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.

Boosting enzymatic fibre processing: efficient production of cellulose-oxidizing enzymes in *Trichoderma reesei*

Authors: Nina Aro[1], Kaisa Marjamaa[1], Stina Grönqvist[1], Jenni Rahikainen[1], Anu Koivula[1], Kristiina Kruus[1,2]

[1]VTT Technical Research Centre of Finland Ltd, Espoo, Finland
[2]Aalto University, Espoo, Finland

Contact: nina.aro@vtt.fi

Introduction

Wood cellulose is actively explored in new material applications. Enzymes are natural catalysts for wood fibre processing: they enable targeted fibre modifications and can reduce consumption of energy, water and harsh chemicals. Lytic polysaccharide monooxygenases (LPMOs) are a group of monocopper enzymes that have an interesting capability to oxidize polysaccharides. They are exploited in commercial enzyme mixtures aimed for total hydrolysis of lignocellulose to platform sugars, due to their capability to enhance the hydrolysis process. Considering cellulosic material applications, LPMOs can modify the surface structure and integrity of pulp fibres, alter the water binding properties and charge and allow fibre functionalization through oxidized groups, which could be utilized in preparation of e.g. nanocelluloses (WO/2015/092146) and regenerated cellulose products (WO19243673A1). Monocomponent LPMOs, in large quantities, are needed to explore the possibilities of LPMOs in modification of cellulosic fibres. Presently, these are not commercially available.

Synthetic biology enables efficient protein production

Trichoderma reesei is a filamentous fungus, which is widely exploited in industrial enzyme production. In optimized conditions it can produce over 100 g/L of extracellular protein, mostly consisting of cellulases and hemicellulases. VTT has developed proprietary protein production strains that have decreased protease activity to improve the stability of the final product, and strains that are deleted for major background enzymes, thus allowing simple purification of the desired end-product. *T. reesei* strains can be genetically engineered to produce a single enzyme or a combination of selected enzymes. The recently developed synthetic expression system (SES) enables production of a highly pure enzyme using a simple and inexpensive carbon source such as glucose (WO2017144777A1).

Bioreactor production of LPMO enzymes in T. reesei

Production of LPMO enzymes in bioreactors was studied using different *T. reesei* strains, promoter systems and cultivation conditions. The analyzed LPMOs included AA9A from *Trichoderma reesei* and AA9C from *Neurospora crassa* (Fig 1), which both could efficiently be produced using a constitutive synthetic promoter and glucose media, leading to grams of LPMO protein (up to 11 g/L) with low levels of contaminating background proteins. In other cases, such as for AA9E from the fungus *Podoposra anserina,* production of LPMOs required use of a conventional cellulase promoter in inducing conditions, and pilot scale protein purification needed to be set up to achieve sufficient amounts of enzyme for application trials. Oxidative enzymes may be challenging in heterologous production, e.g. due to their cofactor requirements and potentially adverse effects on production hosts. Heterologous production of many LPMOs was found to be sensitive towards culture media composition, copper concentration in particular, and cultivation time. Failure in meeting the cultivation requirements leads to degradation of the recombinant protein or even damaging of the host organism.

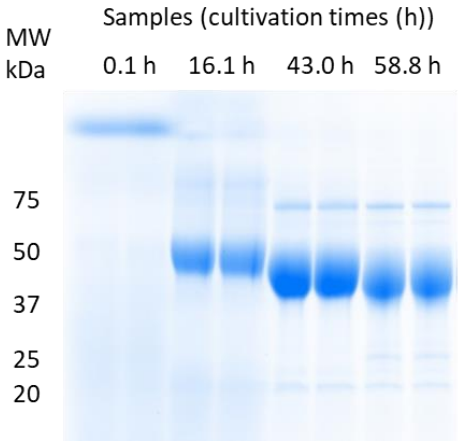

Figure 1. Protein profiles of samples from bioreactor cultivation of *Neurospora crassa* LPMO AA9C in *Trichoderma reesei* using a synthetic promoter and glucose media. The protein band corresponding to the LPMO enzyme is located between molecular weight (MW) standards 37 and 50 kDa. The proteins were analysed with BioRad Criterion Stain free SDS-PAGE gels and imaging system

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 programme under grant agreement N° 773324. We are also grateful for the support by the FinnCERES Materials Bioeconomy Ecosystem"