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# IMAGING TECHNIQUES FOR UNDERSTANDING PHYSICO-MECHANICAL MODIFICATIONS OF LIGNOCELLULOSIC BIOMASS DUE TO HYDROTHERMAL PRETREATMENT

J. PARLATORE LANCHI<sup>1</sup>, J. COLIN<sup>1,2</sup>, P. LU<sup>1</sup>, G. ALMEIDA<sup>3</sup>, P. PERRÉ<sup>1,2</sup>

1 - LGPM, CentraleSupélec, SFR Condorcet FR CNRS 3417, Université de Paris-Saclay, Centre Européen de Biotechnologie et de Bioéconomie (CEBB), 3 rue des Rouges Terres, 51110 Pomacle, France

2 - LGPM, CentraleSupélec, Université Paris-Saclay, 8-10 rue Joliot-Curie, 91190 Gif-sur-Yvette, France

3 - Ingénierie Procédés Aliments, AgroParisTech, INRA, Université de Paris-Saclay, 91300 Massy, France

**ABSTRACT:** Steam explosion is a promising lignocellulosic biomass pretreatment for improving carbohydrates accessibility to enzymatic hydrolysis. The mechanical properties degradation during hydrothermal step is a key parameter for the process efficiency. In this work, two imaging techniques were used to assess changes in ultrastructural characteristics of poplar cell wall caused by hydrothermal pretreatment. Firstly, anatomical changes on the cell wall structure were observed by X-ray nano-tomography. Secondly, chemical changes in the different layers of the cell wall before and after pretreatment were observed by two-dimensional chemical mapping through Raman micro-spectrometry. The comparison between pretreated and raw biomass revealed shrinkage and thinning of cell wall as well as sub-parietal chemical changes.

**Keywords:** lignocellulosic sources, pretreatment, steam explosion, characterization, chemical composition, morphology.

## 1 INTRODUCTION

The production of second-generation bioethanol requires pretreatments for ensuring carbohydrates accessibility to enzymatic hydrolysis. As these processes can represent one of the costliest steps, it is crucial to understand the mechanisms involved in order to ensure a profitable production. Steam explosion appears to be effective for a large variety of lignocellulosic biomass [1]. It consists of two phases: a hydrothermal (HT) treatment, in which the biomass particles are heated under saturated steam (160-270 °C) for a few minutes, followed by a rapid release of pressure to atmospheric conditions [1].

The chemical and anatomical modifications taking place during the HT step change mechanical properties of biomass, highly affect the efficiency of steam explosion and, thus, enzymatic hydrolysis.

In this work, two imaging techniques (Raman micro-spectrometry and X-ray nano-tomography) were used at a cell wall scale, allowing to assess the effect of HT pretreatment throughout the different layers of the cell wall.

## 2 MATERIALS AND METHODS

### 2.1 Sample preparation and hydrothermal pretreatment

Samples used in this work were obtained from a 25-year poplar tree (*Populus euro-americana* 'Koster').

Defect-free blocks with a straight grain angle were cut in the longitudinal direction of the log, in which the wood has less variability. These blocks were then machined to obtain series of samples satisfying constraints of each technique. Two sample sizes were adopted: 8x8x8 mm<sup>3</sup> (radial, tangential, longitudinal) cubes for Raman micro-spectrometry and 1 mm radius cylinders for X-ray nano-tomography.

To ensure a good heat transfer during the treatment, all samples were soaked in water through vacuum/pressure cycles. HT pretreatment was performed at 160 and 180 °C for 0, 10, 20 and 40 minutes under saturated steam conditions in a device specifically developed for this purpose [2]. Even though two sample sizes were used, the treatment can be considered equal in

both cases due to a small characteristic time of heat diffusion (< 15 s) in comparison with treatment duration.

After the treatment, samples followed a specific preparation depending on the imaging technique to which they were submitted.

### 2.2 X-ray nano-tomography

X-ray nano-tomography is a relevant technique for assessing morphological changes on the sample, as it is nondestructive and requires no special preparation. The same sample was observed before and after treatment.

Anatomical changes were observed with cross-sectional slices generated by X-ray nano-tomography (EasyTomXL Ultra 150-160, RX Solutions). To avoid any source of deformation other than the pretreatment itself, treated samples were frozen at - 80 °C immediately after HT pretreatment and then lyophilized.

### 2.3 Raman micro-spectrometry

Treated water-saturated samples were put in a 75 % relative humidity chamber for two days to allow a slow drying and to minimize deformation and cell wall collapse. Samples were then dried in ambient air for two more days.

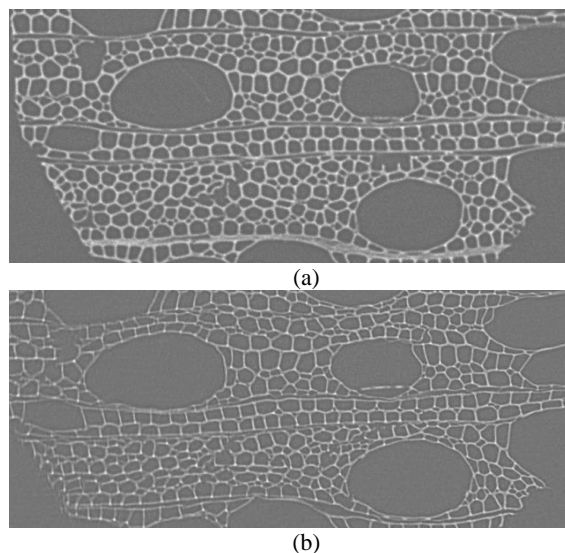
After drying, 16 µm cross-section slices were carefully cut from the cubes using a sliding microtome (Thermo Scientific HM 450). The slices were sequentially washed with distilled water, 0.02 % NaClO solution and distilled water. Samples were maintained in liquid water between a glass slide and a cover slip, sealed together with nail polish to prevent the water from evaporating. The presence of water protects the sample from burning when exposed to the laser.

Chemical mapping was achieved using an Alpha 300R+ confocal Raman microscope (Witec), equipped with a piezoelectric x-y stage and a CCD detector. For each analyzed point of the sample, a spectrum was acquired with a 532 nm laser at 10 mW and an integration time of 0.5 seconds per pixel. To minimize auto-fluorescence generated by HT pretreatment, samples were submitted to a 20 minutes photobleaching prior to the analysis, using the same laser power. Chemical maps were obtained by integrating spectra over specific wavenumbers.

### 3 RESULTS AND DISCUSSION

#### 3.1 Morphological changes

Figure 1 shows nano-tomography slices of the same sample, before and after treatment at 180 °C.



**Figure 1:** X-ray nano-tomography of native (a) and pretreated (b) biomass, with a voxel size of 1  $\mu\text{m}$

From a macroscopic point of view, Figure 1b reveals that the sample suffered a shrinkage through its tangential direction. Comparison between native and pretreated samples reveals a clear reduction in cell wall thickness, which could be linked to a mass loss measured at the macro-scale.

Some deformations and cell wall disruptions can also be observed on the pretreated sample (Figure 1b) as well as the detachment of neighbor cells from each other. As a consequence of these anatomical changes. A weaker cohesion between cells could contribute to the loss of mechanical properties, which is likely to increase the effectiveness of the explosion step.

X-ray tomography is based on the X-ray attenuation caused by the material. The difference in the attenuation level between the two images (Figures 1a and 1b) – evidenced by a difference in the grey level of the cell wall – could be attributed either by a change on the elementary composition of the material or by a change on the density of this material. It is thus conceivable to imagine that the changes suffered by biomass during the hydrothermal pretreatment would affect both its density and elementary composition. Attenuation level analysis through the grey level of images remains a good perspective for further work on the anatomical and chemical modifications of biomass.

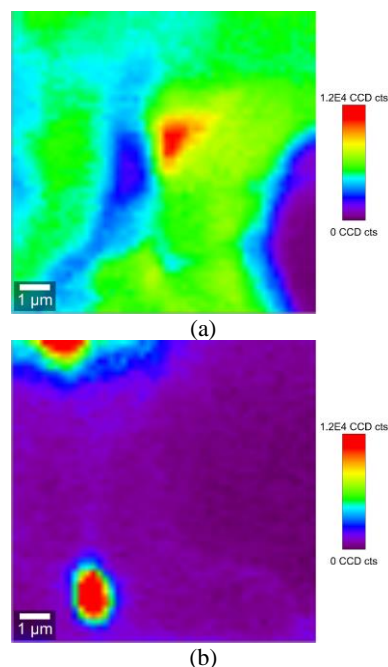
The observed anatomical changes between native and pretreated biomass could be explained by two factors. Firstly, a hydrolysis of structural components, such as cellulose and hemicellulose. Secondly, a changes in the macromolecular organization.

#### 3.2 Chemical changes

To further explain the chemical changes leading to the observed morphological modifications, the use of topochemistry can be very helpful, not only to identify the reactions taking place during this treatment but also

the influence of the microstructure of the cell wall on these reactions.

Raman images of two cells (before and after the pretreatment) are presented in Figure 2. They were generated by integrating around the 1600  $\text{cm}^{-1}$  band, which corresponds to the lignin aromatic skeletal vibrations [3].

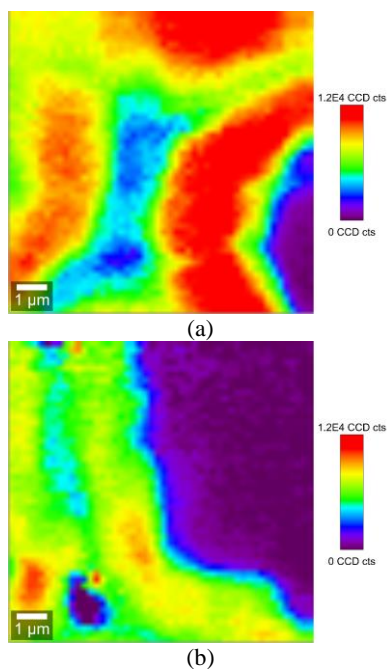


**Figure 2:** Raman images of lignin's spatial distribution in native (a) and pretreated (b) biomass. Intensity scale of Raman scattering is presented on the right

For untreated wood (Figure 2a), as expected, the areas with higher lignin concentration are the cell corner and middle lamella, but some lignin can also be found in the S2 layer. For the pretreated sample (Figure 2b), however, lignin can only be observed in the cell corners.

This could be explained by a migration of lignin inside the cell wall. In fact, once the glass transition temperature is reached, lignin becomes fluid, coalesces and is forced out by hydrostatic pressures within the cell wall. Lignin is then deposited on the cell corner in the form of droplets [4]. In addition to that, lignin chemical modifications could also cause an increasing in sample fluorescence in the region where it deposits. Previous works show that lignin depolymerization prevails over lignin condensation in the case of hydrothermal pretreatment [5], which could explain the obtained results since depolymerized lignin would have an increased mobility, resulting in higher Raman signal and higher fluorescence. That could explain why lignin is not seen as a well delimited area (as in native sample) but as a beam with high intensity.

Holocellulose mapping was possible by integrating around 2894  $\text{cm}^{-1}$ , which corresponds to the CH and CH<sub>2</sub> stretching [3]. Figure 3 presents this integration applied to the same cell areas as Figure 2, for native (a) and pretreated (b) biomass.



**Figure 3:** Raman images of holocellulose spatial distribution in native (a) and pretreated (b) biomass. Intensity scale of Raman scattering is presented on the right

The areas in purple on the right side of both images correspond to the cell lumen and, as expected, present no significant concentrations of holocellulose.

For native biomass (Figure 3a), the change of color – from purple to red – reveals that holocellulose concentration varies progressively between the middle lamella and the S2 layer, where it reaches a maximum.

In the case of pretreated biomass, the holocellulose overall distribution remains the same as in native biomass, even though a general decrease in its concentration is observed. Due to its low thermal resistance, hemicellulose degradation may explain this decrease. It could also be the reason why S2 layer seems to get thinner with the pretreatment.

In the cell corner, the intensity of the 2894  $\text{cm}^{-1}$  band is almost equal to zero. We can assume there is no more holocellulose in this region after pretreatment.

Furthermore, the integration around 2894  $\text{cm}^{-1}$  also includes neighbor less intense pics corresponding to lignin symmetric and asymmetric out-of-plane C-H stretching (2867 and 2922  $\text{cm}^{-1}$ , respectively). As previously observed, the pretreated sample (Figure 3b) presents only a minor intensity at this range of the spectrum. Figure 2b, however, shows a high concentration of lignins in cell corners after pretreatment.

Thus, chemical modifications in lignin out-of-plane C-H stretching could be an additive explanation to the change of 2894  $\text{cm}^{-1}$  band intensity.

The observed microstructural reorganization of the cell wall and hydrolysis of its structural components are helpful for understanding the previously observed reduction on the storage modulus of poplar wood during hydrothermal pretreatment [2].

## 4 CONCLUSIONS

This work showed that, when applied for 2G

bioethanol production, the hydrothermal pretreatment could directly and indirectly improve enzymatic hydrolysis. From a morphological point of view, cell wall disruption and detachment can directly increase the surface available for enzymatic attack. The reduction on cell wall's thickness and cell wall disruption at certain points could represent a reduction on its mechanical properties, and thus, lead to a better efficiency of the following steam explosion step. The migration of lignin from the S2 layer of the cell wall towards cell corners could free cellulose for enzymatic attack.

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7 LOGOS

