

Odorous Sulfur Compounds Emitted during Production of Compost Used as a Substrate in Mushroom Cultivation

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Large-scale composting facilities are known to cause environmental problems, mainly through pungent air emitted by composting material. In air samples taken above stacks set up to prepare compost used as a substrate in mushroom cultivation, several volatile compounds were identified by means of the coupled techniques of gas chromatography and mass spectrography. Among the compounds identified, sulfur-containing compounds [H_2S , COS , CH_3SH , CS_2 , $(CH_3)_2S$, $(CH_3)_2S_2$, and $(CH_3)_2S_3$] are the most conspicuous in causing a nuisance. Quantification of these compounds was performed by concentrating a relatively small air sample on Tenax GC. The sampling method appeared to be very useful under field conditions. During the composting process, the concentration of the volatile sulfur compounds in emitted air ranged from 1 to $35 \mu\text{mol}/\text{m}^3$. The highest concentrations were obtained at the end of the outdoor process. Total sulfur emission amounted to 8.3 mg of sulfur per kg (fresh weight) of compost. The end product still contained 2.58 g of sulfur per kg (fresh weight) of compost. Suggestions about the origin of the volatile sulfur compounds are made.

Among the edible fungi, *Agaricus* spp. constitute more than 75% of the total world production. In The Netherlands, 115,000 tons of mushrooms were produced in 1987 (15). The substrate for the cultivation of *Agaricus* spp. is a specially prepared compost. Straw-rich horse manure and chicken manure are the main constituents. As a result of the steadily growing demand for compost, the amount of horse manure is no longer sufficient and therefore wheat straw has become a more important basic constituent. The process of composting has been described in detail in previous papers (6, 22).

During phase I of the composting process, microbial degradation of readily available organic compounds is stimulated by thorough wetting and mixing of the constituents. On day zero, prewetted wheat straw and horse manure are mixed and set up in a large pile. On day 8, gypsum and chicken manure are added. After thorough mixing, the material is placed in stacks (approximately 1.8 by 1.8 m in cross-section) for 7 more days. In 2 weeks, the outdoor process is completed, resulting in a dark brown product.

In the course of phase I, the temperature rises gradually to an average of 63°C at the end of phase I. Because of heat loss at the outside of the stacks, temperature gradients range from 25 to as high as 80°C in the inner parts of the stacks. As a result of these temperature gradients, air moves through the stacks and water vapor and malodorous compounds are transported to the environment. During phase I, considerable amounts of ammonia are released as a result of ammonification (21), but the identities of other compounds present in the air are still unknown. Miller and Macauley (13) compiled a list of compounds implicated in odors from different composting materials. Among these, especially sulfur- or nitrogen-containing volatile compounds are known to cause a nuisance at very low concentrations (23). The present paper deals with the identification and quantification of several volatile sulfur compounds present in air emitted

from stacks during phase I of the composting process performed to produce a substrate for mushroom cultivation.

MATERIALS AND METHODS

Sampling site. Samples of effluent air above compost stacks were taken. A bottomless stainless steel barrel (1.20 m tall; inner diameter, 0.64 m) was placed on top of the stacks to minimize the influence of wind (Fig. 1). The barrel had an opening at the top (inner diameter, 2.5 cm) and a small sampling tube at the side which ended in the center. After positioning of the barrel, the air inside was allowed to stabilize for at least 15 min. Air temperature and velocity were measured at the top opening with a Wallac GGA-23S thermoanemometer.

Sulfur determination. To measure total organic and inorganic sulfur contents, compost samples were dried at 70°C and milled to pass through a 5-mm (aperture diameter) sieve. For total-sulfur analysis, samples were degraded as described by Tabatabai and Bremner (19). For inorganic sulfur analysis, samples were treated in the same way after being ashed at 550°C . Sulfate was measured by the inductively coupled plasma technique (3). Organic sulfur content was taken as the difference between the total and inorganic sulfur contents. Standard addition of methionine and $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ to samples resulted in recovery of 80 ± 10 and $100 \pm 11\%$, respectively, of the total sulfur estimations. When inorganic sulfur was estimated, the same additions yielded recoveries of 2 ± 8 and $97 \pm 9\%$, respectively. This proves that organic sulfur is completely volatilized by the ashing procedure.

Qualitative analysis of air samples. Large-volume (30-liter) air samples were obtained via the top opening of the barrel with a Dupont P-4000 pump. Air was sucked through charcoal tubes (70 by 4 mm [inner diameter]) consisting of two compartments. The first contained 100 mg of charcoal, and the second contained 50 mg and served as a control to test overloading (25). Tubes were positioned near the center of the barrel and connected with Teflon tubing to the pump.

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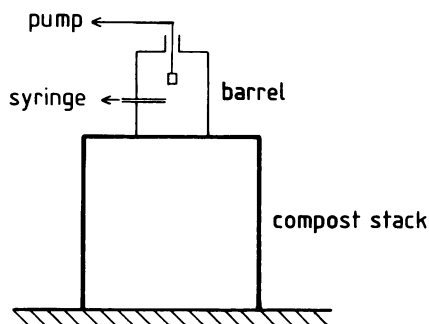


FIG. 1. Schematic diagram of the air sampling procedure used in this study. For more details, see the text.

Contact of the air sampled with any material before entering the tubes was avoided. The sample rate was adjusted to 500 ml/min and was checked before and after sampling. Tubes were closed with Teflon plugs immediately after sampling and stored at 4°C until analyzed. The charcoal was removed from the tubes, and compounds bound to the charcoal were desorbed with a minimal amount of carbon disulfide at room temperature for at least 1 h. Alternatively, benzyl alcohol was used in the same way to examine the occurrence of carbon disulfide in the air samples (10). Liquid samples obtained in this way were injected into a Hewlett-Packard 5790A gas chromatograph using either a flame ionization detector or a VG analytical 7070E mass spectrograph. Details of the gas chromatographic system are summarized in Table 1 (system A). The temperature of the mass spectrograph source and the ionization energy were 190°C and 70 eV, respectively.

Quantitative analysis of air samples. Small-volume (60-ml) air samples were taken from the side opening of the barrel with a Monoject syringe equipped with a Teflon piston. The sampling procedure used was that of Tangerman (20). Excess water was removed by passing the sample through a tube containing $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$. Thereafter, the sample was passed through a tube (80-mm length, 4-mm inner diameter, 6-mm outer diameter) containing 200 mg of Tenax GC (80/100 mesh). To trap volatile compounds, the Tenax tube was cooled in liquid nitrogen (-196°C). The Tenax tubes were kept at -196°C until analysis was performed. Tangerman (20) showed a negligible loss of volatile sulfur compounds on storage of the tubes for 1 week at -196°C . Analyses were performed within 24 h on a Packard 438A gas chromatograph equipped with a packed glass column. The

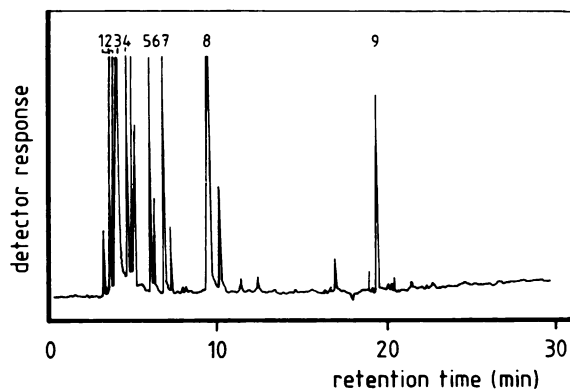


FIG. 2. Typical chromatogram obtained with system A by injection of 1 μl of the carbon disulfide phase after desorption. Unequivocally identified peaks are numbered as follows: 1, propanone; 2, dimethyl sulfide; 3, carbon disulfide; 4, butanone; 5, 3-methylbutanone; 6, benzene; 7, 2-pentanone; 8, dimethyl disulfide; 9, dimethyl trisulfide.

specifications of the two different systems are summarized in Table 1 (systems B and C). The special design of the inlet system of the gas chromatograph enabled replacement of a Tenax tube within seconds (20). As suggested in the literature (4, 18), the flame photometer was calibrated for every compound separately with authentic compounds; a range of 40 pmol to 1.7 nmol was used. Dilutions were made by using the single-rigid-chamber method as described by Barratt (2). To avoid adsorption, all glassware was treated with dichlorodimethylsilane (5% [wt/vol] in toluene) before use.

Chemicals. Dimethyl trisulfide was purchased from Eastman Kodak Co., Rochester, N.Y. All other chemicals originated from E. Merck AG, Darmstadt, Federal Republic of Germany.

RESULTS

Qualitative analysis of air samples. Figure 2 shows results of a typical qualitative analysis with system A (Table 1). Similar analyses were performed by using the mass spectrograph as a detector. The resulting mass spectra were compared with those of authentic compounds to identify the components. As additional evidence, retention times were checked with authentic compounds by using three gas chromatographic systems (Table 2). Analysis of the second section of the charcoal tube showed no signals other than

TABLE 1. Gas chromatographic systems used for identification and quantification of volatile compounds emitted during production of compost used as a substrate in mushroom cultivation

System	Packing	Column specifications				Gas chromatograph specifications			Temp program specifications				
		Length (m)	Outside diam (mm)	Inside diam (mm)	Nitrogen flow (ml/min)	Detector	Detector temp ($^\circ\text{C}$)	Injector temp ($^\circ\text{C}$)	Initial temp ($^\circ\text{C}$)	Initial time (min)	Temp rise ($^\circ\text{C}/\text{min}$)	Final temp ($^\circ\text{C}$)	Final time (min)
A	CP Sil % CB	25	0.45	0.32	1.5	FID ^a	300	270	40	10	10	200	10
B	20% SE-30 on Chromosorb PNAW (60/80 mesh)	1.50	6.0	4.0	90	FPD ^b	190	190	50	3	35	190	3
C	Carbopack B HT100 (40/60 mesh)	2.00	6.0	4.0	90	FPD	190	190	80	1	30	140	27

^a FID, Flame ionization detector.

^b FPD, Flame photometric detector.

TABLE 2. Physical properties^a and retention times of authentic compounds identified in the air above compost stacks during production of a substrate for mushroom cultivation

Compound	Boiling point (°C)	100% recognition level (ppm [μ l/liter])	Odor index at 20°C ^b	Retention time (min)		
				System A	System B	System C
Hydrogen sulfide	-60.7	1	1.70×10^7	x ^c	0.46	0.49
Carbonyl sulfide	-50.2	— ^d	—	x	0.49	0.78
Ammonia	-33.35	55	1.67×10^5	x	x	x
Methanethiol	6.2	0.035	5.33×10^7	x	1.12	1.15
Dimethyl sulfide	37.3	0.1	2.76×10^6	4.01	4.04	2.98
Carbon disulfide	46.3	0.3	1.60×10^6	4.15	1.52	3.31
Propanone	56.2	300	7.20×10^2	3.71	x	x
Butanone	79.6	6	1.54×10^4	4.84	x	x
3-Methylbutanone	94.5	5	—	6.35	x	x
2-Pentanone	102.0	10	2.00×10^3	7.22	x	x
Dimethyl disulfide	109.7	0.006	—	10.11	5.39	7.86
Dimethyl trisulfide	165 ^e	10^{-5f}	—	19.35	7.10	26.1

^a These data are from references 23 and 24.

^b Defined as the ratio between the vapor pressure at 20°C and the 100% recognition level, both expressed in parts per million (microliters per liter) (23).

^c x, not determined or not detectable with the system used.

^d —, Data not available in the literature.

^e Datum from reference 16.

^f Datum from reference 5.

those resulting from carbon disulfide, which was used in the desorption procedure. The carbon disulfide used contained small amounts of benzene (data not shown). Application of benzyl alcohol as a desorption solvent revealed the presence of carbon disulfide in the air sampled. As expected, the benzene signal at 6.63 min was not found in this analysis. The physical and odorous properties of the components identified are given in Table 2. As shown by the values of the odor index, the sulfur compounds were much more readily detected by the human nose than, for example, ammonia or 2-pentanone. For that reason, our attention was focused on the volatile sulfur compounds. To investigate the presence of other volatile sulfur compounds which were not trapped on charcoal tubes because of their low boiling points, small-volume air samples were concentrated on Tenax GC at -196°C. Subsequent analyses on systems B and C revealed the presence of hydrogen sulfide, carbonyl sulfide, and methanethiol in the samples (Table 2). Identification of these compounds was based on their retention times on two different systems in combination with the use of the sulfur-specific detector. Besides the volatile sulfur compounds mentioned, no unidentified peaks were observed.

Quantitative analysis of air samples. All of the volatile sulfur compounds mentioned in Table 2 were measured quantitatively in one run on a gas chromatograph equipped with a flame photometric detector using system C (Fig. 3). A plot of the logarithm of the peak area versus the logarithm of the amount of sulfur injected showed a linear relationship over the range of 40 pmol to 1.7 nmol. The slope of these calibration curves approached the theoretical value of 2 (20).

To estimate the total amount of volatile sulfur compounds emitted during preparation of the mushroom substrate, air samples were taken at different stages of the phase I composting process. Mixing of prewetted straw with horse manure occurred on day zero. Air samples showed distinct changes in the concentrations of the volatile sulfur compounds (Fig. 4). During the first 5 days of the process, dimethyl sulfide was emitted at a 10-times-higher concentration than any of the other compounds. After day 5, the dimethyl sulfide concentration increased by a factor of only about 2, but the concentrations of the other volatile sulfur compounds increased by 1 order of magnitude. The total emission of volatile sulfur compounds per kilogram of com-

post produced was calculated on the basis of the concentrations and the air flow rates through the compost stacks. The results are presented in Table 3. Dimethyl sulfide was the main component emitted during the first half of the process. After day 10, its contribution was exceeded by methanethiol, carbon disulfide, and dimethyl disulfide. Emission of dimethyl trisulfide was small and remained rather constant during the entire process. The total loss of sulfur as volatile sulfur compounds amounted to 8.2 mg of sulfur per kg (fresh weight) of compost.

Total organic and inorganic sulfur analysis ($n = 10$) of the product produced by the phase I composting process revealed 0.33 ± 0.81 and 2.24 ± 0.60 g of sulfur per kg (fresh weight), respectively.

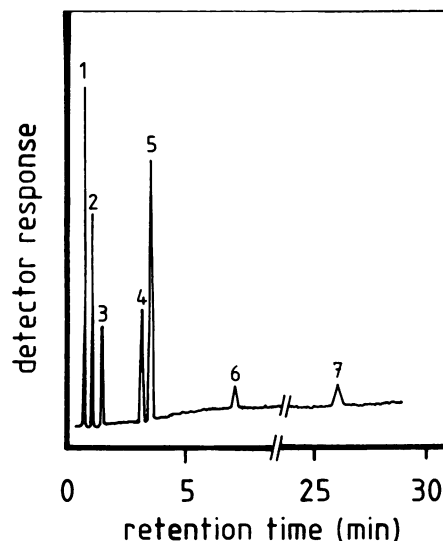


FIG. 3. Chromatogram obtained with system C by direct injection of 0.1 ml of a calibration gas mixture containing 63 pmol of hydrogen sulfide (peak 1), 63 pmol of carbonyl sulfide (peak 2), 63 pmol of methanethiol (peak 3), 100 pmol of dimethyl sulfide (peak 4), 125 pmol of carbon disulfide (peak 5), 85 pmol of dimethyl disulfide (peak 6), and 40 pmol of dimethyl trisulfide (peak 7).

TABLE 3. Emission of volatile sulfur compounds during production of the compost used as a substrate in mushroom cultivation

Processing period (days)	Mean air flow rate (m ³ /m ² per h)	Concn (μmol/kg [fresh wt] of product) of:						
		H ₂ S	COS	CS ₂	CH ₃ SH	(CH ₃) ₂ S	(CH ₃) ₂ S ₂	(CH ₃) ₂ S ₃
0-3.5	4.56	0.3	1.1	2.0	1.6	9.6	0.6	0.7
3.5-7.0	3.97	0.3	1.3	1.7	0.7	6.8	1.3	1.0
7.0-10.5	4.93	11.2	9.8	2.9	10.2	12.0	1.3	0.8
10.5-14.0	7.19	11.1	11.9	24.3	19.8	13.4	26.9	1.6

DISCUSSION

The use of a charcoal adsorption technique, common in environmental and industrial hygiene (25), appeared to be effective in concentrating a large range of different compounds present in air emitted from stacks set up to prepare a mushroom substrate. Application of different desorption solvents provides special advantages, depending on the analytical system used. The sensitivity of the flame ionization detector for carbon disulfide is extremely low, as shown by the rather small solvent peak in Fig. 2. Therefore, the appearance of carbon disulfide in the beginning of the chromatogram does not interfere with the other components present. However, when a mass spectrograph is used as a detector, elution of the solvent used causes a disturbance of the high vacuum of the mass spectrograph, prohibiting mass spectrographic analysis until the vacuum is restored. When special interest is taken in the identity of components in the beginning of the chromatogram, the use of benzyl alcohol as a desorption solvent is highly favorable. Use of benzyl alcohol in system A led to a large solvent peak at a retention time of about 13 min.

Among the compounds identified (Table 2), volatile sulfur compounds exhibited the highest odor index, caused by a high vapor pressure at 20°C and a threshold as low as 10⁻⁵ ppm (10⁻⁵ μl/liter). Organoleptic qualification of the odor of volatile sulfur compounds is often as foul or pungent (13, 23). Therefore, volatile sulfur compounds have a high nuisance potential, even at very low concentrations. Although the charcoal technique may be used for quantification purposes as well, large air sample volumes and elaborate sample preparation are required. Use of a sulfur-sensitive detector and a sophisticated sampling technique allowed reduction of

the air sample volume to 60 ml. No further sample preparations were necessary, and the Tenax sampling tubes could be reused several times without loss of efficiency.

Both the air flow rate through the stacks and the measured concentrations of most volatile sulfur compounds increased during the composting process. This implies an increase in the net release of these compounds by the composting material.

However, the total amount of sulfur (8.2 mg of S per kg [fresh weight]) emitted as volatile sulfur compounds was small compared with either the total organic (0.33 ± 0.81 g of S per kg [fresh weight]) or the total inorganic (2.24 ± 0.60 g of S per kg [fresh weight]) sulfur content of the end product. On the basis of the sulfur analyses of the basic constituents (straw, horse manure, chicken manure, and gypsum) and the amounts needed to obtain 1 kg of phase I compost, organic and inorganic sulfur contents of 0.27 ± 0.41 and 3.09 ± 0.36 g of S per kg (fresh weight) were calculated. As a result of the high standard deviations, no significant loss of either organic nor inorganic sulfur was observed. The high inorganic sulfur content was due to addition of gypsum to the manure mixture (8). Nevertheless, even when gypsum is omitted, sufficient sulfur is present in the composting material to account for the emission reported here. Wheat straw, as the major constituent of the composting material (8), contains 1.3 ± 0.4 mg of S per g of dry matter (*n* = 10). Although its contribution on a sulfur basis is the lowest of all of the constituents used, wheat straw alone accounts for 0.15 ± 0.03 g of inorganic and 0.02 ± 0.05 g of organic sulfur per kg (fresh weight) in the end product. As emission of volatile sulfur compounds cannot be avoided by elimination of sulfur from the material, changes in the process will be necessary to reduce environmental problems.

Production of volatile sulfur compounds from various biologically active systems, including soils (7), breweries (14), and algal mats (26), has been reported. The involvement of microorganisms has been reported (12), and production of volatile sulfur compounds has been stimulated by addition of sulfur-containing amino acids (1). Further studies are needed to resolve the agents and substrates involved in the production of volatile sulfur compounds during production of the compost used as a substrate in mushroom cultivation. To control the production of these compounds and handle the air emitted, indoor composting would be an attractive alternative. Although reports about indoor composting date from the early seventies on (11, 17), only recently were promising results obtained (9).

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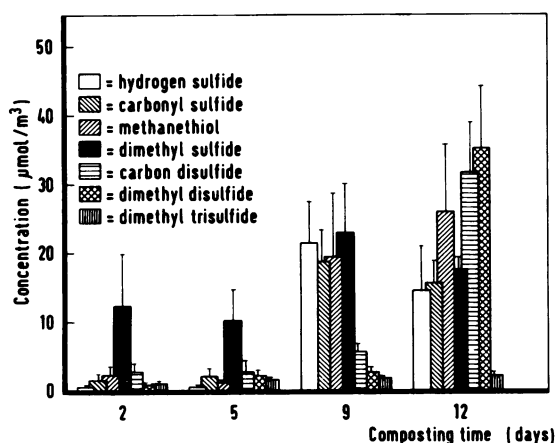


FIG. 4. Concentrations of volatile sulfur compounds in air emitted from compost stacks. The bars represent means of six independent sampling sites, and the standard errors of the means are indicated. Analyses were performed in triplicate at each sample site.

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