylotrophic nature was shown by the ability to grow aerobically on a broad spectrum of reduced C_1-compounds (Table 1). No growth was found on urea, glyoxylate, lactate, pyruvate, malate, succinate, citrate, ribose, fructose, glucose, glycine, serine, β-alanine, asparagine, aspartate, acetate, butyrate, 3-hydroxybutyrate, dichloromethane, carbon disulfide, dimethyldisulfide (DMDS), dimethyltrisulfide (DMTS) and thiosulfate. Unlike many other *Hyphomicrobium* species, strain VS was unable to grow anaerobically on methanol with nitrate as electron acceptor. Growth on ethanol occurred only to a very small extent. However this substrate could be used very well as an additional source of energy and/or carbon since addition of ethanol (10 mM) to methanol (60 mM) cultures resulted in an increase in OD_{600} from 1.6 to 2.2. Yeast extract was stimulatory but did not increase the yield in batch cultures grown on methanol. Growth was observed between pH 6.0 and 8.0 with an optimum at pH 7.0. The temperature optimum was 30 °C.

Growth of Hyphomicrobium VS in batch and continuous culture

The maximum growth rate (μ_{max}) on methanol in batch culture was 0.14 ± 0.01 h^{-1} (n = 6). The yield (Y) amounted to 13.2 ± 0.7 (n = 5) g dry weight $mole^{-1}$. Values found for μ_{max} and YCH_3OH in a continuous culture were 0.12 h^{-1} and 13.4 ± 1.1 (n = 7) g dry weight $mole^{-1}$, respectively, at a dilution rate (D) of 0.1 h^{-1}. Due to toxicity of DMS and acidification of the medium during growth, studies with batch cultures on DMS were not easy to perform. To overcome this problem *Hyphomicrobium* VS was first grown batch-wise in the chemostat on methanol (60 mM) after which DMS addition was started and the D was set at 0.02 h^{-1}. The DMS concentration in the air stream was 100 μmol l^{-1}, which in the absence of consumption would result in an equilibrium concentration of 1.5 mM in the liquid phase. By increasing the air flow-rate addition of DMS was gradually increased within 5 days up to 15 mmol l^{-1} medium. During a cultivation period of 3 weeks the respiration rate for DMS was 244 nmol O_2 min^{-1} mg $protein^{-1}$. Increas-

Table 1. Growth and respiration of *Hyphomicrobium* VS.

Substrate	Growth[a]	Respiration rate (nmol O_2 min^{-1} mg $protein^{-1}$)							
		Methanol grown[b]				DMS grown[b]		DMS[c]	DMSO
		D=0.016	D=0.023	D=0.105	D=0.115	D=0.02	D=0.04	batch	batch
Methanol	+	308	322	381	319	91	127	73	13
Formaldehyde	+	156	303	n.d.[d]	278	113	n.d.	88	n.d.
Formate	+	0	24	n.d.	26	19	n.d.	13.5	n.d.
Ethanol	+/-	298	293	334	260	n.d.	n.d.	n.d.	n.d.
DMS	+	0	0	0	0	244	444–800	151	286
MT	n.d.	0	0	0	0	268	n.d.	106	137
DMDS	-	n.d.	n.d.	n.d.	n.d.	40	17	30	n.d.
DMTS	-	n.d.	n.d.	n.d.	n.d.	44	20	n.d.	n.d.
DMSO	+	n.d.[e]	n.d.	n.d.	n.d.	89	n.d.	n.d.	245
Sulfide	-	894	818	n.d.	1135	374	n.d.	n.d.	n.d.
Methylamine	+	0	0	0	0	0	0	0	n.d.
Dimethylamine	+	0	0	0	0	4	n.d.	6.4	n.d.
Trimethylamine	+	0	0	0	0	0	n.d.	0	n.d.
Thiosulfate	-	n.d.	0	0	0	51	n.d.	51	115

[a] Growth was tested as described under Materials and Methods, + indicates growth on basis of turbidity within a few days.

[b] Continuous cultures limited for the substrate at the indicated dilution rate (h^{-1}). Medium concentration for methanol and DMS were 20 mM and 15 mM, respectively.

[c] Methanol exhausted culture adapted to DMS.

[d] n.d. = not determined.

[e] Respiration of DMSO using batch-grown cells on methanol was found to be zero.

ing the D to 0.04 h^{-1} enhanced the DMS-respiration rate to 444 nmol O_2 min^{-1} mg $protein^{-1}$ after one week. A further increase was observed during the following two weeks of cultivation up to about 800 nmol O_2 min^{-1} mg $protein^{-1}$. A Y_{DMS} of about 16 g dry weight per mole was estimated. Yield measurements were negatively influenced by cell attachment to the wall and inner parts of the chemostat vessel. When the organism was grown on DMSO in batch culture a μ_{max} of 0.065 ± 0.015 h^{-1} (n = 3) was found. The substrate affinity constants (K_s) for DMS and methanol as estimated from the respiration rates at different substrate concentrations were both around 3 μM.

The high affinity for DMS resulted in very low concentrations of DMS in the gas outlet of the chemostat. A value of 2 nmol l^{-1} was found indicating a liquid concentration of 0.03 μM. Neither MT nor H_2S could be demonstrated in the gas outlet. HPLC-analysis revealed that the sulfur from DMS was quantitatively oxidized to sulfate.

Respiration of Hyphomicrobium VS grown under various conditions

Methanol-grown *Hyphomicrobium* VS cells were able to respire the growth substrates methanol, formaldehyde, formate and ethanol, but not the methylated amines, methylated sulfides and DMSO (Table 1). Sulfide however could be respired very actively. In order to see if energy could be derived from its respiration, sulfide was added at 3 mM feed concentration to methanol limited continuous cultures (D = 0.023 and 0.1 h^{-1}). An increase in yield was not observed. Moreover, DMS and MT respiration were not induced. Upon addition of limiting amounts of sulfide (80 μM) during respiration measurements the ratio of oxygen consumed per mole of sulfide added was (1–1.2):1 for methanol-grown cultures, indicating an initial oxidation product at the level of thiosulfate. The same ratio was found both for the methanol plus sulfide- and for the DMS-grown chemostat cultures. However, for the latter cultures the respiration curves were biphasic: the final rates were significantly higher compared to the

endogenous respiration, indicating a slow sequential oxidation of the initial product. Indeed DMS-grown cells respired thiosulfate, although at a slower rate than sulfide. Methanol-grown cells did not respire thiosulfate but this activity could be rapidly induced by the addition of thiosulfate (5 mM) to the chemostat.

After addition of DMS (< 0.15 mM) to a methanol-depleted batch culture the capacity to respire methylated sulfides was observed after a further 4 h incubation period, and a maximum respiration rate of DMS of about 150 nmol O_2 min^{-1} mg $protein^{-1}$ was achieved after 18 h.

Cells grown on DMS in a continuous culture were more versatile in their respiration compared to methanol-grown cells (Table 1). Cells grown on DMSO were also able to respire MT, DMS, thiosulfate and to a much lesser extent methanol.

Enzyme activities

Cell-free extracts of methanol-grown *Hyphomicrobium* VS cells displayed high activities of methanol dehydrogenase, formaldehyde dehydrogenase and formate dehydrogenase (Table 2). Moreover, high activities of malate dehydrogenase, hydroxypyruvate reductase and catalase were measured. This indicates that the methanol assimilation of strain VS is similar to that of other *Hyphomicrobium* species and follows the serine pathway (Harder et al. 1973). DMS-grown cells also possessed the above-mentioned carbon assimilating and dissimilating enzyme activities (data not shown). Extracts from DMS-grown cells further contained a MT oxidase activity of about 200 nmol min^{-1} mg $protein^{-1}$. All attempts to demonstrate in cell-free extracts DMS mono-oxygenase, the enzyme proposed to be responsible for the first step in the assimilation of DMS, were not successful.

Application of Hyphomicrobium VS in air biofiltration

A biofilter (2.5 l volume) with polyurethane as carrier material was started up by inoculation with a DMS-adapted culture of *Hyphomicrobium* VS (Fig. 1). The initial air flow rate in this experiment was set at a space velocity of 6.25 h^{-1} with a DMS concentration of about 1 μmol l^{-1}. Outlet DMS concentrations during start-up were below the detection limit (< 0.6 nmol l^{-1}). After 4 days the concentration was increased to 4 μmol l^{-1} resulting in a transient increase in outlet DMS concentration. After 11 days the space velocity was increased to 24 h^{-1}. The outlet concentration increased

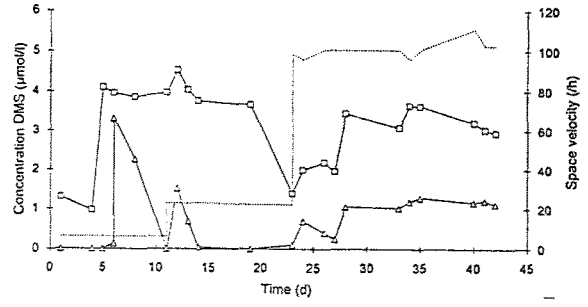

Fig. 1. Removal of DMS from an air stream in a trickling biofilter filled with polyurethane. Symbols: DMS concentration in air inlet (□), and outlet (△). The dotted line indicates the air space velocity.

to 1.5 μmol l^{-1}, but decreased to 0.05 μmol l^{-1} within 3 days. At day 23 the space velocity was increased further to about 100 h^{-1} with a concomitant decrease in the DMS concentration of the inlet to 1.4 μmol l^{-1}. The outlet DMS concentration increased to 0.1 μmol l^{-1} as a result of an increased load. Subsequently inlet DMS concentrations were gradually increased (day 24 to 27: 2 μmol l^{-1}; from day 27: 3–3.6 μmol l^{-1}). This gradual increase resulted in a more or less constant high outlet concentration of 1.1 μmol l^{-1} (from day 27 onwards). The biofilter was now operating at a capacity of 5.3 mmol l^{-1} d^{-1}. No other VSC were detectable in the outlet.

A biofilter of the same dimensions, packed with lava stone, was started by inoculation with a methanol-grown culture of *Hyphomicrobium* VS not yet adapted to DMS (Fig. 2). A high space velocity of 80 h^{-1}, was applied from day 0. The inlet DMS concentration gradually decreased from 2.5 at day 0 to 1.1 μmol l^{-1} at day 20. Rapid adaptation to DMS was obvious from the high removal rate from the first day on (2.3 mmol l^{-1} d^{-1}). Outlet concentrations did not exceed 0.05 μmol l^{-1} from day 15 onwards. An increase in the inlet concentration (at day 24) did not increase the outlet concentration, indicating that the biofilter was not operating at maximum capacity. Compared to the second week during which the filter was operated at the same loading, the removal capacity had clearly increased at the end of the experiment.

Discussion

By using low, non-toxic, concentrations of DMS, we succeeded in the isolation of a *Hyphomicrobium*

Table 2. Enzyme activities in cell-free extracts of methanol-grown *Hyphomicrobium* cells.

Enzyme	Coenzyme	Specific activity (μmol min^{-1} mg^{-1})
Methanol dehydrogenase	DCPIP	0.24
Formaldehyde dehydrogenase	DCPIP	0.17
	NAD^+	0.05
Formate dehydrogenase	DCPIP	< 0.01
	NAD^+	0.22
Malate dehydrogenase	NAD^+	0.49
Hydroxypyruvate reductase	NADH	2.95
Catalase		188

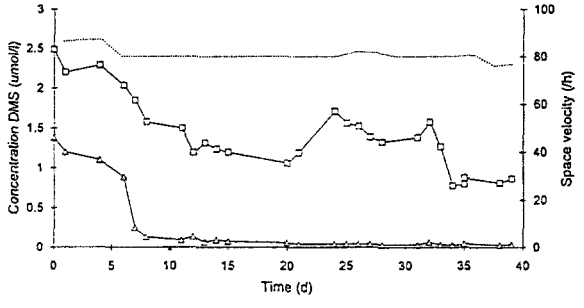

Fig. 2. Removal of DMS from an air stream in a trickling biofilter filled with lava stone. Symbols: DMS concentration in air inlet (□), and outlet (△). The dotted line indicates the air space velocity.

species, designated *Hyphomicrobium* strain VS. Up till now DMS-utilizing *Hyphomicrobium* species were all enriched on DMSO (De Bont et al. 1981; Suylen & Kuenen 1986; Zhang et al. 1991). Besides hyphomicrobia some *Thiobacillus* species have been shown to grow aerobically on DMS. These microorganisms were enriched on inorganic sulfur compounds (Cho et al. 1991a; Kanagawa & Kelly 1986). Many growth characteristics and respiration features of our isolate were similar to *Hyphomicrobium* EG isolated by Suylen et al. (1986) and *Hyphomicrobium* S isolated by De Bont et al. (1981). However, in contrast to these isolates, *Hyphomicrobium* VS was able to grow on methanol. In this respect it seems more related to *Hyphomicrobium* I55 recently isolated by Zhang et al. (1991) which was able to use DMS, DMSO and methanol for growth. Our isolate was even more versatile since growth also occurred on formaldehyde, formate and methylated amines, and to a lesser extent on ethanol. On methanol the growth rate (0.14 h^{-1}) is among the highest reported for hyphomicrobia (Harder & Attwood 1978).

The respiratory enzymes for methylated sulfur compounds of *Hyphomicrobium* VS were not constitutive. In *Hyphomicrobium* EG these enzymes were constitutive, but the levels could be enhanced by addition of sulfide (Suylen et al. 1986). This was not observed for *Hyphomicrobium* VS. Induction of these enzymes was achieved readily by DMS: methanol-grown cells fully adapted to DMS within one day in batch culture. Further, a biofilter inoculated with methanol-grown cells acquired the ability to remove DMS from an airstream within one day. DMS mono-oxygenase activity, the first enzyme in DMS metabolism, was not found in cell-free extracts. Attempts to demonstrate this enzyme in *Hyphomicrobium* EG also failed (Suylen 1988), while in *Hyphomicrobium* S a very low activity (0.001 μmol min^{-1} mg protein^{-1}) could be measured (De Bont et al. 1981). A possible explanation may be that this enzyme needs a functional electron transport chain to provide the reducing equivalents for DMS-monooxygenase.

Sulfide respiration was constitutive and occurred at a high rate. The results indicated that cells quickly oxidize sulfide up to the level of thiosulfate. Thiosulfate is then further oxidized at a lower rate. Indeed, thiosulfate could be identified by HPLC analysis as an intermediate during sulfide respiration (data not shown). Thiosulfate formation was detected in methylamine- plus sulfide-grown chemostat cultures of *Hyphomicrobium* EG (Suylen et al. 1986) and oxidation of sulfide to thiosulfate and elemental sulfur was reported recently for *Hyphomicrobium neptunium* by Sasahira et al. (1991, 1992). However, for the latter organism methylated or

inorganic sulfur compounds were not reported to act as carbon or energy source. Yield data for *Hyphomicrobium* EG demonstrate that energy was derived from S-oxidation to the level of sulfate (Suylen et al. 1986). Oxidation of DMS by DMS-grown *Hyphomicrobium* VS cells resulted in sulfate formation as confirmed by HPLC analysis. Whether the S-metabolic pathway of DMS will gain energy remains to be studied.

Hyphomicrobium VS looks a promising organism for application in biofiltration of air containing DMS. It can easily be cultured at a large scale on methanol. Only a short period for adapatation to DMS was needed. From short-term experiments (circa 45 days) it was concluded that high concentrations of DMS (1–2 μmol l^{-1}) were removed very efficiently at relatively high space velocities (up to 100 h^{-1}): final outlet concentrations obtained were 0.05 μmol l^{-1} (1 ppm). This value is still well above the odor threshold level. However the inlet concentrations applied exceeded that of most industrial air streams, so outlet concentrations below the odor threshold may be achievable at lower inlet concentrations. Thus far efficient removal of volatile sulfur compounds was found by the use of peat biofilters inoculated with *Hyphomicrobium* I55 (Zhang et al. 1991), *Thiobacillus* species (Cho et al. 1991a,b, 1992a) or mixed cultures (Cho et al. 1992b). Removal rates reported for DMS are in the same order of magnitude as our values. All experiments were done with lab-scale reactors, no reports are published on industrial scale. Peat biofilters may have a disadvantage if acidification occurs as a results of microbial degradation of the contaminants present in the waste gas. This problem can be circumvented by spraying the peat with water (Cho et al. 1992a). The use of a biological trickling filter is a good alternative. In this type of reactor the waste gas is forced to rise through a column with inert packing material, on which the suitable micororganisms are immobilized. By the recirculation of water through the system, acid produced is continuously removed. Further, the liquid recirculation allows control of optimal physiological conditions i.e., pH, medium addition. Hence optimal microbial activity can be maintained. The suitability of such a biofilter with immobilized *Thiobacillus thioparus* was demonstrated for the removal of volatile sulfur compounds (Tanji et al. 1989). Although *Hyphomicrobium* was successfully applied for removal of dichloromethane in a trickling biofilter (Diks 1992), use of this organism for removal of volatile sulfur compounds in such reactors was not described before. An extra advantage of *Hyphomicrobium* VS for odor treatment is its ability to degrade methylated amines.

Both polyurethane and lava stone appeared to be good carrier materials for immobilizing *Hyphomicrobium* VS in trickling filters. It was shown that the carrier material had a large effect on the maximum removal rates of volatile compounds in exhaust gas (Kirchner et al. 1987; Tiwaree et al. 1992), Further studies will therefore focus on use of different package materials and long term operation at concentrations equal to those found in industrial exhaust gases.

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