Orientation Dependent FT Raman Microspectroscopy on Hemp Fibers

S. K. Kovur, K. Schenzel, W. Diepenbrock*

Summary: FT Raman microspectroscopy was used for polarization experiments on strained hemp fibre cells. The cellulosic plant fibers were macerated with alkaline and enzymatic solutions. Those cleaned and refined single fiber cells were subjected to micro tensile tests as well as to polarization measurements under the FT Raman microscope. Mechanical parameters of the fiber cells (e.g. E-modulus) were determined and changes in orientation of the –(C–O–C)– structure units of the cellulose were considered with respect to fiber stress and molecular fiber structures. Intensity ratios R_1 and R_2 calculated on the polarized micro FT Raman spectra of the strained fibers describe the order parameter $\langle P_2 \rangle$ and $\langle P_4 \rangle$ allowing the quantitative determination of the orientation of the structure units –(C–O–C)– of fiber cellulose with respect to the fiber cell axis.

Keywords: fiber microstraining experiments; hemp fibers; orientation of cellulose structure units; polarized FT Raman microspectroscopy

Introduction

Natural cellulose fibers of annual plants - like hemp and flax - are increasingly being used for various industrial applications. They are recognised as potential replacement for glass fibers because of their biodegradability and their high strength-to-weight ratio.

The so-called bast fibers consist of different hierarchical microstructures, whereby the microfibrils of cellulose serve as basic units. They are embedded in a matrix of hemicelluloses and form the different cell wall layers of an elementary single fiber cell, which is 10–30 μm in diameter in case of hemp fibres. The single fiber cells are bonded together with pectins and small amounts of lignin framing the next level of microstructure, the technical fibers, with diameters of 50–100 μm. These filaments are fixed together with a pectin-lignin matrix that forms the fiber bundles in the cortex of the plant stems. Thus, bast fibers are bundles of individual strands of fibers held together by a pectin-lignin interface.[1]

The removal of these binding material results in the refining of the fiber bundles, yielding filaments or single fiber cells respectively. An important characteristic of the fiber cells is their composition of highly structured cell wall layers, e. g. primary (P) and secondary (S) ones. The S layer contains the most cellulose mass and with regard to the mechanical support of the plant fibers it is the most important one. In its microstructure one has to differentiate between three additional layers S1, S2, and S3. They are distinguished by their microfibril textures which are represented by different orientation angles of the cellulose microfibrils with respect to fiber cell axis. These microstructural characteristics together with molecular substructures of the fiber cellulose, which may exist in terms of the polymorphic forms, cellulose I and/or cellulose II respectively,[2] strongly influence mechanical parameters of the plant fibers like tensile strength and E-modulus.[3–6]

Martin Luther University Halle-Wittenberg, Department of Natural Science III, Institute of Agricultural and Nutritional Science, D-06099 Halle, Germany
E-mail: karla.schenzel@landw.uni-halle.de

Since the molecular orientation affects the physical properties of macromolecular systems it is very important to understand the mechanisms that govern their orientation and relaxation of orientation. Polarized Raman microspectroscopy is a powerful technique suitable to characterize the orientation of macromolecules since it can provide information about the degree of orientation of chemical groups and structure units in crystalline or semicrystalline polymers. One of its main advantages is the feasibility to record high quality spectra of high spatial resolution. Low diameters of the focused laser beam allow the investigation of single fiber cells. In addition, the second and fourth terms, $\langle P_2 \rangle$ and $\langle P_4 \rangle$, of the Legendre polynomial expansion of the orientation distribution function can be determined from polarized Raman spectra. Thus, for systems of uniaxial or fiber type symmetry a general procedure to determine $\langle P_2 \rangle$ and $\langle P_4 \rangle$ coefficients, the so-called order parameters, has been developed in detail by Bower[7] and Jen et al.[8]

Thereupon, it was the aim of our FT Raman microscopic investigations to follow changes in the orientation of structure units of cellulose microfibrils of hemp fibers due to fiber stress experiments and with respect to changes of the molecular fiber composition and substructures of the fiber cellulose caused by enzymatic and alkaline fiber treatments.

Experimental Part

Plant Material

Hemp (*Cannabis sativa*, L.; *cv*. USO 31) was cultivated at experimental station of the Martin-Luther-University Halle-Wittenberg. After dew-retting the fibers were extracted mechanically from the stems on industrial premises. The extracted fiber bundles, 50–100 μm in diameter, were refined by hand. Following the mechanically isolated fibers were cleaned and refined chemically by alkaline, C_{NaOH} (w/w%) = 6% and 25%, as well as by enzymatic treatments with hemicellulase (*Aspergillus niger*, 1.5 Umg^{-1}) and cellulase (*Aspergillus niger*, 1.3 Umg^{-1}) always after an alkaline treatment using 6% NaOH solution. Finally, the fibers were washed with distilled water and dried at room temperature.

Environmental Scanning Electron Microscopy (ESEM)

ESEM micrographs of the isolated and refined fibers were recorded using the electron microscope XL30, ESEM-FEG (Philips) with different magnifications.Typically it was equipped with environmental SE-detectors. The fiber micrographs were recorded at a pressure of 0.13 kPa and an accelerating voltage of 15.0 keV. Fibers were measured in the wet mode preserving their original characteristics without making the samples conductible.

NIR FT Raman Microspectroscopy

Near Infrared Fourier Transform (NIR FT) Raman spectroscopic measurements were carried out using the BRUKER RFS/100S spectrometer coupled directly to a NIKON microscope, Eclipse E 400. The Nd:YAG laser ($\nu_0 = 1064$ nm) was used for Raman excitation. Raman back scattering was detected by a nitrogen cooled Ge-diode. A rotable $\lambda/2$ plate serving as polarizer was included into the laser pathway between spectrometer and microscope. On the terms indicated the laser was oriented parallel (x) or perpendicular (y). An analyzer – placed into the pathway of the Raman back scattering towards the interferometer – allowed the detection of the polarized scattered radiation along the x and y directions.

Details of the fibre stress experiments under the Raman microscope were described elsewhere.[6] For fiber tensile tests in combination with polarization experiments fiber cells of around 15–30 μm were fixed to a fiber card at a micro straining rig. The axis of the fiber cell was oriented parallel to the electric field vector of the incident laser, as shown in Figure 1.

The fiber straining experiments in combination with polarization investigations require a strong compromise between the

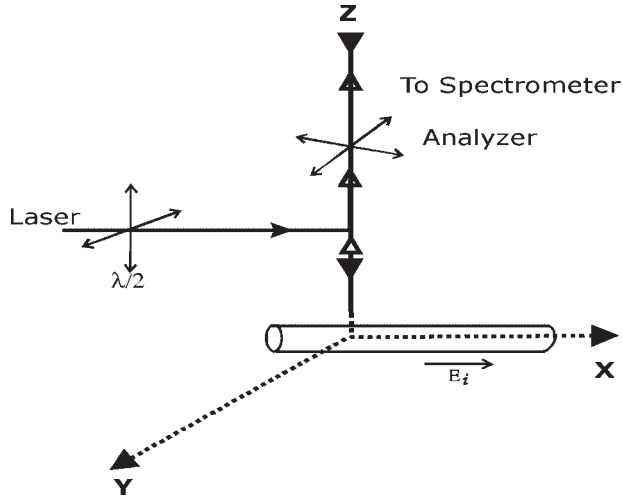

Figure 1.
Coordinate system used for the recording of the micro FT Raman spectra of the hemp fiber cells. E_i = vector of the incident electric field.

need for short spectra accumulation times during the straining process as well as the need for spectra with good S/N ratio and sharp Raman signals to determine real peak positions and peak intensities. By view of this phenomenon each spectrum was accumulated over 120 scans at each level of strain following the wavenumber shift of the Raman signal at 1096 cm^{-1}.

For each polarization measurement a strain increment of 100 μm was chosen. Real time spectra were recorded always before starting the measurement of a new fiber cell and when several spectral changes were observed. Then, the sample position under the microscope were monitored and the fibre position as well as the focal plane were readjusted to get similar spectra.

All micro FT Raman spectra were treated with the Bruker software, OPUS 4.2. The spectra were vector-normalized, baseline corrected and smoothed (9-pt). Raman peak positions and band intensities were determined by curve-fitting (Levenberg-Maquardt algorithm) considering a linear baseline in the frequency range 1050–1150 cm^{-1}.

Results and Discussion

As shown by the ESEM images in Figure 2, typical differences in the microstructure of the fiber bundles were detected due to different alkaline and enzymatical fiber

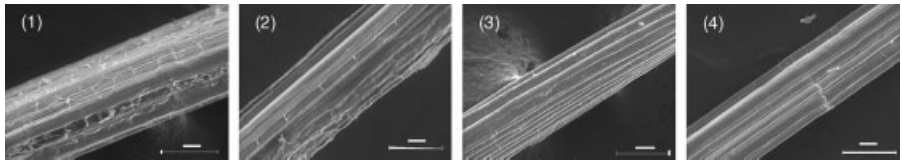

Figure 2.
ESEM images of untreated (1), 6% NaOH (2) and 25% NaOH treated (3) as well as of cellulase treated (4) hemp fibers.

treatments. A surface cleaning and refining process of the fibers bundles was observed and can be followed in the images (1)–(4).

The alkaline and enzymatical fiber treatments caused changes in molecular fiber composition, particularly by removing of small amounts of waxes and remaining parts of lignin from the fiber surfaces. Also the degradation of hemicelluloses and pectines, which serve as matrix materials for the cellulose microfibrils and tie the single fiber cells to a bundle structure, was yielded.

The strongest effect of surface cleaning and fiber refining was achieved if the fibers were treated enzymatically with cellulase afterwards an alkaline treatment with 6% NaOH solution, compare ESEM image (4). A fiber bundle characterized by another substructure of the fiber cellulose is represented in image (3). This bundle was cleanded from waxes and lignin. However the substructure of the cellulose was transformed from cellulose I into the polymorphic form -cellulose II- due to an alkaline fiber treatment using a 25% NaOH solution. The polymorphic transformation followed by FT Raman spectroscopy and microspectroscopy was described earlier.[9] As a result of fiber cleaning and refining the uniaxial morphology of the hemp fiber cells became obvious by the ESEM investigations.

Assuming an uniaxial symmetry structure for the microfibrils of the fiber cellulose the orientation of molecular structure units of cellulose with respect to the fiber axis can be determined quantitatively utilizing polarized micro Raman spectra of the fiber cells.[7,8]

In this work, changes in orientation of –(C–O–C)– structural units of fiber cellulose of hemp fibers were examined with respect to fiber straining and fiber composition by means of FT Raman microscopy. The orientation dependent behaviour of –(C–O–C)– units of cellulose was indicated by intensity changes of the Raman signal of the ν(C–O–C) stretching vibration, as shown in Figure 3 (left). The vibrational mode ν(C–O–C) is mainly assigned to the 1,4-β (C–O–C) glycosidic linkages in the cellulose chains [11] and has already proved its high stress sensitivity for cellulose fibers.[6,12] The highest intensity of the corresponding Raman signal at 1096 cm^{-1} was detected if the electric field vector (E_i) of the incident laser beam was oriented parallel to the fiber axis. By turning the E_i vector to the perpendicular direction, the signal intensity of the ν(C–O–C) mode was decreasing, compare Figure 3 (left). Thereupon a mainly parallel orientation with respect to the fiber cell axis has to be assumed for the glycosidic linkages and the glucopyranose units (GPUs) which form

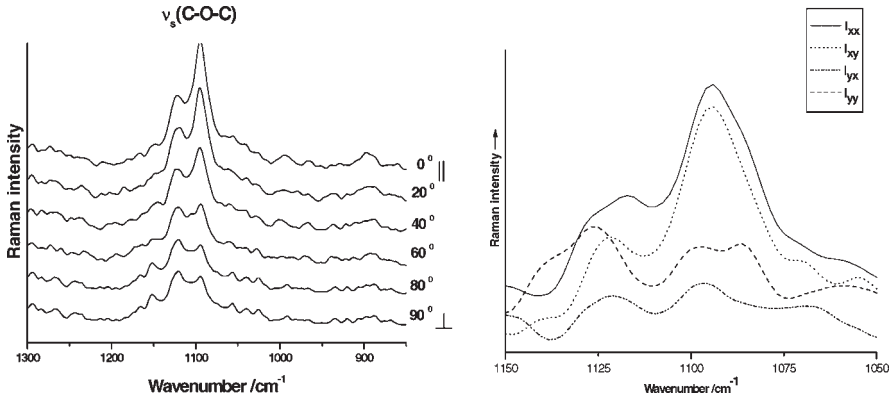

Figure 3.
Orientation dependent FT Raman spectra of the ν(C–O–C) mode of a hemp fiber cell (left) and polarized spectra of the ν(C–O–C) mode (right).

the molecular skeletons of the cellulose microfibrils.

Here not only the orientation sensitivity was surveyed on the strained fiber cells. Additionally polarization experiments in the FT Raman microscope were carried out to determine changes in orientation of the –(C–O–C)– structure units quantitaively. Thus the order parameters $\langle P_2 \rangle$ and $\langle P_4 \rangle$ were determined for these molecular units, always with respect to the level of fiber strain and molecular fiber composition.

Following the works of Pezolet and co-workers on spider silk fibers[10] a quantitative determination of $\langle P_2 \rangle$ and $\langle P_4 \rangle$ for systems of uniaxial symmetry can be yielded from the intensity ratios R_1 and R_2 which are obtained directly from the polarized micro Raman spectra:

$$R_1 = \frac{I_{z(xy)\overline{z}}}{I_{z(xx)\overline{z}}} \quad (1)$$

$$R_2 = \frac{I_{z(yx)\overline{z}}}{I_{z(yy)\overline{z}}} \quad (2)$$

Four polarized micro FT Raman spectra were recorded for the ν(C–O–C) mode of a hemp fiber cell on each level of increasing fiber strain up to fracture. In Figure 3 (right) the spectra are represented illustrating one level of fiber strain. They were identified by their polarization configurations: $Z(XX)\overline{Z}$, $Z(XY)\overline{Z}$, $Z(YY)\overline{Z}$ and $Z(YX)\overline{Z}$, illustrated in Figure 1 and described in accordance with the Porto[13] notation. The intensity ratios R_1 and R_2 were calculated from peak-height intensities of the signal of the ν(C–O–C) mode in the polarized spectra. Average values of the ratios were obtained over 5 samples.

In this manner, changes in orientation of the cellulose structural units –(C–O–C)– of the hemp fibers were followed up with increasing fiber strain and with respect to different chemical compositions of the plant fibers. In Figure 4 the changes are descibed by means of the order parameter $\langle P_2 \rangle$ for the alkaline and enzymatically treated hemp fiber cells. The values of this parameter for the unstrained fibers are in the range between 0.18 ± 0.03 and 0.29 ± 0.03. Knowing that the limiting values of $\langle P_2 \rangle$ are 1 and -0.5 for perfect orientation at $0\,^\circ$ and $90\,^\circ$ from the fiber axis, the obtained values indicate that the –(C–O–C)– units of the unstrained fibers are arranged

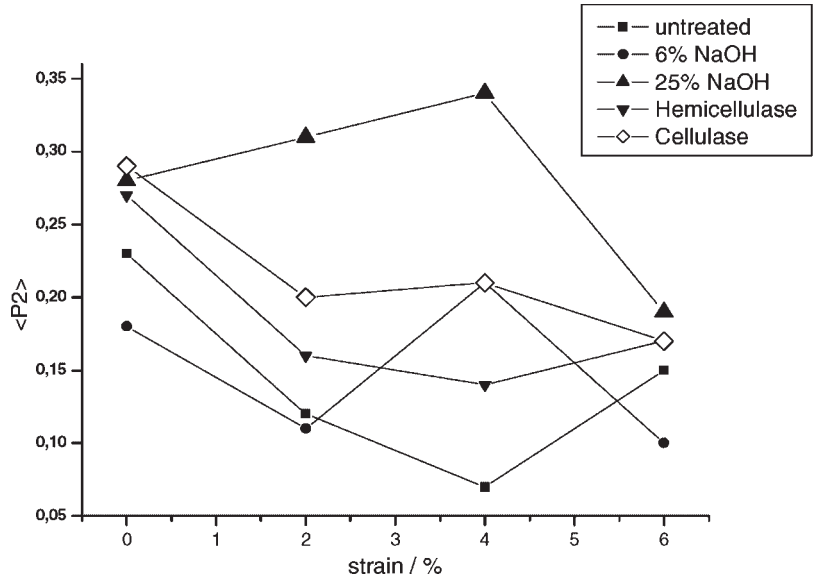

Figure 4.
Changes of the orientation parameter $\langle P_2 \rangle$ of the –(C–O–C)– units of alkaline and enzymatical treated hemp fiber cells with respect to fiber strain.

neither parallel nor perpendicular to the fiber cell axis.

The orientation angle between the fiber axis and the –(C–O–C)– units of unstrained fibers are bigger for the enzymatical and 25% NaOH treated fibers than for total untreated ones. Only unstrained fibers treated with 6% NaOH solution showed a smaller angle and therewith a more parallel orientation of the –(C–O–C)– units to the fiber axis.

With increasing fiber strain the orientation angles of –(C–O–C)– units increase except for fibers treated with 6% and 25% NaOH solutions. For these fibers an increase of the $\langle P_2 \rangle$ values was obtained indicating smaller angles between –(C–O–C)– units and the fiber axes, coincident with a more parallel orientation of the cellulose microfibrils. It was concluded, that the cellulose structures become more isolated and get a higher capability for incorporating mechanical stress in terms of the stretching of the 1,4-β –(C–O–C)– glycosidic linkages owing to the removal of lignin by the alkaline fiber cleaning processes. Thus, a decrease of the orientation angle between –(C–O–C)– units and fiber cell axis was recorded at higher strain levels.

Conclusions

Orientation dependent FT Raman micro spectroscopic experiments were carried out on strained fiber cells of alkaline and enzymatical treated hemp fibers beside morphological investigations by means of ESEM. With respect to chemical and enzymatical fiber treatments a fiber cleaning and refining process was observed in the micro structure of the fibers.

Order parameters of the molecular structure units –(C–O–C)– of cellulose of the alkaline and enzymatical treated hemp fibers were determined with respect to the levels of fiber strain by means of polarization investigations in the FT Raman microscope.

Exemplarily, changes of the $\langle P_2 \rangle$ values were discussed with respect to changes in fiber morphology and chemical fiber composition. It was shown, that the –(C–O–C)– units of the cellulose microfibrils of unstrained fibers lie inbetween a parallel and a perpendicular orientation to the fibre cell axis.

Only the fibers treated with 6% and 25% NaOH solutions showed a decrease in the orientation angle of the –(C–O–C)– units with respect to the fiber axis with increasing fiber strain, indicating the more parallel orientation of the microfibrils at higher strain levels. Here, the cleaned and isolated cellulose structures represent their capability for higher stress incorporation. Thereby, the decrease of the orientation angle was caused.

In contrast, the increase of the orientation angle was observed for enzymatical treated fibers at higher strain levels reflecting the loss of order within the cellulose structures owing to the removal of matrix materials and shortening of cellulose chains by cellulase treatments.

Acknowledgements: Financial support of Deutsche the Forschungsgemeinschaft (DFG), project no. SCHE 415/11-1, 2 is gratefully acknowledged.

[1] H. J. Purz, H.-P. Fink, H. Graf, *Das Papier* **1998**, *6*, 315.
[2] R. D. Gilbert, J. F. Kadla, in: "*Biopolymers from Renewable Resources*", D. L. Kaplan, Ed., Springer Berlin, Heidelberg, New York **1998**, 47–90.
[3] S. Pleasants, W. J. Batchelor, I. H. Parker, *Appita J.* **1998**, *51*, 373.
[4] B. M. Prasad, M. M. Sain, *Mater. Res. Innovat.* **2003**, *7*, 231.
[5] A. Bhatnagar, M. Sain, *J. Reinforced Plastics Composites* **2005**, *24*, 1259.
[6] P. Peetla, K. C. Schenzel, W. Diepenbrock, *Appl. Spectrosc.* **2006**, *6*, 682.
[7] D. I. Bower, *J. Polym. Sci. Phys.* **1972**, *10*, 2135.
[8] S. Jen, N. A. Clark, P. S. Pershan, E. B. Priestly, *J. Chem. Phys.* **1977**, *66*, 4635.
[9] K. Schenzel, S. Fischer, *Cellulose* **2001**, *8*, 49.
[10] M.-E. Rousseau, T. Lefevre, L. Beaulieu, T. Asakura, M. Pezolet, *Biomacromol.* **2004**, *5*, 2247.
[11] H. G. M. Edwards, D. W. Farwell, D. Webster, *Spectrochim. Acta* **1997**, *53A*, 2383.
[12] S. J. Eichhorn, J. Sirichaisit, R. J. Young, *J. Mat. Sci.* **2001**, *36*, 3129.
[13] T. C. Damen, S. P. S. Porto, B. Tell, *Phys. Rev.* **1966**, *142*, 570.

