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Imaging FTIR microscopy – technique for rapid screening of plant cell walls

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1. Introduction

Plant cell walls (CW) are the most abundant, renewable and biodegradable composite on Earth. Cell wall can also be considered as a nano-composite in which cellulose, lignin and hemicelluloses are interconnected in a specific manner. Biopolymers such as cellulose, hemicellulose and lignin, have wide applications in different industries, especially for biofuels and biomaterials [1,2]. By using imaging FTIR microscopy, run in transmission mode and at different polarisation modes (from 0° to 90°), it is possible to follow chemical variability and orientation of cell wall polymers [3]. The orientation of cellulose, xylan and lignin, as essential components of plants, were analysed by iFTIR with regard to the sample axis.

2. Materials and Methods

The purified isolated cell wall material was obtained from maple leaves (*A. platanoides*) by methanol extraction and subsequent purification using a series of solvents (1% Triton X-100, 1M sodium chloride, distilled water, methanol, acetone) [4].

FTIR microscopy measurements were carried out using a Spectrum Spotlight 400 FTIR Imaging System (Perkin Elmer Inc, Shelton, CT, USA). The area of interest was first displayed, using a visible CCD camera to locate the cell wall area, which was then irradiated using mid-IR light (Figure 1). The scanning was carried out in imaging mode using an array detector, providing a pixel resolution of 6.25 µm x 6.25 µm, a spectral resolution of 4 cm^{-1} and a spectral range from 1,800 to 720 cm^{-1}. IR scanning with a polarisation, where the incident IR radiation is polarised by a gold wire grid polariser, in our case, from 0° to 90° polarisation and in relation to the fibre orientation with intervals of 5° were carried out also. In this way, it is possible to investigate, for example, orientations of different polymers in a wood fibre. The IR spectra were processed by the software Spotlight 1.5.1, HyperView 3.2 and Spectrum 6.2.0 (Perkin Elmer Inc., Shelton, CT, USA) [3].

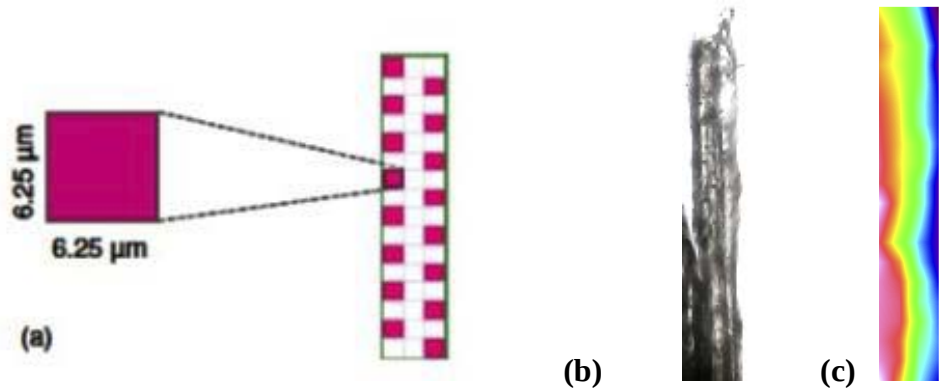

Figure 1. (a) shematic image of the linear array detector containing 16 MCT detectors, also showing one magnified pixel of a 6.25 µm x 6.25 µm, (b) visible image of a part of maple leaves cell wall, (c) IR full-spectral image of the sam part of maple leaves cell wall.

3. Results and Disscusion

From the in-depth study of polymer orientation, three areas from the sample (maple leaf) were selected. The transmission spectra were recorded from 0° to 90° polarisation modes. Figure 1 shows FTIR spectum of cell walls of maple leaves in the region 800–1800 cm^{-1}. Spectral signals related to absorptions from cellulose, xylan and lignin can be identified.

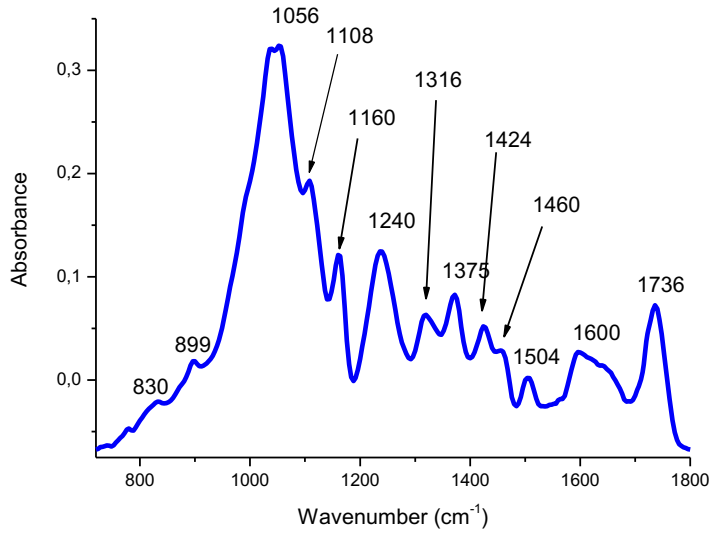

Figure 2. Average absorbance spectrum of maple leaves cell wall.

The relative absorbance spectra are presented (Figure 2) as specific absorption peaks (RA = (I_p – I_{min}) / (I_{max} – I_{min})$RA = \frac{(I_P - I_{min})}{(I_{max} - I_{min})}$) where RA is relative absorbance, I_p is intensity of the absorbed IR radiation at a given angle of the polarisation, I_{max} is maximal intensity observed for a given vibration and I_{min} is minimal intensity observed for a given vibration. These relative absorbance values were presented in relation to the angle of the incident IR polarisation (from 0° to 90°).

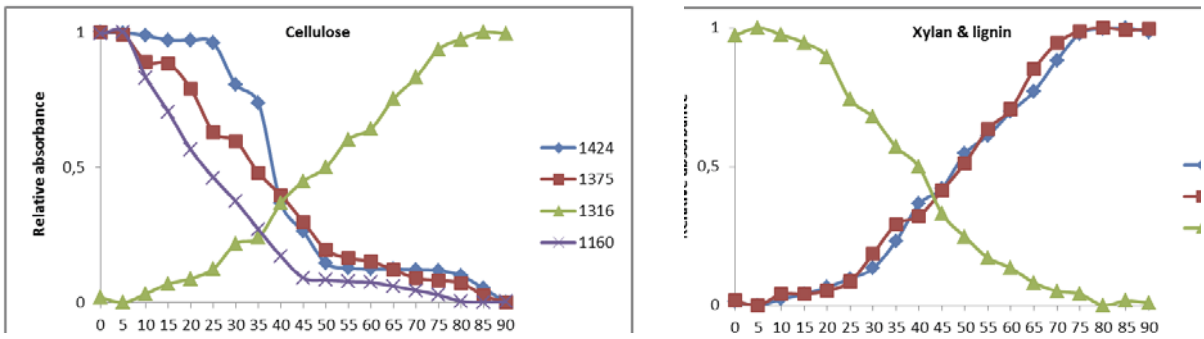

Figure 3. The relative absorbance of IR specific absorption wavenumbers plotted against the polarisation angle for the different polymers (cellulose - left, xylan & lignin – right) for maple leaves.

It is evident (Figure 3 left) that the three cellulose peaks (1160 cm^{-1},1375 cm^{-1} and 1424 cm^{-1}) [3–5] had high absorption levels at low polarisation angles, which is a consequence of a more parallel orientation of the corresponding groups to the CW longitudinal axis. The fourth cellulose peak (the perpendicular signal at 1316 cm^{-1}) had the greatest intensity at a high polarisation angle, due to the perpendicular orientation of the corresponding group (Figure 3 left). For the xylan, the characteristic band signals (1244 cm^{-1}, 1736 cm^{-1}) [5–7] increased with an increase in the polarisation angle. Due to the parallel orientation of these side groups in xylan, an orientation parallel to the longitudinal CW axis is indicated (Figure 3 right). For the lignin, the characteristic band signal (1517 cm^{-1}) [8,9] decreased with an increase in the polarisation angle (Figure 3 right), indicating that lignin is organised in parallel with the longitudinal CW axis.

4. Conclusion

It has been shown that xylan is oriented in parallel to the cellulose and more or less parallel to the axis of a cell wall, in isolated CW fragments from maize leaves. There was also a clear indication of lignin orientation parallel to the longitudinal CW axis. This means that all of these components show strong anisotropic behaviour and organisation.

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