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Micromechanics and ultrastructure of pyrolysed softwood cell walls

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ABSTRACT

Pyrolytic conversion causes severe changes in the microstructure of the wood cell wall. Pine wood pyrolysed up to 325 °C was investigated by transmission electron microscopy, atomic force microscopy and nanoindentation measurements to monitor changes in structure and mechanical properties. Latewood cell walls were tested in the axial, radial and tangential directions at different temperatures of pyrolysis. A strong anisotropy of elastic properties in the native cell wall was found. Loss of the hierarchical structure of the cell wall due to pyrolysis resulted in elastic isotropy at 300 °C. The development of the mechanical properties with increasing temperature can be explained by alterations in the structure and it was found that the elastic properties were clearly related to length and orientation of the microfibrils.

1. Introduction

Wood is a cellular biomaterial with complex properties and various forms of appearance. The tube-shaped lumen of a wood cell (tracheid) is bordered by the cell wall, an intricate structure principally consisting of three biopolymers: cellulose, hemicelluloses and lignin. Cellulose (β-1,4-glucan) forms elementary fibrils with a diameter of 2–4 nm, surrounded by a hemicelluloses matrix [1]. Hemicelluloses consist of short chain polysaccharides with variable structures. Several elementary cellulose fibrils make up a microfibril. The microfibrils, 10–30 nm in diameter, are embedded in a lignin matrix and orderly wound around the cell at a certain angle, referred to as the microfibril angle (MFA) [1]. Novel cellulose fibre-based products derived from wood are successfully used as a reinforcement in polymer composites [2]. The cell wall is composed of several layers, which vary in thickness, MFA and lignin concentration. In general, the stiffness of the cell wall increases with decreasing MFA with reference to the longitudinal direction of the cell [3]. The thickest layer, which determines the mechanical properties of the cell wall, is referred to as S2. The outermost layers (primary cell wall P) and the lignin-rich phase in between two adjacent cells are grouped under the term compound middle lamellae (CML) [1]. This basic architecture forms various wood cells with different properties, due to changes in thickness of the entire cell wall or S2, lignin concentration and MFA in S2 and the ratio of the inner and outer diameters. In softwoods, like spruce and pine, two types of axially oriented cells can be found. Latewood cells, with a smaller lumen and thicker walls, provide stiffness, and earlywood cells, with a wider lumen and thinner walls, undertake the task of water transport. The alternating change in these two cell types results in a regular dark–light contrast and forms the well-known growth rings.

The whole design of the tissue is dominated by the tubular shape of the cells and the complex microstructure of the cell wall, leading to anisotropic mechanical properties, as measured in tensile tests [4]. In Fig. 1 the three basic directions in wood are illustrated. The small size of the structures makes testing of individual cell compounds difficult and suggests nanoindentation methods for measuring the mechanical properties [5]. Nanoindentation has proven to be a valuable tool for testing a variety of biological materials, such as cartilage [6–8], hard tissues [9–11] and even cells [12], helping to clarify the influence of the hierarchical structure, the effects of ageing and the quality of repair tissues [7]. Nanoindentation is also sensitive to the elastic anisotropy, even though the effect is not as pronounced as in uniaxial testing. Indentations in strongly elastic anisotropic metals, like Cu, result in small differences for indentations in different crystallographic directions. This has been studied by Vlassak and Nix, who showed that, due to the complex stress field underneath the indenter, an average elastic stiffness is measured during indentation experiments [13]. In fibre–matrix composite materials, like wood or bone, the fibre orientation can be quite important for the indentation response of the material and a significant elastic anisotropy can be measured by

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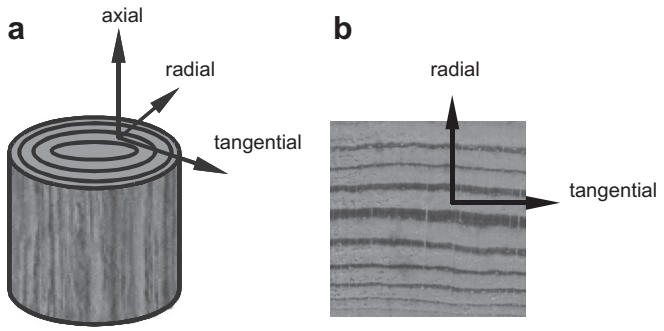

Fig. 1. (a) Principal directions in wood, (b) top-view of a cross-section, perpendicular to the axial direction.

nanoindentation [11,14,15]. Relating the anisotropic indentation response to the elastic constants requires a reverse analysis. However, the method is still quite useful for comparative measurements.

The manifold structures of wood have inspired the design of new functional materials. Novel biomorphous ceramics, as well as Si–SiC composites can be generated from wood templates [16,17]. Fields of application reach from high temperature filters [16] to bone replacement [18]. For the formation of ceramics and composites the pyrolytic conversion of wood into carbonaceous material is required. These porous templates can be infiltrated with melts or be treated with reactive gases to form ceramics. Intensive research has been done on converting wood into monolithic carbonaceous preforms with defined geometry and structure [19], mainly focusing on microstructural changes in the wood cell wall during pyrolysis. Transmission electron microscopy (TEM) showed that the layered microstructure of the wood cell wall disappears during temperature treatment [20], causing degradation of the hemicelluloses and lignin, followed by shortening and reorientation of the microfibrils. Finally, an apparently homogeneous phase surrounding the residual cell lumina occurred at temperatures higher than 325 °C. Broadening of the MFA distribution can be determined from the TEM images via a two-dimensional (2D) Gaussian fit, as described further in Zollfrank and Fromm [20]. The full width at half maximum (FWHM) of the distribution is used to characterize the disorientation of the microfibrils. The mechanical properties of the carbonaceous phase can be determined by nanoindentation change in the temperature regime between 500 and 2000 °C, as investigated by Zickler [21].

In the present work the changes in the ultrastructure of the cell wall during pyrolytic conversion are related to the mechanical properties in the three principal directions of wood (Fig. 1). A transition from a highly structured, heterogeneous biological tissue to a homogeneous, carbonaceous phase was found. We hypothesize a change to isotropic mechanical properties in a temperature regime around 300 °C.

2. Materials and methods

2.1. Pyrolysis

Samples of Scots pine (*Pinus sylvestris* L.) were pyrolysed at different temperatures. In order to minimize the influence of inorganic components of the native wood on the pyrolytic conversion the samples were first extracted for 12 h in toluene:ethanol (2:1) and second for 12 h in ethanol in a Soxhlet apparatus, followed by a 12 h hot water extraction. Afterwards the samples were dried for 12 h at 105 °C [20]. The pyrolysis was performed in a tube furnace (Hareus K1251) flushed with nitrogen, using a heating rate of 2 K min^{-1}. The peak temperature was held for 2 h. Samples were pyrolysed from 200 to 325 °C in steps of 25 °C. The pyrolysed wood samples at every temperature step were cut in the axial, radial and tangential directions. The axial direction is the longitudinal growth axis of the living tree (Fig. 1a). The radial direction is oriented perpendicular to whereas the tangential direction is parallel to the stem surface (Fig. 1b). Additionally, reference specimens of native pine wood were prepared after the same pretreatment.

2.2. TEM imaging

Approximately 1–2 mm segments of heat-treated wood samples were embedded in Spurr's epoxy resin. Ultrathin sections with a thickness of 70–80 nm were obtained from the tangential plane of the wood samples with a diamond knife on an ultramicrotome (Reichert-Jung Ultracut E) and stained for 10 min with 1.0% lead(II) citrate solution. The ultrathin sections were transferred to Cu grids coated with the polymer Formvar® (Plano). Bright field TEM micrographs were recorded on Kodak SO-163 emulsion in a Philips CM 30 microscope operated at 200 keV. TEM micrographs of the same magnification were selected and digitized with a commercial scanner using transmitted light. Images of 512 × 512 pixels were extracted from the digitized TEM micrographs of the S2 region of the cell wall. A fast Fourier transformation (FFT) was performed on the extracted images using Scion image software (beta 4.0.2, Scion Corp.). A 2D Gaussian fit of the FFT images was carried out in a defined circular segment (inner radius 60 pixels, outer radius 160 pixels) over the whole angular range (0–360°) using FIT 2D software (Andy Hammersley, ESRF, Grenoble, France) on the FFT images. This procedure was performed on at least 15 different FFT images obtained from various regions. The raw data from the 2D fit for the cell walls was corrected by subtracting a background file. The resulting data sets providing the frequency of orientation per degree were averaged over the segment width. The measured MFA were corrected according to the inclination angle of the growth direction defined in the TEM micrographs by the CML in the tangential sections. The result was a plot providing the frequency of orientation as a function of deviation from the growth direction, which was taken as a quantitative measure of the MFA of the respective sample.

2.3. Nanoindentation testing and AFM imaging

All samples were embedded in epoxy resin (EpoFix), as well as ground and polished metallurgically. SiC papers up to grade 1600 were used for grinding. Afterwards, the specimens were polished manually with diamond emulsion on a textile washer up to 1 μm crystallite size. The samples were cleaned in a supersonic bath after each polishing step.

Nanoindentation testing was performed using a nanoindenter (MTS Nanoindenter G200) with a Berkovich tip in continuous stiffness mode (CSM). In CSM the major load profile is superimposed with a small sinusoidal oscillation of a given harmonic displacement set prior to testing. The contact stiffness S is calculated from the phase angle between the load and displacement signals [22,23]. The elastic modulus and hardness were deduced from the relationship to contact stiffness and the contact area between indenter and surface, as discussed in detail in Oliver and Pharr [22]. The results are given in terms of the reduced modulus of elasticity E_r, which combines the compliance of the indenter tip and the properties of the sample according to Eq. (1), where E is the Young's modulus and υ is the Poisson ratio.

$$\frac{1}{E_r} = \frac{1-\upsilon^2_{sample}}{E_{sample}} + \frac{1-\upsilon^2_{Indenter}}{E_{Indenter}} \quad (1)$$

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