Improved methods for analyzing (bio)chemical interactions with wood

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The chemical and structural complexity of wood has made it difficult to understand the details of wood structure, its alteration when modified by chemical, biochemical, or genetic means, and its interaction with adhesives and coatings. This limitation has led us to the develop tools that can better measure nanoscale properties. These tools include methods for two dimensional solution NMR, advanced nanoindentation analysis, and quantization of reactive oxygen species. These tools have been used to evaluate chemical and biochemical processes with wood.

Introduction

Understanding the chemical and biochemical processes taking place with wood are often limited by the available analytical tools. Thus, we have placed emphasis on developing tools to better understand the chemical and mechanical changes taking place in some specific processes, such as wood adhesion and biologically modified wood. Many of the tools are useful for studies beyond these initial program areas.

For most wood products, strength is an important property measurement, but we need to know both the chemical structure and mechanical properties for doing structure-property relationships. The cell walls are a nano-composite structure made up of three polymer groupings. Of these groups, the cellulose fibrils are the best characterized and understood. Wood also contains cross-linked lignin polymers made from aromatic monomers. The remaining cellulosics are classified as hemicellulose and contain a variety of sugars that are assembled into different polymeric structures and contain some crosslinks to the lignin network. The nano-composite nature of the wood cells makes separation of these

polymer classes difficult without degradation of the polymers. Thus, properties of isolated components are often different than those in the native cell walls. Improved in-situ analysis of both the wood chemical structure and mechanical properties is vital to improve our knowledge of wood and wood products.

Methods

To better understand the chemistry, we have developed, in cooperation with the University of Wisconsin, a way to prepare wood so that the entire cell wall can be used to obtain high resolution two-dimensional solution nuclear magnetic resonance (NMR) spectroscopy. First, wood shavings are ground to a small size using ball mills made with non-paramagnetic metals and then dissolved in a special solvent mixture. Then the correlations between proton and natural carbon-13 (^{13}C) atoms are measured. The resulting spectra allow separation of signals from lignin aromatic, lignin side chains, lignin methoxy, anomeric carbon, and remaining carbohydrate carbons. Thus, we have the ability to examine many of the polymer structures in wood without separation or modification of these polymers.

Nanoindentation measures mechanical properties of small domains of materials, making the method suitable for measuring wood cell wall properties. However, the cellular properties of wood complicate the data analysis. Joint studies with the University of Wisconsin has led to advanced nanoindentation methods that are suitable for all materials and are very useful in the analysis of wood. These methods allow removal of wood structural compliances from flexing and edge effects, and measurement of broadband creep and broadband elasticity in addition to hardness and elastic modulus.

To address the difficulty of sorting possible reactions during biological decay of wood, we have developed microbeads that provide micron scale maps of reactive oxygen species activity in decaying wood. These fluorescent beads have provided semi-quantitative measurements of oxidative gradients around decay fungi, giving us a better understanding of the mechanisms used by wood decay fungi during early decay.

Experimental

The chemical analysis via the solution-state 2D NMR and other methods allows the determination of how the adhesives, coatings, and modification chemicals

interact with wood. Norimoto separated wood modification into cases of lumen filling and cell wall infiltration. He further separated the cell wall infiltration into simple bulking of the cell wall, reaction with the hydroxyl groups on the polymer chains, and cross linking these chains. Microscopy methods provide information on lumen filling and cell wall infiltration by the added chemicals. This new NMR method provides a measure of whether or not the chemical is reacting with the cell wall polymers. As an example, diisocyanate adhesives provide durable bonds for wood composites, but there has been a debate whether or not the isocyanate actually reacted with the cellulosic and lignin hydroxyls. This NMR analysis allowed us to determine that a small isocyanate molecule reacts with wood only under extremely dry conditions, and that a larger isocyanate molecule reacts even less. Because wood with typical moisture content does not react much with the isocyanate, covalent bonds between the isocyanate adhesive and wood are rare. Therefore, wood-isocyanate covalent bonds do not contribute to adhesive bond durability under normal bonding conditions. Ruling out these options means that the adhesive either simply bulks the cell wall or self-polymerizes to form an interpenetratfing polymer network.

The question of bulking versus self-polymerization can be addressed by measuring the cell wall and middle lamella mechanical properties. Nanoindentation has been used to measure the hardness and elastic modulus on embedded wood specimens. First, we developed a way to make unembedded samples suitable for nanoindentation to eliminate complications from the embedding process. Then, methods were developed to measure the structural compliance effect of wood. The cellular structure can flex, but more critically, the empty lumens create an edge effect, as do the layers in the cell wall. The original materials evaluated using nanoindentation was more uniform than wood and were less sensitive to these effects. Consequently, we developed a way to measure these structural compliances and to correct the measured modulus and hardness values. Having developed ways to determine the structural compliance, we moved on to developing methods for measuring broadband creep and elasticity. These methods were applied to study the isocyanate reactions with wood. From our NMR work, we know that the isocyanate adhesive either simply bulked the cell wall or self-polymerized in the cell wall. The first case would tend to soften the wall while the second would reinforce the cell wall. We found that a monofunctional isocyanate that could only bulk the cell wall did soften it, while the multifunctional isocyanate that could self-polymerize reinforced the cell wall.

The combination of the NMR and nanoindentation methods provides a good route to analyze wood modification processes. Although the methods were developed for and used to understand adhesive interaction with wood, the methods are applicable to many studies on wood and other materials. For example, these methods can be used to study chemical, biological, or genetic modification wood. In fact, the NMR method showed that some the proposed modifications of lignin by a brown rot fungus did not occur, while other, previously unproposed modifications did occur. The nanoindentation methods were first validated on better characterized ceramics, metals, and plastics; thus the methods are applicable to study of most materials.

Another tool to measure processes with wood involves developing a better understanding wood decay. It has been proposed that early stages of wood decay are especially dependent upon the use of reactive oxygen species (ROS). Determining the chemical processes used by fungi during the decay process has been difficult, but we have developed microbead sensors to help understand this. Because these fluorescent beads change color upon ROS exposure, this method allows not only quantization of the ROS, but also the location of the ROS in relation to the structure of the microorganism.

Conclusion

Wood has a very complex chemical composition and morphology that has made it difficult to analyze changes in wood by chemical, biochemical, or genetic processes. This has led us to developing more sophisticated analytical tools for evaluating these processes. The solution state two-dimensional nuclear magnetic resonance method allows the ability to quantify changes in structure of the cell wall polymers. The nanoindentation provides measures of the mechanical properties of the cell walls and middle lamella. These methods have been used to understand the structure–property relationships of wood and wood modified by reaction with an isocyanate adhesive. The NMR method is useful for most wood modification studies, and the nanoindentation methods can be applied to study many materials.

Another tool allows us to measure the reactive oxygen species around fungi during early wood decay. These microsensor beads measure the quantity of oxidant at each location, which we use to create maps of chemical gradients. These gradients are helping us to understand the chemistry of early wood decay.

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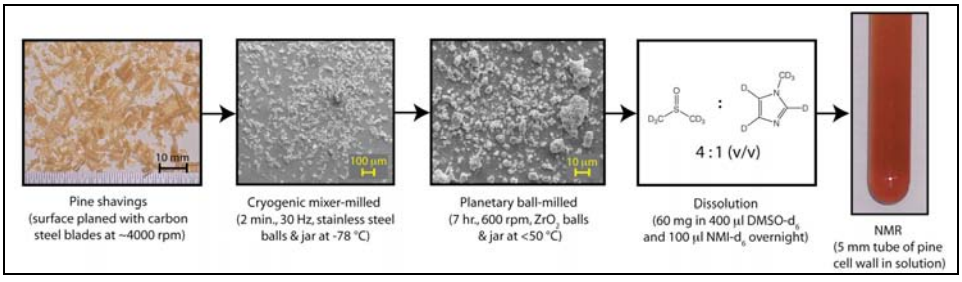

Figure 1. Process for going from wood shavings to a solution for the NMR analysis.

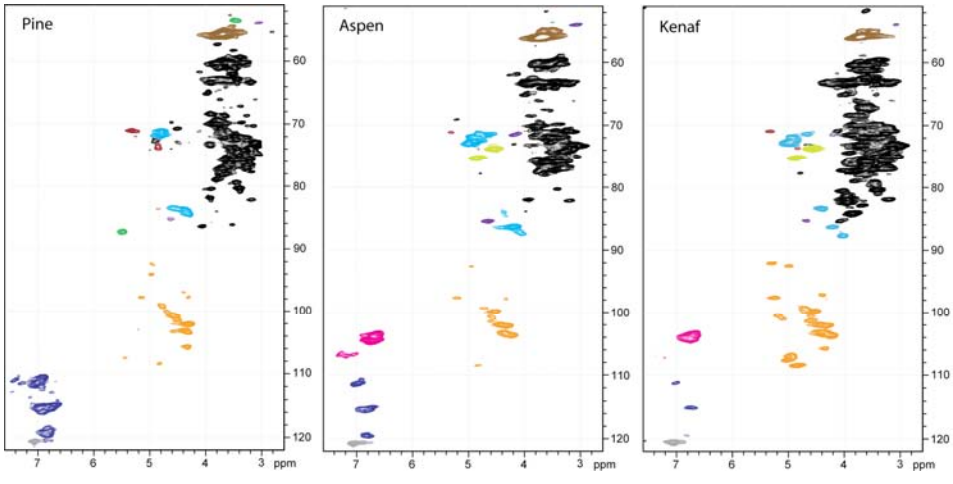

Figure 2. NMR spectra showing differences in spectra between the softwood pine, the hardwood aspen, and the grass kenaf.

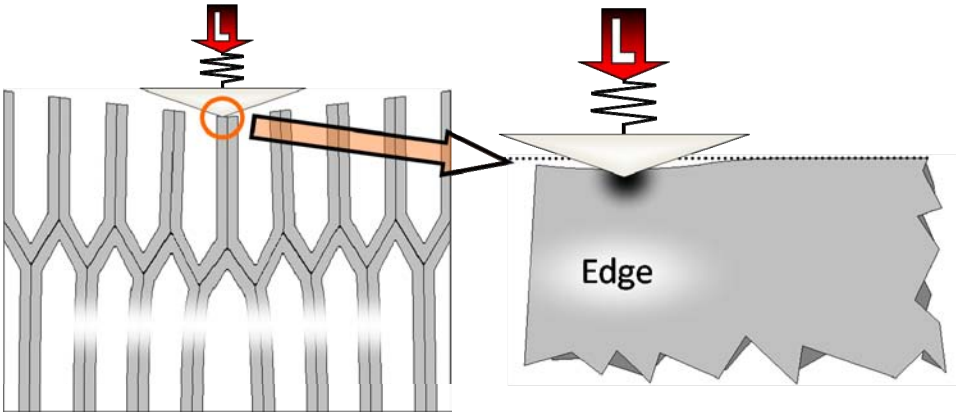

Figure 3. Schematic illustrating the structural flexing on the left and edge effect on the right for the cellular wood structure. (edge picture would be better if left edge was bowed to accentuate deformation.

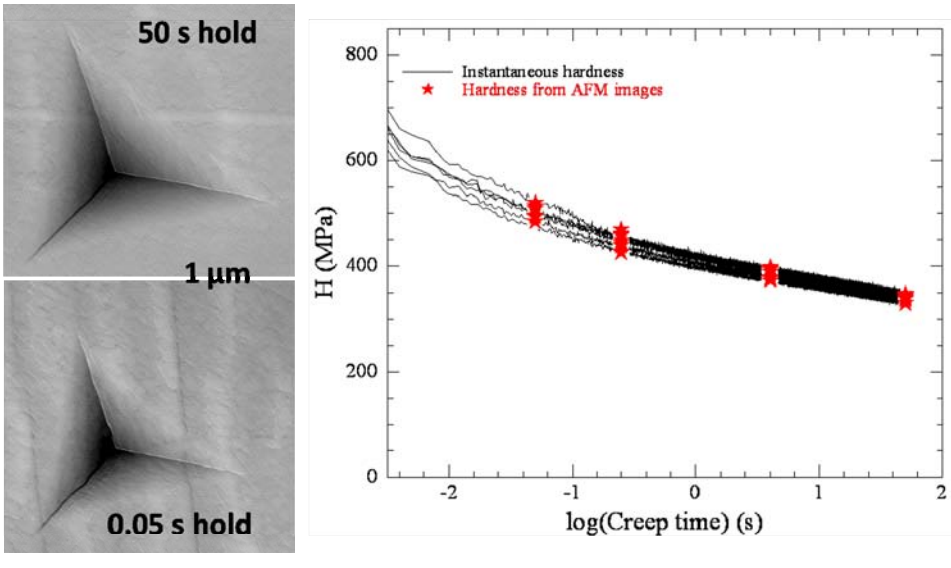

Figure 4. Creep experiments with nanoindenter showing the larger indent on the left for a longer hold time and the creep curve for wood cell wall of pine latewood.

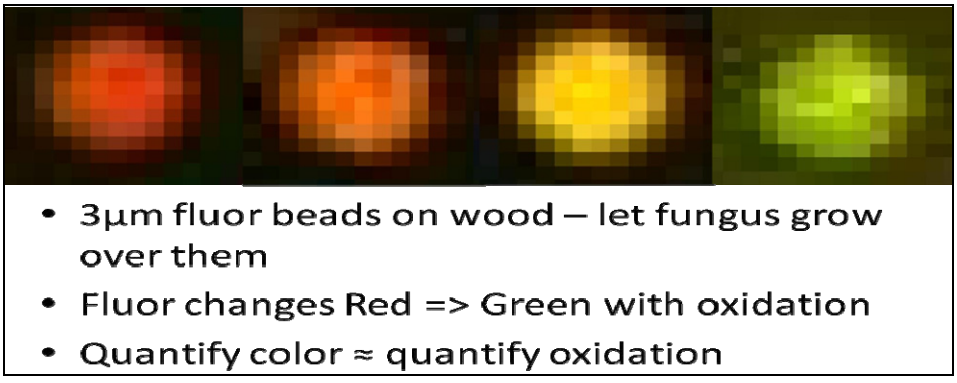

Figure 5. Figure showing the color change from red to green in the microbeads upon exposure to reactive oxygen species.

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