DETECTION OF FUNGAL DEGRADATION AT LOW WEIGHT LOSS BY DIFFERENTIAL SCANNING CALORIMETRY[1]

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ABSTRACT

A thermo-analytical method to detect incipient fungal degradation was investigated. Hybrid poplar (*Populus maximowiczii* × *trichocarpa*) specimens were degraded by the brown-rot fungus *Lenzites trabea* and analyzed at five sequential, 3-day intervals to a weight loss of 5%. To measure the extent of decay, cold water, hot water, and sodium hydroxide solubilities, ethanol-benzene extractive content as well as lignin, holocellulose, and alpha-cellulose were determined. Viscometric analysis was conducted to determine changes in the weight average degree of polymerization (DP_w), and thermal analysis by differential scanning calorimetry (DSC) was performed to determine endothermic transitions in the whole decayed wood, extractive-free wood, and holo- and alpha-cellulose. Chemical analyses provided results consistent with those expected in wood decayed by a brown-rot fungus. DP_w changes of both holo- and alpha-cellulose were significant with regard to decay interval. Analysis of DSC data revealed that this methodology was a reliable means of evaluating fungal degradation in extractive-free wood and holo- and alpha-cellulose preparations from the decayed wood but not the whole wood.

Keywords: Incipient decay, thermal analysis, differential scanning calorimetry (DSC), hybrid poplar, *Populus maximowiczii* × *trichocarpa, Lenzites trabea.*

INTRODUCTION

A relatively rapid means of detecting the effects of progressive fungal degradation of wood at an early stage in the decay process is needed. Biodeterioration caused by fungal organisms at this stage is commonly termed "incipient decay." Wilcox (1978), in a comprehensive review of the literature on decay over the incipient range, interprets a weight loss of less than 10% as an objective representation of wood in the early stages of decay. Even at this slight weight loss, significant strength reduction in wood can occur. At advanced stages of decay, weight loss has long been commonly used to effectively characterize the state of degradation.

Weight loss in decaying wood represents the loss of cell-wall substance as it is metabolized by the fungal organism. This indicator is thus a reflection of fundamental chemical changes occurring in the lignocellulosic complex. Weight loss methods, e.g., ASTM D 1413-76 (1976) and D 2017-71, (1978) are widely used

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to evaluate the effectiveness of wood preservatives to inhibit fungal metabolism and to ascertain the natural decay resistance of wood over extended periods of time. Weight loss because of decay has also been used to determine changes caused by fungi in the basic strength properties of wood, specifically, toughness or impact bending, static bending, compression parallel, and other characteristics even at weight losses as minimal as 1 to 2% (Wilcox 1978). These methods, however, generally require destructive testing procedures on samples of significant size and thus do not lend themselves well to the analysis of a small quantity of the suspect wood.

There are other means available for determining the advent of incipient decay in addition to the standard weight loss methods. A test employed by Safo-Sampah and Graham (1976) utilized the steadily increasing breaking radius of agar-birch sticks decayed by white-, brown-, and soft rot fungi as a measure of the extent of decay. Reliable results were obtained with this method in as little as 2–4 weeks, with a corresponding weight loss of 2% to 6%. A spectrophotometric method resulting from a change in the fluorescent color characteristics of microscopically viewed wood decayed by brown-rot to a weight loss of from 1% to 4% was reported in a sensitive histological technique developed by Krahmer et al. (1982). Wilcox (1983), in evaluating the widely used "pick-test," concluded that as a means of detecting incipient decay this method is sensitive enough to discern wood degradation at 5% to 10% weight loss. Each of the above methods relies on sensing changes in the fundamental physical or chemical properties of the cell wall as decay progresses. Hartley (1958) indicated that weight loss was a suitable basis on which to compare the results of experiments involving wood decay. Kennedy (1958) concluded, however, that alterations in the strength of decayed wood were more closely related to hydrolytic carbohydrate degradation than to weight loss.

The effects of the chemical degradation of wood constituents by selected wood rotting fungi may be followed experimentally by noting progressive increase in alkali solubility products and a decrease in the degree of polymerization (DP) of holocellulose as decay proceeds (Cowling 1961). It is generally held that a reduction in the DP of cellulose due to decay is associated with a concomitant reduction in the residual strength properties of the material.

Thermal analysis describes a number of closely related techniques for measuring changes in selected physiochemical properties of a material as a function of temperature (Wendlandt 1974). While there are several reviews covering the many applications of thermal analytical methods to nondecayed wood (Beall 1972; Beall and Eickner 1970; Schaffer 1966), there are few references on the application of this technique to the analysis of decayed wood.

Isothermal and dynamic thermogravimetric analyses of birch wood decayed by white- and brown-rot fungi over a 12-week period were carried out by Beall et al. (1976). It was found that the degree of thermal instability, as measured by activation energies, was related to the degree of fungal degradation but only after weight losses in this case over 50%. Differential scanning calorimetry (DSC) is a nonequilibrium calorimetric method of examining endo- and exothermic transition energies in materials. This method was employed by Blankenhorn et al. (1980) to investigate thermal changes in aspen wood decayed by white- and brown-rot fungi over a 7-week period. Analysis of the DSC endothermic peak area data, even at low weight loss, revealed that weight loss because of decay was significantly

correlated with changes in the thermal properties of the degraded wood. In both these thermogravimetric and DSC studies, there was the underlying assumption that thermal instability was directly related to changes in DP of the cellulose caused by fungal degradation. This instability may have occurred prior to readily detectable weight loss.

The DSC method appeared to provide a technique whereby fungally induced changes in two major wood constituents occurring at or below a weight loss of approximately 5% could be ascertained. This method employs gram-or-less quantities of wood for testing and is thus essentially nondestructive. In this paper we report changes in the chemical constituents and the DP of brown-rotted wood, extractive-free wood, holocellulose and alpha-cellulose as related to changes in the endotherm peak areas using the DSC method of wood decayed to a low weight loss.

MATERIALS AND METHODS

Wood specimens and decay

All wood samples were removed from the same sapwood growth ring of a single bolt of a hybrid poplar clone NE 388 (*Populus maximowiczii* × *trichocarpa*). Seven, surface sterilized match-stick-size specimens, weighing approximately 0.1 g each, were placed in each of 400 petri dishes containing 30 ml of 3% malt agar medium inoculated with the brown-rot fungi *Lenzites trabea* Pers. ex Fries (Mad. 617). The specimens were supported on U-shaped glass rods embedded in the malt agar medium as previously described by Blankenhorn et al. (1980). The dishes were incubated at 27 C and 80% relative humidity for periods of 3–15 days in the dark, and eighty petri dishes were selected for sampling at each of five 3-day intervals. The specimens in each dish were removed and gently washed under running water to remove surface mycelia. The samples were placed in separate vials and oven-dried to determine weight loss. After weighing, the samples were ground into a meal to pass a 40 mesh and be retained on a 60-mesh standard sieve. It has been shown by Merrill and French (1964) that an experimental error may occur in both alpha- and holocellulose determinations of wood decayed to a 20% or greater weight loss when particle fractions smaller than 60 mesh are not included in the chemical analysis. The wood in our study was decayed to less than 4.7% and did not exhibit the pronounced frangibility one might expect of wood in more advanced stages of decay. Thus, only the 40/60 mesh fraction was analyzed in the current study. The approximate 0.7 g from each dish was stored prior to chemical, viscometric, or thermal analysis. Nondecayed samples served as controls. The general sequence of analysis may be seen in Fig. 1.

Chemical analyses

Random samples of wood meal drawn from specimens from each decay interval, as well as from the nondecayed controls, were analyzed to determine cold water, hot water, and sodium hydroxide solubility. The extractives obtained during the preparation of extractive-free wood (ASTM D 1105-56, 1972), sulfuric acid lignin (ASTM D 1106-56, 1977), holocellulose and alpha-cellulose (ASTM D 1103-60, 1977) were determined. Because of the small sample weights, a semimicro quantity procedure developed by Erickson (1962) for multiple holo- and alpha-cellulose

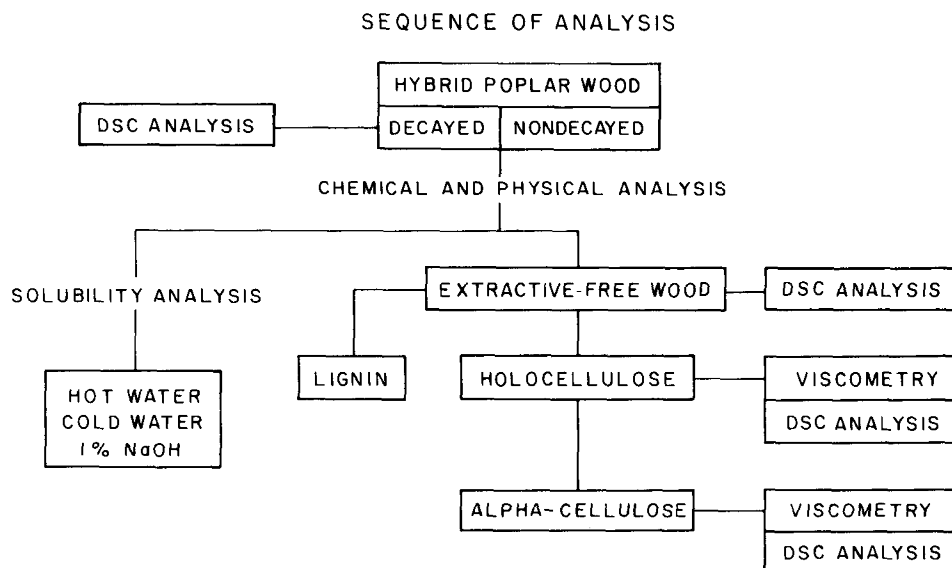

FIG. 1. Sequence of Analysis

determinations was used. In addition, this method was also adapted for use in the other chemical determinations. Thus each ASTM standard method used in this study was modified to accommodate the diminution of sample weights used in the Erickson apparatus.

Cold and hot water extraction.—Both cold and hot water extractive contents were determined using a modified ASTM D 1110-56 (1977) procedure wherein the sample size was reduced to 0.3 g and the volume of distilled water to 25 ml.

Sodium hydroxide solubility.—The NaOH solubility was determined using a modified standard ASTM D 1109-56 (1978). The sample size was reduced to 0.25 g and the volume of 1% alkali to 25 ml. At the end of the extraction period the preparation was sequentially washed with 50 ml distilled hot water, 6.5 ml of 10% acetic acid and 75 ml of distilled hot water.

Extractives derived during the preparation of extractive free wood.—The extractive content derived from an ethanol-benzene, ethanol solvent sequence carried out during the preparation of extractive free wood was determined following a modified ASTM D 1105-56 (1972) procedure. Samples of 0.5 g each were placed in 30-ml medium porosity fritted glass thimbles in the Erickson apparatus. The thimbles were immersed in the ethanol-benzene solution in a heated (60 C) covered, pyrex pan so the level of the solvent was about 4 mm above the surface of the wood meal. At the end of each of four 30-minute periods the solvent was lowered to a level just above the fritted glass bottom and 20 ml of fresh solvent (60 C) was added to the thimble. The contents of the thimbles were stirred every 15 minutes. At the end of 2 hours the thimbles were washed two times in rotation using 30 ml of heated (60 C) 95% ethanol, which was then allowed to drain by gravity. The thimbles were next steeped in distilled water and washed exhaustively with hot distilled water. Finally the excess water was removed by vacuum.

Lignin.—Lignin determinations were carried out on the extractive-free wood

meal using 0.5 g samples. ASTM D 1106-56 (1977) was followed from this point on, but the volumes of sulfuric acid and distilled water were halved.

Holocellulose.—Holocellulose content was determined beginning with 0.5 g samples of extractive-free wood. Ten ml of sodium hydroxide/acetic acid solution were added to each 30 ml stoppered fritted glass thimble. This was followed by 1 ml of 20% sodium chlorite solution, and the thimbles were placed in a heated water bath at 75 C. After 30 minutes, another 1 ml of 20% chlorite solution was added and each thimble was stirred. This process was repeated four times, after which the samples were washed sequentially with a total of 100 ml of 1% acetic acid followed by distilled water.

Alpha-cellulose.—Alpha-cellulose was determined beginning with the holocellulose samples using the Erickson apparatus and a modified ASTM standard D 1103-60 (1977). The thimbles were restoppered, placed in a water bath at 20 C, and 3 ml of 17.5% sodium hydroxide was added to each. The samples were stirred in rotation, and after a period of 5 minutes, 6 ml of 17.5% sodium hydroxide were added and the mixture was again stirred. After a total of 45 minutes, 9 ml of distilled water were added, the stoppers were removed, and the solvent was drawn off by vacuum. The thimbles were washed sequentially with 30 ml distilled water, restoppered, and 5 ml of 12% acetic acid were added. The acid was removed by vacuum after 5 minutes, the samples were washed with 60 ml distilled water followed by two, 20-ml washes of acetone, which also was removed by vacuum.

Viscometric analysis

Samples of both the holocellulose and alpha-cellulose preparations were prepared for intrinsic viscometry determinations for each decay interval according to a modified TAPPI Standard T230 os-76. The samples (0.1 g) were placed in 60-ml serum bottles containing glass beads, the bottle was then closed with a rubber septum and purged with nitrogen through a syringe needle inlet/outlet system. Ten ml of cupriethylenediamine (CED) was introduced into each bottle by syringe and the bottles were shaken until the preparation dissolved. Approximately 30 minutes were required for the holocellulose, while 45 minutes were required for the alpha-cellulose. Aliquots of the dissolved cellulose were transferred to other nitrogen filled, septum-closed bottles containing sufficient 0.5 M CED to give final concentrations of 0.125, 0.100, 0.063, and 0.031% CED. The bottles were again shaken, samples were withdrawn, and efflux times were determined using a nitrogen-flushed size 100 Cannon Fenske standardized viscometer. A plot of reduced viscosity against concentration was extrapolated to zero concentration to yield intrinsic viscosity. The weight average degree of polymerization DP_w was calculated using the K value of Conrad et al. (1951).

Thermal analysis

Samples of approximately 0.003 g of the whole decayed wood, extractive-free wood, holocellulose and alpha-cellulose preparations from each decay interval were tested for thermal transitions in a Perkin Elmer DSC-2B. Each specimen was enclosed in a crimped aluminum sample pan, the specimen chamber was purged with nitrogen and the dynamic temperature programmer was set at 10 K per minute increase across a range of 500 K to 700 K. A sensitivity range of 5 mcal/sec was used to determine the endotherm peak. Baselines were determined

by locating a steady portion, over a range of more than 20 K, and that portion of the baseline was extended until it intersected the exotherm. The peak endotherm area was then determined using a polar planimeter.

RESULTS AND DISCUSSION

Chemical analysis

The results of the chemical and solubility analyses are shown in Table 1. Analyses of variance (ANOVA), significant at the 0.05 level, indicate that changes in the cold water, hot water, and sodium hydroxide solubilities—as well as changes in the ethanol-benzene, ethanol extractive, holocellulose, and alpha-cellulose contents—are highly significant with respect to time interval of the wood decayed to a weight loss of approximately 5%. These observations are consistent with those of Cowling (1961) made over a much longer decay period with greater weight loss. The lignin content was the only chemical constituent that showed no statistically significant change with respect to decay interval as would be expected in brown-rotted wood. This finding is also in general agreement with those of both Cowling (1961) and Kirk (1973). Brown-rot fungi mainly degrade the carbohydrate fraction of the wood leaving the lignin content, as represented by acid insoluble substances, almost undiminished at this incipient stage of decay.

Viscometric analysis

The results of the chemical analyses, particularly the increase in water and 1% NaOH solubilities, strongly suggest that some polysaccharide constituents of the decayed wood had begun to be freed by the end of the first 3-day decay interval. This observation was also verified by viscometric determinations of changes in the DP_w of both the holocellulose and alpha-cellulose fractions (see Fig. 2).

Holocellulose.—The holocellulose content decreased at each time interval as decay progressed. This is consistent with Cowling's (1961) observation that brown-rot fungi rapidly depolymerize cellulose prior to the point where significant weight loss occurs. The products of this hydrolysis accumulate more rapidly than they are consumed. Kirk and Highley (1973), in studying the effect of brown-rot on coniferous woods, reported that glucomannan was removed at a faster rate than cellulose. In addition they suggested that the removal of the depolymerized cellulose may depend on removal of the major hemicellulose component prior to or concomitant with cellulose degradation. They also suggested that the same process could also occur in hardwoods. The rapid initial alteration or partial removal of a large part of the hemicelluloses prior to general cellulose breakdown is a reasonable assumption since these noncellulosic constituents are smaller, noncrystalline heteropolymers which may be the most readily accessible substrates for degradative enzymes.

Alpha-cellulose.—Only a slight decrease in the DP_w of alpha-cellulose over the 2-week decay period was detected. A significant relationship between decay interval and the change in the DP_w of holocellulose was observed; however, the relationship between weight loss and alpha-cellulose was not as pronounced (Fig. 2). Cowling (1961), in discussing the changes in the average DP_w of cellulose in sweetgum sapwood decayed by both white- and brown-rot organisms, observed

TABLE 1. *Extractive solubility means (%) for cold water, hot water, 1% NaOH, and content means (%) of ethanol-benzene extractives, lignin, holocellulose and alpha-cellulose of hybrid poplar sapwood throughout five sequential decay intervals of 3 days each by* Lenzites trabea.[1]

Decay interval (days)	Mean weight loss (%)	Solubility means (%)[3]			Content means (%)			
		Cold water	Hot water	1% NaOH	Ethanol/benzene extractive[5]	Lignin[6]	Holocellulose[7]	Alpha-cellulose[6]
0	0.0 (0.0)[2]	2.03 a[4]	3.08 a	34.65	4.11 a	21.99 a	81.07	47.70
3	0.14 (0.006)	2.08 b	3.17 b	36.81	4.20	21.89	80.01	46.08 a
6	0.27 (0.046)	3.22	4.41	39.51 a	4.78 b	21.56 b	78.70	45.01
9	1.06 (0.082)	3.06	4.74 c	40.89	5.14	21.32	75.85	40.46 b
12	2.78 (0.172)	3.37 c	4.80	40.92	5.01	21.14	73.84	39.18
15	4.67 (0.812)	3.15	4.81	46.95	4.90	21.15	72.46	35.62

[1] Based on oven-dried weight of decayed wood.
[2] Based on nondecayed controls, figure in parenthesis is standard deviation.
[3] Each mean based on 16 samples.
[4] Means with same letter indicate nonsignificant difference between means—Duncan's multiple range test.
[5] Extractives derived during preparation of extractive-free wood, each mean based on 30 observations.
[6] Lignin and alpha-cellulose derived from extractive-free wood, each mean based on 10 observations.
[7] Holocellulose derived from extractive-free wood not corrected for residual lignin, each mean based on 10 observations.

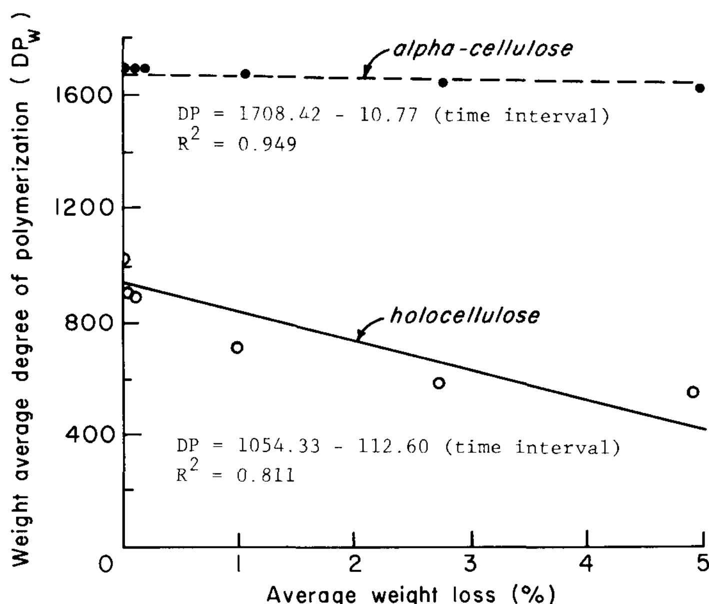

FIG. 2. Weight average degree of polymerization (DP_w) holocellulose and alpha-cellulose as a function of weight loss.

a rapid initial decrease in the cellulose DP_w over a span of approximately 4 weeks. Interpolation of his data indicated an average DP_w of approximately 875 at a weight loss of 4.5%. In our study this precipitous change was not seen over the 2-week experimental duration where the DP_w was 1649 at a mean average weight loss of 4.7%. The slow, gradual decrease of DP_w at this very early stage in the decay process may indicate, under the conditions of the current study, that the depolymerization of cellulose is still restricted by the presence of hemicelluloses and lignins as Cowling and Brown (1969) suggested.

Thermal analysis

The results of both the chemical and viscometric analyses indicate that a portion of the polysaccharide constituents of the decayed wood begin to depolymerize very early in the 15-day decay period. Thus the thermodynamic properties of the wood and cellulosic constituents may also have changed enough so they can be detected by DSC. Figure 3 shows the thermal relationship of the change in endothermic peak areas for decayed extractive-free wood, holocellulose, and alpha-cellulose to decay interval. Figure 4 indicates the relationship between DP_w and the endotherm peak areas for both the holocellulose and alpha-cellulose fractions.

Whole wood.—At a mean weight loss below 4.7%, the application of the DSC analysis to whole decayed wood does not appear to be a useful method in ascertaining the extent of decay. Statistical analyses of either weight loss or decay interval versus the endotherm peak area of the deteriorating wood did not show significance at this incipient stage of decay. Beall et al. (1976) conducted thermogravimetric studies using isothermal and dynamic methods to analyze both white- and brown-rotted whole birch wood samples decayed to an advanced stage