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# Use of Differential Scanning Calorimetry for Structural Analysis of Fungally Degraded Wood

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**This paper assesses the potential use of differential scanning calorimetry for analyzing sound and decayed wood. With sound wood, this method permitted the detection of cellulose, hemicellulose, and lignin components as discrete peaks of combustion at defined temperatures. Characteristic changes in the calorimetric thermogram of birchwood (temperature of maxima, peak height, and peak area) were obtained from wood samples degraded by the basidiomycetes *Fomes fomentarius* and *Piptoporus betulinus*. Additional peaks in the thermograms of white rotted birchwood were assigned to lignin degradation products and to mycelium. Results obtained by the differential scanning calorimetry method are compared with those of chemical determination, with particular emphasis on Klason lignin.**

Recent interest in the ability of some basidiomycetes to degrade the highly polymerized lignin component of wood has focussed on two new areas of research. First, intensive screening programs have started to find white-rot fungi which may be suitable for biotechnological purposes (1, 15). The second topic concerns the biodegradation mechanism, isolation, and characterization of ligninolytic enzymes as well as selection of suitable mutants mainly by using the model fungus *Phanerochaete chrysosporium* (8, 20).

Besides their potential application in biotechnological processes, several white-rot fungi are economically and ecologically important forest pathogens. For instance, the parasitic basidiomycete *Fomes fomentarius* causes extensive destruction of birches (*Betula* sp.) in the forests of Berlin.

The ability of white-rot fungi to degrade lignin is usually determined by growing mycelia on natural substrates such as wood or straw with subsequent chemical analysis of the changes in the content of lignin (especially Klason lignin and soluble lignin) and cellulose (14, 16). Complete degradation can also be followed by using $^{14}C$-labeled lignin or lignin model compounds and measuring the $^{14}CO_2$ production by standard techniques (5, 21). By another indirect method the splitting of the model compound "poly-R" can be determined photometrically by a characteristic color change (3, 6).

In the present study we describe an additional method to directly analyze sound and degraded wood samples by the use of differential scanning calorimetry (DSC). In the calorimeter the specimen and an inert reference sample are subjected to increasing temperatures over the range of 30 to 600°C at a specific heating rate. The differential setup of the instrument minimizes the disturbances by environmental factors. The sequence of endothermic and exothermic peaks of phase transitions appearing in the course of such a temperature program can be displayed as a heat flow-versus-temperature scan. Thermograms obtained in this way may be evaluated with respect to the number of peaks, the temperature at the individual maxima, the quantity of absorbed or released energy, and the change of the heat capacity at each phase transition. However, in some cases, such phase transitions are situated close to one another on the temperature scale and merely appear as a small deformation (shoulder) in the trace of the larger peak (22).

Phase transitions appearing during the heating up of wood specimens are caused by successive combustion of the different wood components at specific temperatures. Since the area under a single peak is directly proportional to the heat of combustion and to the amount of the examined wood constituent in the specimen, DSC can supply valuable information about changes in the relative amounts of cellulose, hemicellulose, and lignin in wood samples. Furthermore, accumulation of intermediates of lignin degradation or enrichment with mycelium may also be detected in the thermograms as additional peaks or enlarged areas. It is therefore possible to determine from individual scans the degree and type of decay of a birchwood sample.

## MATERIALS AND METHODS

Specimens of several different sound woods, *Betula* sp., *Fagus sylvatica*, *Eucryphia cordifolia*, *Picea abies*, and field samples collected from rotting birch trunks bearing fruit bodies of *F. fomentarius* or *Piptoporus betulinus* were used. Suitable material was kindly supplied by the Forstamt Nikolassee (Berlin) or the OSZ Holzverarbeitung or were collected in the Spandauer Forst (Berlin) and in the Chilean rainwoods.

A laboratory decay study of birchwood blocks (DIN Deutsche Industrienorm no. 52,176) inoculated with *F. fomentarius* (CBS 311.82) and *P. betulinus* (CBS 377.51) was executed at 26°C for up to 345 days.

Pure aerial mycelium of *P. betulinus* withdrawn from birchwood blocks and of *F. fomentarius* from field samples of birchwood were collected. The samples were ground with an ultracentrifugal mill (pore size, 0.25 mm; Retsch GmbH + CoKg). After extraction with ethanol-toluene (19 [toluene instead of benzene]), insoluble lignin was determined as Klason lignin (4, 17) and soluble lignin was measured (19). To obtain cellulose, the method of Seifert (13) was used, and for Björkman lignin, the slightly modified procedure described by Pepper et al. (12) was applied.

The following substances were used as references: xylan from larchwood (Sigma Chemical Co.), vanillic acid (Sigma), cellulose MN 21 ff (Macherey & Nagel), indulin (Westvaco).

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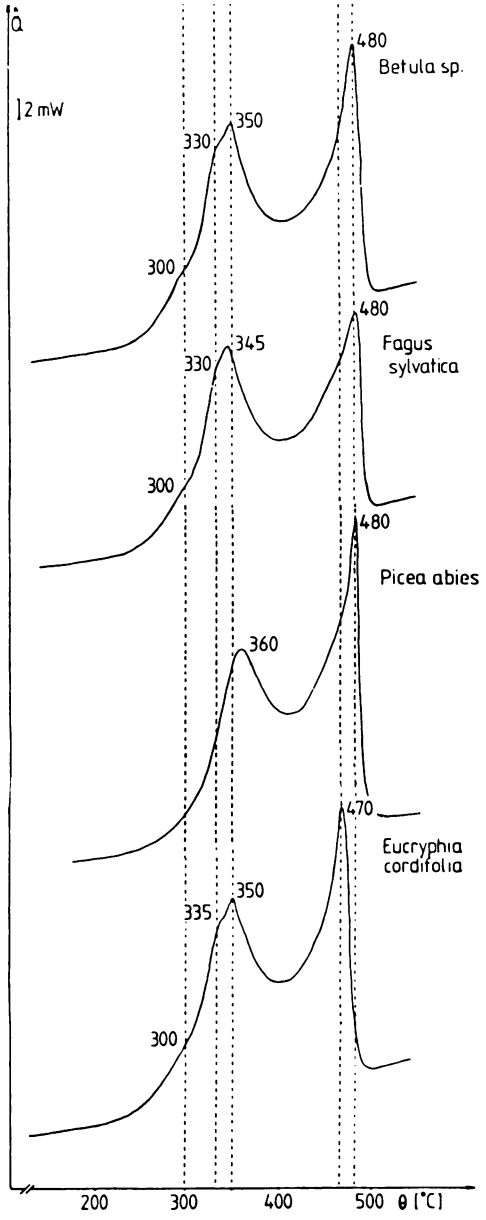


FIG. 1. Thermograms (heat flow, Q̇, versus temperature, θ) of wood samples (3.0 mg each) from sound trunks of *Betula* sp., *Fagus sylvatica*, *Picea abies*, and *Eucryphia cordifolia*.

D-Pinoresinol, dehydrodiconiferyl alcohol, and guaiacylglycerol-β-guaiacylether were kindly supplied by H. H. Nimz. All other chemicals used were analytical grade.

A differential scanning calorimeter (type 910, series 99; Du Pont Co.) with a sensitivity of 50 mV/W in combination with an XY recorder (Servogor XY 743; BBC-Goerz) was used. Experimental conditions for all runs were as follows: initial temperature, 30°C; final temperature, 600°C; heating rate, 10°C/min; recorder settings, *x* axis—1 mV/cm, *y* axis—10 mV/cm. Sample size usually was about 3.0 mg. All runs were done at least twice.

## RESULTS AND DISCUSSION

**Detection and identification of wood components in sound wood.** Thermograms of four different species of intact wood

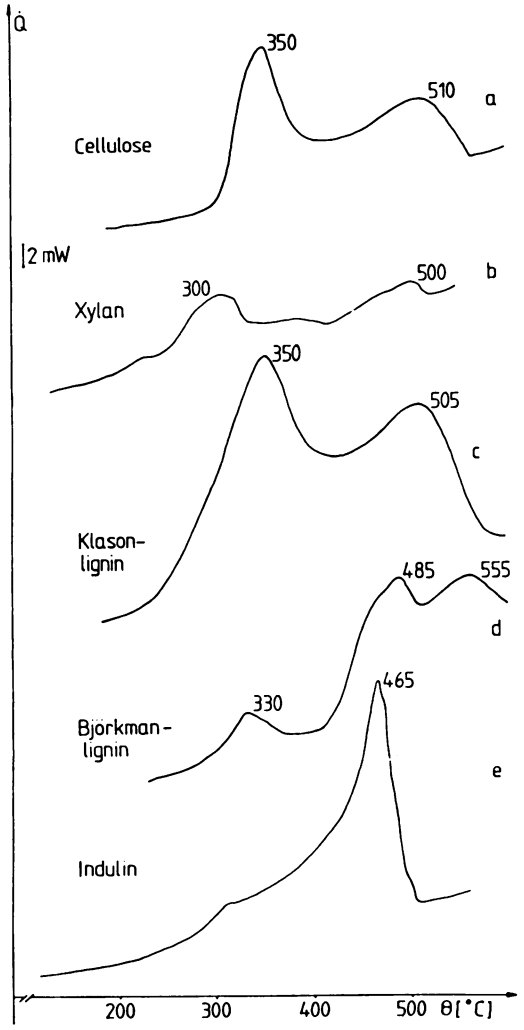


FIG. 2. Thermograms of reference compounds. Sample size: cellulose, 2.5 mg; xylan, 3.0 mg; Klason lignin, 2.4 mg; Björkman lignin, 3.8 mg; indulin, 0.9 mg. Q̇, Heat flow.

are shown in Fig. 1. In each of these thermograms two main peaks and two more or less distinct shoulders appear with their maxima at the temperatures indicated in the graph. The position of these maxima depends to some extent on the sample size and the heating rate of the calorimeter. Under the chosen experimental conditions the maxima of the two main peaks fell within the range of 345 to 360°C and 470 to 480°C, respectively. In addition, two shoulders were found at about 300 and 330 to 335°C. Besides these small variations, the thermograms of sound hardwoods merely differed from each other quantitatively, not qualitatively.

Peaks from the different wood samples were compared (i.e., cellulose, hemicellulose, and lignin). For this purpose, reference substances were run in the calorimeter. With pure cellulose two peaks were obtained with maxima at 350 and 510°C (Fig. 2a). According to Mitchell and Birnie (10) these two peaks can be explained by the presence of volatile and nonvolatile components and their differing temperatures of combustion. As expected, after addition of pure cellulose the peak at 350°C in the birchwood thermogram strongly increased and a shoulder at 515°C became visible.

Xylan is the dominating hemicellulose in birchwood, with

35% of dry weight. Commercial xylan from larchwood shows a maximum at about 300°C and a small peak at 500°C (Fig. 2b). Addition of xylan to a birchwood sample increased the shoulder at 300°C and introduced a small peak at 530°C.

Lignin, constituting about 20% of dry weight in birchwood, has a remarkably high energy content and produced the most prominent peak in the thermograms of wood samples. With three types of lignin prepared by different methods quite distinct thermograms were obtained: Klason lignin exhibited two maxima at 350 and 505°C (Fig. 2c), similar to those found in the thermograms of cellulose. Björkman lignin revealed three maxima at 330, 485, and 555°C (Fig. 2d), while indulin (industrial Kraft lignin) merely showed one prominent maximum at 465°C (Fig. 2e).

This remarkable variation among different lignins is not surprising since Klason lignin particularly, obtained after acid hydrolysis, is surely not identical to native lignin in untreated wood (2). On the other hand, chemical properties of lignin such as the degree of condensation and the quantity of different functional groups are considerably altered depending on the preparation procedure (7).

It is generally assumed that Björkman lignin is more similar to native lignin than Kraft lignin or lignin sulfonate (11). Indeed, the second peak of Björkman lignin at about 485°C corresponds very well to the main peak of intact birchwood at 480°C (Fig. 1), though the other peaks should also be considered.

The high temperature peaks of cellulose (510°C), xylan (500°C), Klason lignin (505°C), and Björkman lignin (555°C) were never observed in intact woods. This could be due to their low energy content in comparison with the main peak at between 470 and 480°C. However, it is more likely that these peaks originate from preparation-mediated artefacts, presumably condensation products. Similarly, the prominent peak of indulin at 465°C indicates that some kind of specific modification in the lignin polymer took place.

Although considerable amounts of residues from other wood constituents were found in the material used as references, three peaks in the thermograms of intact birchwood could be assigned: the shoulder at 300°C to xylan, the peak at 350°C to cellulose, and that at around 475°C to native lignin. The additional peak at 330°C, adjacent to the cellulose peak of intact birchwood (Fig. 1), has the same position as the first peak found in Björkman lignin (Fig. 2d). The origin of these peaks is still unclear and deserves further study. This particularly refers to the pronounced peak at 350°C found in the Klason lignin preparation and formerly attributed to cellulose.

**Analysis of decayed wood samples by DSC.** In a first series

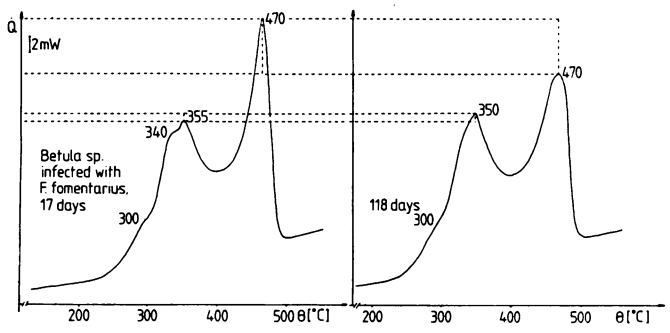


FIG. 3. Thermograms of 3.0-mg samples from sterilized birchwood chips infected with a pure culture of *F. fomentarius* and incubated for 17 (left) and 118 (right) days at 26°C. Q̇, Heat flow.

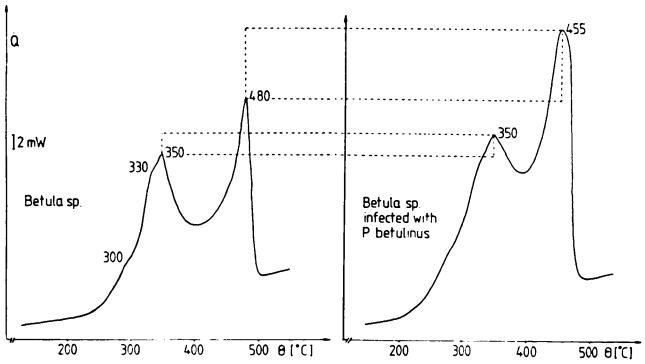


FIG. 4. Thermograms of 3.0-mg samples from sound birchwood (left) and outdoor birchwood (right) infected with *P. betulinus*. Q̇, Heat flow.

of experiments DSC was applied to birchwood samples degraded by either the white-rot fungus *F. fomentarius* or the brown-rot fungus *P. betulinus*. In Fig. 3 the thermograms of birchwood chips after 17 and 118 days of incubation at 26°C with a pure culture of *F. fomentarius* are compared. They show a slight increase of the cellulose peak (350°C) and a strong reduction in the height and area of the lignin peak (470°C). This result is expected for a white-rot decay with preferential lignin degradation. Nevertheless, the relative amount of Klason lignin did not change during 118 days. Starting with 19.1% insoluble lignin (undegraded control), a constant portion of 19.6% was found after 17 and 118 days, while the soluble lignin slightly increased from 2.2 (control) to 2.7% after this time. Therefore, the result of chemical analysis implies a simultaneous white-rot decay.

Brown-rot decay, in contrast, is generally characterized by an increase in lignin content of the decayed wood. As expected, the relative height and area of the lignin peak in the thermogram of a field sample from birchwood infected with *P. betulinus* were strongly increased as compared with intact birchwood (Fig. 4). The same results are given by a laboratory culture of *P. betulinus* on birchwood blocks. Since the brown-rot basidiomycetes are known to selectively remove cellulose from wood while modified lignin accumulates, it is not surprising that the lignin peak in the thermogram was shifted from 480 to 455°C. In this brown-rot sample the content of Klason lignin was increased to 45.5% of dry weight (soluble lignin, 2.6%). Though there are still some discrepancies between the results obtained by chemical and DSC analyses which need further consideration, there is no doubt that white- and brown-rot decay can be differentiated by characteristic changes in the thermogram of a degraded wood. Even the type of degradation of unknown wood decayed by unknown organisms can be determined with DSC (data not shown).

**Detection of mycelium within infected wood by DSC.** Several white-rotted samples from birchwood bearing basidiocarps of *F. fomentarius* were collected in the forests of Berlin and analyzed by the DSC method. Some of these samples showed a dramatic reduction of the lignin peak but had an additional peak with a maximum at 430 to 440°C (Fig. 5a), which was never observed in any sound wood. Since these rotted samples still contained active mycelium, which grew out within a few days after dissecting the trunks, it was suspected that the new peak might be due to the fungus itself. In fact, pure mycelium of *F. fomentarius* produces two main peaks in the thermogram with maxima at about 320 and 435°C (Fig. 5b). When some of this mycelium was

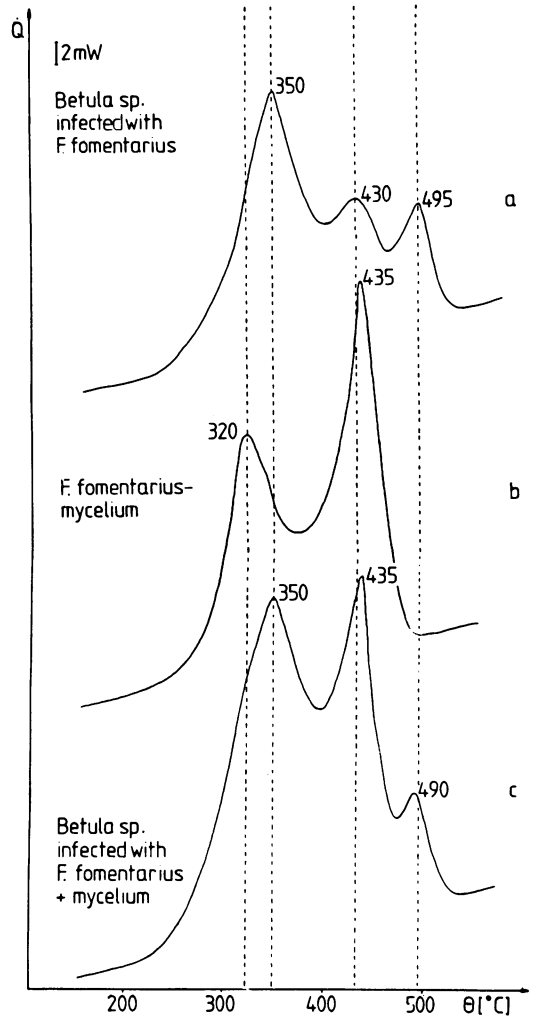


FIG. 5. Thermograms of 3.0-mg samples from (a) birchwood infected with *F. fomentarius* (outdoor specimen BW9 in Fig. 7); (b) pure mycelium of *F. fomentarius*; and (c) parallel sample from (a) with mycelium added from (b) showing superposition of the presumed mycelium peaks at 430 to 435°C. Q̇, Heat flow.

combusted together with the degraded wood sample, the peaks became superimposed with the result that the original cellulose peak at 350°C as well as the new peak at 430°C increased (Fig. 5c). The peak at 490°C assigned to residual lignin compounds remained unchanged.

Though these observations cannot conclusively prove the suggestion that the peak at 430°C is due to mycelial components, it could at least provide a conceivable explanation. Empty hyphae (cell wall residues) are still visible microscopically in a white-rotted wood even after active growth of the fungus has ceased. But the mycelium peak was only observed in samples still containing the living basidiomycete. Hence, the mycelium peak cannot merely be due to persistent cell wall residues, but rather must originate from compounds which disappear when the fungus leaves the wood.

With the brown-rot fungus *P. betulinus* no mycelial residues could be detected microscopically inside wood fibers, either in pure cultures on birchwood blocks or in degraded field samples from birchwood trunks bearing basidiocarps of this basidiomycete. At the same time, no mycelium peak at 430°C was ever found in thermograms of brown-rotted wood samples.

A preliminary conclusion that can be drawn from these observations is that at least in the case of a still active white rot produced by *F. fomentarius* the degraded wood contains a distinct portion of living mycelium which can be detected in the thermogram by an additional peak appearing between the cellulose and the lignin peak.

**Detection of lignin degradation products by DSC.** Thermograms of white-rot samples differed more conspicuously from one another than those of brown rots did. A reduction in content or a significant change in the chemical structure of the lignin or both, indicated by a simultaneous decrease of corresponding peak height and area, as well as an enrichment of mycelium in the wood, can be detected. Thermograms from field samples of various stages of white-rotted birchwood bearing basidiocarps of *F. fomentarius* often were even more complex than those described above. Additional maxima may appear at 400 to 415°C and 500 to 505°C (Fig. 6). Both of these thermograms still show a typical cellulose peak (350°C). The lignin peak at 480°C, characteristic for sound birchwood, had completely disappeared; instead, minor peaks at 505 and 500°C are now visible.

A semiquantitative evaluation of the thermograms on the basis of peak height in sound wood indicates a reduction of the lignin content in the two samples to about 36 and 7%, respectively. Determinations of the Klason lignin content made in parallel gave a value of 107% for the sample in Fig. 6a (BW3 in Fig. 7) and of 126% for the sample in Fig. 6b (BW7 in Fig. 7) as compared with sound birchwood (set 100% for lignin content). The results of chemical analysis and DSC once more show a clear discrepancy: the thermograms indicate that the original lignin has been largely degraded, while the Klason determination points to a simultaneous cellulose and lignin degradation with even a slight enrichment of "insoluble lignin." This discrepancy could tentatively be explained if one assumes that, besides fungal mycelium, some accumulating lignin derivates contribute to

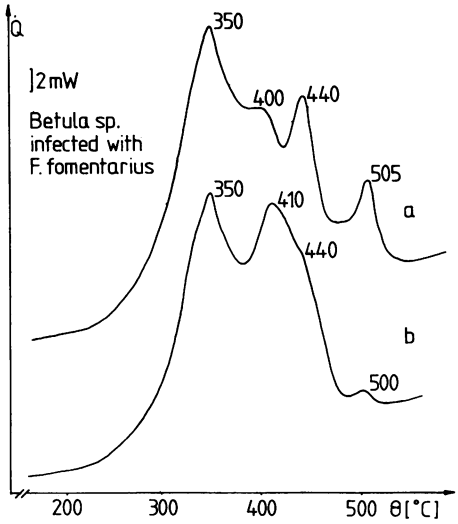


FIG. 6. Thermograms of outdoor birchwood samples infected with *F. fomentarius* showing additional peaks in the intermediate temperature range: (a) 2.8-mg sample of BW3; (b) 3.0-mg sample of BW7 (cf. sound *Betula* sp. in Fig. 1). Q̇, Heat flow.

the peaks in the range of 400 to about 480°C. The Klason method, in this case, would not differentiate between these compounds and the native lignin polymer, while DSC does due to their lowered combustion temperature.

To substantiate this hypothesis, three typical dimer lignin model compounds (D-pinoresinol, dehydrodiconiferyl alcohol, and guaiacylglycerol-β-guaiacylether) were tested in the calorimeter. Of these, only dehydrodiconiferyl alcohol had two characteristic peaks at 420 and 550°C. Addition of the latter compound to the wood sample BW 7 resulted in a shift of the maximum from 550 to 505°C and in increase of the peak at 410°C. Presumably, other oligolignols may be found with peaks like those observed in the degraded wood, which then should also be tested by the Klason procedure.

**Comparative analysis of wood components by chemical separation and DSC.** To estimate the reliability of the chemical and thermodynamic analyses on a broader basis, the results of chemical determinations of Klason lignin and Seifert cellulose were compared with a semiquantitative evaluation of corresponding thermograms. The distance between the thermogram trace and the extrapolated base line at the temperature of a peak maximum was taken as a measure of the heat of combustion and the mass of the corresponding substance.

Peak height is more sensitive to experimental parameters than the area under the curve. Such parameters are, for instance, sample size, homogeneity of the material, and heating rate. With overlapping peaks it is particularly difficult to determine the contribution of each phase transition to the total heat. Therefore, the chosen procedure has still to be refined by mathematical treatment of the thermograms to correct the base-line shift and to determine the true size and the enthalpy change of each transition. Presently, results of different experiments can only be compared under constant experimental conditions as in Fig. 7. Lignin content of nine field samples of evident white rot (*F. fomentarius/Betula* sp.) was analyzed by the chemical and calorimetric methods. Values are expressed as percentage of undegraded wood as a reference (100%). All values determined from the individual thermograms (hatched blocks) indicate a more or less reduced lignin content, while all but two samples (BW10 and BW11) had increased values for Klason lignin versus sound wood. As can be seen, the divergence between the two methods becomes more prominent with decreasing lignin values obtained from the thermograms. The strongest deviation was obtained with the last two samples (BW7 and BW5) included in Fig. 7.

As pointed out above, the Klason lignin fraction is not identical to the original lignin. About 0.8 to 2.4% of dry weight appears as soluble lignin which can be determined photometrically. A loss of Klason lignin during the ethanol-toluene extraction and an increase by other substances is not detectable by standard methods (2). Therefore, quantitative results obtained by the Klason method (including degraded wood) are disputable. Besides degradation, secondary condensation products of lignin radicals and xylose may accumulate and falsify the obtained values (9). Moreover, about 11% of the dry weight of a pure *P. betulinus* mycelium and 19% of *F. fomentarius* mycelium appeared in the insoluble lignin fraction when subjected to the Klason lignin procedure. One can speculate that some unidentified condensation or degradation products of lignin from an infected wood may also pass into the Klason lignin fraction. As a consequence, it is not surprising that the values for Klason lignin content in most of the white-rotted samples were remarkably increased.

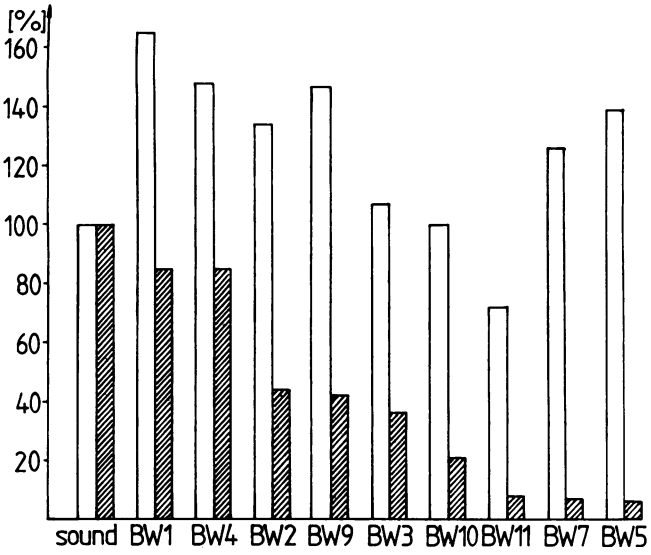


FIG. 7. Lignin content of white-rotted wood samples collected outdoors from nine different birches infected with *F. fomentarius*. White bars represent the values obtained from Klason lignin determinations and hatched bars represent those calculated from thermograms, both expressed as percentage of sound wood.

Likewise, it is difficult to compare the cellulose content from thermograms and Seifert cellulose determinations. The reason is primarily the inaccurate chemical separation. Only 55% was recovered from pure cellulose and just 35% was recovered from sound birchwood.

**Advantages and drawbacks of DSC analysis.** In contrast to the chemical analyses of wood which rely on quantitative separation of individual components, DSC is a nonspecific diagnostic tool for obtaining in a single step the whole pattern of phase transitions which occur during a temperature scan. Without any previous treatment of the extremely small samples, thermograms obtained by one single measurement provide information about all components of a wood sample. The presence of cellulose, hemicellulose, and lignin as well as lignin degradation products and fungal mycelium can qualitatively be detected if the positions of their peak maxima on the temperature scale are known.

For obtaining quantitative values for single components, a correction of the base-line shift during combustion and a mathematical unfolding of the curves are still necessary for a complete separation of superimposing peaks. After such an unfolding it will also become possible to determine calorimetrically the masses of single components of intact wood due to their linear correlation with the heat of combustion.

For a decayed wood this conclusion holds true if the structure of the three main components remains unchanged during degradation as detectable by the position of their peak maxima. Cellulose, for instance, invariably showed a peak maximum at 350°C in almost all wood samples. A smaller area under the peak and hence a decreased heat of combustion can then be interpreted as a true mass loss. If necessary, a portion of a superimposed peak (e.g., mycelium) has to be subtracted from the main area. In the case of lignin, concentrations are particularly difficult to obtain from the heat of combustion since different peak maxima are found depending on the type and degree of wood decay. For brown-rot degradation the lignin maximum was usually shifted about 25°C downwards; for white rots a shift of about 35°C

upwards was also observed. Therefore, calculations of the total lignin content, native as well as modified, are only possible when the physicochemical causes for these temperature shifts are known.

In conclusion, the results of this study have shown that DSC analysis is a very sensitive and useful method for obtaining further insight into the constitution of sound wood and its characteristic changes during biodegradation. Since the danger of introducing artefacts is extremely small, as compared to chemical analysis, the price of an effective differential scanning calorimeter will be justified. Preliminary results are encouraging, and we anticipate that DSC will find its place in the analysis of wood and its decay as it has already done in many other fields of scientific and industrial life sciences.

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