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ANALYSIS OF THE COMPONENTS OF LIGNOCELLULOSE DEGRADED BY *AGARICUS BISPORUS* AND *PLEUROTUS OSTREATUS*

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ABSTRACT

Derivative thermogravimetry (DTG) and differential scanning calorimetry (DSC) were used to identify the changes in the components of mushroom compost and straw degraded by *Agaricus bisporus* and *Pleurotus ostreatus*, respectively. The differences in enzyme-release patterns of the two fungi were correlated with changes in the polysaccharides and lignin content of the two substrates. The results from the DTG and DSC analysis of the lignocellulosic materials suggest that the pyrolysis peaks and the energy release pattern may be correlated to the degradation of the microbial biomass, plant polysaccharides and lignin by fungi.

INTRODUCTION

The application of thermal analytical techniques for the determination of several kinetic parameters associated with the chemical reactions which occur upon heating has been extensively reviewed by Manche and Carroll [1]. However, the application of such methods to lignocellulosics did not start until the late 1950's [1–3]. Thermogravimetry is now widely used for studying wood pyrolysis [4–7]. Differential scanning calorimetry has been used for determining changes in enthalpy associated with transformation during heating and this provides direct information relating to the changes [8].

Beall [5] reported that pyrolysis and combustion of wood and wood components were related to their molecular structure. The thermogravimetric profiles of wood depends not only on the cellulose content but also on the changes in lignin and hemicellulose [4,9,10]. Microbial attack on lignocellulosic materials results in enzymatic depolymerisation, suggesting that degraded lignocellulosics have reduced thermal stability [3], and thermal stability could be related quantitatively to the degree of fungal degradation. This was further supported in a recent report on the degradation of woody

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materials like flax shives by fungi [11]. Generally, woody plant materials have larger proportions of lignin than those found in cereal straw. Basidiomycetes such as *Pleurotus ostreatus* are able to oxidise the lignin–polysaccharide complex directly without prior chemical or biological treatment [12], whereas *Agaricus bisporus* normally grows on a degraded lignocellulosic substrate prepared by composting wheat straw.

The use of differential scanning calorimetry to determine changes in enthalpy has been reported for chemically-modified celluloses [13,14] but reports on the changes caused by fungal degradation of lignocelluloses are limited [15].

The aim of the present study was to investigate the thermal degradation pattern of mushroom compost and wheat straw degraded by *A. bisporus* and *P. ostreatus*, respectively, and to correlate these thermograms with changes in the components of the substrates.

MATERIALS AND METHODS

Fungi and substrates

The fungi used in this investigation were *Agaricus bisporus* and *Pleurotus ostreatus*. The mushroom compost used was commercially prepared from wheat straw (stored for nearly 6 months) and chicken litter (100 : 1) (Reen Compost, Reen). The pasteurised compost was inoculated with *A. bisporus* (U_3 strain, Darmycel), then bagged (7 kg) and the bags arranged in three groups. Each group contained 13 bags, and three bags were sampled randomly from the three groups. The bags were incubated at ca. 23° C and the tops sealed so as to maintain high humidity. The compost was cased with a mixture of lime and sphagnum moss (pH 7) after 18 days and the casing watered regularly. The temperature in the growth chamber (Fitotron 600H, Fisons) was then reduced to 18° C and the humidity was maintained near 85–90% by adjusting the water sprayer. Mushrooms were harvested every 5–6 days for four flushes. Wheat straw similar to that used for the compost was used as a substrate for *P. ostreatus*. The straw was ground to pass a 20 mesh screen, the straw powder (20 g) was wetted with deionised water (20ml) in flasks and the flasks sterilized for 30 min at 15 psi. They were then inoculated with three 2mm diameter plugs cut from the growing edge of a 5-day old culture plated on agar plates (MA, Oxoid), and incubated at 24° C in near darkness. The flasks were arranged and sampled in the same way as described for mushroom bags. During the incubation period of 5–8 weeks, mushroom compost and straw powder samples were taken every 5 or 7 days for analysis of degraded substrate components, the presence of polysaccharide degrading enzymes and for thermal profiling. A sub-sample was taken from every replicate compost (200 g) and straw (20 g) sample and half

of each of the sub-samples was dried at 85 °C for 18 h. Later all the samples were ground in a Glen Creston mill to pass through a 40 mesh screen.

Enzyme assay

The fresh compost and straw samples (25 g) were homogenised with 0.3M KCl (150 ml) for 2 min, the extracts clarified and dialysed against cool tap water overnight. The enzyme activities of polygalacturonase, pectin-lyase, cellulase and xylanase in the extracts were measured by the release of reducing groups from their respective substrates, i.e. sodium polypectate (5g l^{-1}), carboxymethyl cellulose (1 g l^{-1}) and xylan (1 g l^{-1}) buffered with either Tris–HCl at pH 7 and 8.5, or sodium acetate–acetic acid at pH 5 [16].

Substrate solutions (2 ml) were mixed with 1 ml of enzyme extract, incubated at 30 °C for 1 h and the concentration of the reducing groups determined colorimetrically with 3,5-dinitrosalicylic acid [16]. Laccase activity was assayed by incubating the substrate (2 ml of 2,6-dimethoxyphenol) with 1 ml of enzyme extract at 39 °C, stopping the reaction with 1.0 ml dimethoxy sulphoxide and measuring the absorbance at 468 nm [17]. An increase in absorption of 0.01 min^{-1} was considered equivalent to a unit of laccase activity.

Analysis of the lignocellulosics

Amounts of lignin present in 1 g samples were determined [18]. The samples were also analysed for acid-detergent fibre, neutral detergent fibre [19,20] and ash content. Cellulose and hemicellulose was calculated as the differences of ADF–lignin and NDF–ADF, respectively. Each treatment was repeated three times.

Thermal analysis of the substrates

The samples (3.4–4.0 mg) were analysed by derivative thermogravimetry (DTG) and differential scanning calorimetry (DSC). The following substrates were used as references, xylan from larch wood, pectin (Sigma Chemical Co), cellulose (cotton) and lignin from wood (Westvac Co.).

Differential thermogravimetry

Thermogravimetry (TG) and derivative thermogravimetry (DTG) of the mushroom compost and straw samples were analysed by Perkin–Elmer TG S2 at a heating rate of 20 °C min^{-1} and air flush rate of 10 mm min^{-1}. The experimental conditions were similar to those reported by Sharma and Kernoghan [21]. The DTG curve is a plot of dw/dt vs. time. Each sample was analysed at least twice.

Differential scanning calorimetry

The instrument comprises sample and reference holders mounted on separate heating coils. The DSC system was flushed with air at 40 ml min^{-1}. A control unit (Du Pont 990, Thermal Analyser) programs the system's temperature at a preset uniform rate of 20 °C min^{-1} while keeping the sample and reference temperatures equal. The differences in energy were amplified and recorded.

RESULTS

Characteristics of the mushroom compost and straw

After the compost was degraded by *A. bisporus*, a high level of xylanase activity was detected 14 days from the start of incubation and the activity remained high thereafter. Laccase activity was maximal after 35 days and coincided with the development of primordia. Cellulase activity increased just before casing but fell to lower levels during fruiting (Fig. 1). As the level of laccase activity fell, the level of cellulase activity rose, reaching high levels at fruiting, which was then maintained throughout the subsequent cropping. The levels of polygalacturonase and pectin-lyase were below 50 μg ml^{-1} throughout the experimental period.

The colonisation of straw by *P. ostreatus* started with the enzymatic depolymerisation of pectin and hemicellulose and maximum activities of polygalacturonase, pectin-lyase, and xylanase were detected after 20, 35 and 20 days, respectively, from the start of incubation. Laccase activity was maximal after 30 days of incubation and cellulase activity also peaked after 30 days (Fig. 1).

The chemical analysis of the pasteurised compost indicated that the main components were cellulose (21%), hemicellulose (7%) and lignin (23%). Similarly, the following were the main components of undegraded straw: cellulose (40%), hemicellulose (28%) and lignin (17%) (Fig. 2). At the end of the incubation periods, *A. bisporus* had degraded nearly 50% of the available cellulose, lignin and hemicellulose present in the compost. However, less than 30% of the available cellulose and hemicellulose and only 40% of the lignin were degraded by *P. ostreatus*.

Thermal analysis of the mushroom compost and straw

Although small amounts of residues from other wood constituents were detected as minor peaks in the materials used as references, the main DTG peaks were at 238°, 267°, 362° and 475 °C for pectin, hemicellulose, cellulose and lignin, respectively. Similarly, the DSC thermograms of the

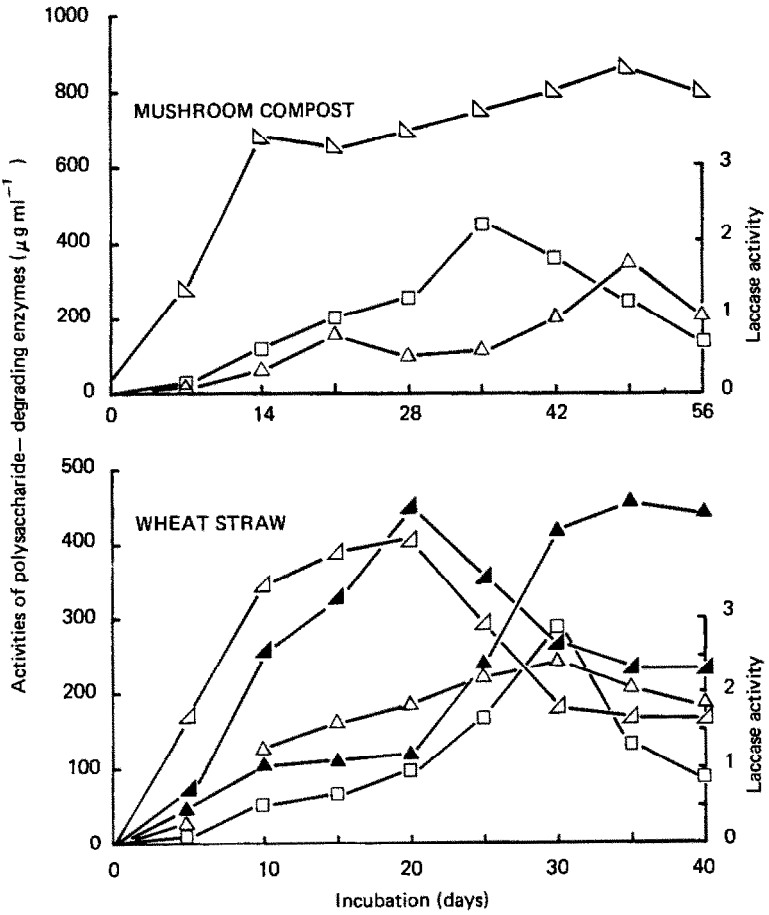

Fig. 1. Changes in the concentrations of cellulase (△), xylanase (◺), and laccase (□) during the colonisation of mushroom compost by *A. bisporus* and of polygalacturonase (◢), pectin-lyase (▲), xylanase (◿), cellulase (△) and laccase (□) during the degradation of straw by *P. ostreatus*. (The levels of polygalacturonase and pectin-lyase were low during the colonisation of mushroom compost).

reference samples were at 250°, 280°, 350° and 475°C for the same materials.

Derivative thermogravimetry

After the preliminary weight loss of moisture at 50–85°C, the straw and mushroom compost decomposed and pyrolysed at 1.5% min^{-1} at about 215–330°C, this representing loss of polysaccharides [11]. This was followed by a minor weight loss peak at about 430–440°C (Figs. 3 and 4). An increase in incubation time correlated with the decline in the weight loss in this region from 52% to 32% (±2) for mushroom compost compared to a

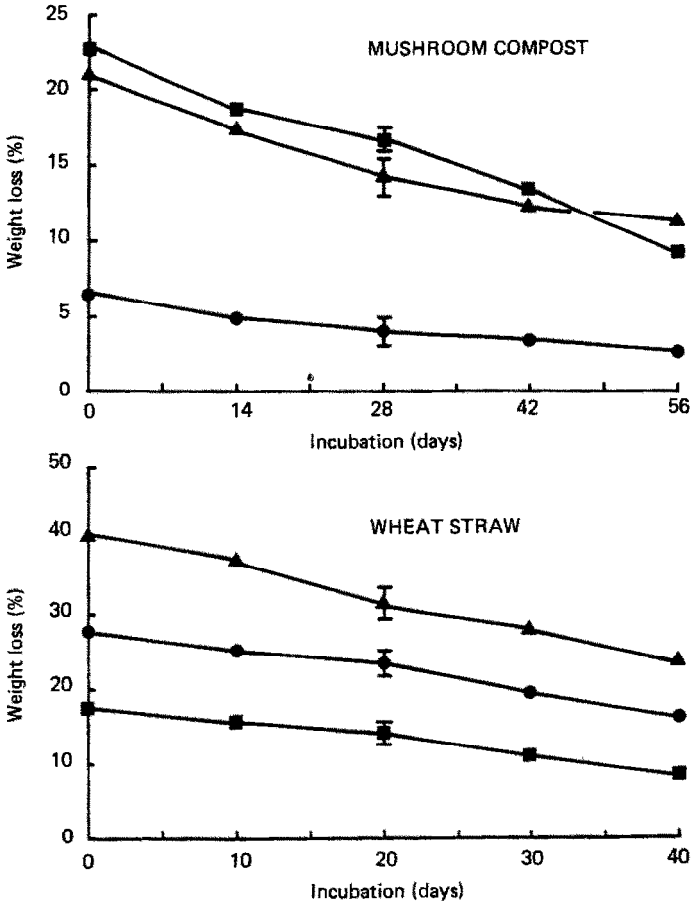

Fig. 2. Changes in the cellulose (▲), hemicellulose (●) and lignin (■) contents during the degradation of mushroom compost and wheat straw by *A. bisporus* and *P. ostreatus*, respectively.

reduction of 64% to 48% (±2) for wheat straw. This was followed by a third weight loss peak at about 430–440 °C (Figs. 3 and 4). By contrast at the end of the experimental period, the minor peak at near 435 °C was absent from the thermograms of both substrates. At ca. 510 °C, the lignin–humus complex present in undegraded compost was pyrolysed at the rate of 0.75% min^{-1} and the lignin present in the straw decomposed and burnt off at the rate of 1.7% min^{-1} at about 478 °C. The progress of fungal colonisation was detected as weight loss from 20 to 7% (±2) for compost, compared to a decline of 19 to 12% (±2) for straw. A small weight loss peak for straw appeared at 500 °C after 30 days of incubation. At higher temperatures, weight loss in mushroom compost was detected at ca. 780 °C, the remaining 22% was left as char. The amount of char left after pyrolysing compost increased with the progress of substrate decomposition and after 56 days

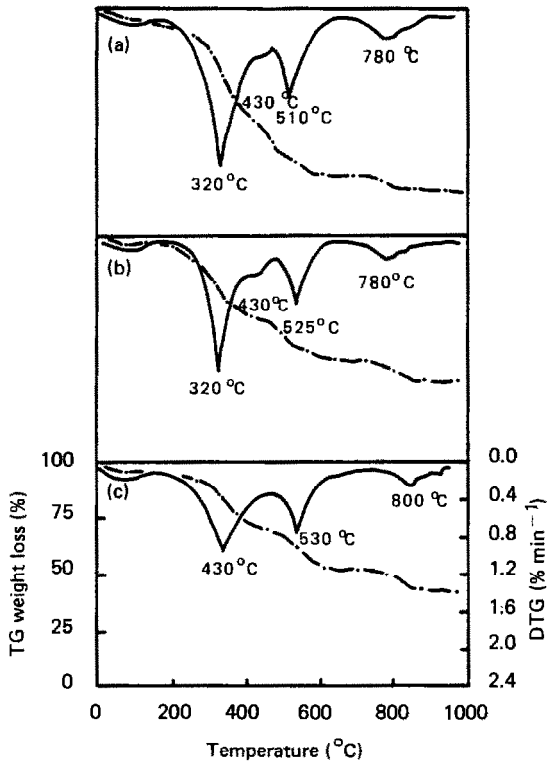

Fig. 3. The thermal analysis of compost; undegraded (a) and degraded by *A. bisporus* after 28 days (b) and 56 days (c) of incubation, showing TG weight loss (———) and DTG profiles (·–·–·–) of the components.

incubation, the residual char was as high as 37%. However, the amount of char left after thermal analysis of degraded straw remained unchanged even after 40 days incubation. With increases in incubation time the three main pyrolysis peaks of compost (320°, 510° and 780°C) shifted to higher temperatures (Figs. 3 and 4). In contrast, the main peaks of the pyrolysis of degraded straw remained unchanged at the end of the incubation period.

Differential scanning calorimetry

The preliminary endothermic reaction of releasing moisture from the uninoculated mushroom compost was followed by exothermic reactions at 325°, 400° and 503°C, the last being joined by a shoulder at 490°C. At the end of degradation of the compost by *A. bisporus*, the DSC curve was similar to that for the unspawned compost, except for the loss of the peak at 400°C and the shoulder at 490°C, and a general reduction in the areas inside the individual exothermic peaks (Fig. 5). The DSC curve of undegraded straw

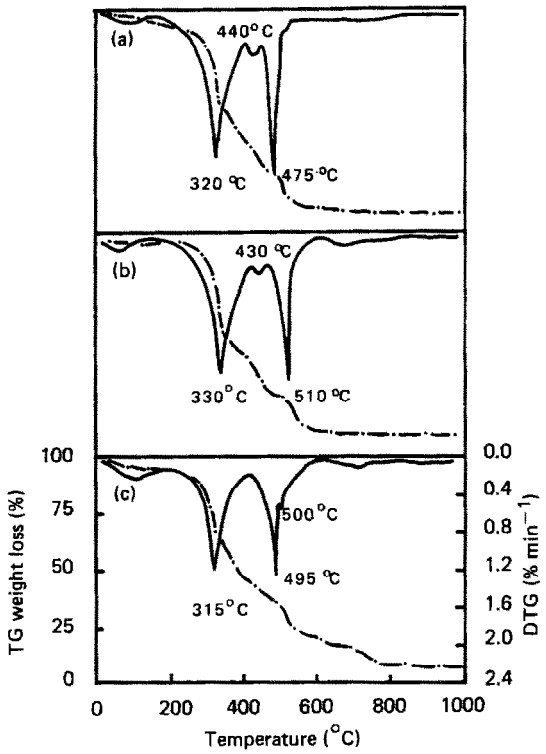

Fig. 4. The thermal analysis of straw; undegraded (a) and degraded by *P. ostreatus* after 20 days (b) and 40 days (c) of incubation, showing TG weight loss (———) and DTG (·–·–·) profiles of the components.

had peaks at 325°, 415° and 460°C. However, after the degradation of the straw by *P. ostreatus*, the peak at 415°C was missing (Fig. 5).

DISCUSSION

The inherent difficulty in the study of plant materials is that they are normally complex and heterogenous, at both macroscopic and molecular levels [11]. Mushroom compost and wheat straw are chemically complex materials consisting mainly of three polymeric materials; cellulose, hemicellulose and lignin. The two test fungi produced different quantities of polysaccharide- and lignin-degrading enzymes. The laccase enzyme was at a high level during the stage of maximum lignin decline in the compost, and cellulase levels increase rapidly at the maturation of the first flush of mushrooms. The results confirmed earlier reports on the enzyme profiles of cellulase and laccase released during colonisation of mushroom compost [22,23]. Although hemicellulose is a minor component of mushroom compost, it may still be an important substrate as indicated by the high xylanase

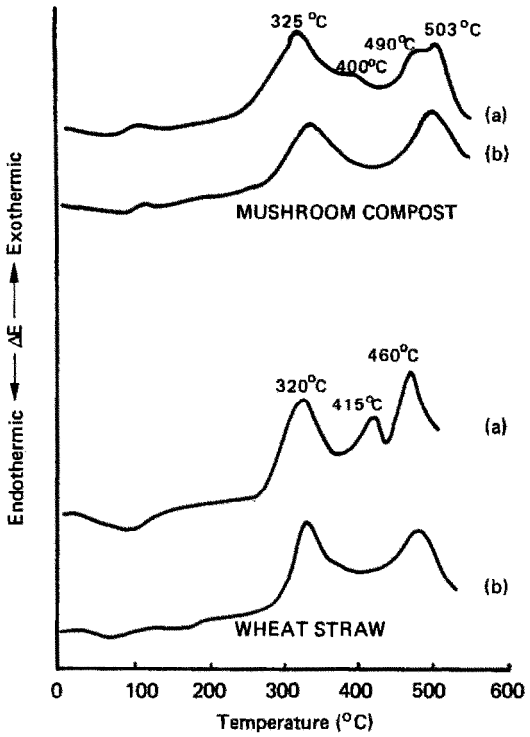

Fig. 5. The DSC curves of compost; undegraded (a) and degraded (b) by *A. bisporus* after 56 days of incubation, and wheat straw undegraded (a) and degraded (b) by *P. ostreatus* after 40 days of incubation.

activity released during the colonisation of compost and the fruiting stage. However, Turner and co-workers [22] did not investigate the levels of hemicellulase present during the spawn run casing and fruiting.

The changes in the polysaccharide and laccase enzymes produced by *P. ostreatus* during the degradation of wheat straw where broadly similar to those reported earlier [12].

The thermal behaviour of these lignocellulosic materials has been assumed to represent a summation of the individual components of the polysaccharide and lignin components [24]. The cellulosic contribution normally predominates due to the larger proportion of cellulose in the substrates tested and to its higher reactivity. This investigation has shown that undegraded straw begins to loose significant weight at ca. 180–330 °C, followed by a less active pyrolysis stage at ca. 390–550 °C. The pyrolysis of hemicellulose and cellulose at ca. 300 °C was followed by a small peak at 430 °C and this DTG peak may represent the thermal degradation of residual xylan which exists in close association with lignin [12]. At near 500 °C, carbonates may be formed and the peaks between 750 and 890 °C may represent the decomposition of the carbonates. Arima [14] studied the

pyrolytic cleavage of various lignins by DSC in a nitrogen atmosphere, and showed that the method of lignin preparation strongly influences the peak positions.

The thermal profiles of lignocellulose recorded by DTG and DSC depend not only on the cellulose content but also on the changes in lignin and hemicellulose [4,9,10]. The DTG profiles changed when *P. ostreatus* degraded the cellulose and lignin in preference to hemicellulose. *A. bisporus* degraded the cellulose, hemicellulose and lignin in equal proportions. However, mushroom compost contains other substrates, including bacterial cell walls, which *A. bisporus* can degrade. The microbial biomass present in peak-heated mushroom compost can be as high as 10% of the dry weight [25] and this may have contributed to the higher weight loss of the mushroom compost during pyrolysis at ca. 200–350 °C compared with the total polysaccharide content (cellulose and hemicellulose) determined by chemical analysis. This was confirmed by the results of experiments carried out on mixtures of straw and microbial biomass. Such mixtures produced three active pyrolysis peaks and the primary and secondary weight loss peaks could be correlated with the changes in the proportion of straw and biomass. The difference between % weight loss in the primary peak and total polysaccharide content (cellulose and hemicellulose; as determined by fibre analysis) represents the microbial biomass present. The determination of microbial biomass on leaf litter and compost has been attempted and most of the methods are complicated and cumbersome [26]. Microbial biomass consists of mucopolysaccharides and protein and differs from plant cell walls which are mainly cellulose, hemicellulose and lignin [27]. Consequently, during fibre analysis for plant polysaccharides, the microbial cell wall fractions were not detected. At the end of DTG analysis, 20% of the sample of compost was left as char, compared with 8% for straw, confirming that mushroom compost contains a higher proportion of inorganic materials.

The use of DSC for detailed analysis of wood degraded by fungi was reported by Reh et al. [15]. The peak near 325 °C in both mushroom compost and straw may be assigned to a composite polysaccharide peak, is similar to the polysaccharide peak of the thermogravimetric analysis. On undegraded mushroom compost and straw, the main exothermic peak near 325 °C was followed by small peaks at 430 °C and 440 °C, respectively. The origins of these small peaks are not clear. However, they may represent hemicelluloses which exist in close association with lignin and are degraded preferentially compared with the hemicellulose fraction that exists in association with cellulose. These peaks are missing from curves recorded on degraded mushroom compost and straw, suggesting that the fungal colonisers have utilised those compounds as substrate. The last peaks of mushroom compost and straw represent lignin–humus complexes or lignin, respectively. A semi-quantitative evaluation of the thermograms recorded before and after incubation indicates a reduction in the peaks representing

polysaccharides and lignin. The exothermic effects of the complete combustion curves changed as a result of fungal degradation of the constituent components.

CONCLUSIONS

Thermal analysis was used to identify changes in lignin, hemicellulose and cellulose which occur during incubation of mushroom compost. The decline in weight loss of compost samples taken at different incubation periods was correlated with the changes in the proportions of the lignocellulose components. This suggests that thermogravimetry, allied with fibre analysis, can be used to determine the rate of breakdown of the polysaccharides and perhaps also changes in microbial biomass and lignin fraction during preparation of mushroom compost. However, the mode of sample preparation and the consistency of the experimental conditions are extremely important.

Experiments are underway to determine the relationship between thermal analysis patterns, other related tests and mushroom yields from different types of compost.

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