The project 'From fundamentals to valorization: Enzymatic oxidation of cellulosic fibres and underlying mechanisms (FunEnzFibres)' aims at unraveling the potential of lytic polysaccharide monooxygenases (LPMOs) in modification of wood cellulosic fibres for material solutions, in particular textile fibres and nanocelluloses. The project is carried out by VTT Technical Research Centre of Finland Ltd, Norwegian University of Life Sciences (NMBU) and University of Natural Resources and Life Sciences (BOKU, Austria) in collaboration with a network of industrial partners.

FUN ENZ FIBRES

Detailed characterization of enzymatically treated cellulosic fibres

Authors: Irina Sulaeva[1], David Budischowsky[2], Antje Potthast[2]

[1]Wood K plus – Competence Center for Wood Composites & Wood Chemistry, Kompetenzzentrum Holz Gmb. Altenberger Straße 69, 4040 Linz, Austria
[2]University of Natural Resources and Life Sciences, Vienna; Department of Chemistry, Institute of Chemistry of Renewables. Muthgasse 18, 1190 Wien, Austria

Contact: antje.potthast@boku.ac.at

Introduction

The increased use of renewable materials will be one of the key aspects in the future bioeconomy. To succeed in reaching this goal, reasonably priced and sustainable raw materials are needed. Wood cellulose has a great potential in replacement of fossil-based materials in textiles, packaging and plastics. However, for these usage options to be realized, entirely new approaches and tools to modify cellulose are needed. Recently discovered lytic polysaccharide monooxygenases (LPMOs) are enzymes that oxidize cellulose in the crystalline parts, thus representing a novel type of enzyme activity with the capability of modifying also the most recalcitrant celluloses [1]. While LPMOs have yielded remarkable improvements in enzymatic conversion of lignocellulosics to sugars, their obvious ability to engineer fibers has been scarcely explored. The major reason for that is the absence of analytical tools capable of providing a detailed profile of the changes in the chemical structure of cellulose at different fiber layers.

This has become the main research objective of the present study, which focuses on the development of analytical methodology for a detailed fiber analysis across the fiber section, from surface to the fiber core, controlling both – changes in carbonyl group distribution and molar mass of cellulose.

Analysis of the insoluble cellulose fraction after LPMO treatment

Conventionally, the efficiency of LPMO action on cellulosic substrates is estimated by analysis of the solubilized, low molar mass degradation products using advanced analytical techniques, including UPLC combined with mass spectrometry [2]. The residual insoluble cellulose fraction is usually characterized by microscopic techniques. This analysis, however, does not provide a detailed (quantitative) information on structural changes of the polymeric cellulose chains at the molecular level. To close this information gap, a previously described approach for monitoring the changes in carbonyl content of cellulosic materials [3] was adjusted to meet the needs of LPMO-treated fiber characterization. The following analytical approach was developed:

1. Fiber labelling with a carbonyl-selective fluorescent label;
2. Gradual fiber dissolution across the fiber section (from outside to inside) with sample collection after different dissolution times;

3. Analysis of the dissolved fractions by gel permeation chromatography (GPC) in combination with fluorescence, multi angle light scattering (MALS), and refractive index (RI) detection.

As fiber dissolution occurs from the outer surface of the fiber and not from the internal lumen side, the inner fiber layers are getting dissolved at later stages. Therefore, the gradual dissolution approach with sample collection after various dissolution times, implying increasing sampling "depths", allows for a layer-by-layer analysis of the fiber. The optimized analytical protocol provided a detailed profile of the changes occurring at the cellulose fiber across the fiber section - from the fiber surface to the core. Changes in molar mass and carbonyl group distribution of cellulose polymer chains were monitored at the same time. Figure 1 provides an example of the molar mass distribution plots that correspond to the different layers of the cellulosic fibers treated with LPMO. As evident from the curves, this particular treatment affects predominantly the outer layers, which are more severely degraded: the molar mass distribution curves shift to higher molar masses with the increase of fiber dissolution time, i.e. when going toward the interior of the fiber structure.

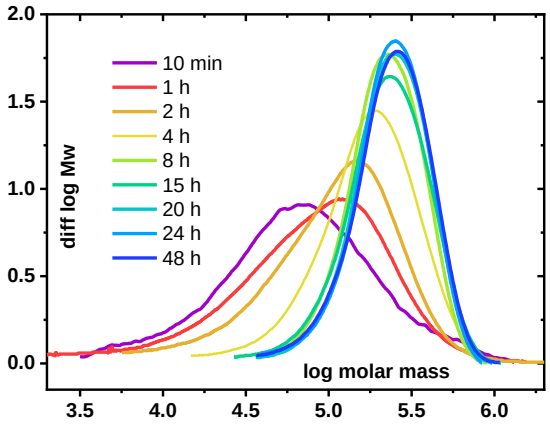

Figure 1. Molar mass distribution plots for different fractions of the same cellulose sample collected after different dissolution times. The graph demonstrates the increase of molar mass values with increasing cellulose dissolution time and sampling depth across the fiber section, which confirms the surface-predominant mode of action of LPMO.

This analytical approach was shown to be highly efficient in the analysis of the LPMOs' mode of action. It bears a great potential to promote commercial application of LPMOs in upgrading of cellulosic fibers and nanofibrillated celluloses.

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The project FunEnzFibres is supported under the umbrella of ERA-NET Cofund ForestValue by the Academy of Finland, the Research Council of Norway and the Austrian Federal Ministry of Agriculture, Forestry, Environment and Water Management (BMLFUW). ForestValue has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement N° 773324.

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