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Evolution of Volatile Sulfur Compounds during Laboratory-Scale Incubations and Indoor Preparation of Compost Used as a Substrate in Mushroom Cultivation

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Volatile sulfur compounds are known to be produced during the preparation of compost used as a substrate in mushroom cultivation. Because they cause odor problems, attempts have been made to reduce the production of these compounds. The influences of temperature and various additions on the production of volatile sulfur compounds from composting material were tested on laboratory-scale preparations. The production of H_2S, COS, CH_3SH, and $(CH_3)_2S$ was proven to be a biological process with an optimal temperature that coincides with the optimal temperature for biological activity. The formation of CS_2 and $(CH_3)_2S_2$ was shown to be a nonbiological process. The emission of volatile sulfur compounds during the indoor preparation of mushroom compost appeared to be remarkably reduced (about 90%) as compared with the emission during the conventional outdoor process. Introduction of this indoor composting process would result in a significant reduction in environmental pollution.

Recently we reported the evolution of volatile sulfur compounds during the preparation of compost, used as a substrate for the edible mushroom *Agaricus bisporus* (7). The smell of many of these compounds is described as pungent (17, 25), and therefore the emission of volatile compounds by composting facilities is a source of complaints by people living in the surroundings and, furthermore, an environmental stress. The production of volatile sulfur compounds from many different biological systems was reported, e.g., surface-ripened cheeses (8), the ruminant intestinal tract (20), and marine environments (5, 15, 16). The role of microorganisms in these processes has been reviewed in detail (4, 13). Sulfur-containing amino acids, mainly methionine and to a lesser extent cysteine, were shown to be the precursors (2, 16). In the marine environment dimethyl sulfonium propionic acid, an osmoregulatory compound in algae, is the precursor for the production of dimethyl sulfide (DMS) (5).

Furthermore, the breakdown of volatile sulfur compounds by various bacteria was demonstrated (14, 19, 21, 23). Since compost stacks are a very complex environment, it may be anticipated that simultaneous production and consumption of volatile sulfur compounds occurs. Reduction of the emission of these compounds can be achieved by decreasing the production rate or by increasing the degradation rate or both. Further insight into the precise origin of the volatile sulfur compounds and the influence of the process parameters of the composting process on the production and consumption rate of these compounds is needed to develop a composting regimen, which would result in minimal emission of volatile sulfur compounds.

The outdoor composting process presently in use was described in detail previously (7, 11). The constituents are mixed and placed in piles for about 2 weeks. No temperature or aeration control is performed during this outdoor stage (phase I). Phase I is followed by an indoor treatment of the composting material in bulk (phase II). In this phase, both temperature and aeration are under close control (10). The resulting material is suitable for inoculation with the edible mushroom *A. bisporus*. Recent research revealed that the duration of phase I could be reduced by 1 week. Omission of the treatment in windrows during phase I followed by a standard phase II treatment resulted in a substrate from which a normal yield was obtained. This indoor preparation of mushroom substrate is called indoor composting (12).

The aim of these investigations is to extend our knowledge of the production of volatile sulfur compounds during the composting process. Therefore the effects of the incubation temperature and different additions on the production of these compounds by composting material under well-defined laboratory conditions were studied, and the amount of volatile sulfur compounds emitted during the newly developed indoor composting process was quantified.

MATERIALS AND METHODS

Compost samples. Compost samples were obtained from a commercial composting facility. Compost samples taken at the end of phase I and at the end of phase II will be referred to as phase I compost and phase II compost, respectively. Subsamples were randomly picked from different stacks representing the same stage of composting and thoroughly mixed before analysis or compost incubations.

Compost incubations. Subsamples (10 to 100 g of fresh weight [FW]) were incubated in sealed 0.5- or 1-liter serum bottles for 24 h at the temperatures indicated. Methionine and potassium sulfate were added by mixing the chemicals as dry powder with the compost (3.4 mmol kg $[FW]^{-1}$). The occurrence of sulfate reduction was tested under waterlogged conditions (3) with 0.05 M phosphate buffer (pH 7.2).

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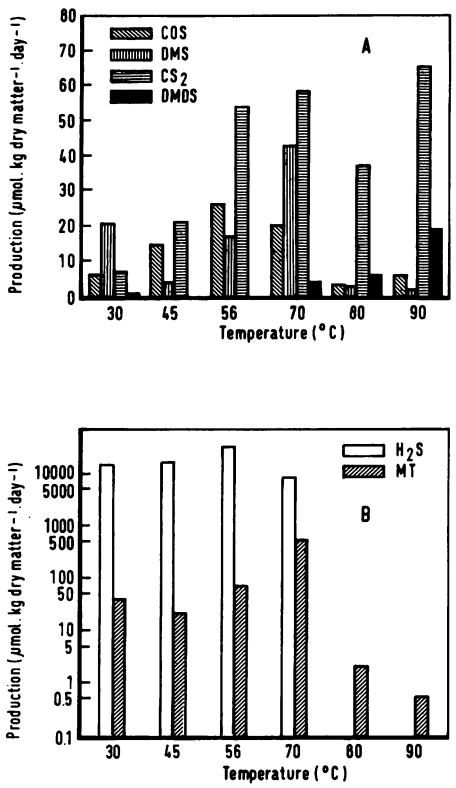

FIG. 1. Temperature dependence of the production of volatile sulfur compounds by phase I compost (25 g [FW] per 0.5-liter bottle).

Sodium molybdate, a specific inhibitor of sulfate reduction (1, 22), was added to a final concentration of 20 mM. Sterilization was performed at 121°C for 1 h. Efficiency of sterilization was checked by recording oxygen consumption and carbon dioxide production versus time. After treatment neglectable oxygen consumption and carbon dioxide production were observed within 24 h (data not shown), indicating complete inactivation of the aerobic microorganisms.

Indoor composting. Indoor composting was performed in the experimental tunnels described by Gerrits (10, 12). Straw-rich horse manure, used as the basic constituent, was prewetted for 5 days; then gypsum and chicken manure were added (25 and 100 kg 1,000 kg [FW]$^{-1}$, respectively). After the material was mixed well, it was placed in containers containing 1,000 kg (FW) each. Four containers occupied one tunnel corresponding to 825 kg (FW) m^{-2}. Immediately after filling, the temperature was allowed to rise to 56°C and kept at this level for 8 h. Then the temperature was lowered and maintained at 45°C for 6 days. In total the indoor composting process took 160 h. Temperature was controlled by regulating the supply of fresh air; no external heating was used. During the entire process an air recirculation rate of 140 m^3 m^{-2} h^{-1} through the composting material was maintained. As shown by Gerrits (12), mushroom yields from indoor compost did not differ from yields obtained from conventionally prepared compost.

Analytical procedures. The dry weight content of the compost samples was calculated on the basis of the weight loss after drying at 105°C. The gas chromatographic analysis of air samples and calibrations with authentic volatile sulfur compounds were essentially as described previously (7). Injections were performed either directly or after concentration of a large volume of the gas phase (60 ml) on Tenax tubes (7, 24). Oxygen and carbon dioxide were analyzed with a gas chromatograph equipped with a thermal conductivity detector (6).

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

RESULTS

Phase I compost samples were incubated at different temperatures, and the production of volatile sulfur compounds was measured after 24 h. Because of slight fluctuations in dry matter content of the different compost samples used, the results are expressed as μmol kg of dry matter^{-1} day^{-1}. The average results are presented in Fig. 1. Maximum production of H_2S and methanethiol (MT) was 10 to 1,000 times higher (Fig. 1A) than the maximum production of the other volatile sulfur compounds (Fig. 1B). All compounds except CS_2 and dimethyl disulfide (DMDS) showed a maximum production between 56 and 70°C. Over the temperature range tested, CS_2 and DMDS showed increasing production with increasing temperature. For phase II compost samples a similar tendency was observed (data not shown). To test the involvement of biological processes in the production of volatile sulfur compounds, compost samples were sterilized before incubation. The production was measured for phase I and phase II compost samples at 56 and 45°C, respectively. In Table 1 the results are presented together with those obtained with untreated compost, which served as a control. The production of all volatile sulfur compounds except CS_2 and DMDS dropped dramatically upon sterilization for both composts. DMTS was not found in measurable quantities in any sample.

Table 2 shows the effects of the addition of an organic or inorganic sulfur compound on the production of volatile sulfur compounds by phase I compost. The addition of potassium sulfate resulted in a slight increase in the produc-

TABLE 1. Production of volatile sulfur compounds by phase I and phase II compost (25 g [FW] per 0.5-liter bottle) with or without sterilization

Compost sample	Sterilization (1 h, 121°C)	Incubation temp (°C)	Production rate[a] (μmol kg [dry wt]$^{-1}$ day^{-1})						
			H_2S	COS	MT	DMS	CS_2	DMDS	DMTS
Phase I	−	56	31,300	26	69	17	54	0	0
Phase I	+	56	0	6	0	1	49	9	0
Phase II	−	45	9,300	16	140	20	12	0	0
Phase II	+	45	1	1	0	0	13	4	0

[a] Values are means of three experiments.

TABLE 2. Relative influence of additions on the production of volatile sulfur compounds by phase I compost at 56°C (25 g [FW] per 0.5-liter bottle)

Addition	Relative increase in production rate[a]			
	H_2S	MT	DMS	DMDS
Sulfate	2.0	1.8	3.9	1.0
Methionine	1.5	27	1.0	2.8

[a] Values are expressed as ratios between the production obtained with and without the indicated addition. Rates for COS, CS_2, and DMTS were not determined.

tion of H_2S and DMS, whereas the addition of methionine resulted in a 27-fold increase in the production of MT. Simultaneously the production of DMDS increased about three times (Table 2). To elucidate whether the high production obtained for H_2S was due to the presence of sulfate-reducing bacteria, sodium molybdate was added to a final concentration of 20 mM in a phosphate buffer (50 mM, pH 7.2) (Table 3). Blank incubations were performed with a equal amount of buffer alone. The amount of buffer was sufficient to create a waterlogged situation for all of the compost particles, so intense contact between the inhibitor and the microorganisms was ensured. The influence of the addition of buffer alone is small, as can be seen when the results are compared with those for phase II without buffer addition (Table 1). No H_2S was found in incubations to which sodium molybdate was added. Furthermore, the production of MT was slightly reduced. Another indication that anaerobic processes play an important role in the production of volatile sulfur compounds came from time-dependent measurements of the oxygen concentration in bottles with various amounts of phase I or phase II compost per bottle. These experiments revealed that oxygen was completely used within 6 h when 100 g (FW) of compost was present. In the presence of 10 g (FW) of compost, oxygen was still present after 24 h at a level of about 10% (data not shown). The production of volatile sulfur compounds during these incubations is shown in Fig. 2. The observed production of H_2S and MT was at least 3 orders of magnitude higher under anaerobic conditions. A similar tendency was observed for carbonyl sulfide (COS), DMS, and CS_2, although the effect did not exceed 1 order of magnitude.

Temperature has proven to be an important parameter in the observed production of volatile sulfur compounds on a laboratory scale (Fig. 1). During indoor composting, the temperature is kept at values below the optimum temperature for the production of volatile sulfur compounds. To test the influence of the indoor composting regimen on the total production of volatile sulfur compounds, air samples were collected during three composting trials at different intervals and analyzed for volatile sulfur compounds. In Fig. 3 concentrations in the effluent air from one trial are presented as a function of the process time. DMS was found at highest concentrations over the entire process. MT was only detectable during the first 2 days, whereas DMDS and DMTS were already below the detection limit (<0.3 μmol m^{-3}) after 1 day. No H_2S was measured in any of the air samples. From the concentrations of volatile sulfur compounds and the fresh air supply rate, the total production per compound was calculated, and averages of three independent trials are

TABLE 3. Influence of sodium molybdate on the production of volatile sulfur compounds by phase II compost at 45°C measured in a waterlogged situation

Addition[a]	Production rate (μmol kg [dry wt]$^{-1}$ day^{-1})						
	H_2S	COS	MT	DMS	CS_2	DMDS	DMTS
None	1,050	1	108	3	1	0	0
Molybdate (20 mM)	0	3	42	5	0	0	0

[a] Addition to 0.05 M phosphate buffer (pH 7.2).

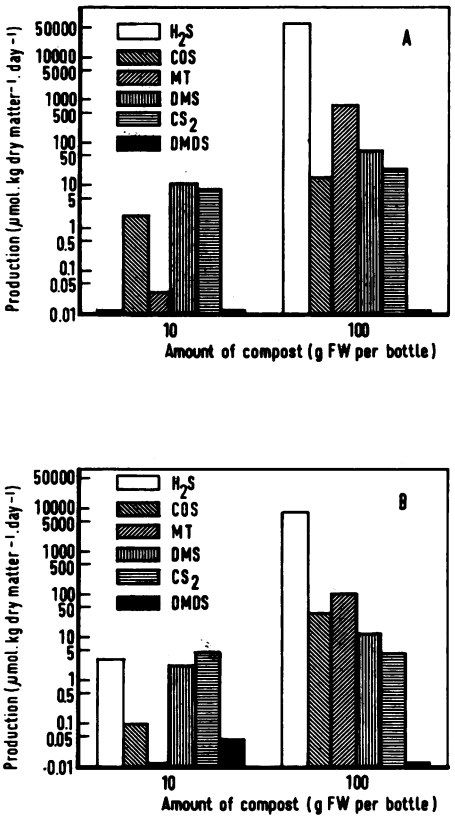

FIG. 2. Production of volatile sulfur compounds by phase I (A) and phase II (B) compost, starting with 10 or 100 g (FW) of composting material in a 1-liter bottle.

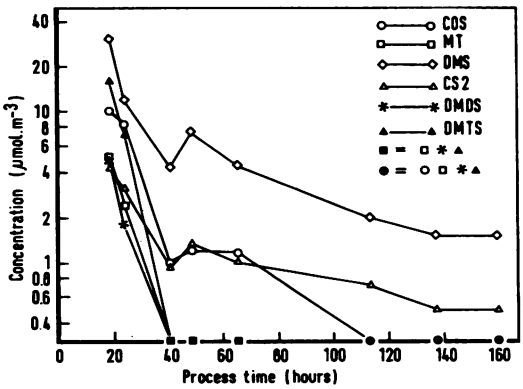

FIG. 3. Concentrations of volatile sulfur compounds in the effluent air of an indoor composting process versus process time. A typical pattern is shown. Air samples were analyzed in triplicate.

TABLE 4. Mean production of volatile sulfur compounds during indoor preparation of a substrate for *A. bisporus*

Method	Production (μmol kg [FW]$^{-1}$) of:						
	H_2S	COS	MT	DMS	CS_2	DMDS	DMTS
Indoor composting	0	3.2 ± 1.2	0.6 ± 0.5	10.2 ± 3.6	2.4 ± 0.6	0.5 ± 0.4	1.1 ± 1.3
Windrows[a]	22.3	21.7	30.0	25.4	27.2	28.2	2.4

[a] Values are from reference 7.

presented in Table 4. The total emission of sulfur during the indoor composting process amounted to 23 μmol of S kg (FW)$^{-1}$. The main contribution to the emission of sulfur is made by DMS (45%).

DISCUSSION

The optimal temperature for the production of H_2S, COS, MT, and DMS coincides with the optimal temperature (50 to 56°C) of biological activity determined by oxygen consumption and glutamate mineralization (6). The biological origin of these compounds is further confirmed by the observation that heat treatment strongly decreased their production (Table 1). The production of CS_2 and DMDS increased with increasing incubation temperature, indicating nonbiological formation. In several other complex biological systems, similar observations were made (2, 15, 20). The microbial origin of H_2S production is further proved by the abolishment of the production of H_2S by molybdate, a specific inhibitor of sulfate-reducing bacteria (1, 22) (Table 3). Since calcium sulfate is a constituent of the composting mixture (11), the availability of sulfate is never the limiting step in the formation of H_2S (Table 4). Although chicken manure, another constituent (11), is relatively rich in protein (18), an increase in the production of MT was observed when methionine was added. The same result was reported for other systems (2, 3, 8, 13). Apparently, anaerobic conditions favor the formation of volatile sulfur compounds. In accordance with this, the presence of oxygen strongly reduced the production (Fig. 2). Previously, considerable amounts of volatile sulfur compounds were shown to be produced during the composting process in windrows (7). In this study we show that during indoor composting the concentration of volatile sulfur compounds decreased dramatically (Fig. 3). Presumably, the high recirculation rate of the air through the composting material during indoor composting prevents the occurrence of an anaerobic zone in the inner part of the composting stack (9) and in this way contributes to a reduction in the production of volatile sulfur compounds (18). Even when anaerobic microenvironments cannot be avoided completely, the recirculation of the emitted air enables close contact between the volatile sulfur compounds formed and the composting material, possibly enhancing breakdown. Preliminary experiments in which small amounts of H_2S were added to compost showed a rapid disappearance of H_2S (data not shown). Whether this is due to sorption to the composting material or to microbial breakdown or both is unclear and is the subject of further research.

Recently, a total emission of volatile sulfur compounds of 256 μmol of S kg (FW)$^{-1}$ was found during the outdoor preparation of compost used as a substrate in mushroom cultivation (7). The highest emissions (217 μmol of S kg [FW]$^{-1}$) were observed at the end of the process, when the material was stacked into windrows. In contrast, the total emission of sulfur during the indoor composting process amounts to 23 μmol of S kg (FW)$^{-1}$. Replacing the windrow section of the outdoor process by the indoor process described here will result in a total emission of sulfur during the preparation of mushroom substrate of 62 μmol of S kg (FW)$^{-1}$. This overall reduction of 76% would implicate a significant decrease in environmental pollution caused by composting facilities. Furthermore, the indoor preparation of mushroom substrate enables a better control of process parameters, resulting in a more constant quality of the end product and mushroom yields comparable to those obtained with a traditionally prepared substrate.

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