Biochemical characterization of a cellulolytic and xylanolytic AA9 LPMO by *Thermothelomyces thermophila* and its utilization for production of fifth generation cellulose-based products

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Introduction

Nanocellulose is one of the most important lignocellulose-derived value added products in the emerging market of biobased polymers. Nanocelluloses are usually divided into two main types: cellulose nanocrystals (CNCs) and nanofibrillated cellulose (NFC). The isolation of nanoscale cellulose upon employment of milder, environmentally friendly processes is particularly attractive. Biocatalysis is a promising approach due to targeted and substrate-specific activity, selectivity, mild and non-toxic chemistry. Enzymatic treatment of ligninocellulose has very recently been reported to be used in order to produce nanofibrillated cellulose. The enzymes that take part in this proccess are hemicellulases, cellulases, and auxiliary enzymes. The newly discovered lytic polysaccharide monooxygenases (LPMOs) are gaining attention due to their implication in nanocellulose production. **In this study we report the heterologous expression and production of a novel fungal C1-acting AA9 LPMO from *Thermothelomyces thermophila* and its use in a novel multi-step enzymatic process for the isolation of nanocellulose from organosolv pretreated hardwood biomass.**

Methods

- ✓ Cloning, expression and production of *Tt*AA9 LPMO gene from *Thermothelomyces thermophila*.
- ✓ Purification and characterization of AA9 LPMO auxilliary enzyme.
- ✓ Design of a four step enzymatic process for the isolation of nanocellulose, including cellulases, hemicellulases and LPMO pre- and post-treatment.
- ✓ Characterisation of nanofibrillated cellulose.

Results

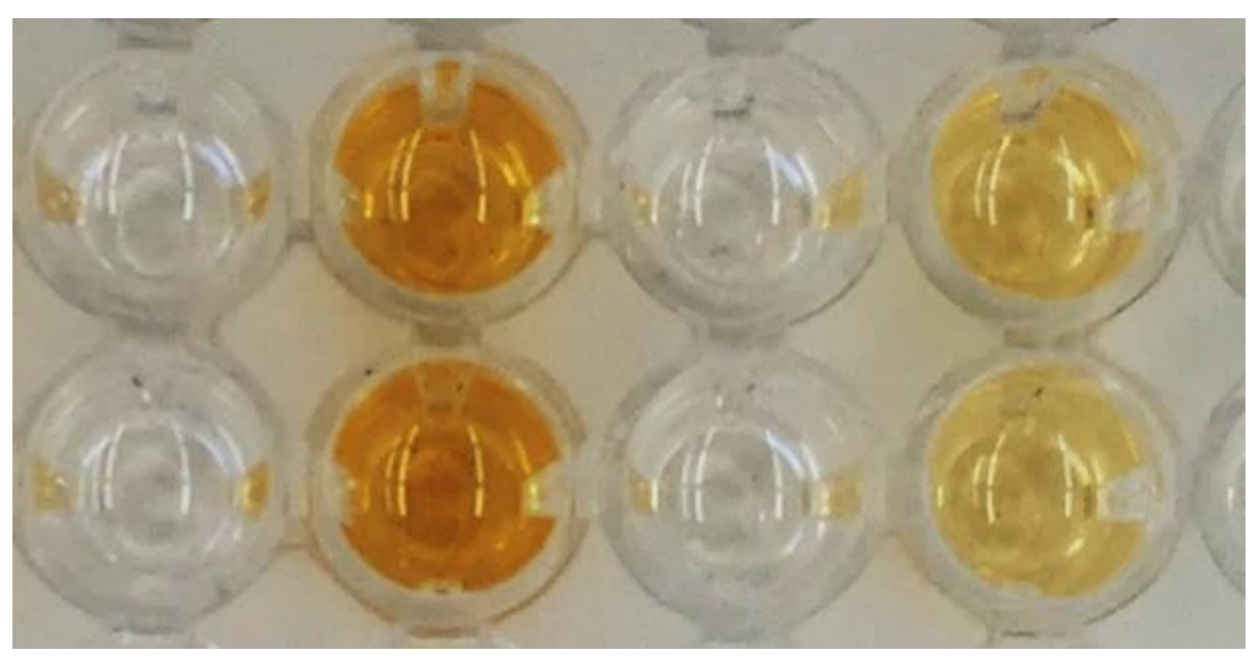
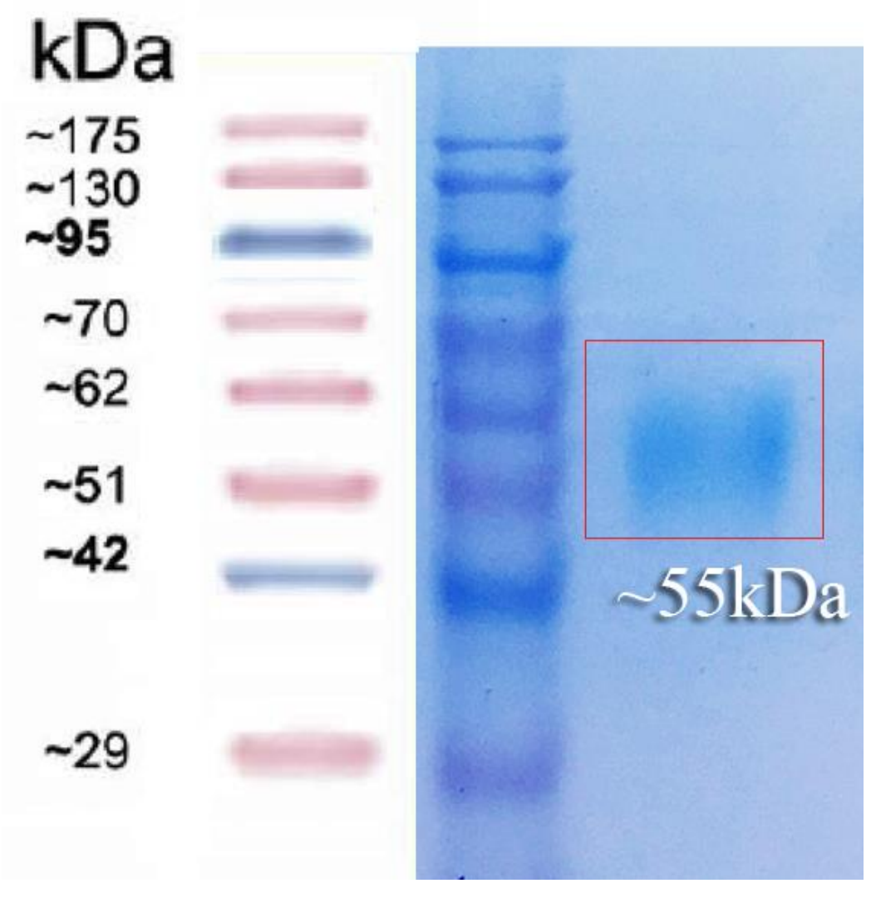


Figure1: Characterization of enzyme's properties with using 2,6 DMP assay.

T optimum (50^{o}C), pH optimum (pH 6)

T stability ($t_{1/2}$=7.76 h, 70^{o}C), pH stability (pH 4-8).

Figure 2: Heterologously expressed AA9 LPMO enzyme of 55 kDa molecular weight.



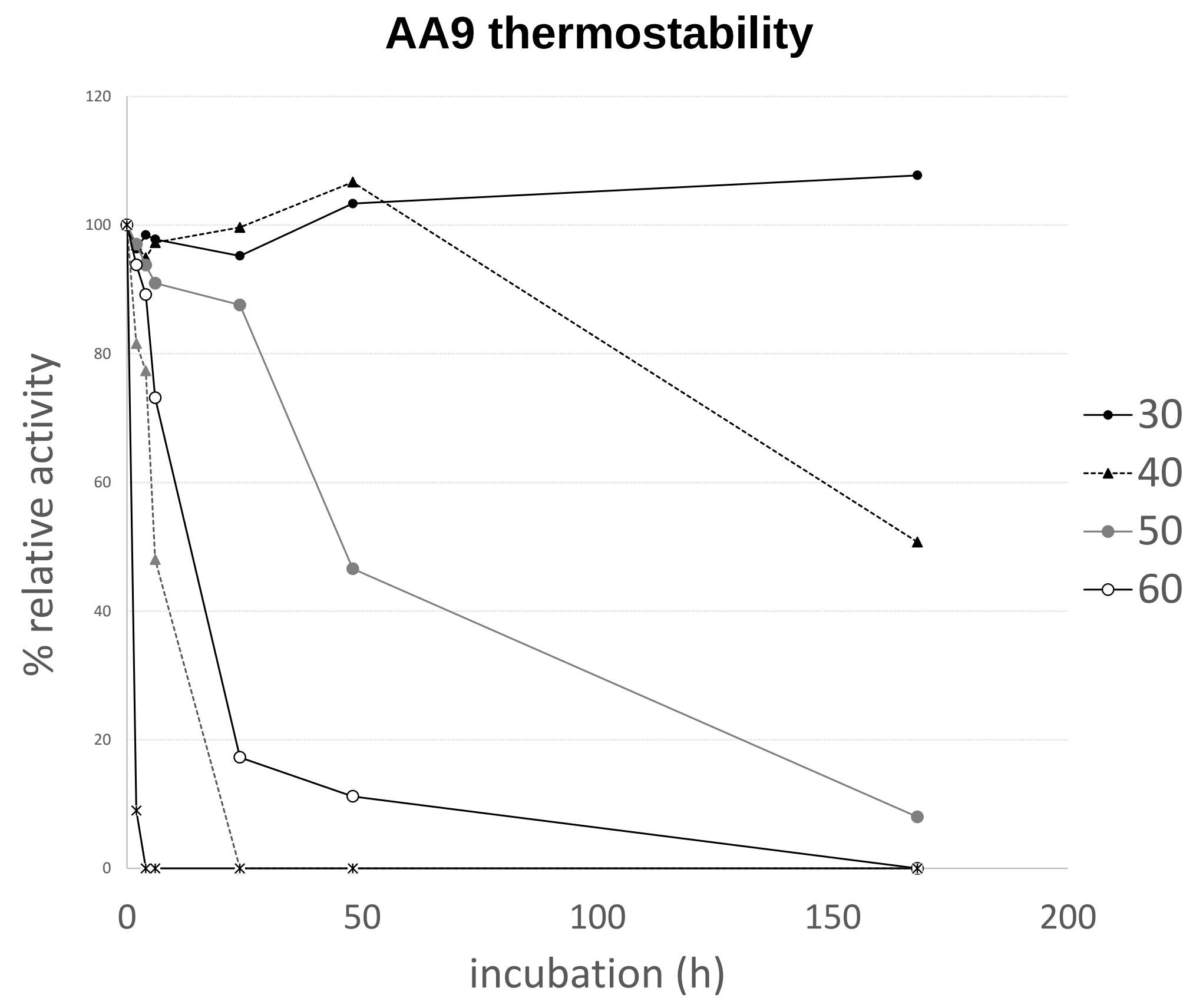


Figure 3: Highly thermostable enzyme at 20 °C - 60 °C.

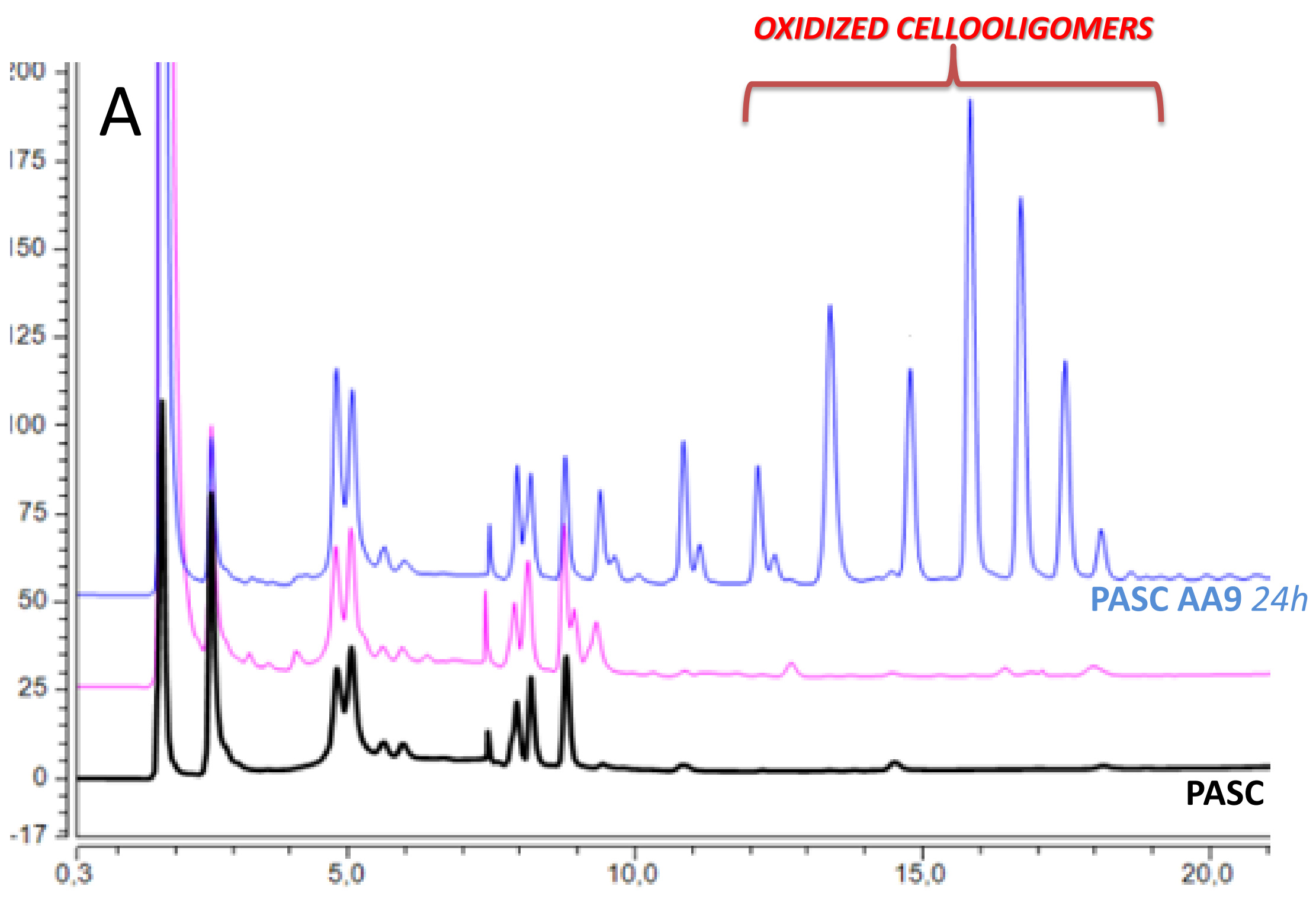
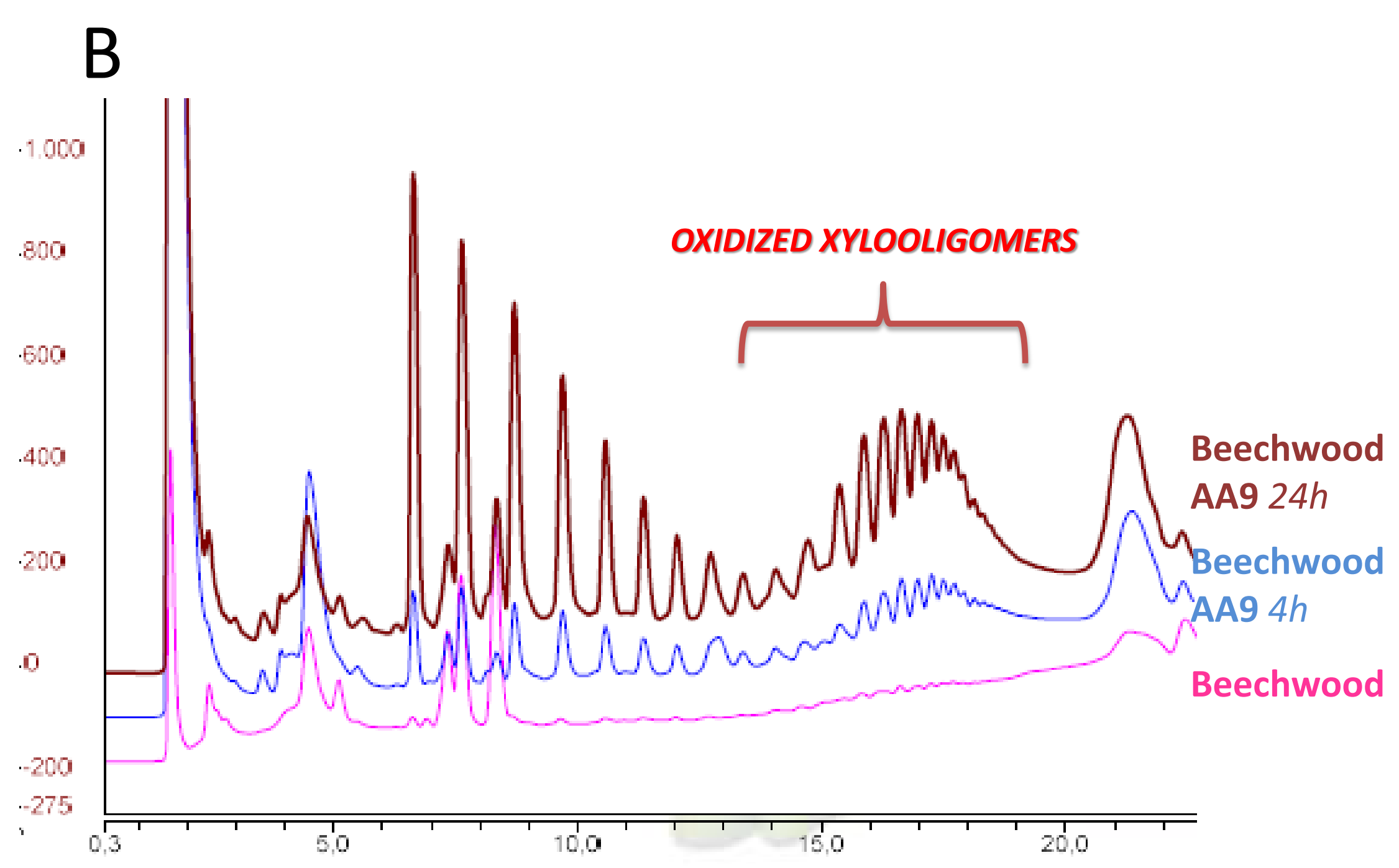


Figure 4A: AA9 LPMO cellulolytic activity on PASC



4B: AA9 LPMO activity on beechwood xylan

Conclusions

- ✓ *We characterized a highly thermostable fungal AA9 C1-acting lytic polysaccharide monooxygenase LPMO from Thermothelomyces thermophila with high activity.*
- ✓ *The apparent redox potential of the enzyme was calculated at 220 mV vs Ag|AgCl, KCl sat.*
- ✓ *AA9 enzyme of 55kDa has cellulolytic activity on substrates as PASC and organosolv pretreated hardwood biomass .*
- ✓ *AA9 has xylanolytic activity on beechwood and bircwood xylan.*
- ✓ *The production of nanocellulose was achieved by a novel four step enzymatic treatment.*
- ✓ *We demonstrate the first totally green and eco friendly process of nanocellulose production.*

Nanocellulose production process →

1. *Treatment with AA9*
2. *Treatment with hemicellulases*
3. *Treatment with cellulases*
4. *Post Treatment with AA9*

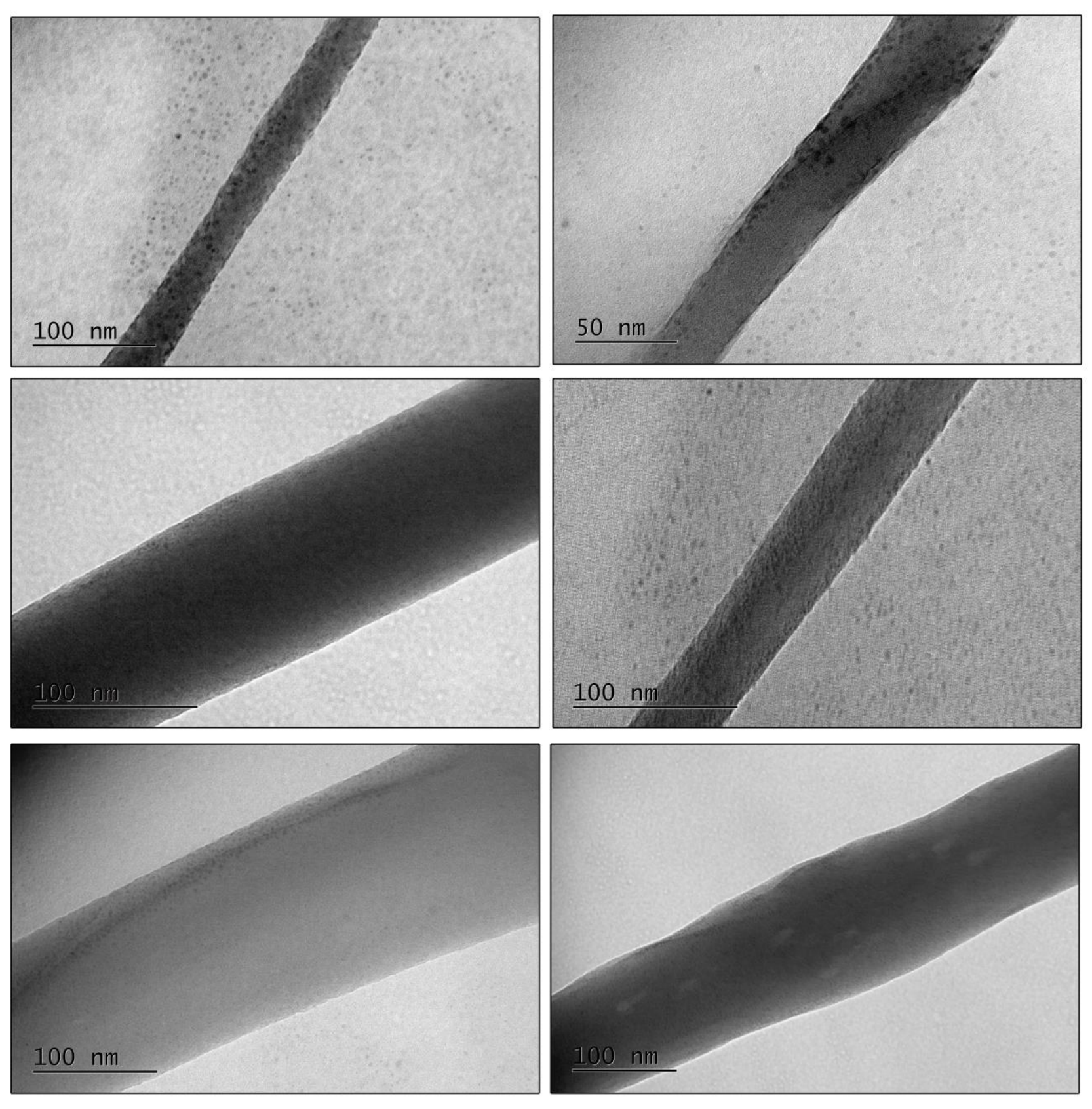


Figure 6: TEM microscopy
Diversity of diameter of nanofibers.

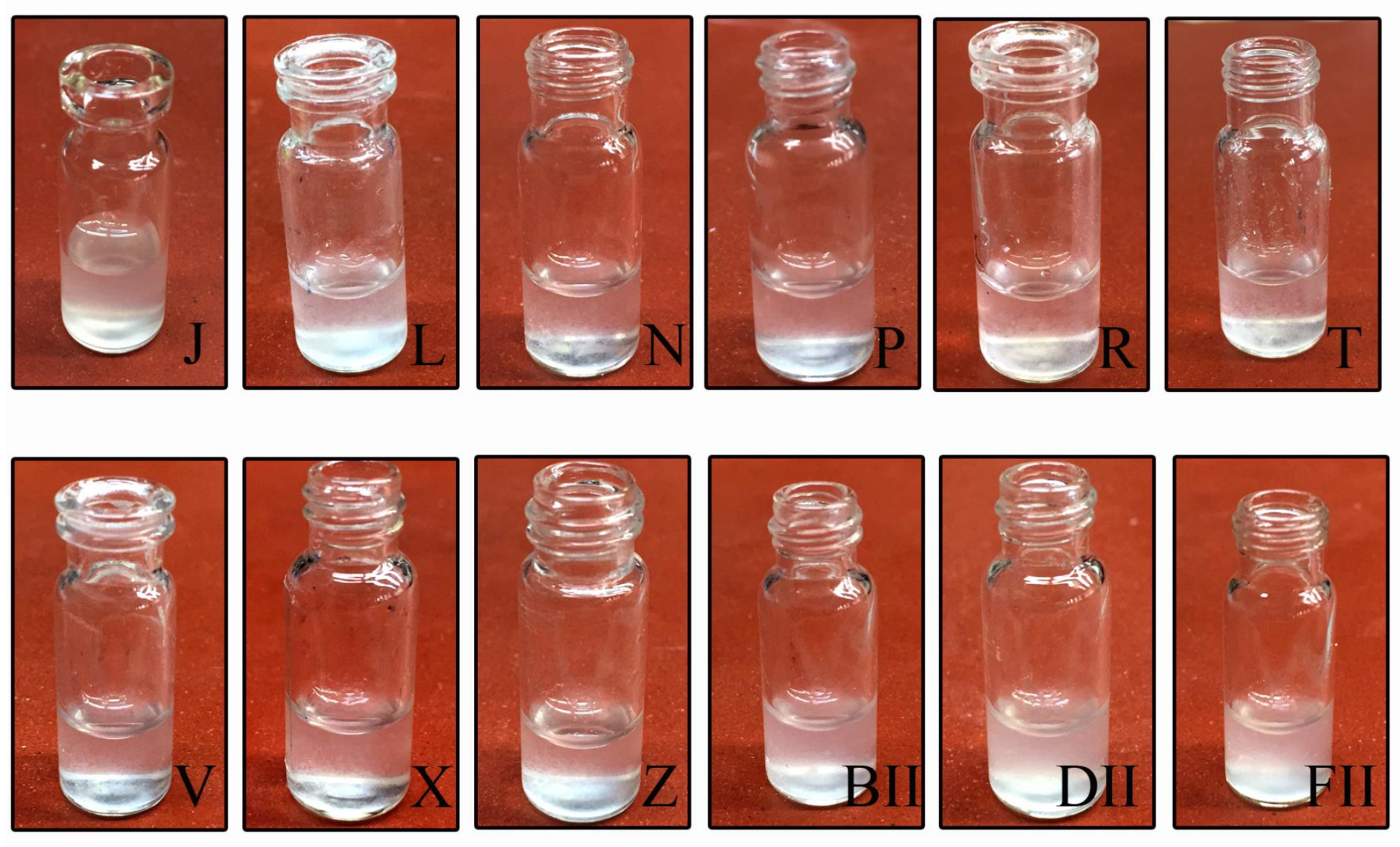


Figure 7: Nanofibrilated Cellulose products of different enzymatic combinations.

References: Anthi Karnaouri, Blanca Jalvo, Philipp Moritz, Leonidas Matsakas, Ulrika Rova, Oliver Höfft, Georgia Sourkouni, Wolfgang Maus-Friedrichs, Aji P. Mathew, and Paul Christakopoulos
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