CONFOCAL FLUORESCENCE MICROSCOPY AS A NOVEL METHOD TO EVALUATE THE DEW RETTING DEGREE OF HEMP STEMS

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The use of plant fibers, as hemp, has spread to new high-value applications intended to replace synthetic fibers. However fiber quality required for industrial uses is a critical point and one major challenge concerns the retting step. This first stage during fiber production consists in the separation of cellulosic fibers bundles from plant stems, thus facilitating their mechanical extraction and preserving fiber quality [1]. Dew retting is mostly used in Europe and consists in dropping off stems on the soil after harvest. This natural process is not yet fully characterized and highly climate-dependent which makes it difficult to control [2]. The aim of this study was to characterize the dynamics of hemp retting under controlled environmental condition and provide indicators of the progression of dew-retting degree for an improved management of the process. In this respect, confocal fluorescence microscopy (CFM) makes it possible to visualize the changes in stem tissues and microorganism colonization upon retting.

Hemp stems portions were incubated at 15°C and 60% air moisture over 42 days, with a 12-hour lighting cycle per day and a 6-mm rain once a week. Changes in the stem tissue architecture, chemical composition, and microorganism colonization were characterized to assess the retting degree, using different approaches as FT-IR spectroscopy and CFM. Histological hemp sections were hand-cut using a razor blade in the transverse and longitudinal plane prior to examination with a Leica TCS SP8 spectral confocal microscope using both UV (408 nm) and red (638 nm) excitations. Then 2D and 3D observations were carried out to visualize microorganism colonization and changes in stem tissue architecture.

CFM observations showed i) a progressive microbial colonization on the stems surface (Fig. 1a, b) and ii) a separation of the fibers bundles from each other after retting, indicating an advanced degree of retting (Fig. 1c). These results are confirmed by FT-IR spectra analysis which revealed chemical alterations.

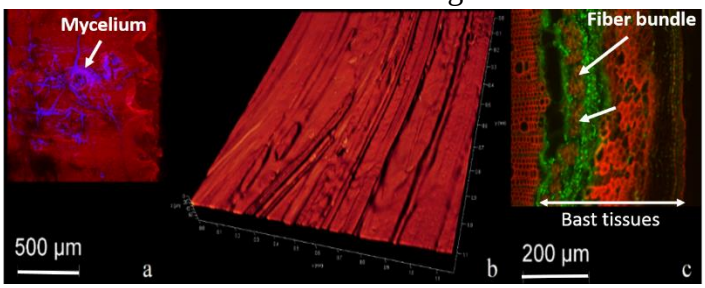

Figure 1 : Autofluorescence pictures at 408 nm, 480-540 nm emission and 638 nm excitation, 650-750 emission (a) retted hemp stem surface − (b) 3D reconstruction of unretted hemp stem surface – (c) retted hemp stem section

In conclusion, hemp retting degree can be characterized over time by CFM. Quantification of these histological changes will be related to the chemical changes and used to develop a model of the retting process and evaluate the impact of environmental conditions on this natural process.

[1] J. Müssig; Industrial Applications of Natural Fibres: Structure, Properties and Technical Applications, Part II. (John Wiley & Sons, 2010)

[2] Z. Jankauskienė; et al., "Chemical composition and physical properties of dew- and water-retted hemp fibers", *Industrial Crops and Products,* **75,** 206‑211 (2015)