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DIFFERENTIAL SCANNING CALORIMETRY AS A COMPLEMENTARY TOOL IN WOOD BIODEGRADATION STUDIES

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SUMMARY

Lignin degradation by white-rot basidiomycetes merits particular interest for several reasons. Since chemical analysis of biodegraded wood is questionable, the differential scanning calorimetry could be valuable as additional tool, particularly for lignin analyses. With intact wood the Klason-lignin content agrees with the results of DSC determination. But the more the decay of wood proceeds the more the results of the two methods diverge from each other. Therefore the chemical preparations were examined for possible artifacts, e.g. inclusion of decay products, cellulose or hemicelluloses into the Klason-lignin fraction. This could be excluded. Although the results of the Klason- and the DSC-method diverge, neither of the two methods seems to be wrong. It is therefore suggested that the energy-content of the residual lignin in white-rotted wood decreases dramatically, and that such changes can not be detected by the chemical lignin determination but only by the physical DSC analyses.

INTRODUCTION

Since some time biodegradation of wood and especially of lignin by basidiomycetes has found increasing interest. The main topics are: Basic research concerning factors and mechanisms involved in lignin and cellulose degradation as well as characterization of ligninolytic enzymes.

- Special screening programs for efficient white rot fungi useful for biotechnological purposes.

- Wood decaying basidiomycetes in forest ecosystems as at least one aspect of the complex phenomena in "Waldsterben".

One of the problems in wood degradation studies, is the difficulty to quantitatively separate cellulose, hemicelluloses, and lignin from one another. This becomes particularly questionable in wood structurally modified by wood degrading basidiomycetes (white- or brown-rot fungi). The usual procedure to measure the lignin content is the Klason-method, but this is known to transform the native structure of lignin by sulfuric acid and heat treatment of samples. Besides solubilization of some of the lignin, secondary condensation products with xylose are formed and up to 20% of the fungal mycelium appears in the insoluble "Klason-lignin" fraction. By using this method, we observed that most of the white rot probes had a higher lignin content than the intact reference wood.

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This would indicate that cellulose was preferentially degraded. Moreover, Klason-lignin merely gives an over-all quantity. No informations about the quality of lignin can be obtained, and there is no detectable difference between Klason-lignin obtained from sound or decayed wood. To overcome these difficulties, the suitability of differential scanning calorimetry (DSC) for wood analysis, especially for determinations concerning lignin modification and degradation was investigated and the results were compared with that obtained in parallel with the Klason method.

The physical DSC method has several advantages:

- Reproducible DSC-thermograms can be obtained without any pretreatment of the sample, besides mechanical desintegration.
- The small quantity of the sample can be selected in that way, that local differences within decayed wood as in "mottled rots" can be analyzed separately without loss of reproducibility of the thermograms.
- A single thermogram simultaneously presents information about all components of the sample, if they differ in their temperature of combustion. Any changes of characteristic temperatures can be detected and from the height of individual peaks a rough estimation of energy and mass of a substance can be obtained. Thus, type and degree of decay of a wood probe as well as the chronological sequence can be judged.
- The DSC-method makes it possible to differentiate between lignins of different quality in contrast to the Klason-method. For instance, thermograms of brown rotted wood show that maximum temperature of combustion of lignin becomes shifted towards lower temperatures, mostly from 480°C (sound wood) to 450°C. This agrees with the known fact that brown-rot lignin is structurally modified. On the contrary, in thermograms of white rotted woods the lignin peak was often shifted from 480°C up to 510°C while the peak height was lowered. Moreover, several white-rot samples showed additional peaks besides the three main ones of cellulose, hemicelluloses, and lignin. These peaks may be attributed to degradation products of lignin.

Values for the lignin content of identical white-rot samples estimated by the Klason- and DSC-method differ from each other the more wood decay proceeds. With the Klason-method an increased lignin content was often obtained, while DSC indicated a reduction as compared to sound wood. To find out the reason for this remarkable discrepancy the following questions were examined:

- Since Klason- and DSC-lignin values diverge from one another in degraded wood, the question is if a content of 20% Klason-lignin in sound wood can be reproduced by the DSC-method?
- Would degradation products assumed to be responsible for the additional peaks in the thermograms enter into the Klason-lignin fraction?

- Are some cellulose and/or hemicellulose components measured as Klason-lignin?
- Why does lignin prepared by the Klason-method always produce two DSC peaks?

MATERIALS AND METHODS

Field samples collected from rotting birch trunks bearing fruit bodies of Fomes fomentarius were used. The samples were ground with an ultracentrifugal mill (pore size 0.25 mm; Retsch GmbH & Co. KG). After extraction with ethanol-toluene (1 (toluene instead of benzene)), insoluble lignin was determined as Klason-lignin (2, 3). Indulin AT (Westvaco) and sound birchwood (Forstamt Nikolassee/Berlin) were used as references. Partially ashed wood was produced by ashing grounded birchwood at 280°C, 300°C, and 325°C in a muffle oven (type Jumolan M, Gerhardt).

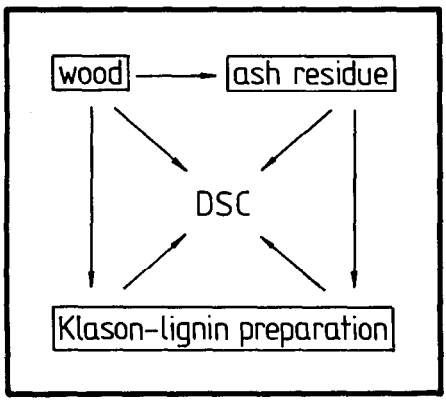

Fig. 1. Scheme of sample treatment. The ground wood was partially ashed, Klason-lignin from this and the untreated wood was prepared and from all samples DSC-thermograms were run.

A differential scanning calorimeter (type 910, series 99; Du Pont Co.) with a sensitivity of 50 mV/W in combination with a 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, 2.0 or 3.0 mg.

RESULTS AND DISCUSSION

The thermogram of intact Betula spec. shows a cellulose peak with a maximum at 350°C, a hemicellulose shoulder at 330°C and a lignin peak at 480°C (Fig.2d). The thermogram obtained with a sample ashed at 300°C (39% residue) gave a redu-

ced cellulose peak (Fig. 2c). By further ashing (33% residue) only a shoulder is left (Fig. 2b), while in an ash residue of 21% the cellulose peak was completely eliminated (Fig. 2a). Only lignin remained. Its amount agrees very well with the 20% of Klason-lignin in sound birchwood.

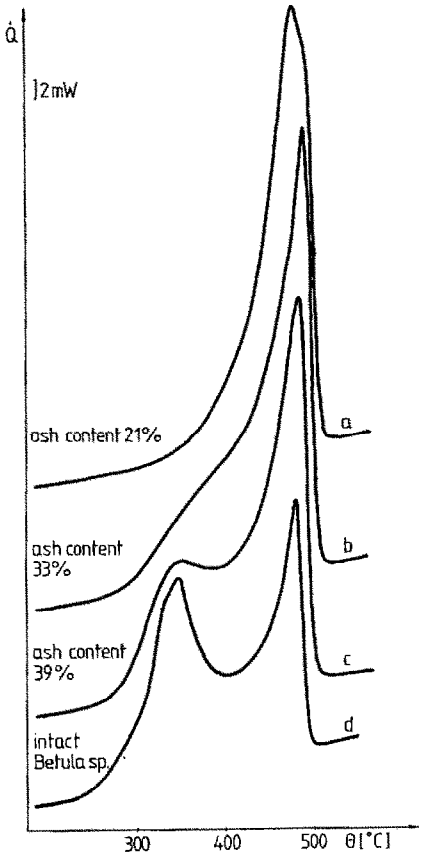

Fig. 2. Thermograms (heat flow, $\dot{Q}$ versus temperature, θ) of intact and partially ashed birchwood samples. Sample size: intact Betula sp. 3.0 mg; probes with 39%, 33% and 21% ash content, 2.0 mg.

If Klason- and DSC-lignin correspond to each other, the question arises, why this is not true for degraded wood. A white-rot sample of Betula sp. with a very reduced lignin peak at 475°C (Fig. 3a) had a Klason-lignin content of 26%, this is 137% of the value of sound wood. Figure 3b shows the thermogram of the Klason-lignin fraction. This is typical for nearly all Klason-lignin preparations tested so far. The presence of an additional peak with a maximum at 410°C leads to the question if this peak influences in some way the Klason-lignin value. To

eliminate an eventual contribution of cellulose a parallel wood sample was ashed at 300°C and the Klason-lignin content in the residue was determined (Fig.3c and 3d). For comparison a probe of sound wood was treated in the same way (Fig. 4a-d). The partially ashed sound wood sample which now consisted only of lignin (peak at 460°C in Fig. 4c) had a Klason-lignin content of 100% (peak at 520°C, Fig. 4d). The partially ashed white-rot probe in Figure 3c still showing one additional peak at 420°C had a Klason-lignin content of 55% and a maximum at 510°C (Fig. 3d). In conclusion, the additional peak appearing at 410°C (Fig.3a) is not measured as Klason-lignin. Tests with other white-rot probes agree with this result. By comparing the peak heights of "true lignin" and the "additional peaks" appearing in thermograms of different white-rot birches with the Klason-lignin content of these probes a relationship is found.

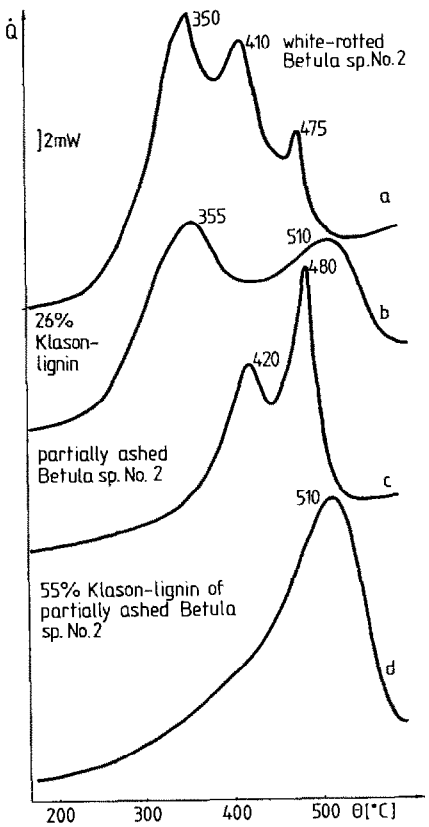

Fig. 3. Thermograms (heat flow, $\dot{Q}$ versus temperature, θ) of a white-rotted Betula sp. No. 2 (3.0 mg) (a), its Klason-lignin (3.0 mg) (b); the ash residue (2.0 mg) (c) and its Klason-lignin (3.0 mg) (d)

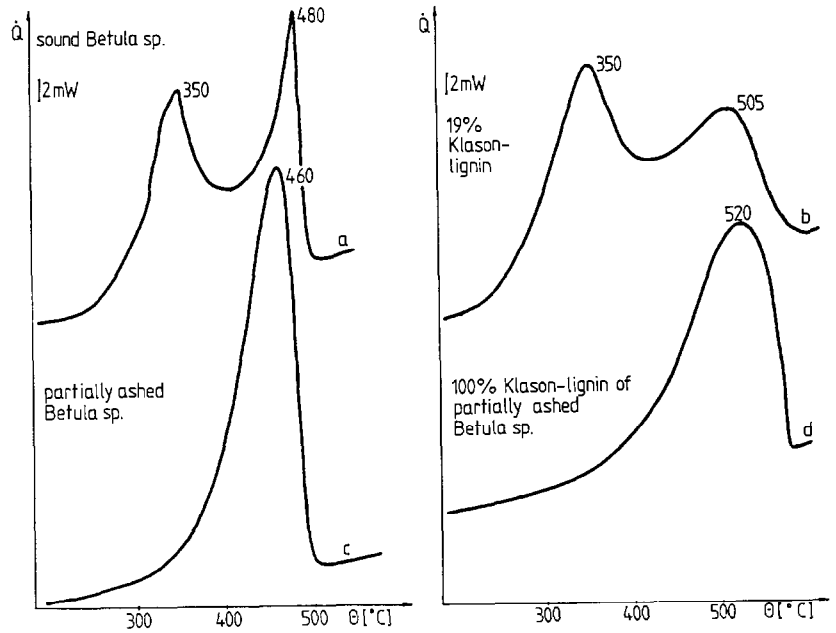

Fig. 4. Thermograms (heat flow, $\dot{Q}$ versus temperature, θ) of sound Betula sp. (3.0 mg) (a), its Klason-lignin (3.0 mg) (b), the ash residue (2.0 mg) (c) and its Klason-lignin (3.0 mg) (d).

If probes are arranged in a sequence of decreasing DSC-lignin also Klason-lignin values decrease (4). If probes are, instead, arranged following the decreasing sum of lignin plus "additional products", no relationship with the Klason-lignin content can be found. Besides that, there seems to be a reverse relation in the thermograms between the increase of the "additional product (s)" and the decrease of the "true lignin". The thermogram of Klason-lignin derived from a partially ashed wood has only one peak (Fig. 3d, 4d) and the Klason-lignin of untreated sound wood or white-rotted wood has two peaks (Fig. 3b, 4b). Since the first of these peaks appears at about the same temperature as cellulose, the question arises, if some cellulose or hemicelluloses become included into Klason-lignin.

Indulin AT, an industrial Kraft-lignin has only one main peak at 465°C (modified lignin) and a hardly detectable amount of cellulose (Fig. 5a). Determination of Klason-lignin gave a value of 85%, but the thermogram of this Klason-lignin preparation once more showed two peaks of equal size very similar to other samples (e.g. Fig. 3b, 4b, 5b). Remarkably, the DSC-thermogram of partially ashed indulin without any cellulose had the same area under the curve as the thermogram of corresponding Klason-lignin. Partially ashed indulin

(Fig. 5c) consists of 99% Klason-lignin. These results show, that the two peaks in thermograms of Klason-lignin contain no or negligible amounts of cellulose. Only 0.4% of industrial cellulose and 1.1% of pure xylan are measured as Klason lignin.

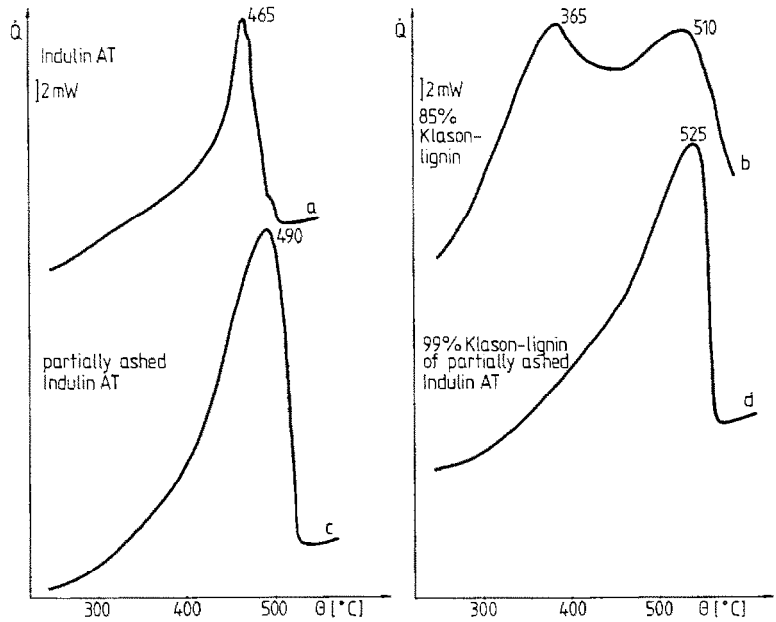

Fig. 5. Thermograms (heat flow, $\dot{Q}$ versus temperature, θ) of indulin AT (0.9mg) (a), its Klason-lignin (3.0 mg) (b), partially ashed indulin AT (2.0 mg) (c) and its Klason-lignin (3.0 mg) (d).

The two peaks of Klason-lignin are reduced to a single one by previous ashing (Fig. 3d, 4d, 5d). Thus, the conclusion can be drawn, that the first peak at 350°C obtained from Klason-lignin is not an inpurity but a lignin derivate produced by the Klason procedure. Accordingly, by using a less concentrated sulfuric acid (ca. 4% instead of 72%), the first peak becomes smaller (not shown). Until now, no other substance could be found, that entered into the Klason-lignin fraction beyond the real lignin. Therefore, the reasons for the progressive divergence of Klason- or DSC-lignin values in white rots are still obscure. But one should not forget that in a thermogram it is not the peak height but the area under the curve that represents the quantity of heat, and thus corresponds to the mass of the substance under question. These values have to be calculated now. It is possible, that the decreasing lignin peaks of rotten woods represent

no, or not only, a decrease of the mass but (also) a lowering in the energy content of the components. Further studies on this subject are in progress.

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