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Improving Pulp Production with Raw Material Changes

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IMPROVING PULP PRODUCTION WITH RAW MATERIAL CHANGES

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ABSTRACT

The ideal raw material for pulp production would be wood with a low content of easily fragmented lignin, a high content of crystalline cellulose, and the presence of pulping catalysts. Tree breeding and genetic manipulation offer ways to achieve these goals and improve the selectivity of pulping. In the area of breeding, there has been considerable interest in a naturally occurring mutant loblolly pine tree that is deficient in cinnamyl alcohol dehydrogenase (CAD). The absence of the CAD enzyme leads to a different pool of precursors for lignin production. The precursors possess fewer sites for polymerization that lead to a less branched, lower molecular weight lignin. In comparison to a normal 12-year-old loblolly pine, wood from a 12-year-old CAD-deficient loblolly pine is much more easily delignified under soda, kraft, and soda/AQ conditions. In the area of genetic manipulation, we are attempting to increase the content of anthraquinone pulping catalysts in hardwoods that already produce low levels of these materials. The strategy used might also lead to trees with less lignin.

INTRODUCTION

Chemistry and process changes will continue to provide improvements in the economics and environmental impacts of pulp production; however, the changes will likely be incremental. In contrast, changing the lignin structure and/or reducing the lignin content of woods offer excellent opportunities for dramatically reducing costs, energy consumption, and environmental impacts, while increasing pulp yields. Manipulation of lignin in trees has been achieved in poplar by genetic transformation (introduction of foreign genes); potential pulping benefits have been shown in small-scale cooks.[1,2] However, genetic transformation of commercial softwoods is not yet possible on a routine basis. In addition, potential regulatory problems could slow the large-scale implementation of genetically engineered trees. Alternative approaches are to crossbreed trees for improved traits or identify natural variants of elite trees that contain reduced lignin levels and altered ratios of lignin bonds. Such trees could likely be multiplied to provide future generations of superior wood and pulp-producing softwood trees. This report outlines two approaches to developing an improved wood raw material: (1) using CAD-deficient trees and (2) increasing the level of natural pulping catalysts in trees.

CAD-Deficient Trees

Softwood lignins are principally derived from coniferyl alcohol (**1**).[3,4] Oxidation of coniferyl alcohol to a radical, followed by coupling one radical form with another, leads to the lignin polymer network. The preferred coupling involves union of an O_4-radical with a C_β-radical; about 50% of the interunit linkages in lignin are of this type (Figure 1).[5] Several other linkages are also present in varying amounts, including C_5-C_5 (Figure 1), C_1-C_β, C_β-C_β, C_5-O_4, etc.[5]

Coniferyl alcohol is formed in the plant by reduction of coniferaldehyde (**2**), a step that requires the enzyme cinnamyl alcohol dehydrogenase (CAD).[6,7] CAD-deficient pines have been obtained through crosses of trees that have a mutant gene, the *cad-n1* allele, found in breeding stocks of loblolly pine. Homozygous *cad-n1* trees are obtained from well-defined crosses and are almost *totally deficient* in CAD activity.[8,9] Lignins from CAD-deficient trees are built up from unusual monomers. Analysis of milled wood lignins by NMR techniques[9] and pyrolysis-GCMS[10] indicates that it contains elevated levels of coniferaldehyde (**2**), vanillin (**3**), and dihydroconiferyl alcohol (**4**) and is low in coniferyl alcohol (**1**) (Figure 2). Relative to normal pinewood, lignin in these CAD-deficient trees contains fewer C_β-O_4 linkages and a high number of C_5-linkages.[11]

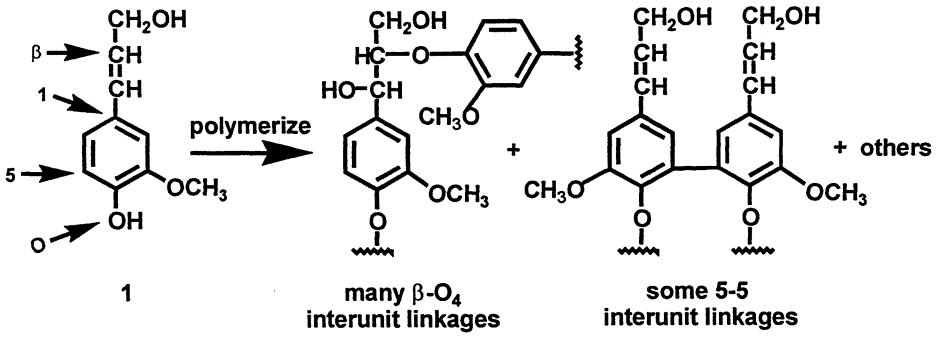

FIGURE 1. Polymerization of normal lignin monomer building blocks.

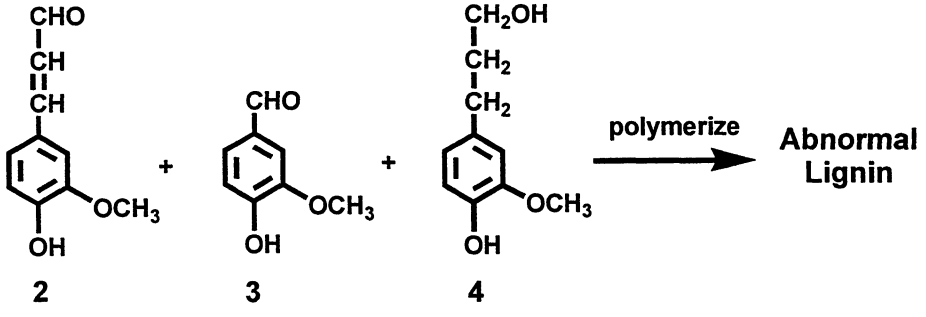

FIGURE 2. The unusual building blocks for lignin production in CAD-deficient trees.

Coniferyl alcohol (**1**), the building block of normal lignin, has four available radical reaction sites (O_4, C_5, C_1, and C_β). Linkages involving C_β are not possible with CAD-deficient lignin precursors **3** and **4**, and probably they are low in frequency with precursor **2**, as Kim *et al.* have recently reported.[12] Since C_β-linkages are precursors to C_α-linkages, the latter should also be low in CAD-deficient lignin. One can speculate that C_1-linkages will also be more infrequent since the aldehyde group at C_1 in the precursor **3** and the saturated side chain at C_1 in precursor **4** will not be easily lost following radical coupling to these sites.

Except for coniferaldehyde (**2**), the unusual CAD-deficient precursors only have two reactive radical sites (O_4 and C_5). Lapierre has shown that O_4-C_5 linkages are more abundant in CAD-deficient wood.[11] Bonding at these sites would result in no cross linking and few residual phenol groups. However, it is known that CAD-deficient lignin has a higher number of phenolic groups than control wood.[11] Because of the low number of reactive sites and the lack of active C_β-sites in the precursors, the lignin in totally CAD-deficient pines will likely be less cross linked, be inhibited in polymer growth, and have a lower molecular weight. These factors, and its higher phenolic content, would facilitate dissolution in alkali. In contrast, its high abundance of pulping-resistant C5-linkages will hurt dissolution. Thus, the pulping and bleaching reactivity of CAD-deficient wood was uncertain and obviously important to the future development of superior trees.

We compared the pulping behavior of two 12-year-old loblolly pine trees, one with normal wood (control) and the other *cad-n1* homozygous (totally CAD-deficient). The trees were grown on the same site and were derived from the same parents. Their lignin contents were 29.5% and 28.5%, respectively. The CAD-deficient tree was much less straight and smaller in size than the control tree; it also contained relatively high amounts of compression wood lignin (~10%).[9,11] Roughly 30% of the lignin was removed from CAD-deficient wood by mild alkaline treatment at room temperature, compared to 10% for the wild type.[13]

Because of a very limited supply of CAD-deficient wood, we developed micro-pulping and bleaching procedures. The pulping studies were conducted with 0.5 g of small wood chips in 4-mL pressure vessels with a high (7:1) liquor-to-wood ratio to ensure that the swelled chips were surrounded by liquor. For many of the cooks, the

chemicals charged into the reactor represent the absolute amounts of NaOH and NaSH that would have been present in a 4:1 cook. Performing 7:1 liquor-to-wood-ratiocooks this way meant that NaOH and NaSH *concentrations* were less than normally employed in a typical 4:1 cook. Therefore, some cooks were also done at standard concentrations but with higher absolute amounts of chemicals.

Soda pulping of CAD-deficient and normal wood with 18% active alkali at several different H-factors showed that CAD-deficient pines are much more easily pulped (Figure 3). The relatively low response of the normal wood to changes in the H-factor is probably related to the lower concentration of NaOH available in the 7:1 liquor-to-wood-ratio cooks.

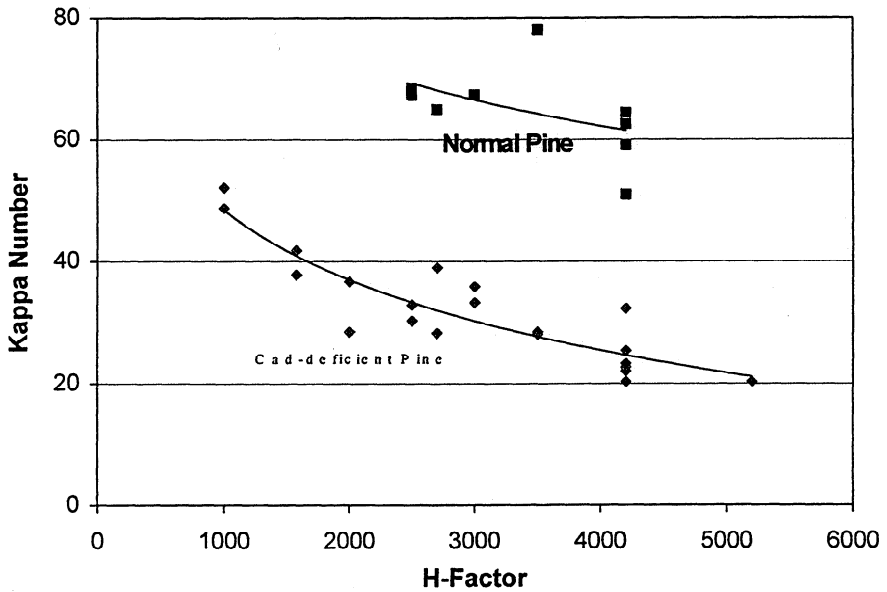

FIGURE 3. Relationship between kappa number and H-factor for soda cooks done with 18% active alkali and a liquor-to-wood ratio of 7:1.

For kraft pulping, we again observed that the CAD-deficient wood delignified more easily than normal wood when the concentrations of NaOH and NaSH were moderate (Figure 4); however, if the concentrations were high, the difference in the degree of delignification were not as large. Delignification occurs in three stages: initial, bulk, and residual. We interpret our results to mean that, in the CAD-deficient wood, the initial and bulk-phase reaction rates are quite fast, but the residual-phase rate is slow, similar to normal wood. For harsher cooks, each wood delignifies to similar residual lignin levels. The pulp yield was ~15% lower with the CAD-deficient wood for soda, kraft, and soda/AQ cooks.

Next, we compared the bleachability of CAD-deficient and normal pulps that had been produced in a similar manner and had identical 29-kappa numbers. Both pulps were produced using 7:1 liquor-to-wood ratio, 20% active alkali, and 33% sulfidity; however, the CAD-deficient wood was cooked at *less than half* the H-factor (790 vs. 1800) of the control wood. The unbleached CAD-deficient and normal pulps (2 g each) were treated in an identical manner with a $D_oE_1D_1E_2D_2$ bleach sequence. There was no difference in the D_1-brightness values for the CAD-deficient and normal pulps; both were 65.3 ± 0.1. The measured brightness values after the D_2 stage were problematic because the CAD-deficient pulp pad had a relatively low opacity.

With a higher amount of 5-5 lignin linkages, CAD-deficient wood should be more difficult to delignify than normal wood. But just the opposite is found. A possible contributing reason for the easy lignin removal could be a lower molecular weight lignin; it would take fewer cleavages to get water-soluble pieces. Indeed, the molecular weight of milled wood lignin obtained from CAD-deficient wood was analyzed and found to be ~2/3 that of normal pine.

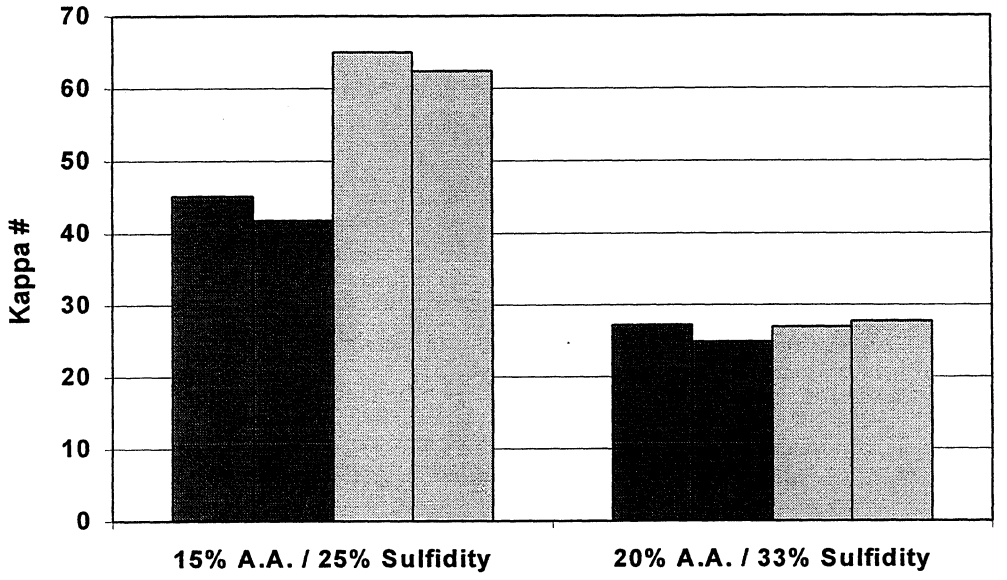

FIGURE 4. Relationship between kappa number and chemical concentrations for duplicate CAD-deficient (dark-colored bars) and normal (light-colored bars) loblolly pine pulping, using a 7:1 liquor-to-wood ratio and a 2000 H-factor.

Pulping Catalysts in Trees

Anthraquinones (AQs) are naturally present in many plants, including teak, which contains methylAQ (~33%) and other AQs that together equal the pulping catalytic level of ~0.7% AQ.[14] Teak pulps very easily and its extracts catalyze the delignification of pine.[14] Because of its scarcity and cost, teak is not a suitable raw material for pulp production. If typical pulpwoods could be encouraged to produce high levels (~1%) of suitable AQs, there would be a large positive impact on pulp mill costs, productivity, and environmental outputs.

Increasing AQ levels in trees is easier when trees already contain the biosynthetic pathway and necessary genes for producing AQs. Therefore, we screened a variety of trees for their AQ contents.[15] Wood samples were reduced to a small size and extracted with an organic solvent; the extracts were then concentrated and analyzed by gas chromatography-mass spectroscopy. Low levels of AQ and anthrone components were detected using a sensitive selected-ion monitoring technique. Ten out of seventeen hardwood samples examined contained AQ-type components; however, the levels were typically below ~6 ppm. Such components were not observed for the few softwood samples that were examined.

We are interested in increasing AQ levels in trees through genetic engineering. Towards this end, we have isolated the gene for isochorismate synthase (ICS). The enzyme converts chorismic acid to isochorismic acid and catalyzes the first committed step in the biosynthesis of anthraquinones.[16-18] In higher plants, chorismic acid plays a central role for the synthesis of a number of aromatic compounds, including lignin precursors.[19] A higher level of isochorismate synthase will increase its competition for chorismic acid and, therefore, increase the flux of chorismic acid into AQ biosynthesis while at the same time decreasing the flux of chorismic acid into lignin biosynthesis.

We have successfully overexpressed an Arabidopsis ICS protein in E. coli and have performed experiments to deliver the ICS gene into model cottonwood plants via Agrobacterium infection. We are now in a position to test the hypothesis that this gene is the rate-limiting enzyme in anthraquinone biosynthesis. After the transformed plants increase in size, we will be analyzing for increased levels of AQs.

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