WITHIN-FIBER NONUNIFORMITIES OF MICROFIBRIL ANGLE

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ABSTRACT

The pattern and extent of variation of microfibril angle of macerated spruce fibers were investigated by confocal laser scanning microscopy. All measurements supported the idea that the orientation of the microfibrils is not uniform along the radial wall of earlywood fibers. Microfibrils had an approximately circular form of arrangement around the bordered pits (inside the border). Between the bordered pits, lower microfibril angles were measured than in the other parts of the fiber. This phenomenon was interpreted by assuming the existence of crossed microfibrils in these zones. Variation of microfibril angle in earlywood fibers was observed only in the vicinity of the bordered pits, not in the nonpitted zones and tangential walls. Within the latewood fibers, microfibril angle was approximately uniform, even close to the pitted areas. The average orientation of simple pits in the crossfield region was consistent with the mean microfibril angle of the fibers; however, some of the measurements showed a highly variable arrangement in the areas between the simple pits.

Keywords: Wood fiber, microfibril angle, latewood, earlywood, bordered pit, crossfield, confocal microscope.

INTRODUCTION

The major part of wood fiber wall consists of cellulose microfibrils, which are embedded helically in the hemicellulose and lignin matrix. The angle between the direction of cellulose microfibrils and the longitudinal axis of the wood fiber is considered as the microfibril angle. Mechanical tests (Page and El-Hosseiny 1983) and models (Cave 1969; Harrington et al. 1998) show that the mean microfibril angle of the S_2 layer (MFA), which is the thickest layer of the wood fiber wall, highly affects the fiber's mechanical properties. Also natural heterogeneities of wood fibers like simple pits, bordered pits (when a border arches out over the pit, normally observed in earlywood fibers), and crossfield zones (wall areas between a wood fiber and a ray cell) affect these mechanical properties. In a recent model, the nonlinear behavior of single wood fibers under tension was explained by MFA nonuniformity and other heterogeneities (Navi and Sedighi-Gilani 2004).

Methods to determine the mean MFA of wood fibers are: X-ray diffraction (Reiterer et al. 1998), orientation of pit apertures (Hiller 1964; Cockrell 1974), soft-rot cavities (Anagnost et al. 2000), polarized light microscopy (Page 1969; El-Hosseiny and Page 1973), direction of crystals of iodine (Bailey and Vestal 1937; Senft and Bendtsen 1985), and confocal laser scanning mi-

croscopy (CLSM) (Batchelor et al. 1997; Jang 1998). Validation and improvement of methods were achieved by comparing the different measurement techniques (Jang 1998; Anagnost et al. 2000; Bergander et al. 2002; Lichtenegger et al. 2003). Khalili et al. (2001) and Bergander et al. (2002) studied the variability of MFA in different annual rings. Also Bergander et al. and Anagnost et al. (2002) showed that there was no correlation between the MFA and morphological features such as fiber width or thickness.

Recently detailed analysis of MFA on single wood fiber using the soft-rot cavity method (Khalili et al. 2001; Anagnost et al. 2002), X-ray micro-diffraction technique (Lichtenegger et al. 2003), and improved iodine method (Wang et al. 2001) confirmed that MFA of a wood fiber is not always uniform. Anagnost et al. determined variable MFAs on radial wall with bordered pits and Khalili et al. nonuniformity even in nonpitted areas of earlywood tracheids. Wang et al. showed the multiple lamellae nature of the S_2 layer and some changes in MFA within its different depths. Lichtenegger et al. compared the results of X-ray micro-diffraction technique and the orientation of pit apertures (which had usually been assumed to indicate the MFA). Their study showed a strong correlation in the results of latewood and compression wood fibers and a large discrepancy in earlywood fibers.

The main objective of this study was to determine the form and extent of nonuniformities of MFAs along the spruce fibers by CLSM. This technique is based on fluorescence dichroism of the fiber wall when they are dyed with congo red. The results were compared in different parts of earlywood and latewood fibers to understand the comprehensive pattern of microfibrils within the single wood fibers.

MATERIALS AND METHODS

Small cubic pieces of spruce wood were saturated in deionized water to facilitate chipping thin layers (about 100 μm) with a microtome. The spruce chips were macerated in a mixture of acetic acid (50%), hydrogen peroxide (10%), and distilled water (40%) for 48 h at 70°C. This maceration removes the middle lamella (bonding medium that contains a high percentage of lignin and holds the fibers together) and facilitates the isolation of single fibers. Maceration has no effect on the orientation of cellulose microfibrils although it could degrade the lignin and hemicellulose components of the cell wall. After rinsing, the macerated fibers were stained with 0.05% Congo red solution for 30 min at 70°C. Then the dyed fibers were peeled out one by one and placed on a microscope slide.

A Carl Zeiss LSM 310 confocal laser scanning microscope, equipped with an argon laser (excitation at 488 nm) and a rotating half-wave plate turning the plane of polarization of the laser beam, was used for measurement. Observations were performed with an X60 oil-immersion objective with a numerical aperture of 1.4. To stop the movement of the immersed fibers in oil, they were fixed by sticking their ends to the slide. To avoid errors due to the convexity of the fibers, they were pressed with a glass cover slip to flatten their top surface.

For each measurement of MFA, a small area of the fiber wall (less than 50 μm^2) was chosen and scanned at each 10° over the plane of 180°. The changes of the fluorescent intensity I, in each step were plotted against the angle of polarization. The sinusoidal changes of fluorescent intensity fit the equation:

$$I = A\ cos^2(P - \theta) + I_{min} \qquad (1)$$

where A is the amplitude of the curve, I is the difluorescence pixel intensity, I_{min} is the minimum difluorescence intensity, θ is the mean MFA of the chosen area, and P is the angle of excitation polarization. MFAs of the chosen area were obtained by fitting the measured data (fluorescent intensities) to Eq. (1), (Fig. 1). In each measurement, to ensure that the MFA was measured in the S_2 layer, the cross-sectional images of the fiber close to the chosen area were scanned using the blue excitation (488 nm). Then the microscope position was refocused on the middle of the upper wall thickness (Fig. 2a, b). As the S_2 layer is the thickest layer of the cell wall, making up about 70–80% of the wall

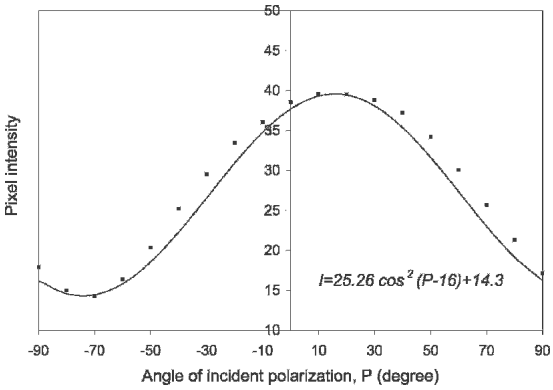

FIG. 1. Fluorescent intensity curve for the marked area in Fig. 2b. MFA = 16° gives the best fit to Eq. (1).

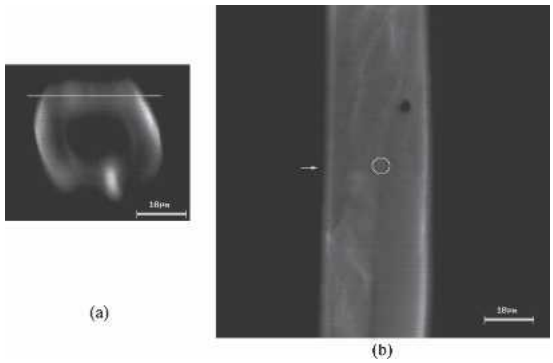

FIG. 2. Cross-sectional image and location of the focused area: (a) cross-sectional image of a latewood fiber indicating the focused area is located in S_2 layer; (b) focused area for microscopic scanning.

thickness (Fengel 1969), we could be sure that measurements in the middle of the wall thickness were in the S_2 layer.

Variation of MFA in the areas between the bordered pits of 25 earlywood fibers and inside the border of the pits of 20 earlywood fibers was studied. As was explained before, to make precise measurements and avoid fiber movement in the oil (using the oil-immersed objective), the fibers were first fixed on the slide. So after measuring the angle on one of the fiber walls, it was impossible to turn the fiber to continue the measurements on the other walls. Hence we found some fibers that were occasionally placed cornerwise on the slide. After the upper surfaces were pressed softly, by a glass cover slip, two adjacent tangential and radial walls were flattened, and the appropriate surface for measurements was obtained. By this method, local MFAs were measured on the radial and tangential walls of 5 earlywood fibers. In latewood to study the variability of microfibril orientation, MFAs of different parts in 10 latewood fibers were studied. Also the measured MFAs of the 12 crossfield areas in earlywood and latewood fibers were compared with the orientation of pit apertures in crossfield areas.

RESULTS AND DISCUSSION

Detailed measurements on the radial walls of earlywood fibers showed that MFAs were considerably variable in the vicinities of the bordered pits. All measured MFAs between the bordered pits of 25 tested earlywood fibers were smaller than the mean MFA of the fibers in nonpitted zones (Fig. 3a, b). This reduction in MFAs was limited to the central band of the radial wall (zones A and B), and outside this band the microfibrils followed the mean MFA of fiber in nonpitted zones. To understand if the variation

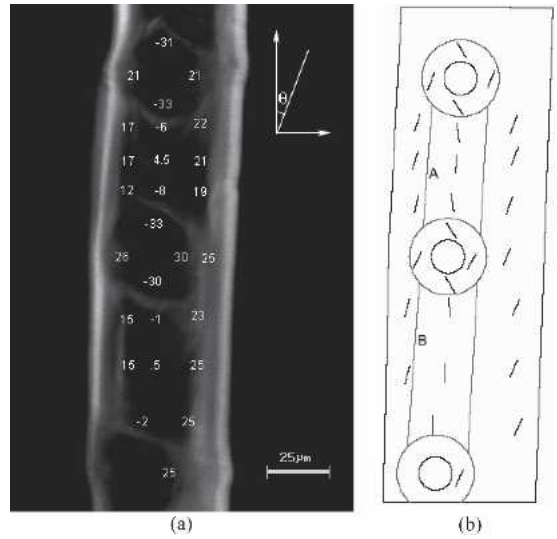

FIG. 3. Variation of MFA in the radial wall of an earlywood fiber: (a) Measured MFAs and their locations; (b) plotted MFAs as measured in (a); lower MFAs are observed in the central band between bordered pits, marked as area A and B.

of MFA in the vicinity of the bordered pits extends to the tangential walls, MFAs of tangential wall in some areas close to the bordered pits of radial wall were measured (Fig. 4). Analyzing the measured MFAs showed that the variation of MFAs was limited to the radial wall, and the microfibrils in the tangential wall even close to the bordered pits followed the mean MFA of the fiber.

A closer look at the bordered pits area showed that the microfibrils had a special pattern of distribution around the pits and inside the borders (Fig. 5a, b). MFAs in zones A and B (in this sample about 30° relative to longitudinal axis) were more than the mean MFA of the fiber (24°), and in the zones C and D had the opposite direction relative to the fiber longitudinal axis (−36°). In the zones E, F, G, and H, the measured MFA always had values smaller than the mean MFA of the fiber (in this sample −7°, −12°, and 14°). However, when we measured the local angle of smaller areas like, G_1, G_2, H_1, and H_2 larger values were obtained, which were negative in zones H_1 and G_2 (−65°, −77°) and positive in zones G_1 and H_2 (63°, 69°). Pattern of microfibrils distribution around the bordered pits could be predicted by measuring MFAs in numerous points inside the border. The microfibrils' approximate path, which is plotted through the measured MFAs in Fig 5b, resembles the 'circular orientation' of microfibrils that has been reported earlier (Harada 1965; Khalili et al. 2001), although it doesn't have a 'perfectly' circular form.

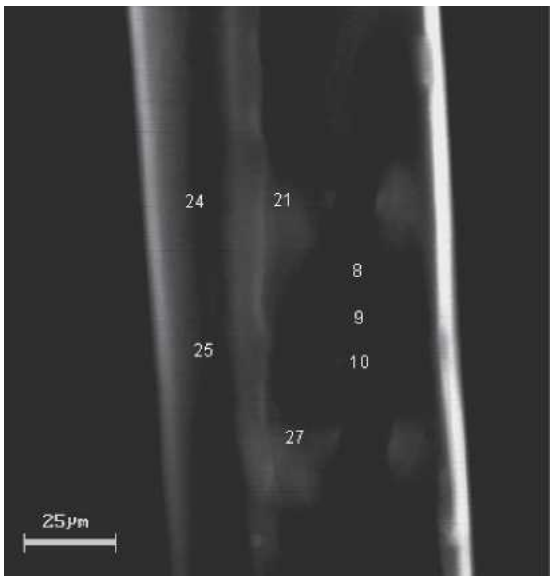

FIG. 4. Measured MFAs in the tangential wall, radial wall, and between two bordered pits.

In the central band of the earlywood radial wall, the measured MFAs were always less than the mean MFA of the fiber (Fig. 3a, b). Even the small negative values (relative to longitudinal axis) were common in this region. There are two possible eventualities to explain this phenomenon, the unidirectional pattern of microfibrils (Fig. 6a) and the existence of the crossed microfibrils in this region (Fig. 6b). In an area with two planes of crossed microfibrils, in each step of measurement the measured fluorescent intensity is emitted from two different directions and the measured MFA would be the resultant of the two directions (Fig. 7). In fact frequent existence of crossed microfibrils in the soft-rot fungi results led us to doubt whether microfibrils in this zone have such a pattern (Khalili et al. 2001). The origin of the crossed microfibrils between the bordered pits could be explained by analyzing the variation of MFAs inside the border of bordered pits (Fig 5a, b). In most of the earlywood fibers, the measured MFAs in the top and bottom regions of the bordered pits had large negative values (S-helical direction). It is possible that the S-helical microfbrils inside the border continue over the pit border into the area between two pits, and the coupling with the Z-helical microfibrils in this region makes a crossed form. However, as direct observation of the cellulose microfibrils paths is not possible with CLSM, the noncrossed unidirectional pattern of microfibrils can't be rejected by this method.

Measuring the local MFA of the small areas between the crossfield pits usually showed a uniform microfibrils distribution (Fig. 8a). However, in some of the fibers, the measured MFAs between the pits were variable and even turned to very small or negative values (Fig. 8b). Orientation of pit apertures in crossfield areas of earlywood fibers and measured MFA in these

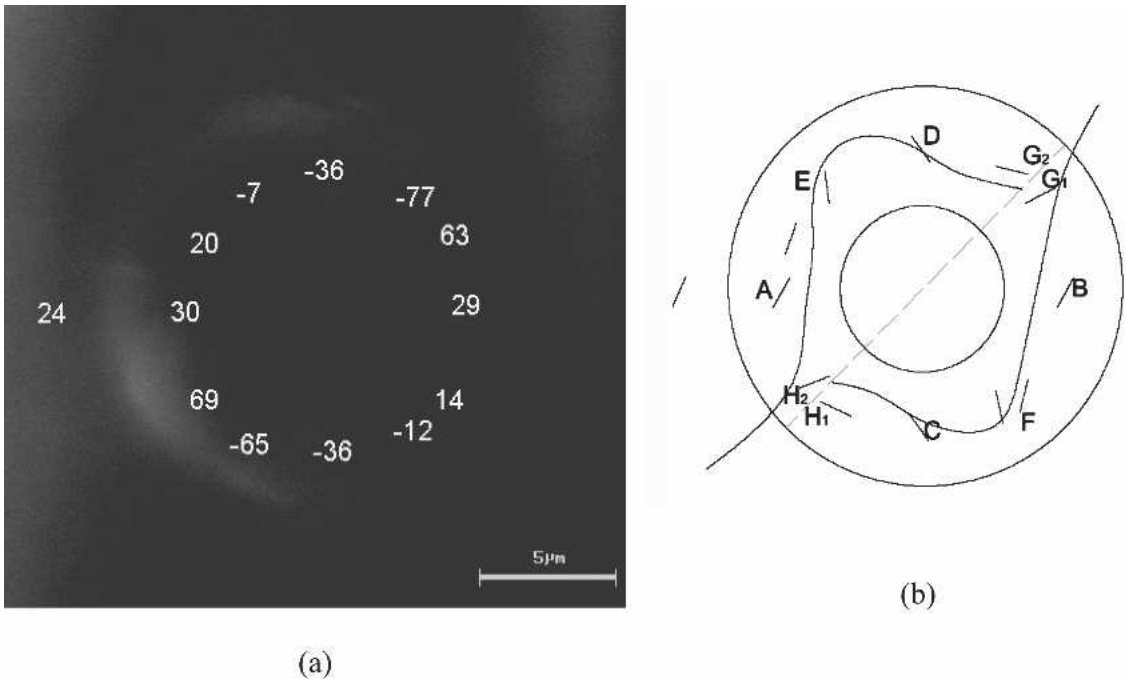

FIG. 5. Pattern of variation of MFA inside a bordered pit: (a) measured data; (b) schematic sketch.

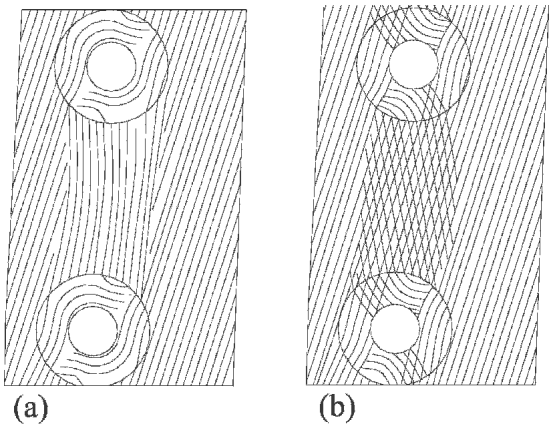

FIG. 6. Two proposed patterns of microfibrils in the vicinity of two bordered pits based on the measured data: (a) crossed microfibrils' assumption; (b) unidirectional microfibrils' assumption.

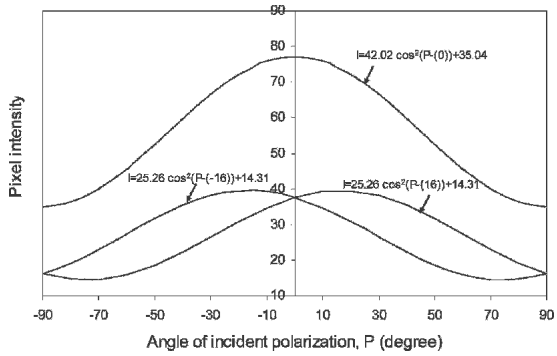

FIG. 7. MFA of an area with two crossed planes of microfibrils (MFA = 16°, −16°) is the resultant of the two directions (MFA = 0°).

zones were usually consistent (Fig. 8a) and showed that measuring the pits' orientation could be considered as a rough and rapid way to measure mean MFA of the fibers.

The MFAs' variation within the latewood fibers was much smaller than the variation in earlywood fibers. Even close to the steep narrow pits of the latewood fibers (which have been usually observed adjacent to the ray cells), the parallel helical arrangement of microfibrils was uniform (Fig 9a, b).

CONCLUSIONS

Using CLSM to measure the mean MFA of wood fibers is not a new technique. However, in

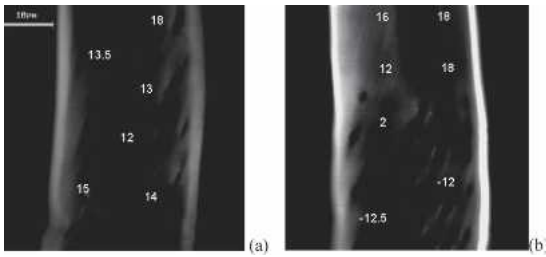

FIG. 8. Variation of MFA in crossfield region of two earlywood fibers: (a) measured MFAs were not highly variable; (b) MFAs were different within the areas between the pits.

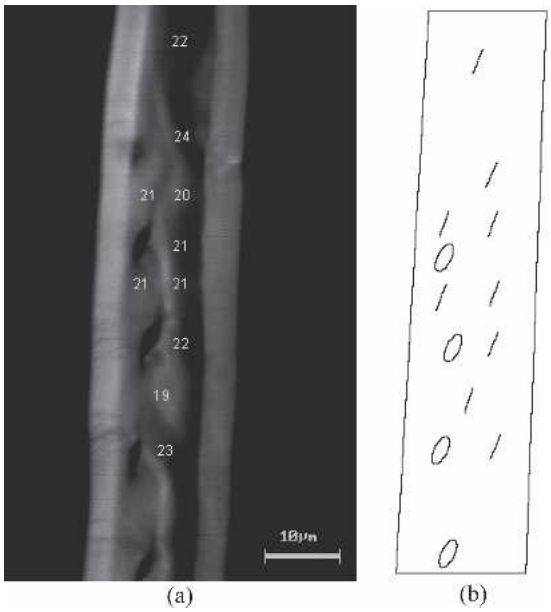

FIG. 9. MFA uniformity in latewood fibers: (a) Measured MFAs and their locations; (b) plotted MFAs as measured in (a).

this study we used it for the first time to investigate the probable '*variation*' of MFA within the individual wood fibers. By focusing on arbitrary small areas along the wood fibers, special not-yet-investigated points, like those inside the border of pits or crossfield zones, were investigated. MFA was highly variable within the radial wall of earlywood fibers, especially in the vicinity of the bordered pits. On the other hand, in the tangential wall of earlywood and in the whole latewood fibers, MFA was approximately uniform. However, a large percentage of the radial wall surface in earlywood fibers is between the bordered pits and variation of MFA in these regions could have an important influence on the fiber mechanical behavior. Indirect observation of MFA with CLSM doesn't show the continuous paths of cellulose microfibrils along the fiber. Nevertheless, the microfibrils' paths could be estimated by measuring MFAs in numerous points along the fiber. CLSM has the advantage of giving the MFA of each area of the fiber, which is the subject of the study. Although measuring the MFA by CLSM is a difficult and time-consuming process, this characteristic makes it a reliable technique for micro-structural study of wood fibers.

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