From fundamentals to valorization: Enzymatic oxidation of cellulosic fibres and underlying mechanisms (FunEnzFibres)

Forest Value mid-term seminar
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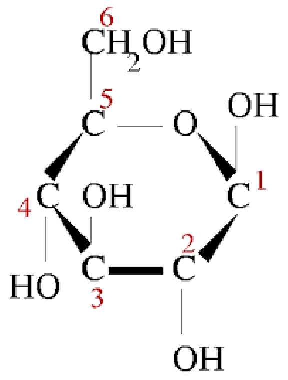
Cellulosic fibres are versatile raw materials

Oxidative modification of cellulosic fibres

- **Oxidation modifies cellulose and cellulosic fibre properties**
  - Molecular level:
    - New functional groups
    - Reduction of molar mass
  - Fibre level:
    - water retention
    - surface charge
    - fibre strength
    - fibrillar interactions



Biocatalytic oxidation of cellulosic fibres

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- **Lytic polysaccharide monooxygenases (LPMOs)**
  - Catalyze oxidation of various polysaccharides (and oligosaccharides): <u>cellulose</u>, <u>hemicelluloses</u>, chitin, starch
  - Different LPMO variants → different oxidation specificities
  - Potential in targeted oxidative modification of cellulosic fibres → FunEnzFibres

Crystal structure of Tr AA9A extended catalytic domain 5O2W, Hansson et al. 2018 J. Biol. Chem. 292: 19099–19109

From fundamentals to valorization: Enzymatic oxidation of cellulosic fibres and underlying mechanisms (FunEnzFibres)

- **Objectives:**
  - To **explore the potential of LPMOs** in oxidative modification of cellulosic fibres.
  - To develop **sustainable refining and dissolving processes.**
- **Consortium:**
  - VTT Technical Research Centre of Finland Ltd
  - Norwegian University of Life Sciences (NMBU)
  - University of Natural Resources and Life Sciences (BOKU, Austria)
- **Industrial advisory board:** MetsäFibre, Essity, UPM, Novozymes

Project structure

- **WP1 Selection, production and characterization of enzymes and substrates**
- **WP2 Development of analytical methods for LPMO modified fibres**
- **WP3 Enzymatic oxidation of cellulosic substrates**

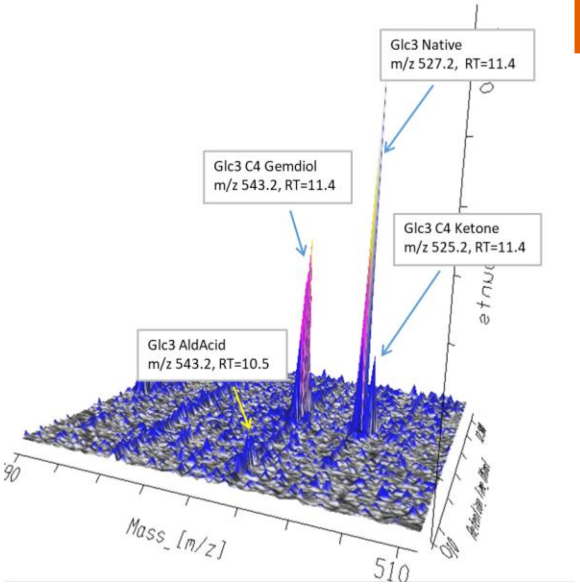
- Production and purification of enzymes
- Activity of LPMO-variants towards cellulosic fibres

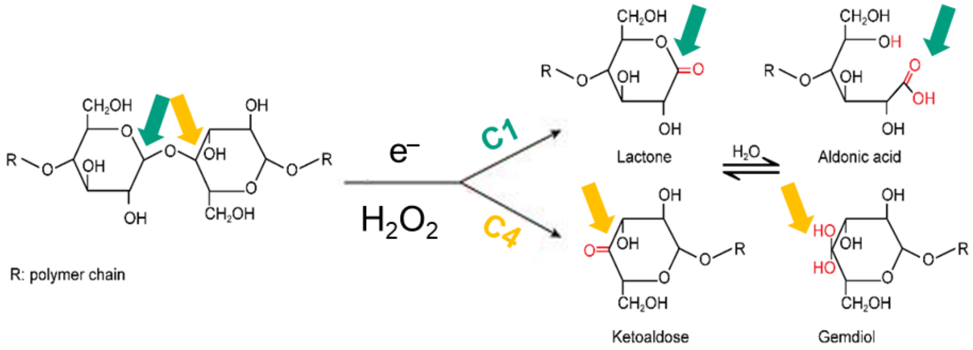
- Methods to detect and localize oxidized sites in cellulose

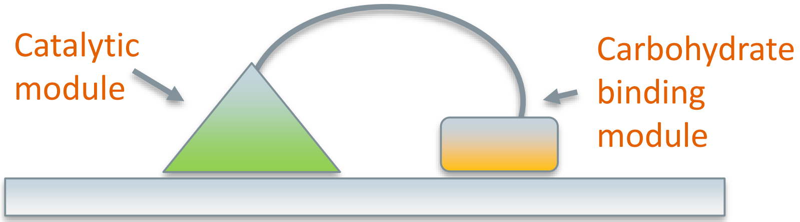
- Optimisation of the reactions
- Application trials

Properties of LPMO variants

- 13 LPMOs from fungi
  - ➢ With different oxidation specificities
    - 6 at C1
    - 5 at C4
    - 2 at both
  - ➢ With different modular structures (5 with CBM)

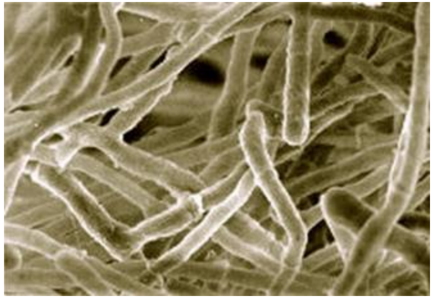


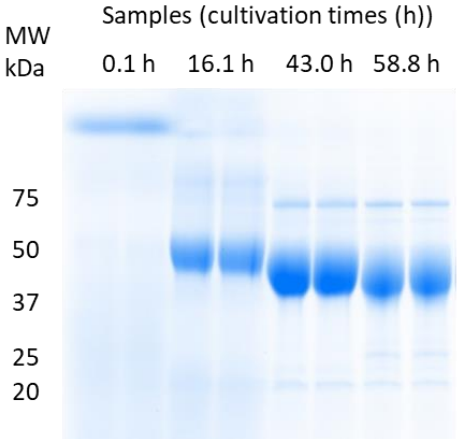




Scaling up of the heterologous production of LPMOs

- Up to 10 g/L production was achieved in bioreactor production, using synthetic promoter and *Trichoderma reesei* as production host



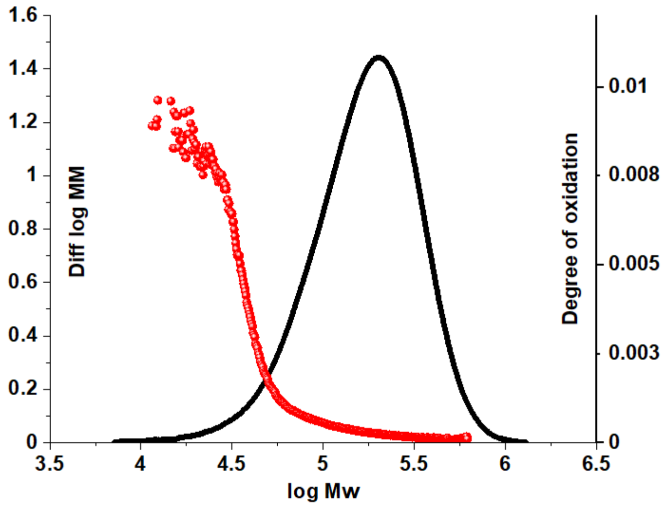


➢ **Enzymes with different oxidation specificities available in gram quantities**

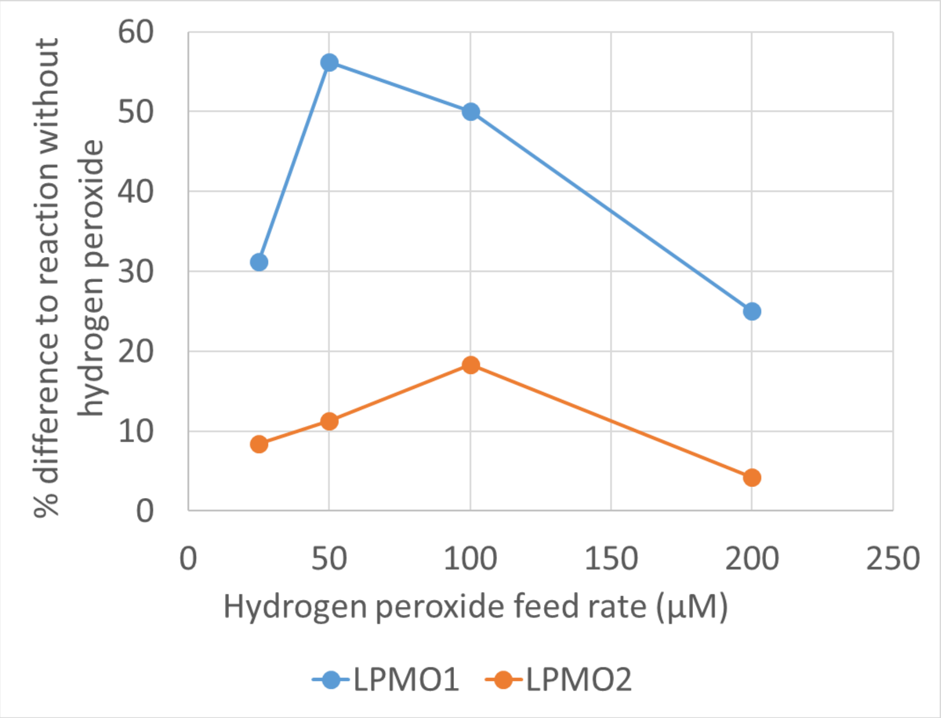
https://doi.org/10.1016/j.biotechadv.2020.107583
https://forestvalue.org/wp-content/uploads/2020/06/FunEnzFibres-stakeholder-oriented-article-1_FF.pdf

Analysis of oxidation products in cellulosic fibres

- Labelling oxidized sites with fluorescent markers, followed by SEC-MALLS
  - ➢ Quantification of oxidized sites
  - ➢ Molar mass distribution of cellulose
  - ➢ Detection of oxidized sites in different molar mass fractions



Reaction conditions affect the level of oxidation



- LPMO catalyses cellulose oxidation in presence of reductant and hydrogen peroxide
- Oxidation can be controlled by feeding rate of hydrogen peroxide
- Response and optimum concentrations of hydrogen peroxide are enzyme dependent

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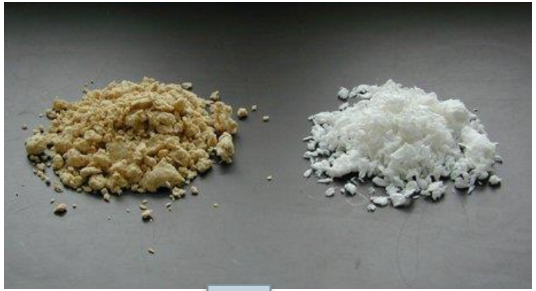
Conclusions so far

- Diversity of LPMOs can be exploited in targeted oxidation of cellulosic fibres
- Synthetic biology enabled production systems can result in high level of LPMO production with low interfering background activites
- Fluoresecent labeling combined to SEC-MALLs enables detailed characterization of the oxidized sites in fibres
- LPMO catalysed oxidation can be controlled by feeding rate of hydrogen peroxide

Oxidative fibre processing

High fibre consistency mixing
combined to LPMO treatment



Improved
dissolution
New functional
groups

Better fibrillation
New material
properties

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