PROTECTION OF WOOD FROM DECAY FUNGI BY ACETYLATION—AN ULTRASTRUCTURAL AND CHEMICAL STUDY[1]

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ABSTRACT

At acetylated weight gains of 15% or above, ultrastructural evidence of wall decomposition was lacking and hyphal cells appeared to be "starved." Blockage of action of fungal catalysts appears to be the primary protection mode of the acetylation technique. The maximum acetylation treatments inhibited consumption of wall polymers and prevented bore-hole formation. Hyphal penetration of cell walls did not proceed by mechanical forces alone; rather, the process was dependent upon chemical action in advance of hyphal tips. A comparison of colonization habits and holocellulose consumption by decay fungi in acetylated woods suggests that the activity, synthesis, or both of lignin-degrading catalysts of the white-rotter is dependent on prior or simultaneous breakdown of carbohydrates.

Keywords: *Fraxinus americana* L., *Pinus taeda* L., *Liriodendron tulipifera* L., brown rot, white rot, acetylation, cell walls, bore-hole formation, hyphae, wood decay, *Coriolus versicolor*, *Gleophyllum trabeum*.

INTRODUCTION

Rowell (1975) defines chemical modification of wood as a chemical reaction between some reactive part of a wood component and a simple chemical reagent, with or without a catalyst, to form a covalent bond between the two. Hydroxyl groups on cellulose, hemicelluloses, and lignin are the most abundant reactive sites in wood; and covalent bonds of the carbon-oxygen-carbon type (ethers, acetals, and esters) are the ones of major importance.

Wood modification techniques are currently receiving interest for the prevention of wood decay, since in some applications they may have advantages over the conventional broad spectrum-type preservatives. Because of their toxicity, conventional preservatives such as pentachlorophenol and creosote are the subject of growing environmental concerns. With wood modification, nontoxic compounds are fixed into the wood structure and the toxicity problems may be avoided.

Fungal enzymes, like most others, are highly specific, their mode of action depending on the substrate molecule, or a portion of it, fitting the active site of the enzyme in a lock-and-key type of relationship (Lehninger 1970). Through chemical modification, the substrate molecule's configuration is changed so that

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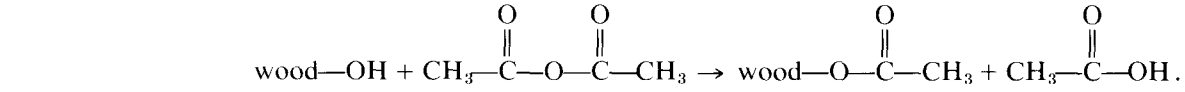
it is excluded from active sites of enzymes, and degradative reactions cannot proceed. One such modification system, which has received considerable attention in this regard, is acetylation. Wood acetylated to weight percent gains (WPG) above 17 has been found to be resistant to decay by a variety of white-rot and brown-rot fungi (Tarkow et al. 1950; Goldstein et al. 1961; Ozolina and Svalbe 1966). Working with wood of lumber thickness, Goldstein and coworkers (1961) found uncatalyzed acetic anhydride in xylene at 100–130 C to be the optimum acetylation conditions:



All the weight gain in acetylation can be directly converted into units of hydroxyl groups blocked, since this technique is a single-site reaction (one acetyl per reacted hydroxyl group). The above acetylation system results in a good distribution of acetyl groups not only in cellulose and hemicelluloses, but in lignin as well (Rowell 1975). Thus, acetylated wood may be both an attractive and viable alternative to broad-spectrum preservatives where decay conditions are not severe or where environmental risks are concerned. This study, then, will evaluate the effects of decay fungi (chemical and ultrastructural) on woods acetylated to various levels of protection (WPG's) in an attempt to better understand the initial stages of cell-wall decomposition and the method of protection of an acetylation technique.

MATERIALS AND METHODS

Acetylation with acetic anhydride and acetyl content determinations

Blocks 1.0 cm × 1.0 cm × 0.5 cm (axial direction) were cut from the outermost sapwood of *Liriodendron tulipifera* L. (yellow poplar), *Pinus taeda* L. (loblolly pine), and *Fraxinus americana* L. (green ash), so that the three opposite faces were in transverse, tangential longitudinal, and radial longitudinal planes. The never-dried specimens were solvent-exchange dried, then oven-dried, weighed, and immersed in a solution consisting of 25% (V/V) acetic anhydride in xylene. A vacuum was drawn on this system for 30 min, after which the samples soaked in the acetylation media for 1 h at atmospheric pressure. The apparatus used was similar to that described in AWPA M10-74. Each block was immediately weighed and transferred to a 500-ml round-bottomed flask containing the above concentration of acetic anhydride in xylene. The solution was heated under reflux and samples were removed from the reaction at intervals of 1, 5, and 29 h. Following reaction, the blocks were washed in water until free acid was undetectable (three to four days), air-dried for seven to ten days and finally oven-dried and weighed. Selected samples were steam sterilized at 121 C for 30 min, transferred to soil-block decay chambers (AWPA M10-74) previously inoculated with either *Gleophyllum trabeum* (Pers. ex Fr.) Murr. (Madison 617) or *Coriolus versicolor* (L. ex Fr.) Quel (Madison 697), and incubated at 25 C and 70% relative humidity for a period of six weeks. After six weeks' decay, paired blocks were removed (some

used in EM studies), cleaned of surface mycelium, and dried to a constant weight. Weight losses due to decay were determined. In order to determine the relationship between WPG and degree of acetyl substitution, an acetyl determination was conducted by the transesterification method (Browning 1967).

Tribromoacetyl bromide synthesis, acetylation, and microdistribution

It is desirable to know which areas of the wood cell wall are being modified in order to assess more accurately the protective action of the acetylation technique. A search of the literature revealed that wood could be acetylated with tribromoacetyl bromide and that the tribromoacetyl groups introduced should be readily detectable in the SEM by EDXA (Energy Dispersive X-ray Analysis). This compound was synthesized by the method of Yocum and Joullie (1966). Acetylation proceeded when wood blocks were treated with a mixture of 25% (V/V) tribromoacetyl bromide in xylene and triethylamine (equimolar quantities with tribromoacetyl bromide) for 1 h at 55 C.

Blocks of the three wood species were prepared and vacuum impregnated with the above mixture in a manner identical to that in the previous section. Following reaction (1 hr at 55 C), the blocks were washed in water for five to seven days until free acid was undetectable, oven-dried, and WPG's calculated. Specimens with microtomed transverse faces were affixed on aluminum specimen holders, coated with carbon, and analyzed by EDXA on a JSM-2 scanning electron microscope operated at an accelerating voltage of 25 kV. Cell walls (all cellular types) were analyzed for Br at the S_1-S_2 and S_2-S_3 interface regions for a total of 200,000 X-ray counts each.

Since three bromines are present in each acetyl substitution, the percent acetyl add-on was calculated from the results of a bromine analysis (conducted on matched specimens by Mikroanalytisches Laboratorium am Inst. f. Physikalische Chemie, Vienna).

Electron microscopy of acetylated woods

Decayed blocks of acetylated wood were transferred directly from decay chambers to fixative and, with a razor blade, transverse and tangential longitudinal pieces (measuring 2 mm square) were cut from the blocks' centers. Samples were fixed in 6.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) at 4 C for 15 h and postfixed in unbuffered aqueous 2% $KMNO_4$ at 4 C for 48 h. Acetylated samples (three WPG classes) of woods lacking fungal exposure were carried through the same procedures. Fixed material was embedded either in a methacrylate mixture (80% n-butyl and 20% methyl methacrylate plus 1% benzoyl peroxide) or epoxy and sectioned at a thickness of 600–900 Å. Methacrylate sections were collected on parlodoin and carbon supporting films and were either observed directly or following resin removal with xylene vapors. Additional sections were platinum shadowed (resin removed) or poststained with 2% $KMNO_4$ for 24 h. Only specimens from the low and medium acetylation levels were examined with a transmission electron microscope operated at an accelerating voltage of 80 kV.

Decayed acetylated blocks to be prepared for scanning electron microscopy were removed from culture bottles, excess surface mycelium was removed, and the blocks were allowed to air-dry. Blocks microtomed to provide smooth trans-

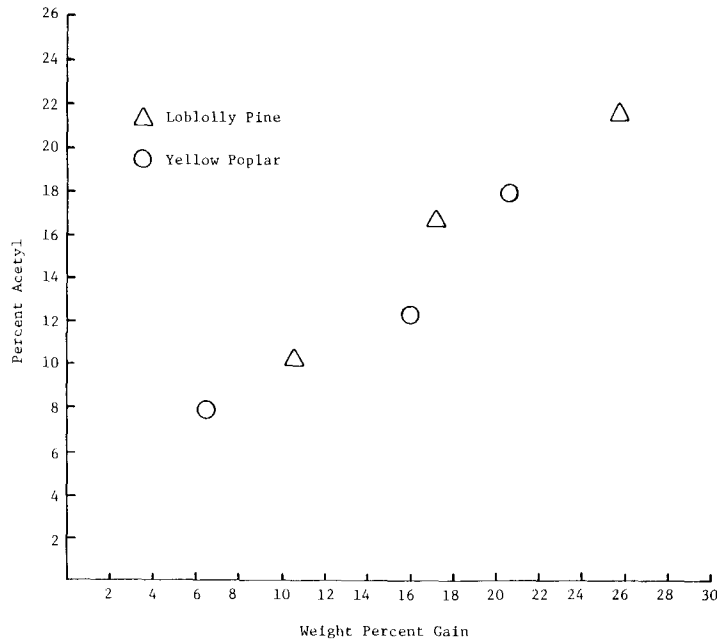


FIG. 1. Relationship between WPG and acetyl content in acetylated (25% V/V acetic anhydride in xylene) loblolly pine and yellow poplar.

verse and tangential surfaces were affixed to specimen holders, coated with gold on a sputter coater, and examined in an SEM operated at an accelerating voltage of 25 kV.

Fungal viability in acetylated wood

Small pieces of brown-rotted and white-rotted wood were aseptically removed from the centers of decayed, acetylated blocks and plated out on 2% malt agar. Five specimens were used for each combination of species, acetylation level (WPG), and test organism, and allowed to incubate for one to two weeks. Viability was expressed as the number of plates in which the fungus grew from wood to the malt agar surface.

Utilization of carbohydrates

A carbohydrate analysis was conducted on nonacetylated decayed samples, decayed acetylated woods, and sound-wood controls. A slight modification of the method of Borchardt and Piper (1970) was employed using a gas chromatograph with a flow rate of 60 cc/min and operated isothermally at 195 C.

Colonization of acetylated and unmodified wood

Degree of colonization in all three levels of acetylated as well as nonacetylated wood was measured after six weeks' exposure to each decay fungus. Decayed wood was embedded in 100% n-butyl methacrylate (plus 1% W/V benzoyl peroxide), sectioned at 14 μm on a sliding microtome, and stained for hyphal dif-