Probing Xylan-Specific Raman Bands for Label-Free Imaging Xylan in Plant Cell Wall

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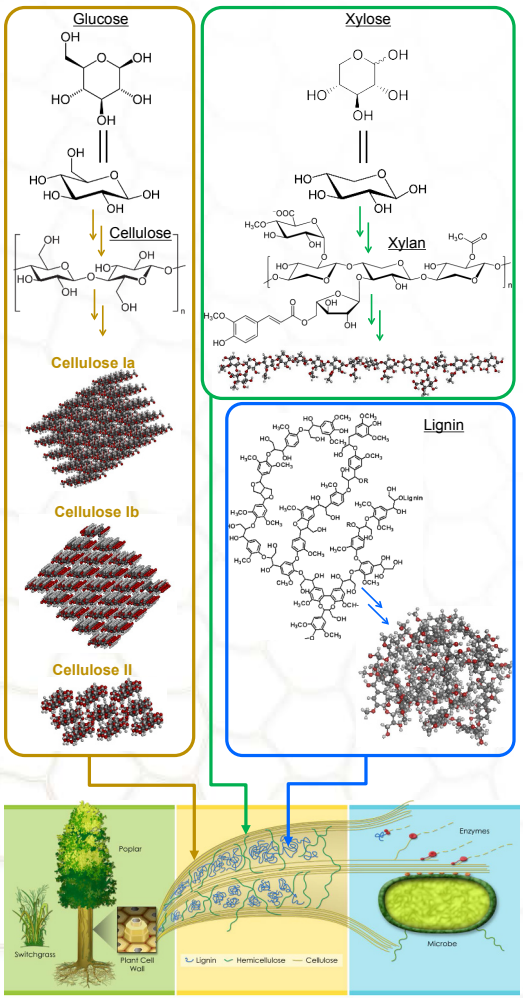
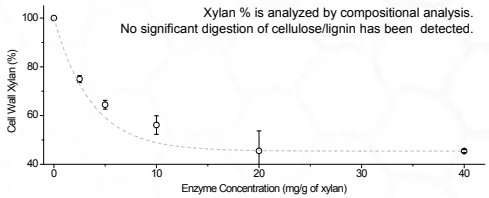
Abstract: Xylan constitutes a significant portion of biomass (e.g. 22% in corn stover used in this study). Xylan is also an important source of carbohydrates, besides cellulose, for renewable and sustainable energy applications. A currently used method for the localization of xylan in biomass is to use fluorescence confocal microscopy to image fluorescent dye labeled monoclonal antibodies that specifically bind to xylan. However, with the rapid adoption of Raman-based label-free chemical imaging techniques in biology, identifying Raman bands that are unique to xylan is critical for the implementation of the above label-free technique for *in situ* xylan imaging. Unlike lignin and cellulose that have long been assigned fingerprint Raman bands, specific Raman bands for xylan remain unclear. The major challenge is the cellulose in plant cell walls, which have chemical units highly similar to that of xylan. Here we report using xylanase enzymes to specifically remove xylan from the feedstock. Under varying degrees of xylan removal, with minimum impact to other major cell wall components, i.e. lignin and cellulose, we have identified Raman bands that could be further tested for chemical imaging of xylan in biomass *in situ*.

Structural Considerations for Raman Spectroscopy

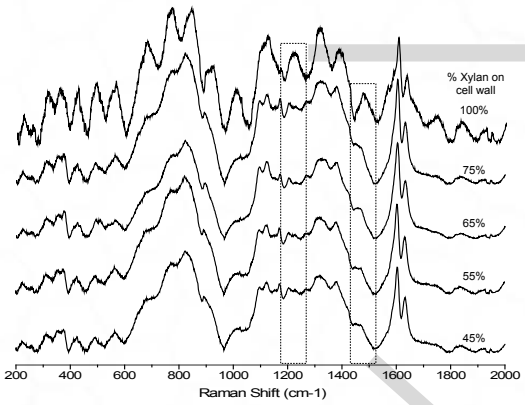
Raman spectra of plant cell walls arise primarily from the three major components: lignin, cellulose and hemicellulose (mostly xylan). Lignin's primary Rman contribution is located at 1600 cm^{-1},with other small bands. Although cellulose and xylan have highly similar chemical substituent groups, the groups have different organization and local environments. Cellulose has highly ordered structures (such as commonly found cellulose Ia, Ib and II). These highly ordered structures distribute energy among the vibrational modes differently than found in the disordered structures such as in xylan. To separate cellulose and xylan in the Raman spectrum, we developed an enzymatic assay that removes significant xylan with minimum impact on cellulose and lignin.



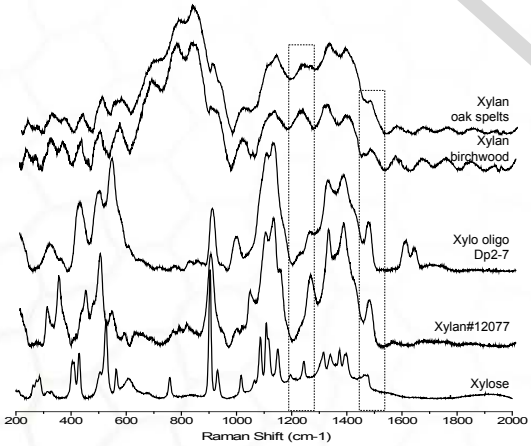
Controlling the Amount of Xylan in Cell Wall



Impact of Cell Wall Xylan Concentration Change to Raman Spectrum



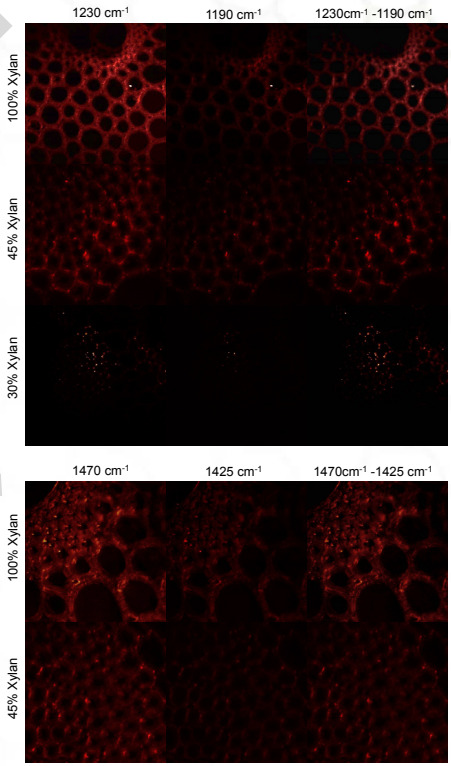
Raman Spectrum of Xylan Model Compounds



Raman Bands that are Sensitive to Xylan Concentration

Band (cm^{-1})	Possible Raman Mode	Previously Published Band Assignments
1750	1750 ν(C=O) ester	Hemicellulose (Xyloglucan, glucomannan) and pectin. Plant Methods 2014, 10:14
1460-1470	1459 δ(CH_2), δ(COH)	Cellulose and hemicellulose, Plant Methods 2014, 10:14.
	1463	Glucomannan, Applied spectroscopy, vol 51, number 11, 1997, 1648-1655.
	1471	Xylan, Applied spectroscopy, vol 51, number 11, 1997, 1648-1655
	1461 δ(CH_2) scissors	Cellulose II, Cellulose 8: 49-57, 2001
	1472 δ(CH_2), δ(COH)	Cellulose, Cellulose-Biomass Conversion, chapter 8.
	1430-1500 HCH bending and some COH	Cellulose, IPC technique series number 220.
	1475 or 1477 HCH and HOC bending	Cellulose, IPC technique series number 226.
1220-1230	1234 τ(CH_2), δ(HCC), δ(HOC), δ(COH)	Cellulose I, Cellulose 8: 49-57, 2001.
	1247 δ(CH), δ(COH)	Xylan, Applied spectroscopy, vol 51, number 11, 1997, 1648-1655.
	1256 δ(CH), δ(COH)	Hemicellulose, Plant Methods 2014, 10:14.
1010	1010 CC and CO stretching	Cellulose, IPC technique series number 226.

Test on Organosolv Pretreated CS Rind with Some Candidate Raman Bands



Conclusion: Our results have shown that there are several Raman bands that are sensitive to xylan concentration. They are highly promising for xylan detection. We have tested a few on pretreated CS, and further testing is in progress for more variety of samples.

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