The origin of recalcitrance in sugar cane hybrids with contrasting lignin contents and chemically pretreated materials

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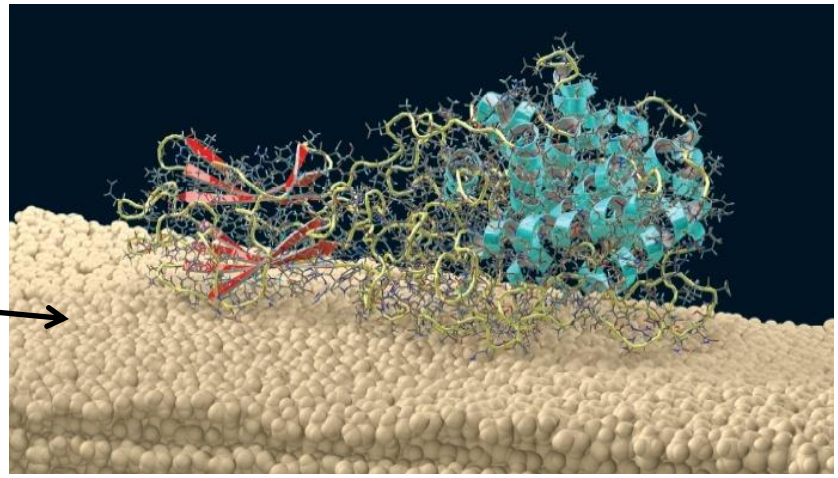
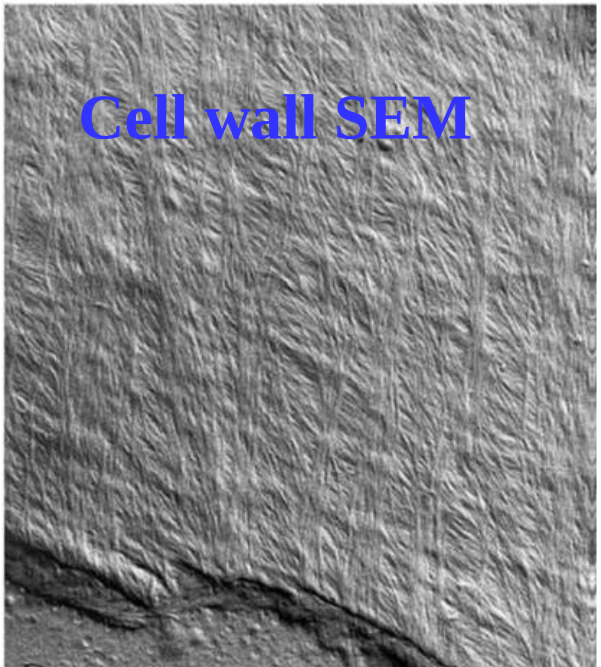
Introduction - Lignocelulose recalcitrance

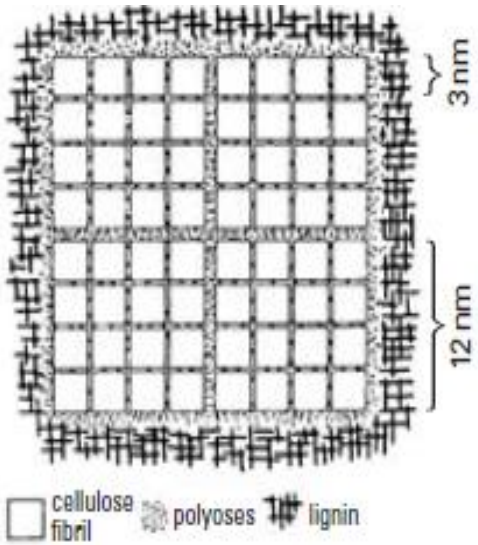
Cellulose is not "clean" as shown in this figure

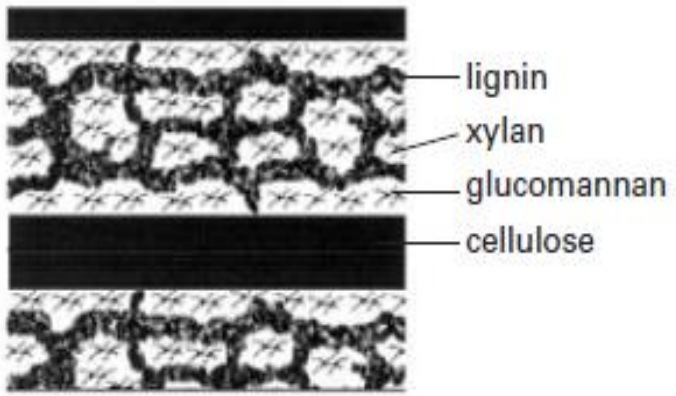


Lignin and hemicelluloses are the major components limiting enzyme infiltration into the cell walls

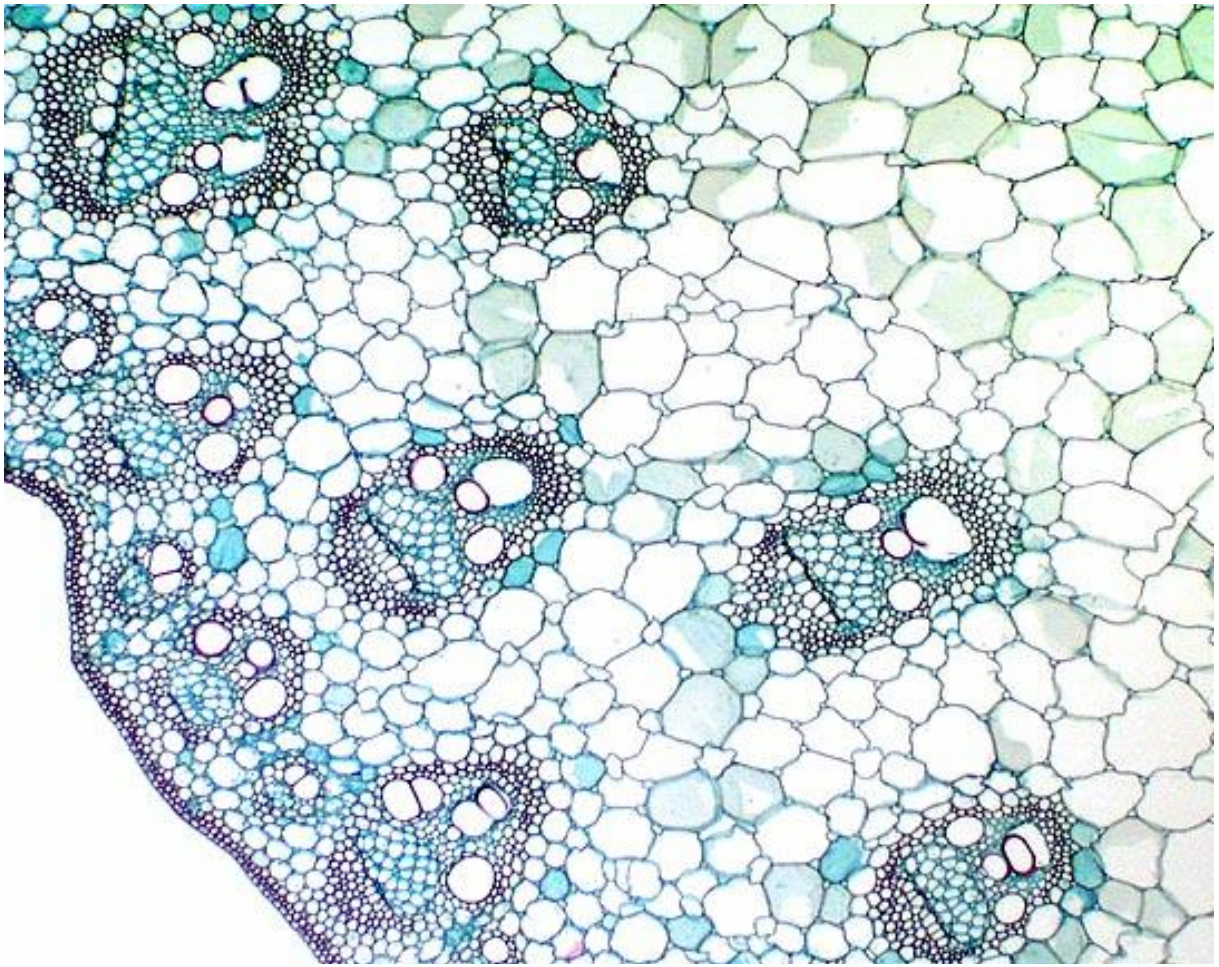


Cell wall models



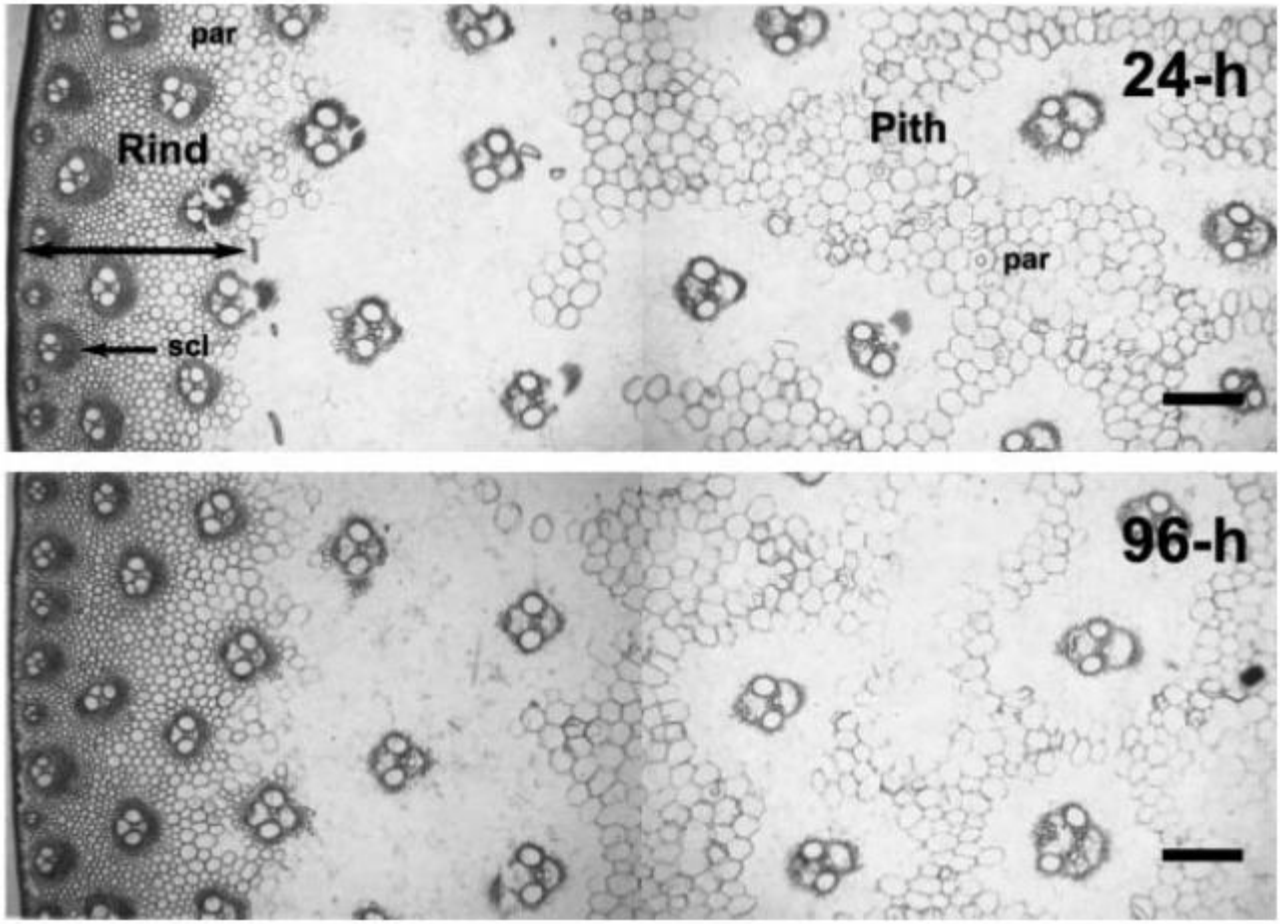


Is recalcitrance variable in different cell walls?



Grass non-wood

Cell wall digestibility in mature maize stalks

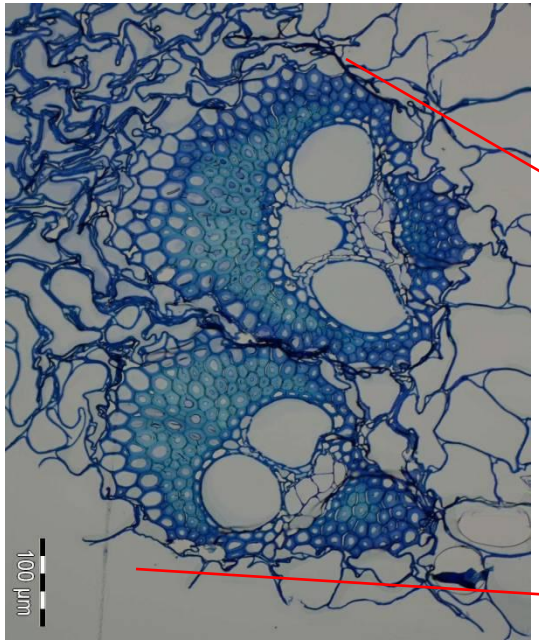


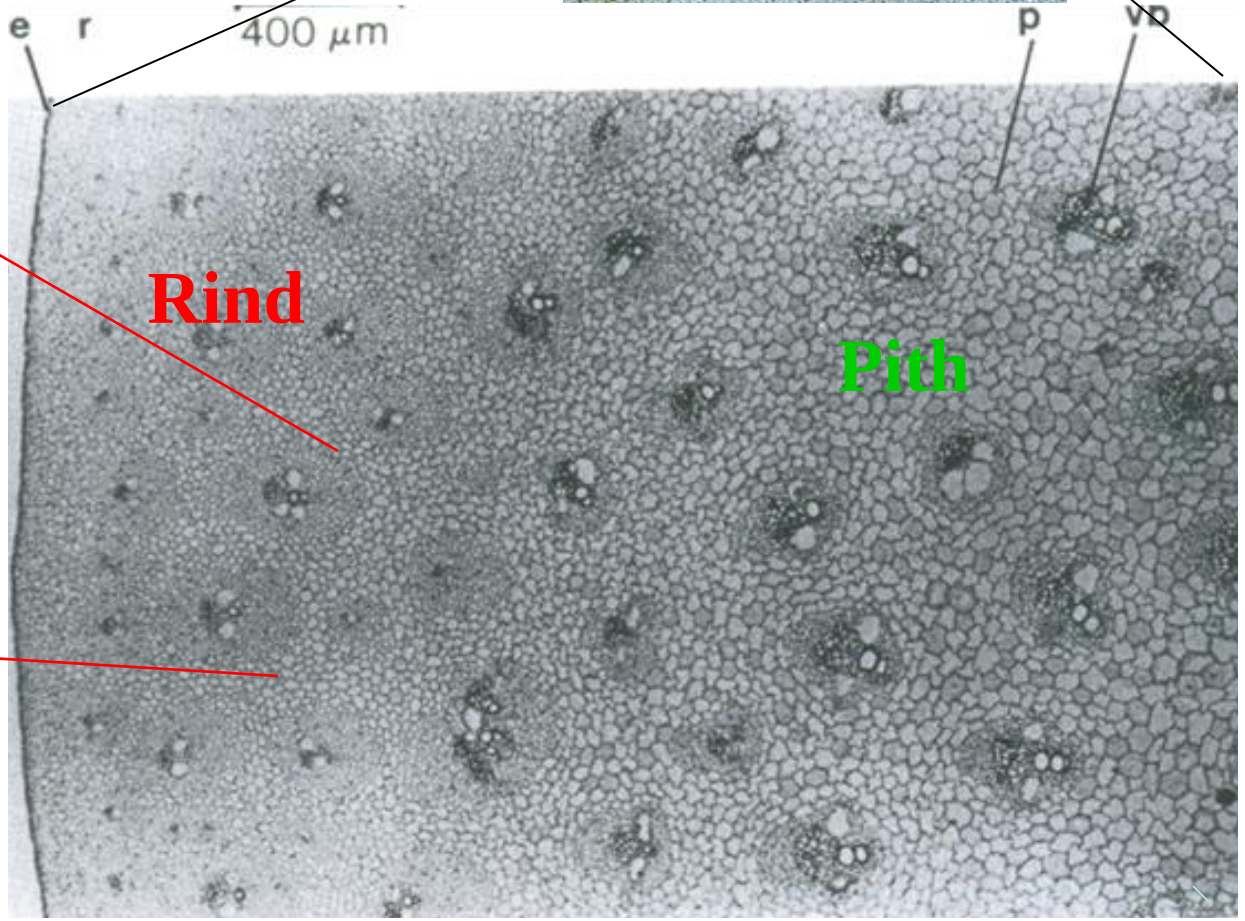
Stem internode tissues of maize at full physiological maturity

>> after *in vitro* degradation by rumen microbes for 24 or 96 h

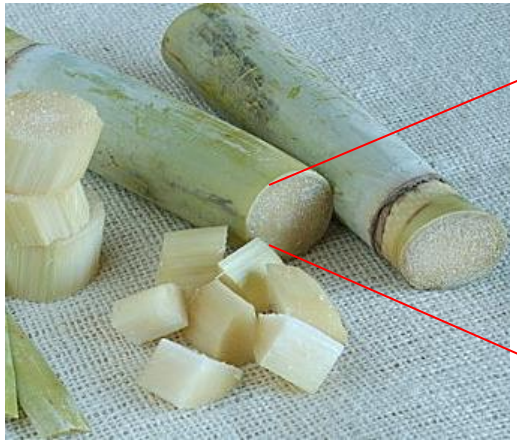
Jung and Casler. Crop Sci. 46:1801-1809, 2006.

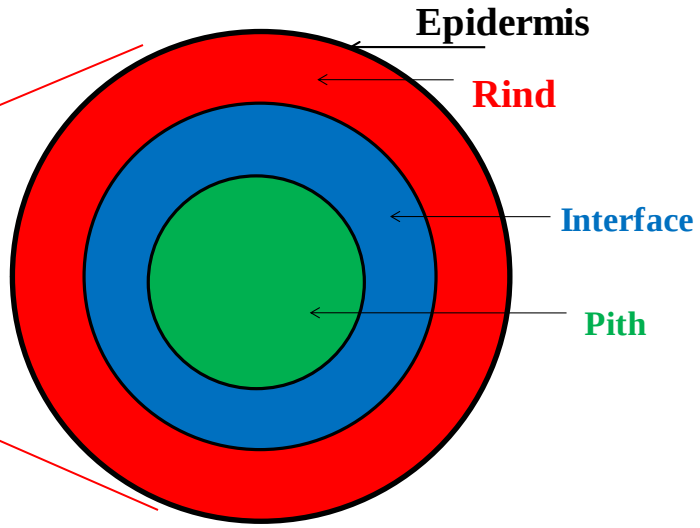
Cell anatomy in sugar cane

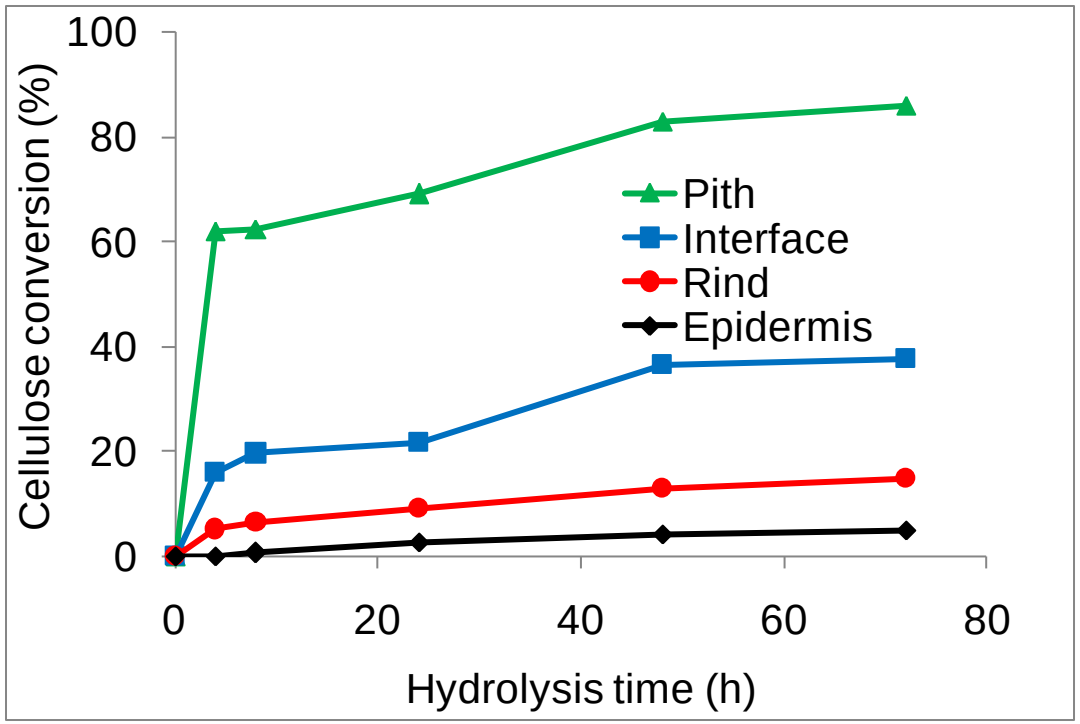




Enzymatic hydrolysis of varied sugar cane regions in an internode



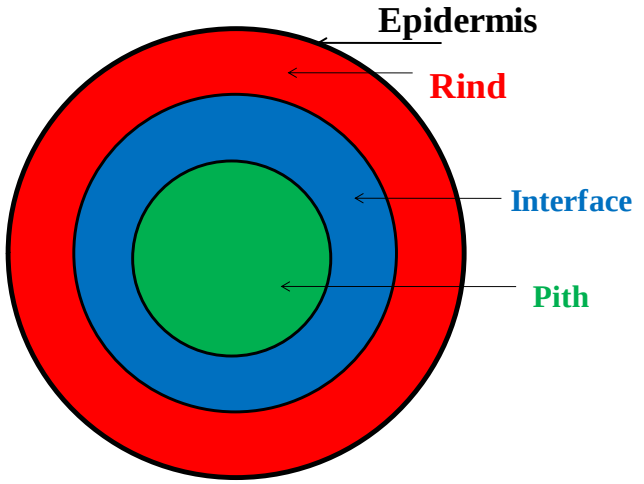


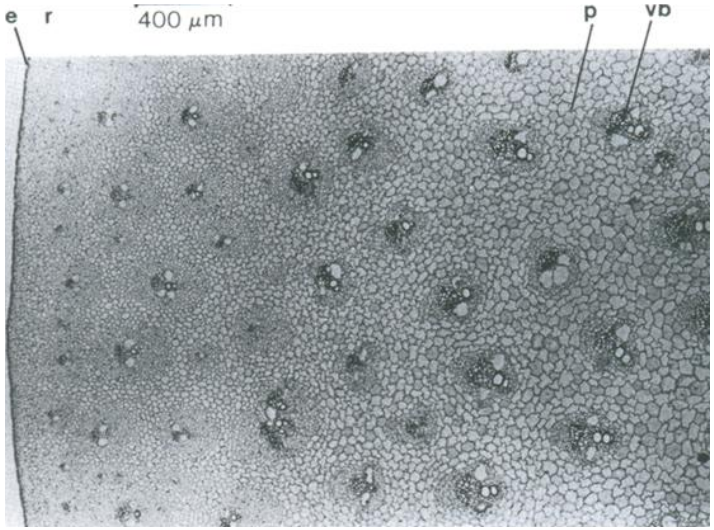


What is different in the evaluated sugar cane regions?

Frequency of vascular bundles in varied fractions of a sugar cane internode

Sugar cane region	Number of vascular bundles/area
Pith	6 ± 1
Interface	8 ± 1
Rind	13 ± 2
Epidermis	Only fibers





Volumetric and mass proportions of each sugar cane region in a sugar cane internode

Sugar cane region	Volumetric proportion (%)	Dry mass proportion (%)
Pith	10	4.7 ± 0.7
Interface	29	12 ± 2
Rind	49	47 ± 1
Epidermis	12	37 ± 1

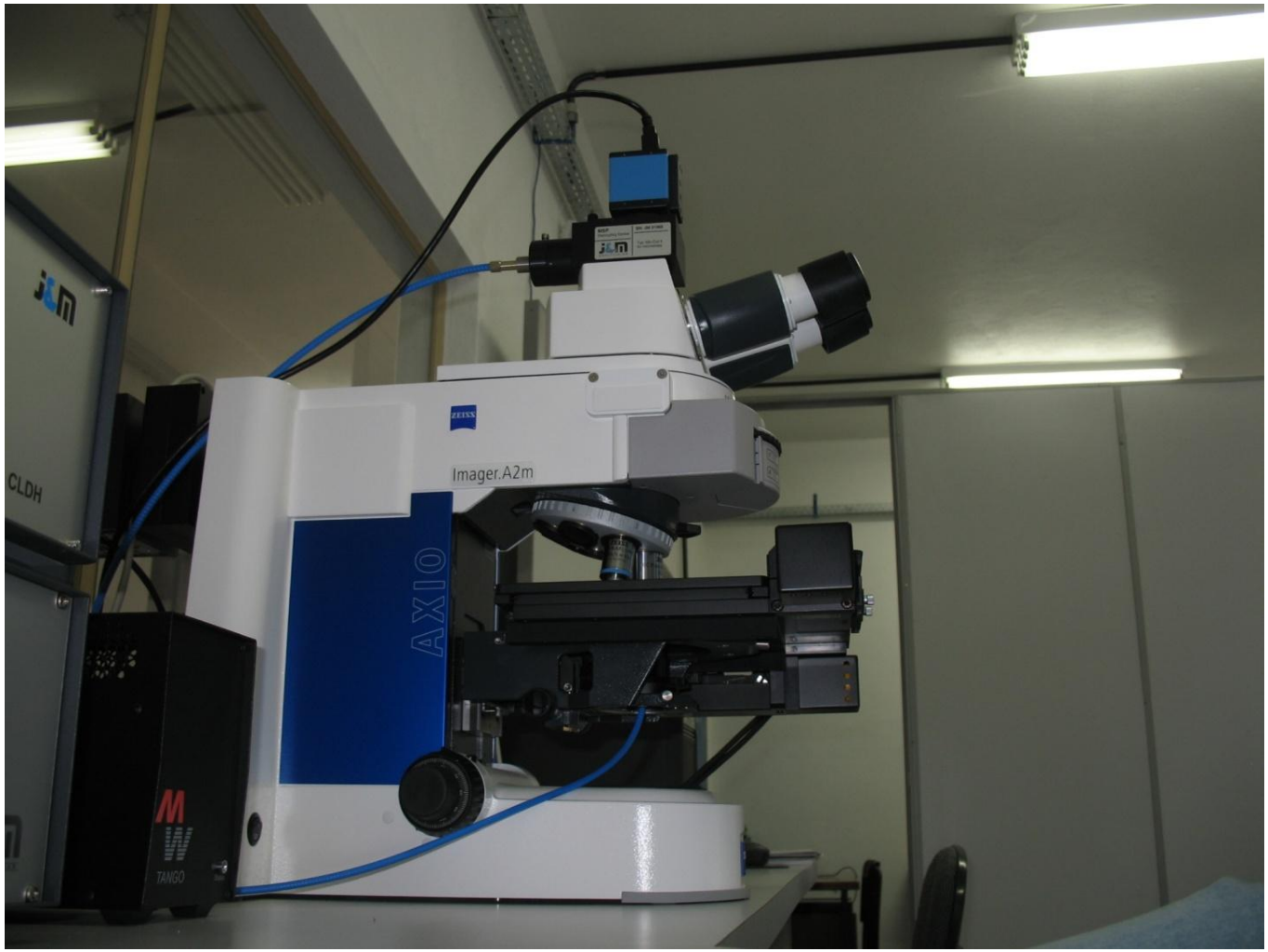
Chemical composition of varied fractions in a sugar cane internode

Sugar cane region	Chemical composition (%, w/w)						
	Extractives	**Glucan**	**Xylan**	**Arabinosyl**	**Acetyl**	**Insoluble lignin**	**Soluble lignin**
Pith	2.9 ± 0.2	49.3 ± 0.7	15.4 ± 0.2	0.1 ± 0.1	2.4± 0.2	**14.8 ± 0.1**	**2.4± 0.1**
Interface	3.0 ± 0.1	43.8 ± 0.1	18.8 ± 0.1	1.4 ± 0.1	3.2 ± 0.1	20.4 ± 0.1	2.4 ± 0.3
Rind	1.3 ± 0.1	42.0 ± 0.5	20.4 ± 0.2	1.1 ± 0.1	3.0 ± 0.2	19.8 ± 0.1	2.0 ± 0.3
Epidermis	3.1 ± 0.1	42.4 ± 0.5	19.6 ± 0.2	1.2 ± 0.1	2.6 ± 0.2	20.4 ± 0.1	1.9 ± 0.2

	Hydroxycinnamic acids (%, g/100g of in natura bagasse)					
	Released by mild-alkaline treatment			Released by severe-alkaline treatment		
	Ferulic	Coumaric	Sum	Ferulic	Coumaric	Sum
Pith	0.49 ± 0.05	1.4 ± 0.2	1.9 ± 0.2	**1.5 ± 0.1**	**5.3 ± 0.7**	**6.8 ± 0.7**
Interface	0.50 ± 0.08	2.0 ± 0.4	2.5 ± 0.4	1.6± 0.1	8.1 ± 0.8	9.7 ± 0.9
Rind	0.48 ± 0.06	2.1 ± 0.2	2.7 ± 0.1	1.4 ± 0.1	6.7 ± 0.4	8.1 ± 0.5
Epidermis	0.34 ± 0.08	1.3 ± 0.4	1.6 ± 0.5	1.6 ± 0.1	8.1 ± 0.8	9.7 ± 0.9

UV-microspectrophotometry was used to map lignin and hydroxycinnamic acids in these cell walls

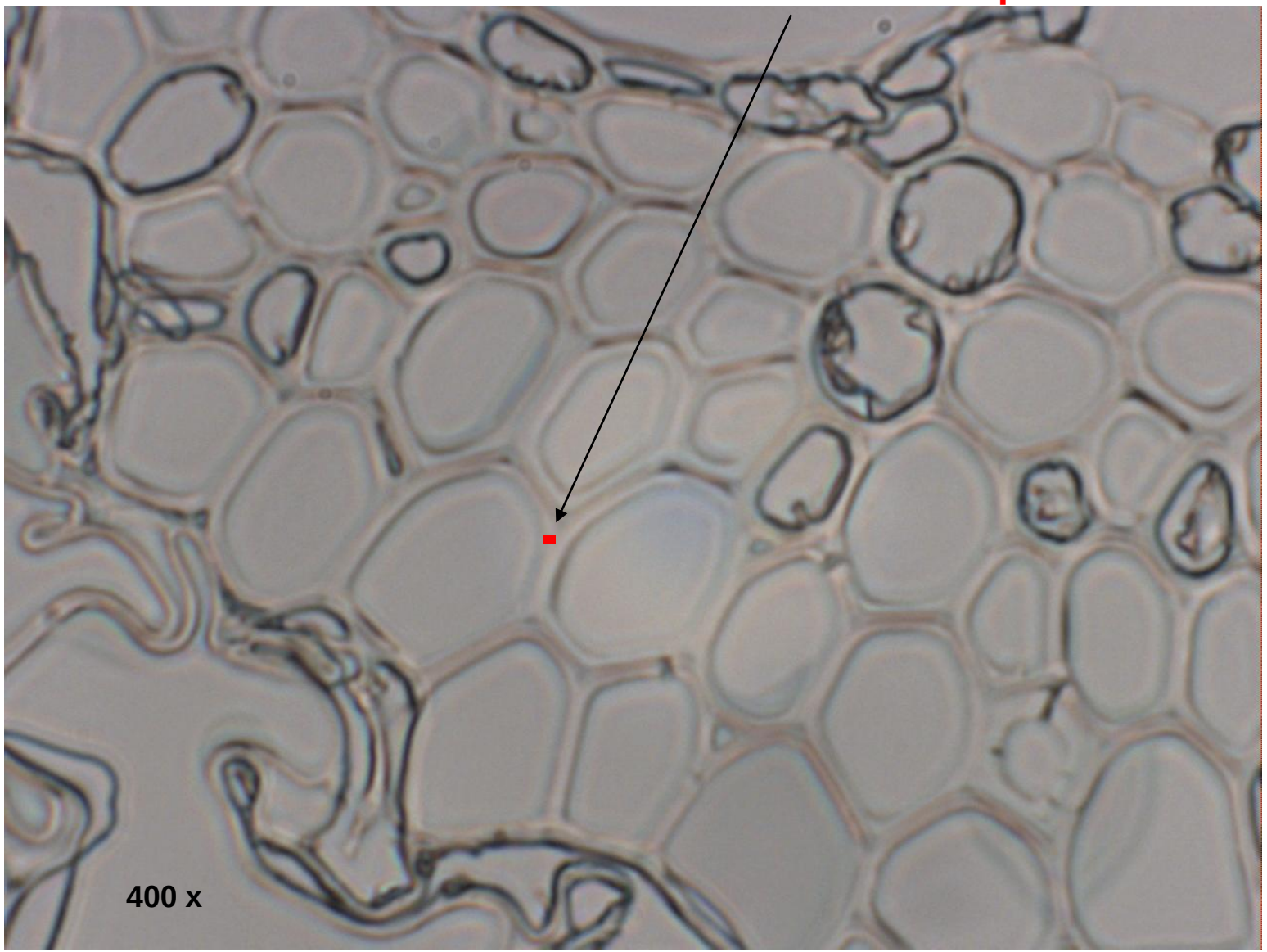
UV-microscope



Fibers from sugar cane rind observed in the UV-microscope

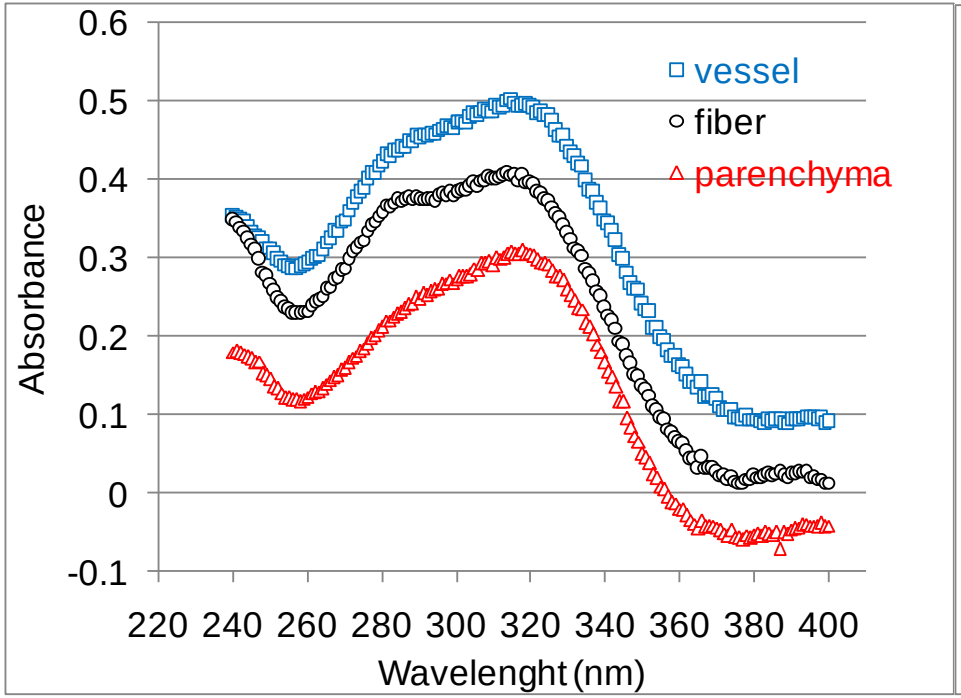
1 µm-thick cut fixed in epoxi resin

Example of a selected point (1 µm^2) to record the UV spectrum

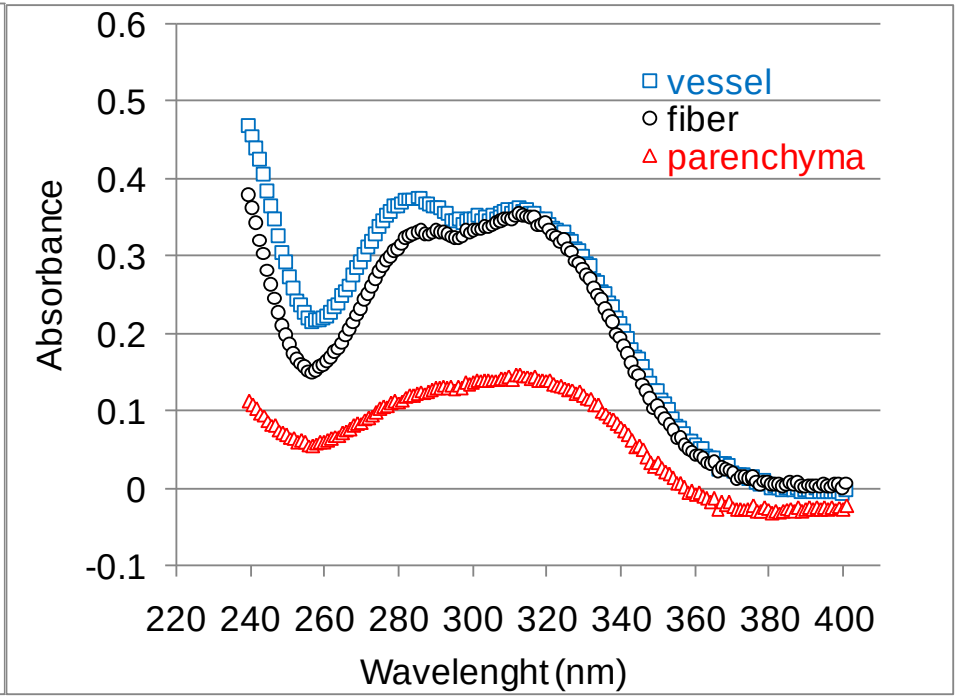


Average spectrum from different cells in sugar cane

Rind

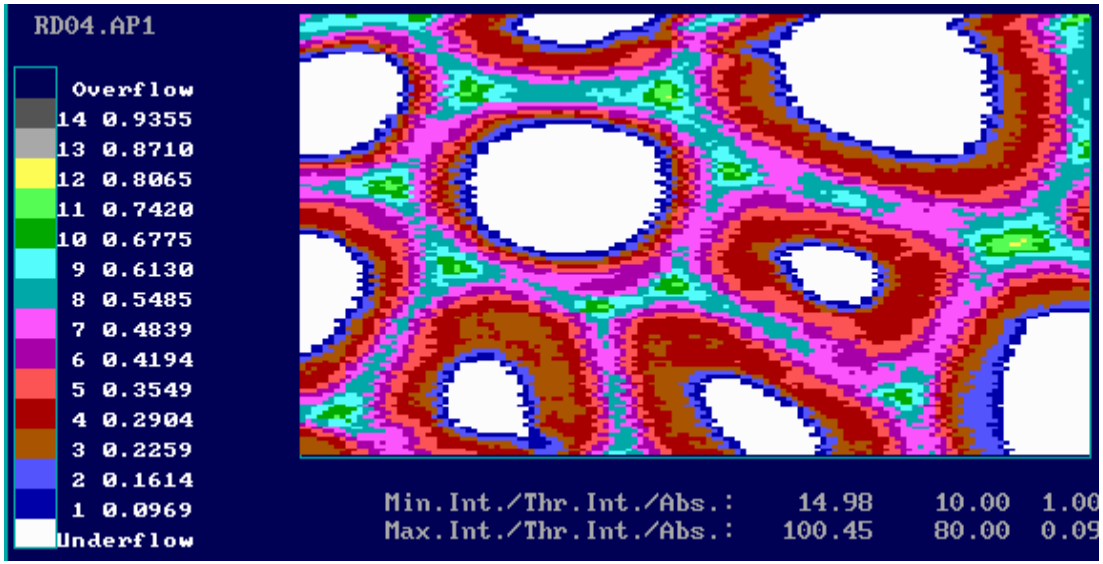


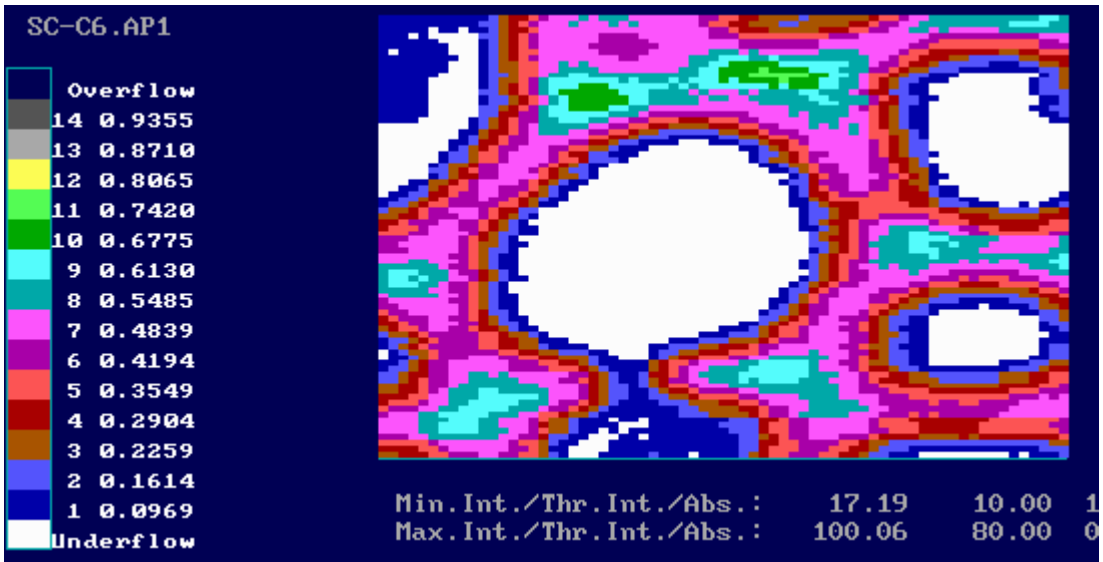
Pith



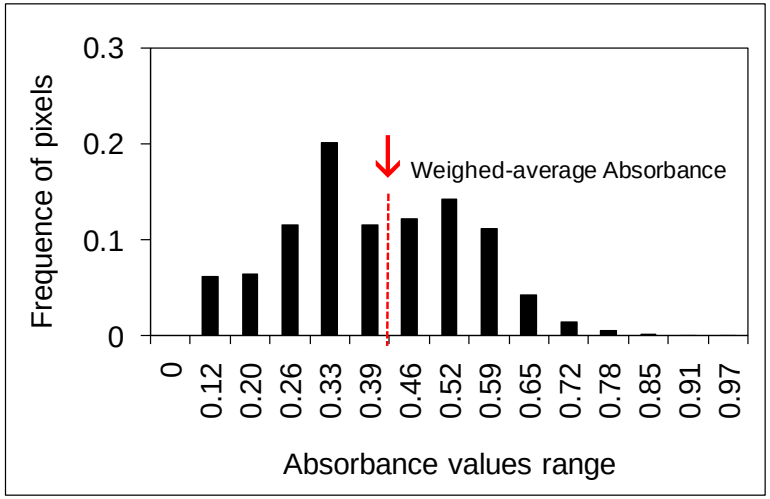
280 nm > aromatic ring substituted with oxygenated groups
315 nm > aromatic ring conjugated with alfa-carbonyl or alfa-beta unsaturated groups >>> in grasses, correspond to ferulic and coumaric acids

Topochemical distribution of lignin





Frequency histograms



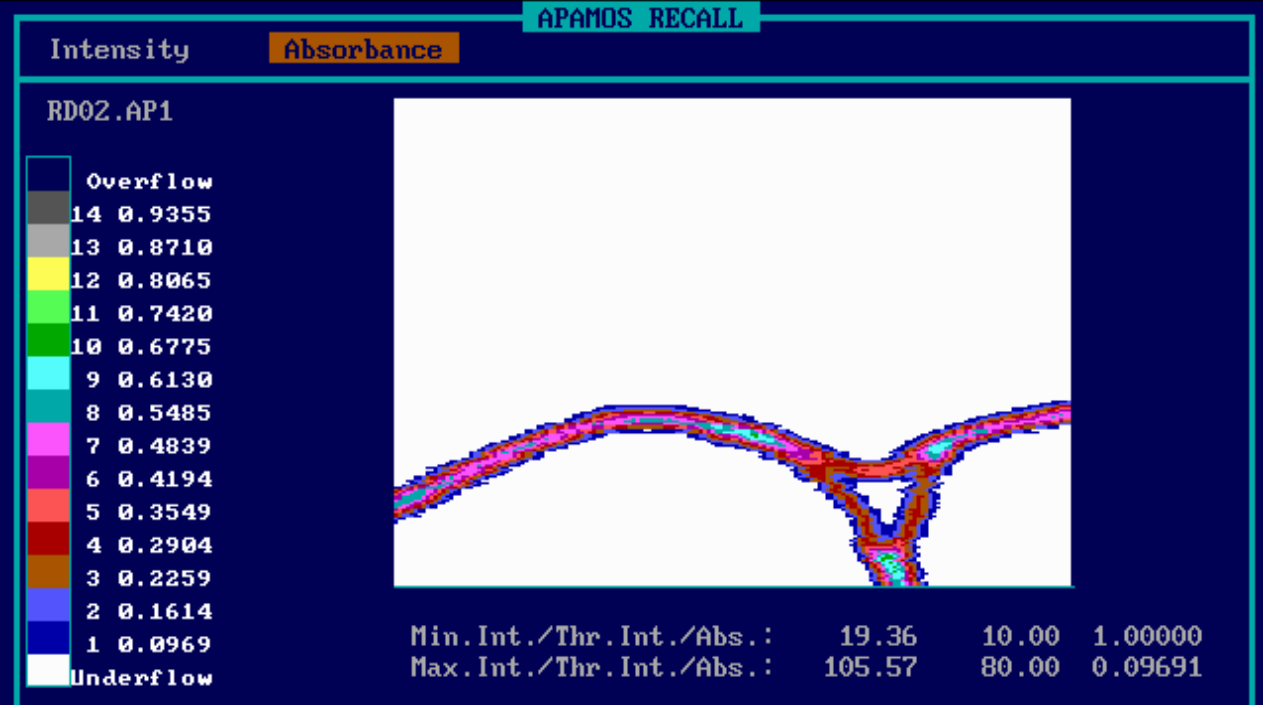
Rind fibers

$Abs_{280\ nm}$=0.40

Pith fibers

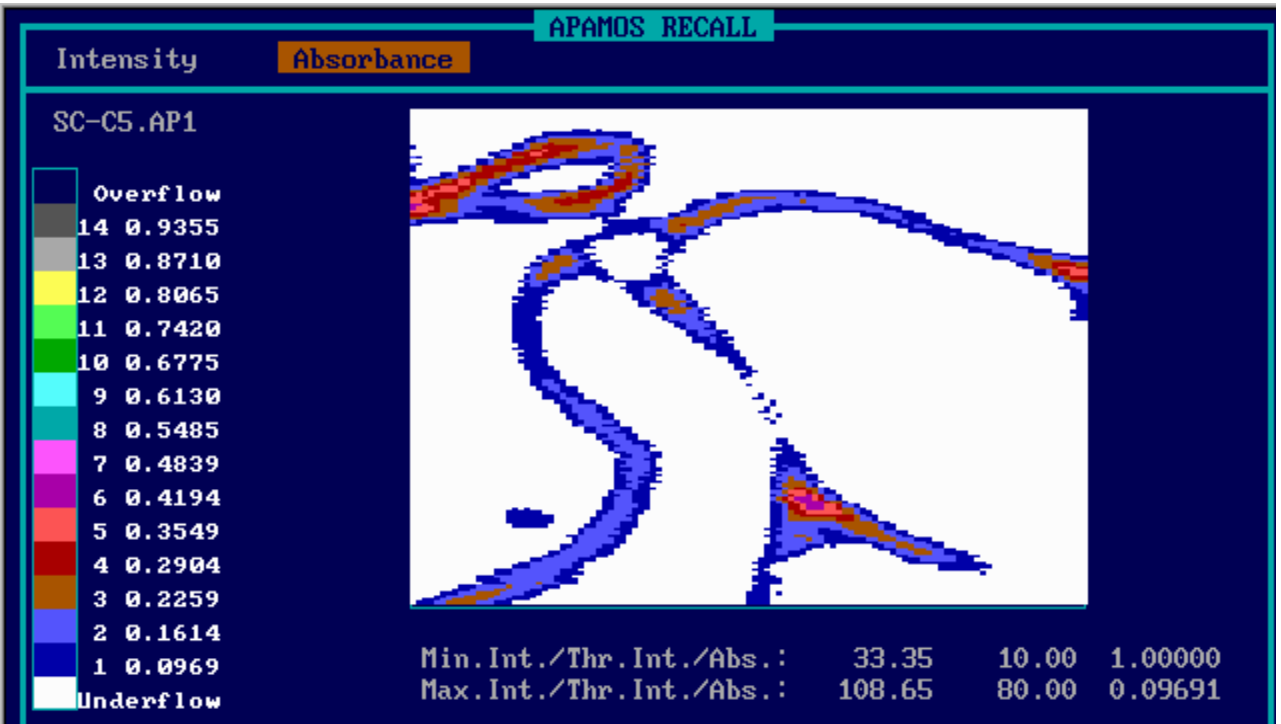
Abs_{280nm}=0.39

Similar lignin contents



Rind Parenchyma

Abs_{280nm} = 0.31



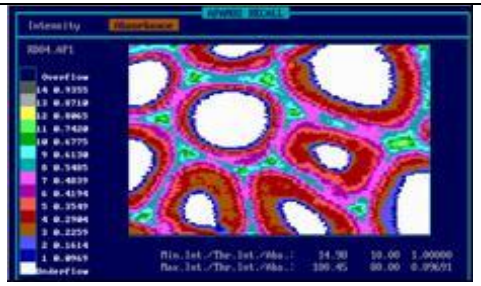
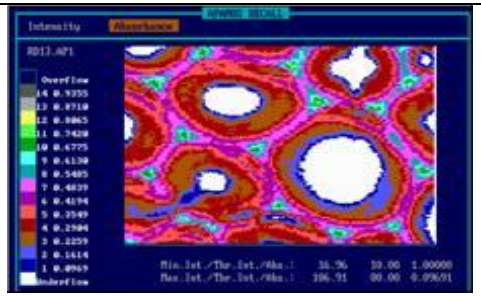
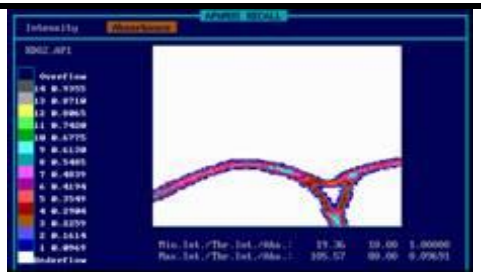
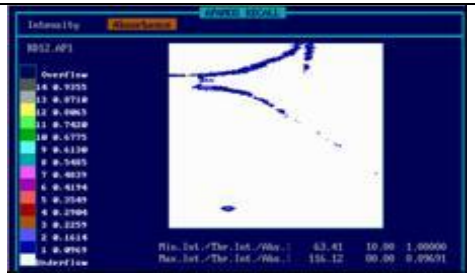
Pith Parenchyma

Abs_{280nm} = 0.18

Lower lignin content in pith parenchyma

UV-images from sugar cane internode cells

>> after chlorite-induced delignification

Cell type	Rind
Untreated fibers	 a
1-h chlorite/ acetic acid treated fibers	 c
Untreated parenchyma	 e
1-h chlorite/ acetic acid treated parenchyma	 g

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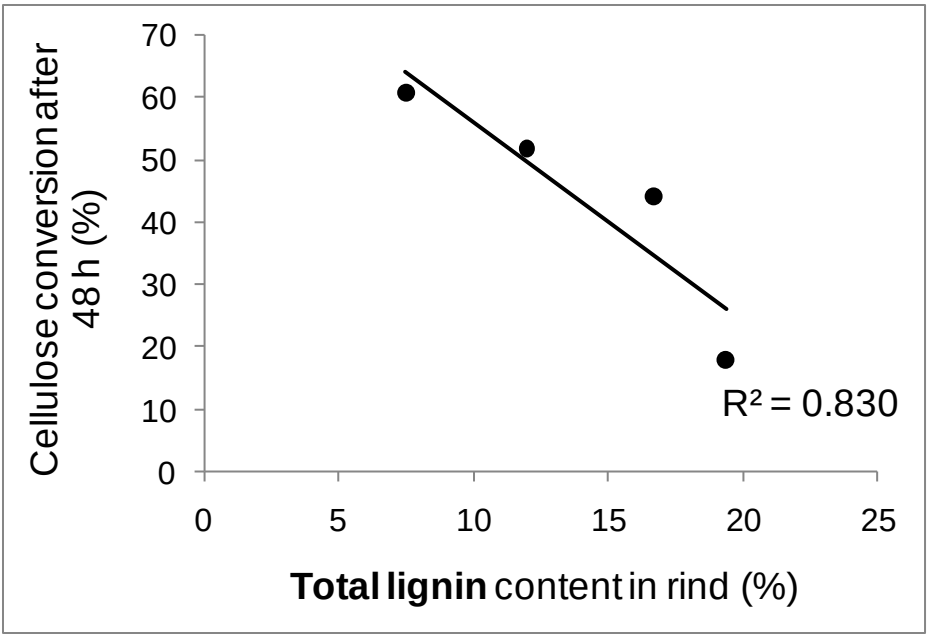
Biotechnol Biofuels 4:7, 2011

The initial question: - Why different cells present varied recalcitrance?

Correlation of enzymatic hydrolysis efficiency of the rind with the lignin contents and UV-microspectrophotometric data



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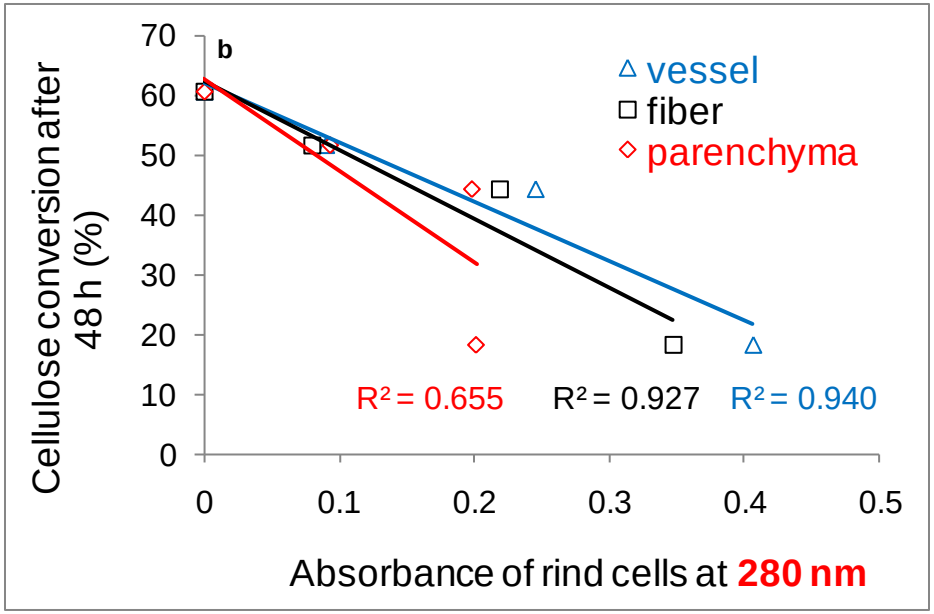
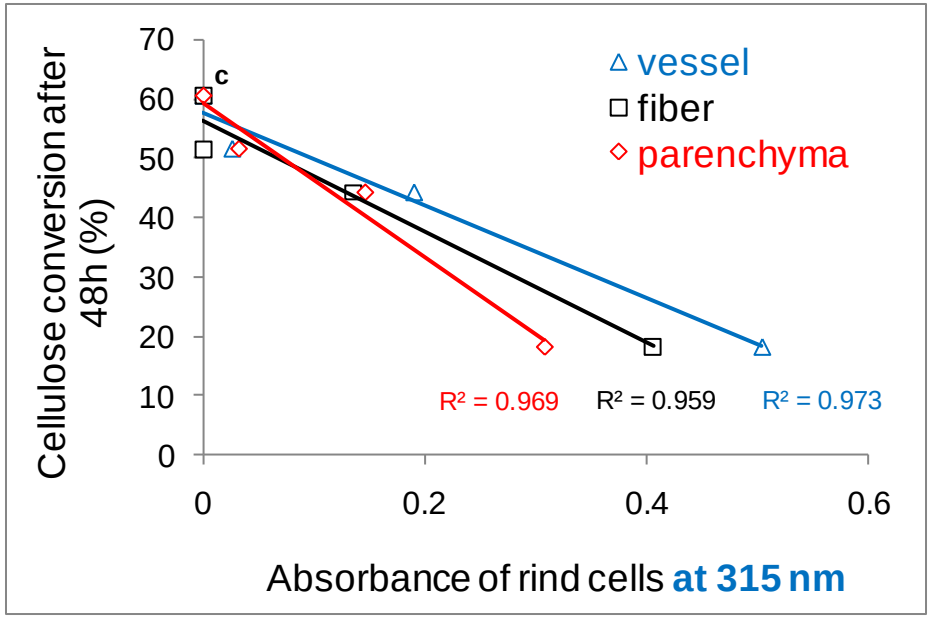




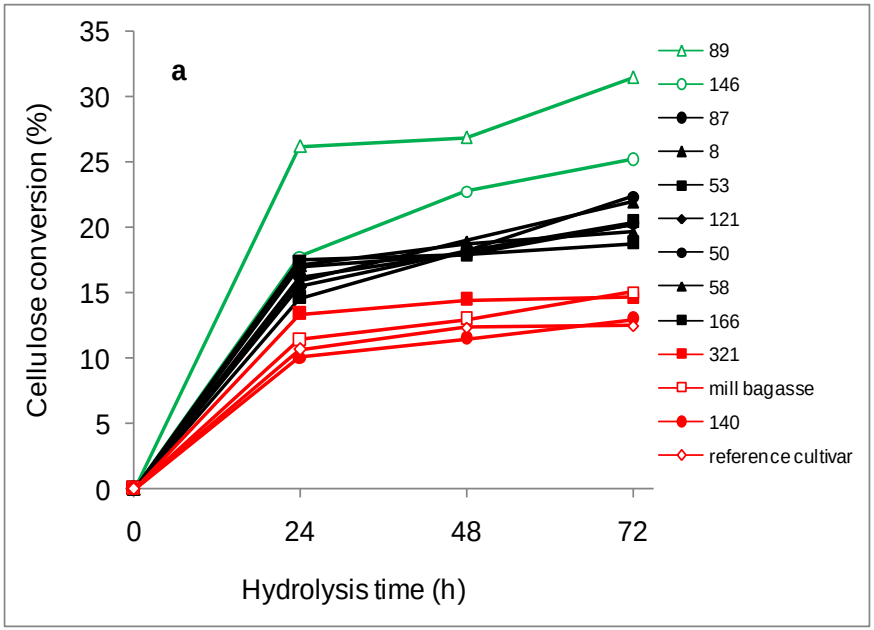
Experimental hybrids selected for reduced lignin contents

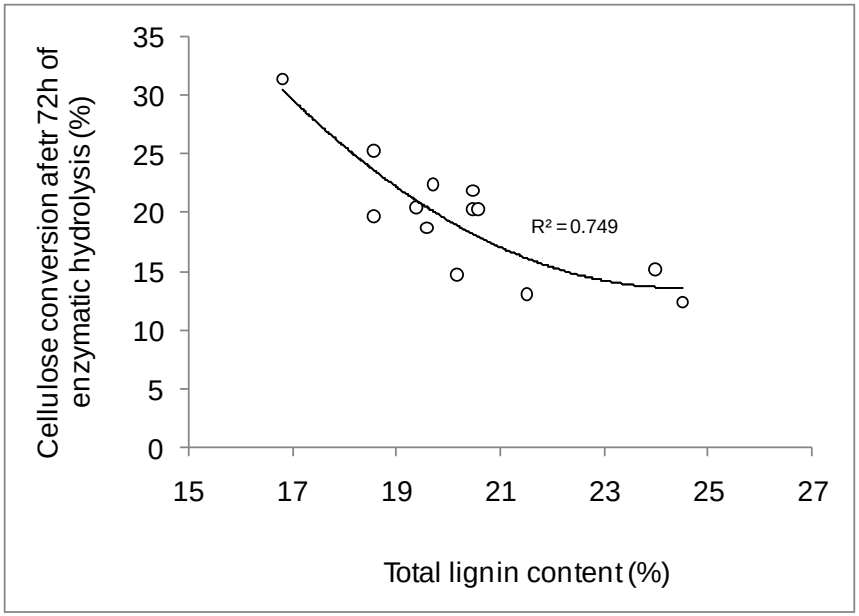
Hybrids obtained by M.H.P. Barbosa, Departamento de Fitotecnia da Universidade Federal de Viçosa

Chemical composition of sugar cane hybrids ranked by their lignin contents

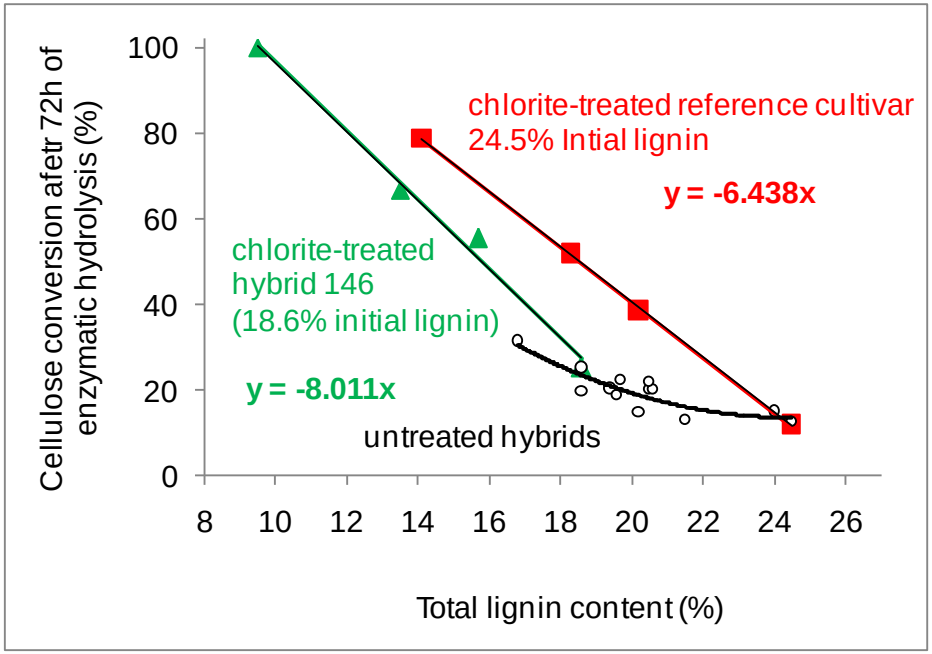
Clone	Total lignin	Hemicellulose	Glucan	Extractives	Sum
89	16.8 ± 0.1	27.3 ± 0.3	40.3 ± 0.1	7.5 ± 0.1	92.0
146	18.6 ± 0.1	31.6 ± 0.8	40.9 ± 0.3	2.4 ± 0.1	93.5
58	18.6 ± 0.1	26.3 ± 0.1	40.9 ± 0.3	2.6 ± 0.2	88.4
53	19.4 ± 0.5	27.1 ± 0.4	42.2 ± 0.5	2.7 ± 0.1	91.3
166	19.6 ± 0.5	31.5 ± 0.1	43.2 ± 0.4	1.9 ± 0.1	96.2
87	19.7 ± 0.1	27.3 ± 0.8	42.2 ± 0.3	3.9 ± 0.4	93.1
321	20.2 ± 0.4	31.0 ± 1.0	40.4 ± 0.5	1.6 ± 0.3	92.9
50	20.5 ± 0.1	30.0 ± 2.0	42.0 ± 1.0	2.5 ± 0.1	94.6
8	20.5 ± 0.4	26.6 ± 0.7	40.0 ± 1.0	3.2 ± 0.4	90.0
121	20.6 ± 0.1	28.2 ± 0.5	39.0 ± 1.0	4.9 ± 0.2	92.9
140	21.5 ± 0.2	27.0 ± 0.3	38.2 ± 0.5	5.1 ± 0.5	91.8
Mill bagasse	24.0 ± 0.1	26.0 ± 1.0	42.0 ± 2.0	2.2 ± 0.4	93.8
Reference cultivar	24.5 ± 0.5	25.2 ± 0.4	38.2 ± 0.2	2.6 ± 0.1	90.5

Direct enzymatic hydrolysis of the studied sugar cane hybrids

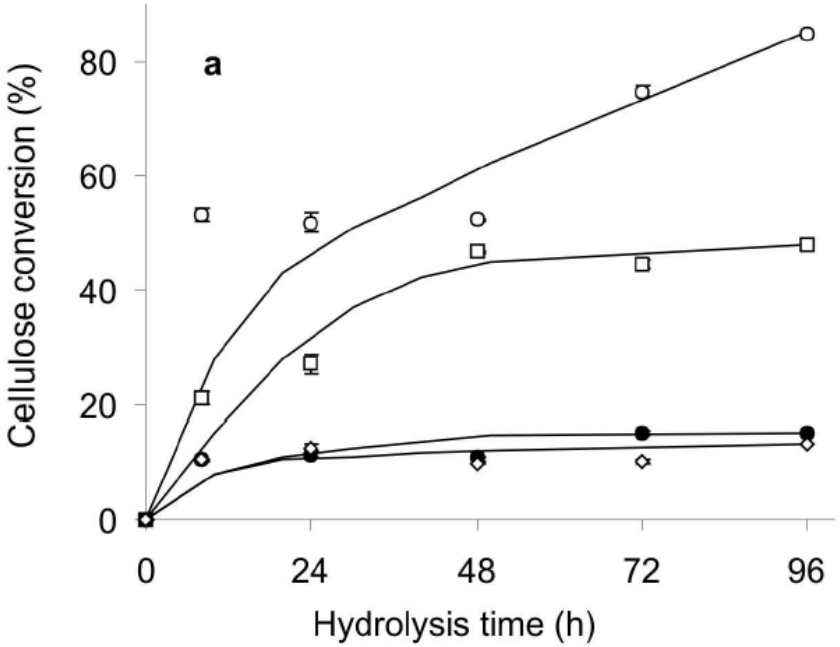




Lignin removal is necessary to reach hydrolysis levels above 70%



Enzymatic hydrolysis of alkaline-sulfite pretreated sugar cane bagasse

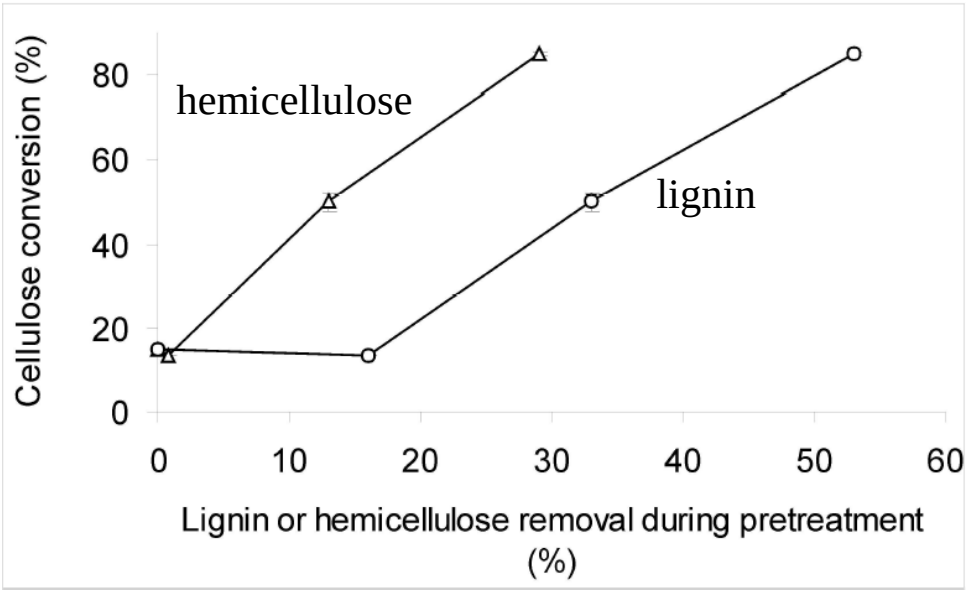


Alkaline-sulfite treated + RMP

Alkali-treated + RMP

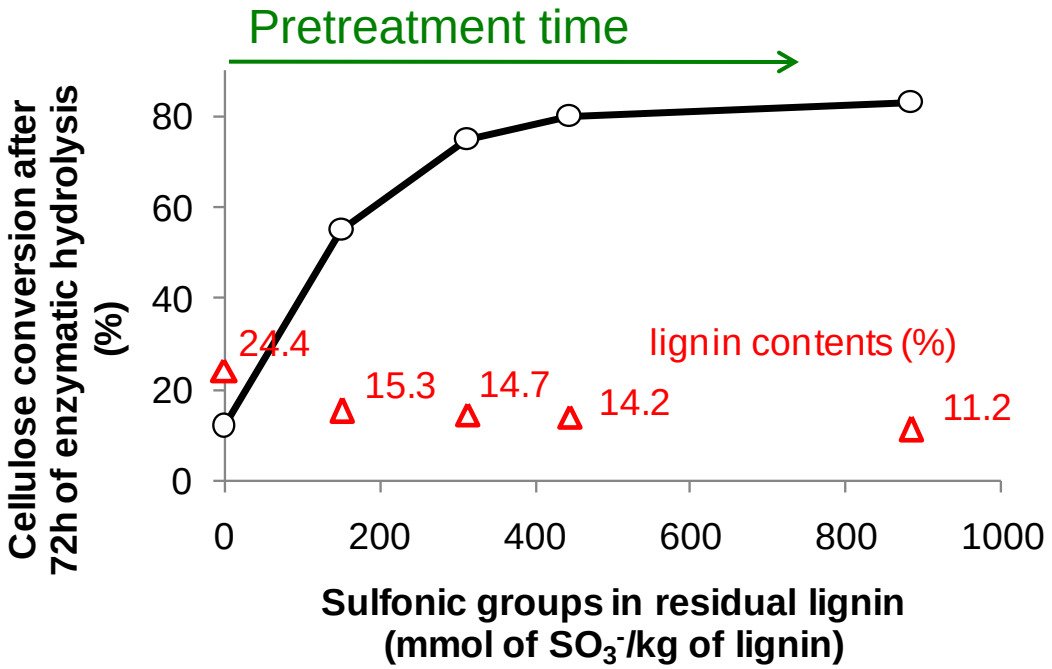
RMP treated

Untreated

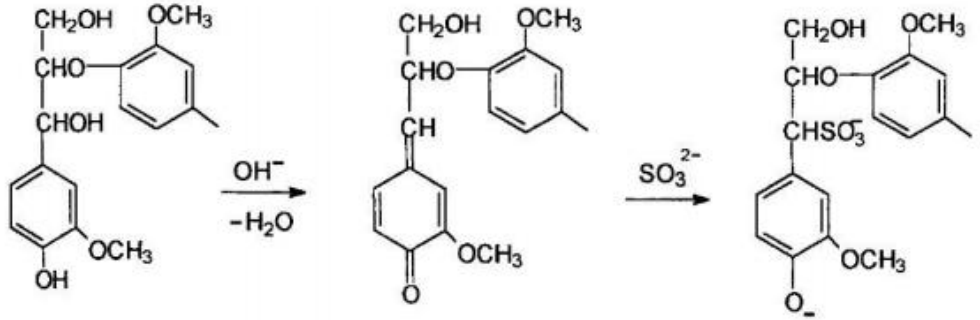


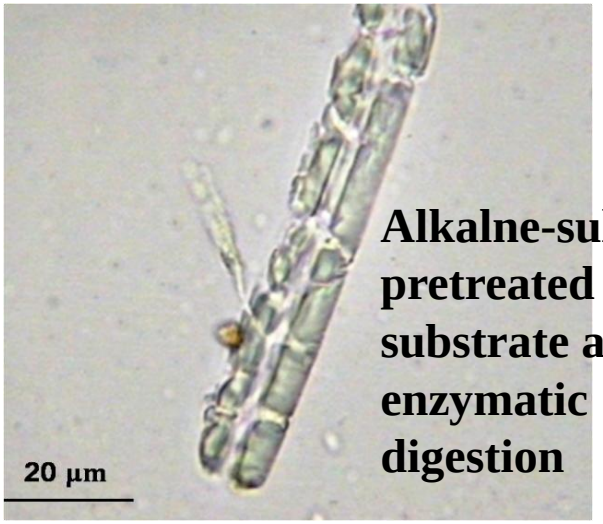
Mendes et al. Progress Biotechnol, 27:395-401, 2011

Not only lignin removal is important, sulfonation of residual lignin also increases swelling and porosity

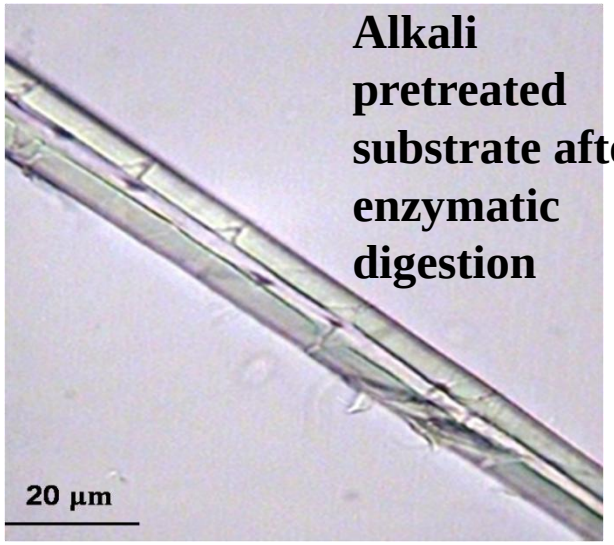


Sulfonation of lignin





Alkalne-sulfite pretreated substrate after enzymatic digestion



Alkali pretreated substrate after enzymatic digestion

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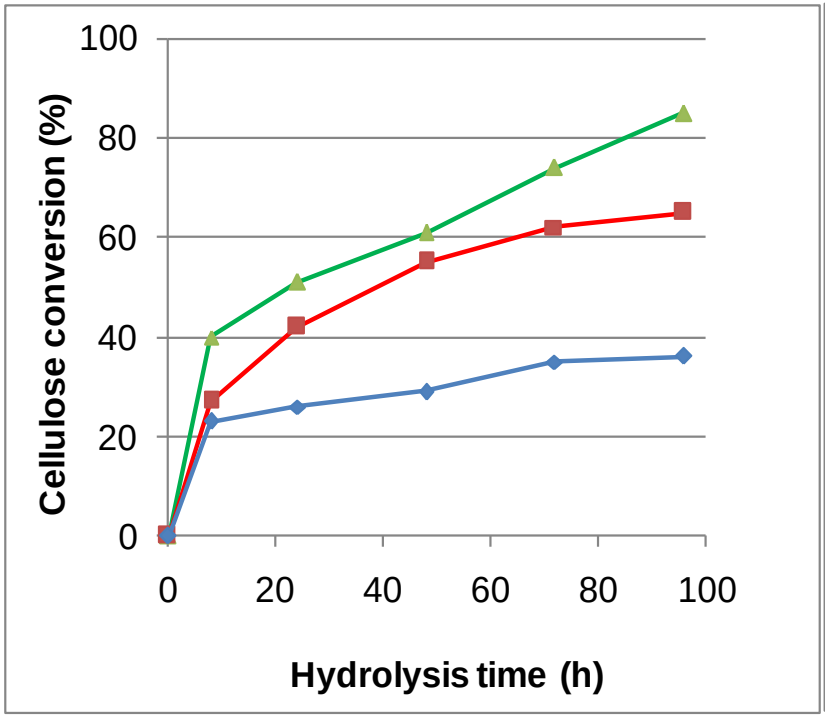
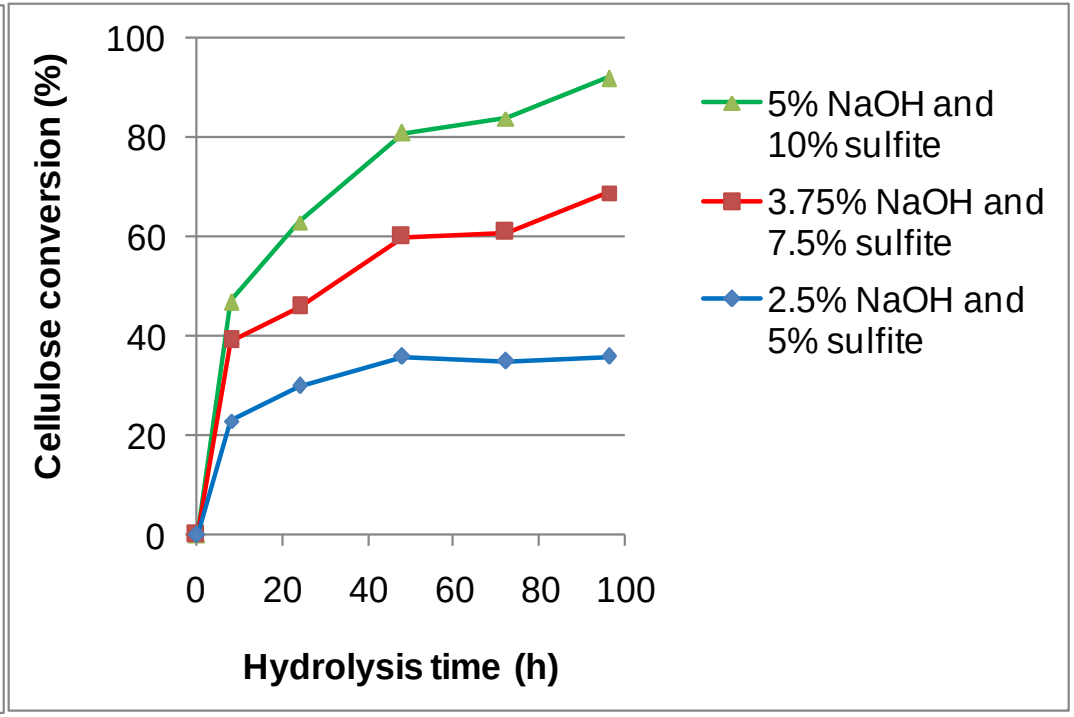
Enzymatic hydrolysis of alkaline-sulfite pretreated samples

Mill bagasse x hybrid with low lignin content

Mill bagasse; Initial lignin 24.0%



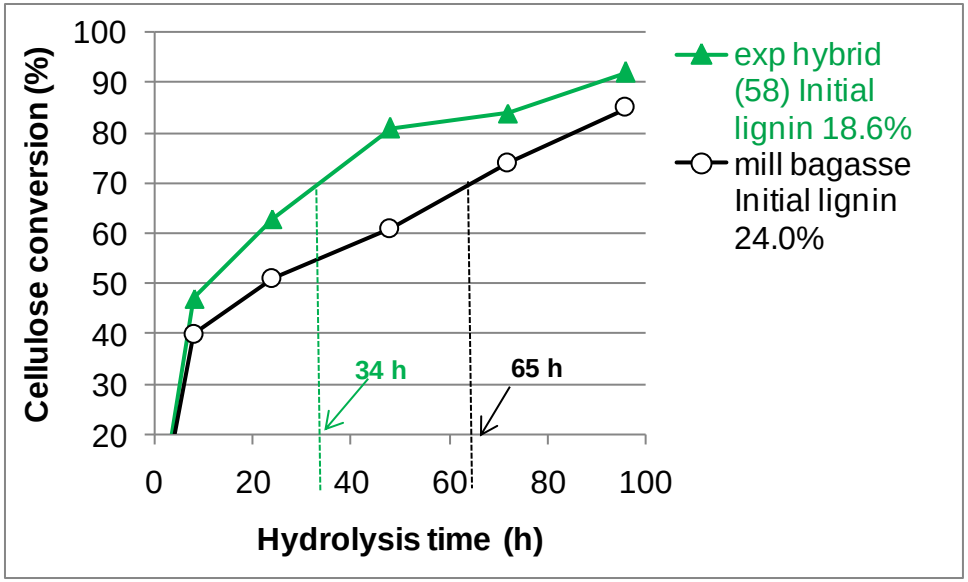
Exp hybrid 58; Initial lignin 18.6%

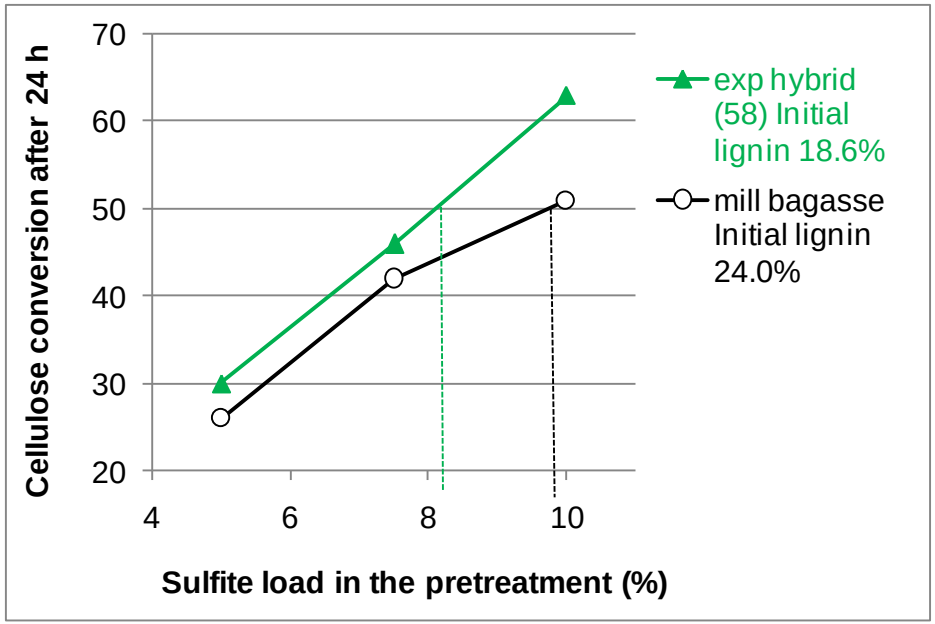


Evaluation of chemical loading in the pretreatment

Possible benefits for using samples with reduced initial lignin contents

Hydrolysis time in the enzymatic step can be reduced →





← Chemical loading in the pretreatment can be reduced

Conclusion

- Lignocellulose recalcitrance is related to the limited porosity of the cell walls that hinders enzyme infiltration

- Lignin and hemicellulose are major components limiting the access of enzymes into the cell wall ultrastructure

-There is a significant variation of recalcitrance in different cell types of sugar cane

- UV-microspectrophotometric analysis showed that there was good correlation between the absorption levels of the cell walls with their recalcitrance.

- Sugar cane hybrids with reduced lignin contents required milder pretreatments or give substrates that were more efficiently hydrolyzed in shorter reaction time

Acknowledgement

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