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Biomass and Biological Activity during the Production of Compost Used as a Substrate in Mushroom Cultivation

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The production of a suitable substrate for the cultivation of the common white button mushroom, *Agaricus bisporus*, is referred to as composting. High microbiological activity causes temperatures of the composting material to rise as high as 80°C. At stacking, an optimal oxygen consumption rate of 140 μmol of O_2 h^{-1} g (dry weight)$^{-1}$ was found in the compost at 50°C, whereas the oxygen consumption rate of the end product was lower at all temperatures tested. No significant differences were observed between biomass content and mineralization rate of ^{14}C-labeled glutamate of the two composts. Biomass content was shown to be a major function of both temperature and the sampling site position in the stack. On the basis of the results reported here, a minimal composting time of 3.3 days for the phase I process was calculated. Further suggestions are made to reduce the time necessary for the production of a substrate for *A. bisporus* considerably.

The commercially cultivated white button mushroom *Agaricus bisporus* (Lange) Imbach and the closely related *A. bitorquis* (Quelét) Saccardo are grown on a special substrate, prepared from horse manure, wheat straw, and broiler chicken manure. This paper will focus on the outdoor composting process (phase I), whereas for the total preparation of a suitable substrate for *A. bisporus* a second treatment (phase II) is needed (10, 13). Traditionally, the substrate for mushrooms is called compost, although the duration of the process is much shorter than the time needed for the preparation of compost from sewage sludge or domestic refuse (1, 7, 18). Including a prewetting period of the straw of 14 days, the phase I process only takes 28 days. Nevertheless, in many aspects the preparation of mushroom substrate is comparable to the other composting processes mentioned. In all of these processes, the breakdown of solid organic matter by microorganisms is the crucial step. This can be accomplished either aerobically or anaerobically. As we have shown recently, considerable concentrations of methane are present in the air evolving from the composting material (5), indicating the presence of anaerobic microenvironments. It was calculated that at least 3.5% of the loss of dry matter was achieved by anaerobic breakdown. Although anaerobic microenvironments cannot be avoided in static compost piles, the major part of the loss of dry matter is due to aerobic breakdown, which results in the production of carbon dioxide, water, biomass, and considerable amounts of heat. Because of the insulating character of the composting material and the absence of forced air movements, heat is transferred only slowly to the outside of the stacks. As a result, a steady increase in temperature is observed, and depending on the dimensions of the stacks, temperatures as high as 80°C are reported (8, 11). Since the majority of the microorganisms isolated so far from this composting material are not extremely thermophilic, it is unlikely that maximum biological activity is exhibited at this high temperature. In general, composting is considered to be an aerobic process (11), and as indicated above the contribution of anaerobic breakdown in the process described here is only small. Therefore, biological activity can be measured as oxygen consumption rate.

The oxygen consumption rates of garbage and sludge mixtures increase logarithmically with temperature from 20 to 70°C, as reported by Schulze (23). This finding was confirmed by Jeris and Regan (16) with mixed refuse for temperatures up to 60°C. Above 60°C, a sharp drop in oxygen consumption rates was observed, demonstrating the inhibitory effect of elevated temperatures on the biological activity. A similar profile was found by McKinley and Vestal (18), who used the mineralization rate of ^{14}C-labeled glutamate by municipal sewage sludge extracts as a measure for biological activity. Optimal activity was found at a range of 35 to 45°C, while the mineralization rate drops to zero at 65°C. The formation of biomass during the composting process is believed to contribute to the selectivity of the substrate for *Agaricus* spp. and, as these fungi are known to produce lytic enzymes, the biomass will serve as a nutrient source (9, 24). A negative correlation is reported between biomass content and temperature above 45°C (18), another indication that high temperatures may be unfavorable during composting.

The aim of the present investigation was to describe the production of compost used as a substrate in mushroom cultivation in terms of changes of biomass content and biological activity and of the correlation of these parameters with temperature.

MATERIALS AND METHODS

Compost samples. The compost used in this investigation originated from a large commercial compost farm, where the total outdoor composting process takes 28 days. On this farm the recipe of Gerrits (13) is used. Wheat straw is the major source of organic matter in the composting material. It consists mainly of cellulose, hemicellulose, and lignin, 36.0, 25.3, an 11.0% of the dry matter, respectively (19). On day 0, wheat straw and broiler chicken manure are mixed and a

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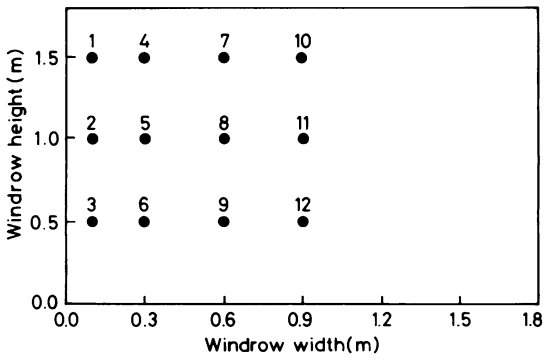

FIG. 1. Cross section of a windrow. Sampling sites are indicated by numbers.

prewetting period of 14 days starts. Horse manure is added on day 14 and water is supplemented for 7 more days. On day 21, gypsum and additional broiler chicken manure are mixed through the composting material. Simultaneously, the material is stacked in windrows with a cross section of approximately 2.0 by 2.0 m and the composting process is continued for 7 days, during which the material is mixed twice to increase homogeneity (13).

Biomass estimations were performed on compost samples obtained at different stages of the process. The correlation between temperature of the sampling site and the biomass content was studied in samples taken at different positions through a cross section of the windrow (Fig. 1) on day 23 of the process just before the first mixing. The temperature of the composting material was measured with an Impac Tastotherm D 700 thermometer prior to the collection of the samples. Oxygen consumption and mineralization experiments were performed with compost taken at days 21 and 28 of the process. These samples are referred to as "compost at stacking" and "phase I compost," respectively. After collection, the compost samples were transferred immediately to the laboratory in plastic bags without any precaution to prevent cooling. Upon arrival, each sample was mixed thoroughly and divided into subsamples for further analyses.

Oxygen consumption of compost. A subsample of 100 g (fresh weight) of compost was transferred into a 1-liter serum bottle and incubated in a stove for at least 1 h at the desired temperature. Then the gas phase was replaced by air, which was conditioned prior to use by passage through plastic tubing (10 m, 6-mm inner diameter) and two water-filled impingers kept at the desired temperature. The bottle was closed with a rubber stopper. As a trap for carbon dioxide produced by the compost, a cellulose dialysis tubing (Visking; diameter, 1.4 cm; length, 10 cm) containing 5 ml of 4 M sodium hydroxide was attached to the stopper inside the bottle. Any contact of the dialysis tubing with the compost was avoided. After closing the bottle, a connection was made with one end of a gas burette by inserting a hypodermic needle through the stopper. The other end of the gas burette was connected to a water-filled reservoir. Volume changes of the gas phase were measured with the water in the gas burette and in the reservoir at the same level (25). Decrease in volume was taken as oxygen consumption.

Oxygen consumption of compost extracts. Subsamples of 40 g (fresh weight) of compost were shaken with 1 liter of tap water for 10 min. Large solid particles were discarded after decantation of the liquid. The liquid phase thus obtained is referred to as compost extract. Oxygen consumption in the extract was measured with a biological oxygen monitor model 53 and a bath assembly 5301 (Y. S. I. Incorporated) by the method of Hemrika-Wagner et al. (14). No buffer or exogenous substrate was added to the extracts.

Mineralization rates of compost extracts. Compost extracts were prepared as described above. To 2 ml of extract 0.1 ml of L-[U-^{14}C]glutamate (3.7 kBq; 0.38 nmol) was added in stoppered vials. The mixture was incubated at various temperatures. The vials were equipped with a disposable center well containing 0.3 ml of ethanolamine-ethylene glycol (1:2, vol/vol) to trap $^{14}CO_2$ produced. The evolution of $^{14}CO_2$ was linear with time over at least 120 min. Routinely, incubations were terminated after 60 min by the addition of 0.5 ml of 3 M perchloric acid to the medium, followed by a second incubation for 18 h at 4°C to ensure complete volatilization of CO_2. Radioactivity of $^{14}CO_2$ was measured in 10 ml of toluene-methanol (2:1, vol/vol) containing 0.4% Omnifluor by means of a Philips model 4400A liquid scintillation counter. All counts (counts per minute) were corrected to disintegrations (disintegrations per minute) by using the channel-ratio method. Mineralization rates are expressed as nanomoles of glutamate mineralized per hour of incubation per gram (dry matter) of compost extracted.

Microbial biomass determination. Biomass formed during composting has been measured in several ways, including direct counts, ATP content, and total extractable lipid phosphate content (18, 24). Here, total extractable lipid phosphate estimations are preferred because of the higher reproducibility (18, 26). Total lipids were extracted from wet samples containing approximately 6 g of dry matter. Water was added depending on the moisture content of the sample up to a total volume of 20 ml. The chloroform-methanol extraction procedure of Bligh and Dyer (3) was used. After digestion in 30% perchloric acid for 2 h at 180°C, inorganic phosphate was measured colorimetrically at 830 nm, using the molybdate blue reaction (2). Results are expressed as micromoles of PO_4 per gram (dry weight) of compost.

Analytical procedures. The gas phase was analyzed as described by Hutten et al. (15), using a Pye Unicam model GCV gas chromatograph equipped with a thermal conductivity detector. Dry-matter content was calculated after drying the compost samples to constant weight at 70°C, resulting in an average dry-matter content of compost at stacking and phase I compost of 24.8 and 27.4%, respectively.

Chemicals. Gases were obtained from Hoek Loos, Schiedam, The Netherlands. Omnifluor was purchased from New England Nuclear, Dreieichenheim, Federal Republic of Germany. L-[U-^{14}C]glutamate was obtained from the Radiochemical Centre, Amersham, United Kingdom. All other chemicals used originated from Merck, Darmstadt, Federal Republic of Germany.

RESULTS

A representative illustration of oxygen consumption by phase I compost at different temperatures is shown in Fig. 2. Sterilized compost (30 min at 121°C), used as a control, showed a negligible change in volume during incubation. Gas chromatographic analyses of the gas phase, after cessation of the volume changes of the oxygen uptake experiments with untreated compost, showed the absence of both oxygen and carbon dioxide. When the sodium hydroxide solution was replaced by water during the incubation, only small volume fluctuations were observed. As a result, only a negligible net volume change over the whole incubation period was measured. After incubation, the presence of

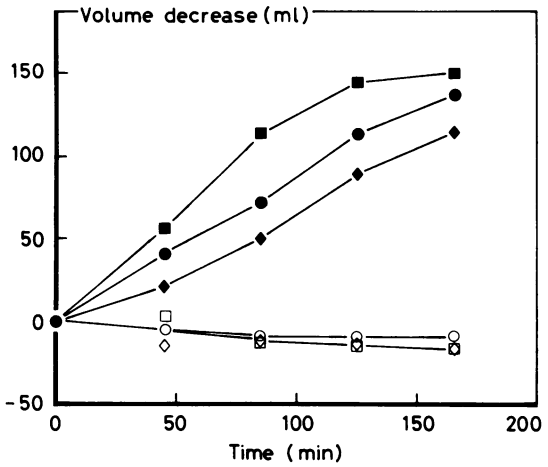

FIG. 2. Volume changes obtained by incubation of phase I compost at various temperatures before (closed symbols) and after (open symbols) sterilization: ●, ○, 40°C; ■, □, 55°C; ◆, ◇, 60°C.

carbon dioxide and the absence of oxygen in the gas phase of this control were confirmed by gas chromatographic analyses.

To determine whether the rate of carbon dioxide uptake by the sodium hydroxide solution in the dialysis tubing was sufficient to trap all carbon dioxide released during incubations, empty bottles were flushed with N_2-CO_2 (80:20%, vol/vol). Volume changes caused by carbon dioxide uptake from the N_2-CO_2 atmosphere ranged from 7 to 20 ml/min over the temperature and time range tested (data not shown). As the volume changes during the experiments with untreated compost never exceeded the rate of 2 ml/min, it is concluded that the uptake capacity of the carbon dioxide trap was sufficient. From the slope of the oxygen uptake curves (Fig. 2), the oxygen consumption rate at a given temperature can be calculated per gram of dry weight. The averages of experiments with both compost at stacking and phase I compost are shown in Fig. 3. The oxygen consumption rate of the compost at stacking is higher as compared with phase I compost. Compost at stacking showed an maximum oxygen consumption rate at 50°C of about 140 μmol of O_2 g (dry weight)$^{-1}$ h^{-1}. Above this temperature oxygen consumption rates declined, reaching a level of 55 μmol of O_2 g (dry weight)$^{-1}$ h^{-1} at 70°C. Compost samples taken at the end of phase I showed an increase in oxygen consumption rate at temperatures up to 40°C; above 40°C, the oxygen consumption rate remained stable at a level of about 50 μmol of O_2 g (dry weight)$^{-1}$ h^{-1}.

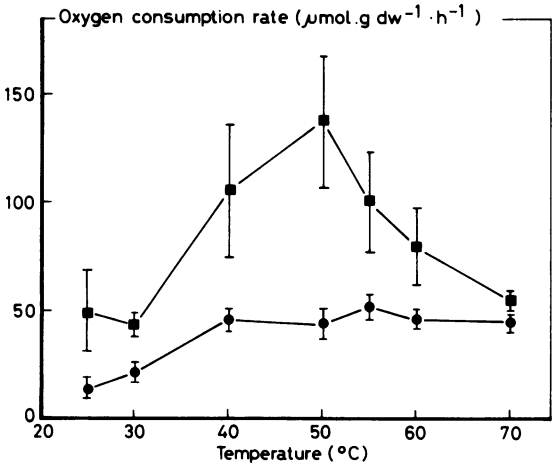

FIG. 3. Average oxygen consumption rates of compost at stacking (■) and phase I compost (●) at various temperatures. Bars represent standard error of the mean. The number of measurements range from 4 to 8. dw, Dry weight.

TABLE 1. Oxygen consumption rate of aqueous compost extracts after various treatments measured at different temperatures

Treatment and temp (°C)	Oxygen consumption rate (μmol of O_2 g [dry wt]$^{-1}$ h^{-1})[a]			
	Compost at stacking		Phase I compost	
	−KCN	+KCN	−KCN	+KCN
Extraction				
25	28.4	0	29.0	6.6
50	63.5	18.9	49.7	15.2
70	0.4	0	0	0
Extraction + centrifugation (15 min, 13,000 × g, 4°C)				
25	0	0	2.7	0.4
50	5.3	1.3	1.8	1.2
70	0	0	0	0
Extraction + centrifugation + boiling (15 min)				
25	0	0	0.1	0
50	0.7	0	0	0
70	0	0	0	0

[a] Corrected for oxygen consumption by the electrode.

The influence of different treatments on oxygen consumption rates of extracts prepared from compost at stacking and phase I compost is summarized in Table 1. At 25 and 50°C, the same tendency was observed for the oxygen consumption rates of the extracts as for the untreated compost samples, although the absolute value of the extracts are lower. After centrifugation, which removes the major part of the microorganisms present, the oxygen consumption rate of both extracts was reduced over 90% at 25 and 50°C. After an additional heat treatment (15 min at 100°C), the extracts showed a negligible oxygen consumption rate. At 70°C no significant oxygen consumption was observed for both extracts independent of the treatments applied. The influence of potassium cyanide, known as a strong inhibitor of the terminal step of the cytochrome-mediated respiratory pathway, was studied at a final concentration of 0.5 mM. At 25 and 50°C, the addition of potassium cyanide reduced the oxygen consumption rate over 70%. Subsequent addition of benzohydroxamic acid (12.5 mM) did not alter the oxygen consumption rates significantly (data not shown). Benzohydroxamic acid is known as an inhibitor of the cyanide-insensitive respiratory pathway (6, 22).

The temperature dependency of the mineralization of ^{14}C-labeled glutamate by phase I compost extracts is shown in Fig. 4. The highest mineralization rate was observed at a temperature range of 50 to 55°C, with a steep decline at higher temperatures. Mean mineralization rates of extracts prepared from compost taken at different stages of the

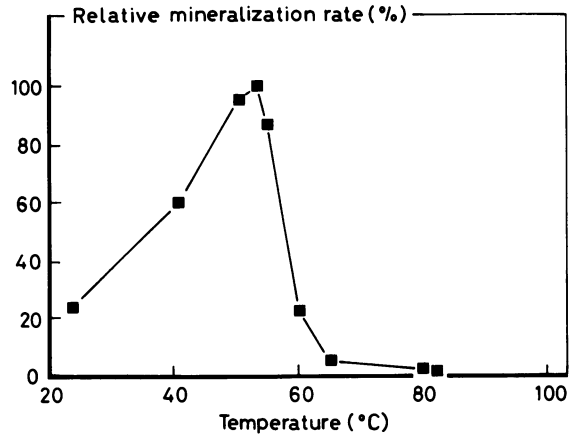

FIG. 4. Temperature dependence of the ^{14}C-labeled glutamate mineralization rates by phase I compost extracts. Mineralization rate at 55°C was set to 100%.

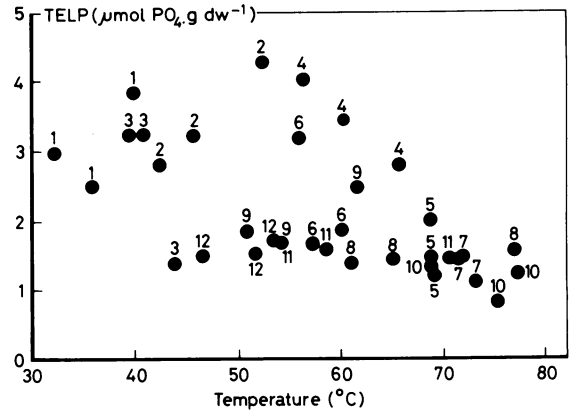

FIG. 5. Biomass content versus site temperature of compost samples taken 2 days after stacking. Numbers refer to the sampling position as shown in Fig. 1. TELP, Total extractable lipid phosphate; dw, dry weight.

process are summarized in Table 2. Taking into account the high standard deviations caused by heterogeneity of the material, no significant differences were observed.

Total extractable lipid phosphate analyses were used as a method to study the biomass content of the composting material. Figure 5 shows the biomass content versus the temperature of the sampling site. The data of three independent experiments are included. In general, two levels of biomass content were observed corresponding with the origin of the compost samples. Samples taken at locations 1 to 4 (Fig. 1) covered the temperature range of 32 to 65°C but, with only one exception, possessed a biomass content higher than 2.3 μmol of PO_4 g (dry weight)$^{-1}$. Temperatures of the other sites were within the range of 43 to 78°C, while the biomass content hardly ever exceeded 2 μmol of PO_4 g (dry weight)$^{-1}$. The average results of biomass estimations performed on samples taken over the entire composting process are presented in Fig. 6. From the start of the prewetting period up to day 18 an increase of biomass was observed. Later in the process no significant changes in biomass content were measured. As a result the end product, phase I compost, contained a sixfold-higher biomass content (2.4 μmol of PO_4 g [dry weight]$^{-1}$) when compared with the starting material.

DISCUSSION

Microbiological activity and biomass content of the composting material changed during the production of compost used as a substrate in mushroom cultivation. Both parameters showed a correlation with temperature. Microbiological activity of untreated compost was measured by oxygen consumption. The optimal temperature of the oxygen consumption rate of compost at stacking (Fig. 3) coincides with the optimal temperature of the mineralization rate of phase I compost extracts (Fig. 4). This temperature optimum (50°C) is somewhat lower than the value of 59°C reported by Jeris and Regan (16) for mixed-refuse composting, but equals closely the reported optimal temperature for municipal sewage sludge composting (18). Oxygen consumption rates of extracts are lower than those observed with untreated compost. This is unexpected since the gas-liquid barrier for oxygen diffusion is omitted and probably due to only partial extraction of the microbial population. It is known that cellulolytic microorganisms strongly adhere to fibers (17). Nevertheless, as the same tendency in oxygen consumption rate is observed in extracts compared with untreated compost, a practical system is obtained for testing the influence of different treatments or additives or both. The dramatic reduction in oxygen consumption rates after centrifugation of the extracts or the addition of potassium cyanide demonstrated the involvement of biological systems in this process. That the addition of benzohydroxamic acid had no effect on the oxygen consumption rate indicates that cyanide-insensitive respiration does not play an important role in the observed oxygen consumption (22).

TABLE 2. Mean mineralization rates of [^{14}C]glutamate of various aqueous compost extracts at 55°C

Compost sample	*n*	Mineralization rate (mean ± SD, nmol of glutamate g [dry wt]$^{-1}$ h^{-1})
Day 21	5	6.7 ± 1.9
Day 24	12	7.2 ± 3.3
Day 28	7	5.4 ± 5.3

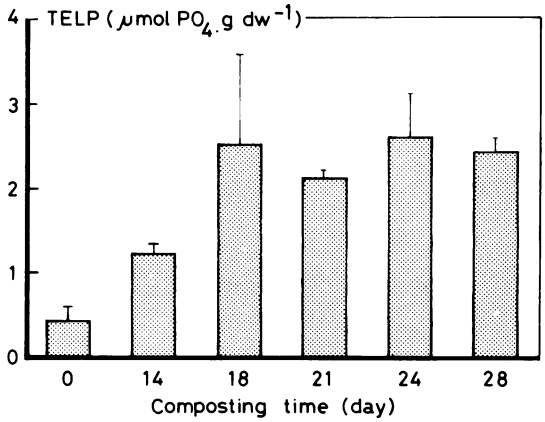

FIG. 6. Mean biomass content and standard deviation at different stages during the production of compost used as a substrate in mushroom cultivation. TELP, Total extractable lipid phosphate; dw, dry weight.

Elevated temperature is one of the most extreme environmental stresses to which organisms are exposed (4). Biological activity of compost extracts, measured as oxygen consumption and mineralization of glutamate, dropped to zero at 70°C. In untreated compost an oxygen consumption rate of 50 μmol of O_2 g (dry weight)$^{-1}$ h^{-1} was observed at this elevated temperature. The prolonged biological activity may be due to the poor heat transfer of the composting material, resulting in a lower actual temperature on microscale. Furthermore, chemical reactions cannot be excluded, but until now only vague suggestions are made (21).

Another illustration of the detrimental influence of the high temperature on biological activity is the low biomass content observed in samples originating from high-temperature sampling sites (Fig. 5). Below 65°C, data can be divided into two groups, depending on the position of the sampling site. The first group consists of samples from positions 1 to 4 (Fig. 1) and is high in biomass content, whereas the biomass content of the second group (samples from positions 5 to 12) does not exceed 2 μmol of PO_4 g (dry weight)$^{-1}$. As shown by Randle and Flegg (20), oxygen concentrations in compost tend to drop steeply over a distance as small as 0.5 m during the first days after stacking. Therefore, the low biomass content of the second group is a result of the lack of oxygen in the corresponding part of the stack.

Leaving the prewetting period out of consideration, the actual production of phase I compost takes 14 days or more (10, 13). Temperatures of the composting material as high as 70°C are reached within 1 or 2 days (13). As a result, the composting process will be slowed down due to thermal inactivation of essential microbial enzymes. As the oxygen consumption rate of phase I compost is only 25% of the oxygen consumption rate of compost at stacking, the highest microbiological activity is exhibited immediately after mixing of the constituents. Since compost at stacking and phase I compost do not differ from each other in terms of biomass content (Fig. 6) or mineralization rate of glutamate (Table 2), the observed lower value of oxygen consumption rate is most probably due to depletion of substrate. Readily accessible compounds will first be degraded, and as a result an accumulation of recalcitrant compounds such as cellulose and lignin and biomass in the composting material will occur. From the observations reported here it is highly plausible that the composting process can be shortened. Taking the maximum oxygen consumption rate of 140 μmol of O_2 g (dry weight)$^{-1}$ h^{-1} and assuming that 30% of the dry matter should be degraded during the composting process (12), a minimal composting time of 3.3 days can be calculated. Performing the phase I composting process at a temperature not exceeding 55°C would reduce the composting period by a factor 4 to 5.

Increase of the selectivity of the substrate for *A. bisporus* is a major objective of the composting process described here. During the composting process biomass is formed out of readily accessible compounds (Fig. 6) and *A. bisporus* is able to digest a broad range of substrates, including microbial cell wall compounds (9). But, as the biomass content does not increase during the last 10 days of the process, continuation of the process after day 18 seems to be meaningless. Nevertheless, a continuous succession of microorganisms will occur and an accumulation of killed microorganisms and microbial metabolites which can act as nutrient source for *A. bisporus* cannot be excluded. Additional research is needed to confirm the desired minimal fraction of dry-matter loss during the composting process, especially with respect to mushroom yield. Active biomass is assumed to be a key index of compost selectivity, but chemical and physical factors (e.g., straw weakening and increase of the water-holding capacity) may also be important.

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