Enzymatic modifications of plant cell wall biomimetic models

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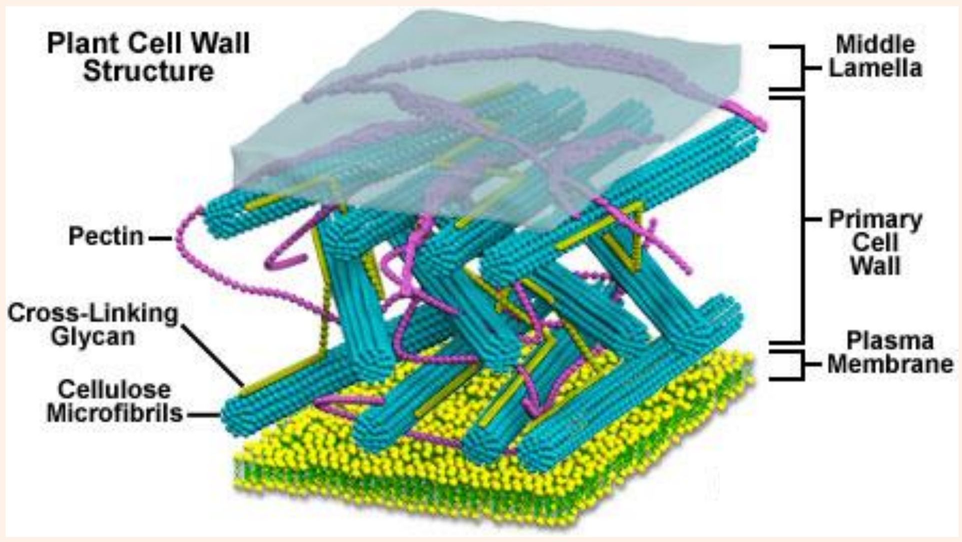
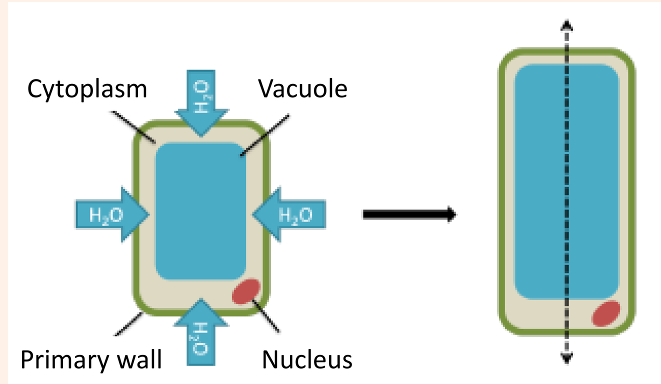
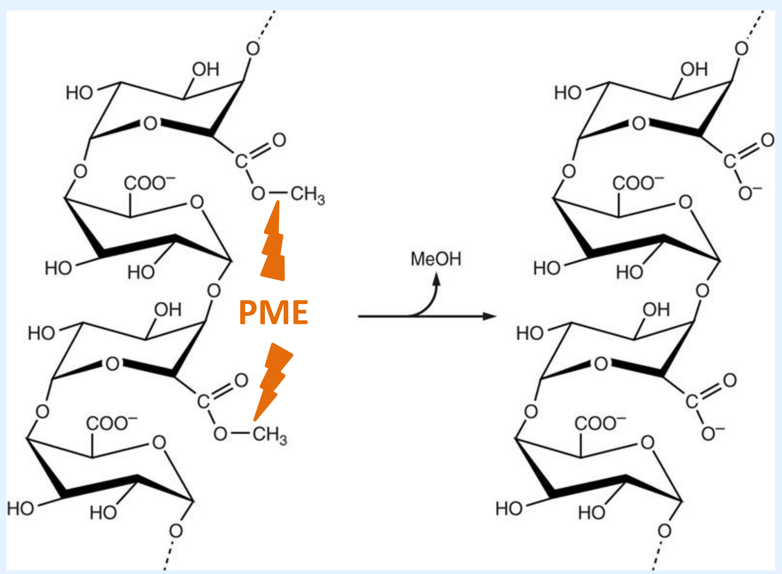
Context



Plant cell walls (**PCW**) are highly hydrated polysaccharides composites: **hemicelluloses** and **pectins** are entangled around **cellulose** fibrils.[1]
The **anisotropic growth**[2] of plant cell walls is not fully understood yet. During cell elongation, the wall is constantly remodeled and undergoes high stresses because of **turgor pressure**.
Biomimetic films made of the three building blocks of the PCW could help to modelize the role of enzymes during remodeling and elongation of plant cell walls.
A focus is made on enzymes remodelling pectins, especially **pectin methylesterase** (**PME**). PME is applied on these biomimetic films in order to follow the consequences on mechanical properties and on the morphology of the films.



Enzymatic reactions follow-up



Pectin methylesterase (PME) is an enzyme expressed during PCW growth.
PME lower the **degree of methylesterification** (**DM**) of the pectin, which is essentially composed of galacturonic acid chains.
Depending on PME sources, block or random demethylesterification can be performed.

The reaction leads to **methanol** formation.[4] Its titration allows to follow the reaction and to determine the DM.

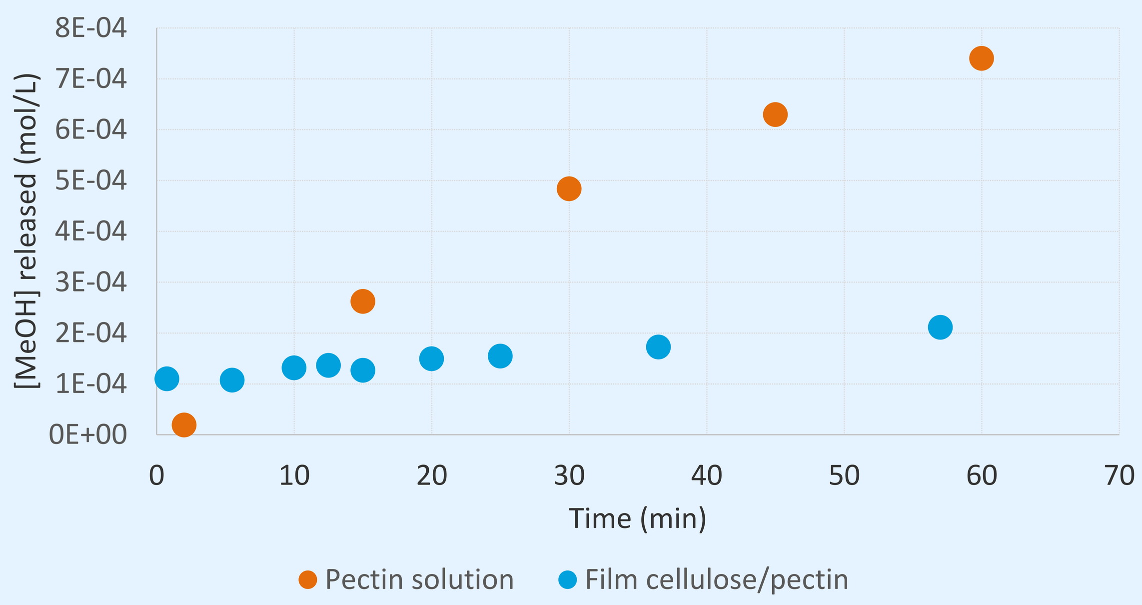
$$CH_3OH + O_2 \xrightarrow{\text{Alcohol oxidase}} CH_2O + H_2O_2$$

$$CH_2O + 2\ MBTH \xrightarrow{Fe^{3+}/H^+} \text{Blue formazan (620 nm)}$$

Reaction conditions

Pectin
- Initial DM : 71%
- Homogalacturonan: 75%
- 0,4 mg/mL

Aspergillus aculeatus PME
- 2 µL/mL
- pH 6
- 30° C



Measured PME activity after 1h reaction

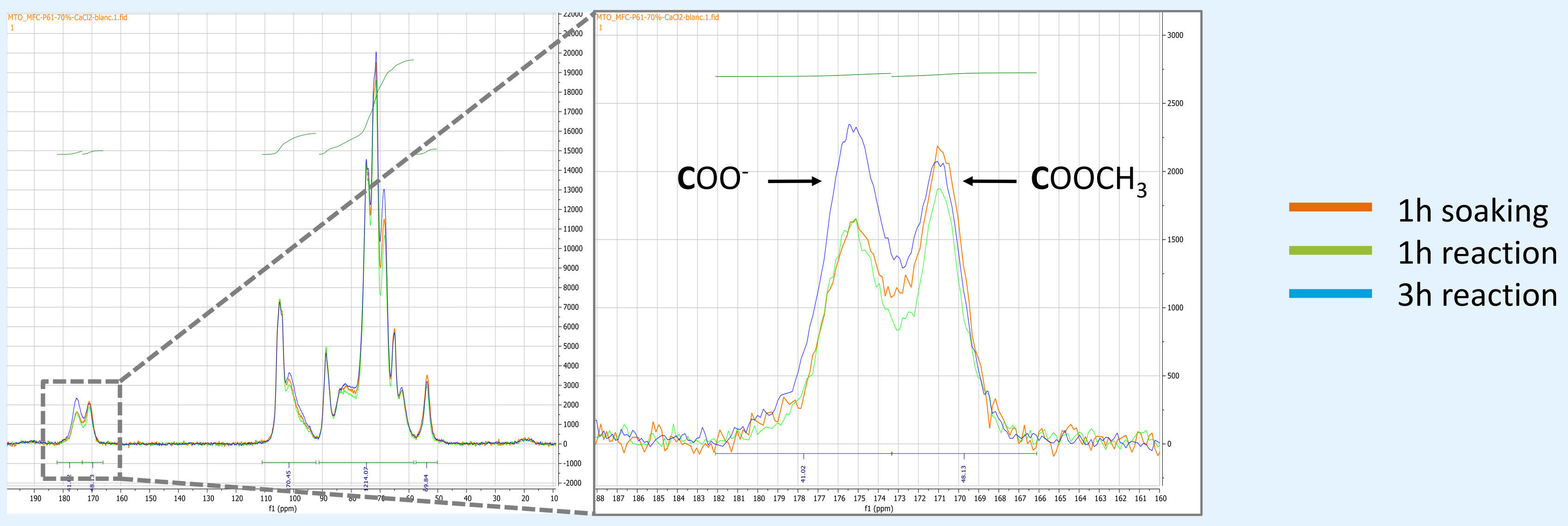
Pectin solution :

6,2 $nmol_{MeOH}/min/\mu L_{PME}$

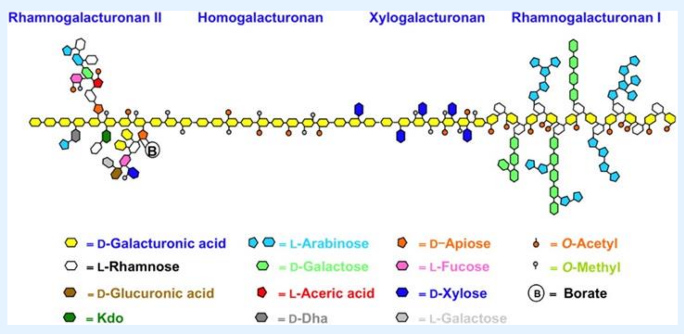
Film :

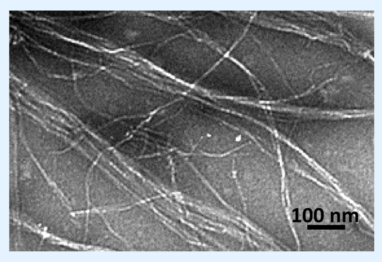
1,9 $nmol_{MeOH}/min/\mu L_{PME}$

As expected, the enzyme activity is lower on the film as it is a solid substrate and the enzyme has to diffuse inside the material.
The final DM after reaction is checked with **CP-MAS NMR** spectroscopy. It corroborates the DM measured thanks to methanol titration.

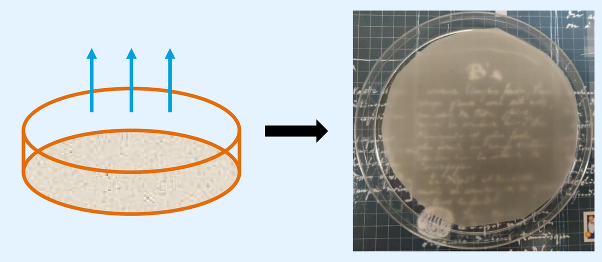


Films preparation





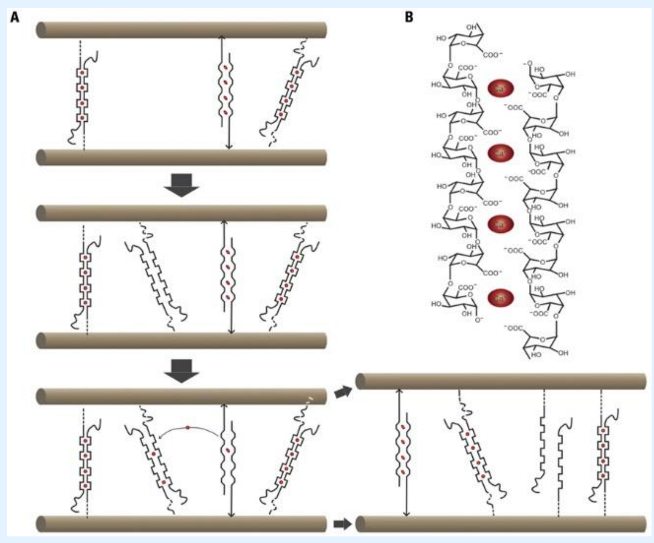
Solvent casting



Biomimetic films are prepared from solvent casting, at room temperature and relative humidity, from suspensions of cellulose microfibrils (**MFC**) mixed with pectins at a concentration of 2,5% w/w. Pectins used contains mainly homogalacturonans (70-80%) in their structures.[3]

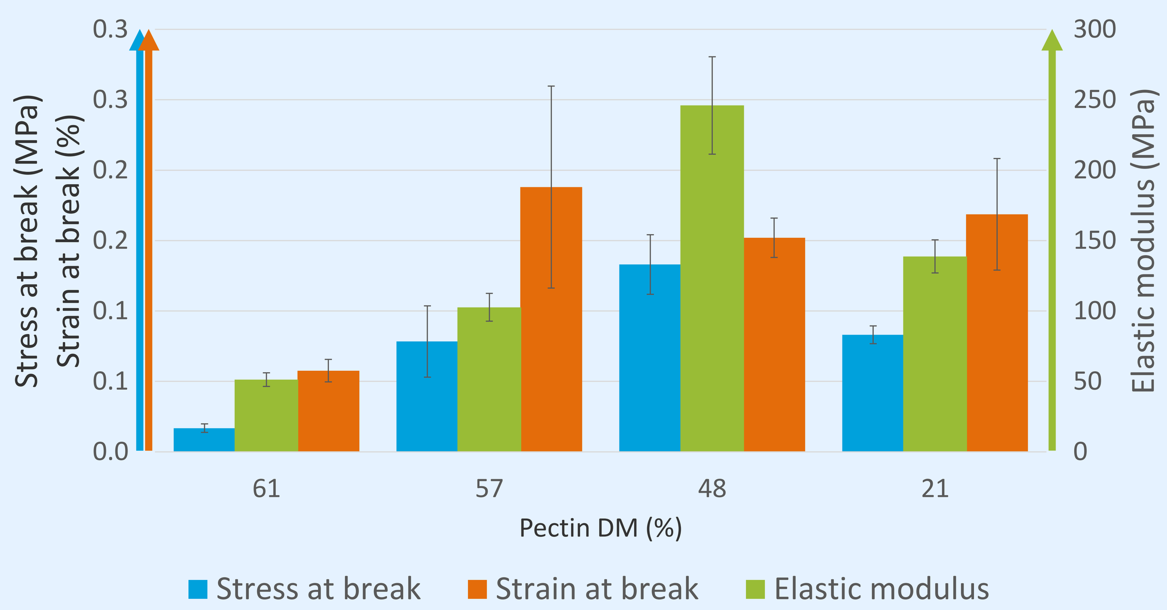
Impact on mechanical properties

Lowering the DM of pectins allows the formation of complexes between carboxylic acids groups and **calcium ions**.[5] These **crosslinks** between pectin chains lead to gelation in solution. Here this gelation is expected to increase the strength of the biomimetic films.



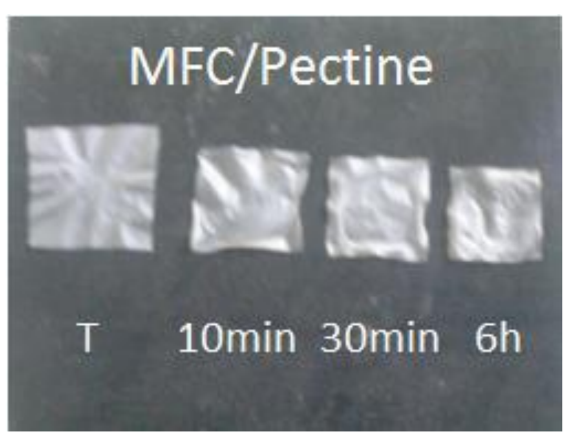
Tensile properties of the films in **wet state** are measured after PME reactions. PME concentration is steady and time of reaction is increased.

Reaction time (h)	Final DM (%)
No reaction 1h soaking	61
1	57
3	48
24	21



Up to 3h reaction (DM = 48%), decreasing the DM of pectins in the films leads to increasing strength, even for low variation of DM.
For longer reaction times, the soaking of the film is responsible for the solubilisation of some pectins. This competes with the crosslinking effect.
The reaction time needs to be steady and the PME concentration has to be adjusted to achieve a targeted DM.

[6]



Conclusion and perspectives

New biosourced and biodegradable materials can be designed with **tuneable mechanical properties**.
These biomimetic films are created from **plant cell wall building blocks**. Enzymatic activity and impacts on these models can be measured. A next step is to incorporate a third building block, **xyloglucan**, into this model.
The outlook of the overall project is to link biomimetic films deformations caused by enzymatic stimuli with observed deformations in plant growth.

References

[1] M. W. Davidson, 2015
[2] H. Radavidson, 2016
[3] J. Harholt et al., 2010
[4] J. A. Salas-Tovar et al., 2017
[5] A. Peaucelle et al., 2012
[6] S. Hongo et al., 2012