

# Pré-tratamento de materiais lignocelulósicos

## Sumário e objetivo da aula

### **1. Qual a finalidade do pré-tratamento?**

- Porque enzimas apresentam ação limitada na biomassa in natura?

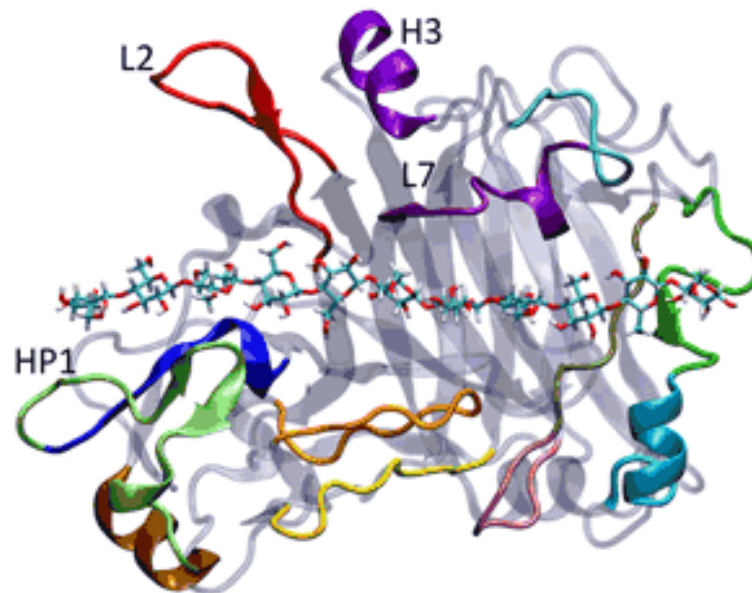
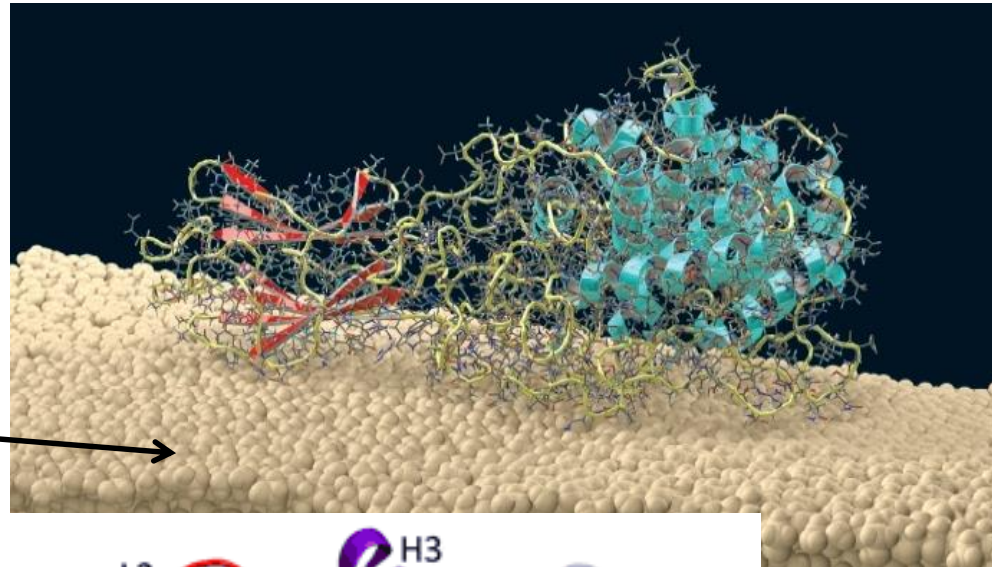
### **2. Fundamentos e tipos de pré-tratamento**

### **3. Eficiência de hidrólise após o pré-tratamento**

### **4. Correlações entre remoção de componentes no pré-tratamento e eficiência de hidrólise**

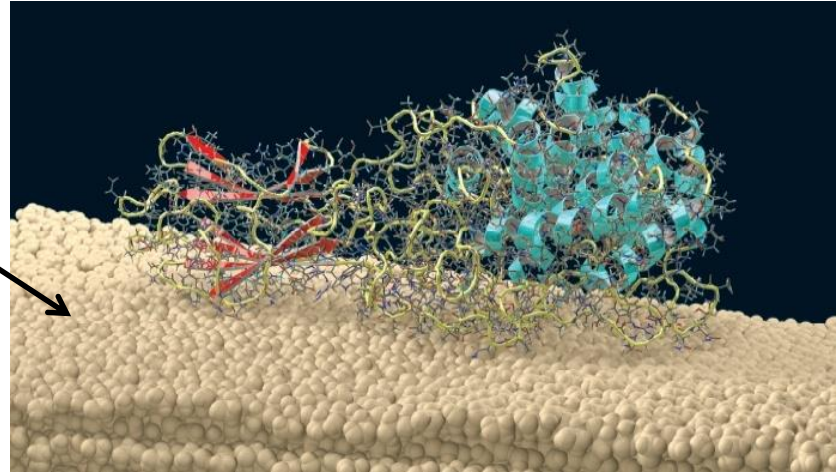
# Enzymatic hydrolysis of polysaccharides is a key step in biomass conversion

Is cellulose free and “clean” as shown in this figure?

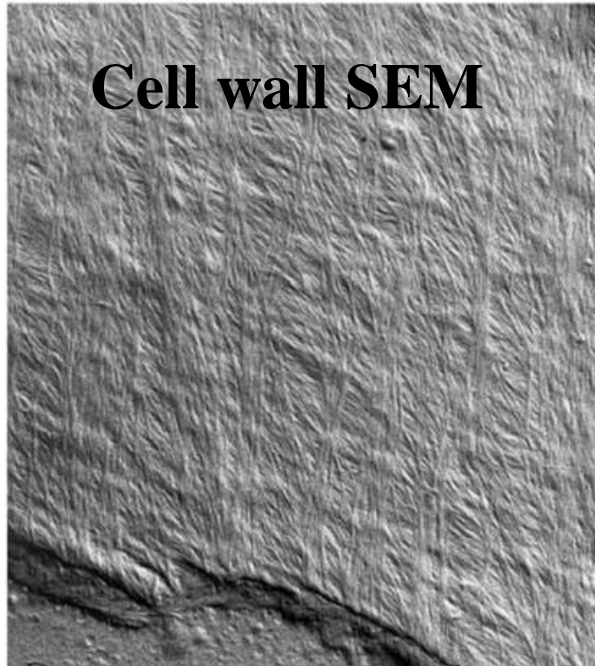


# Recalcitrance in lignocellulosic materials

Cellulose is recalcitrant by itself  
*(but cellulose is not "clean" as shown in this figure)*

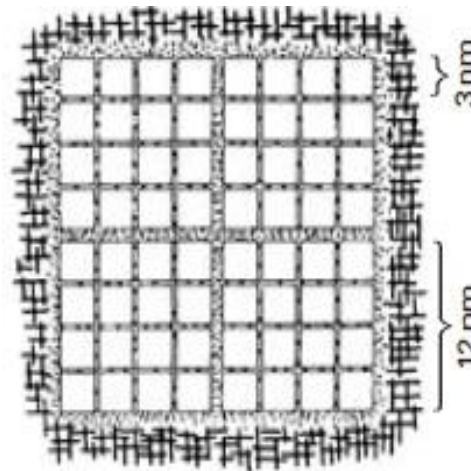


**Lignin and hemicelluloses involve the cellulose nonofibrils in the cell walls causing the primary recalcitrance**

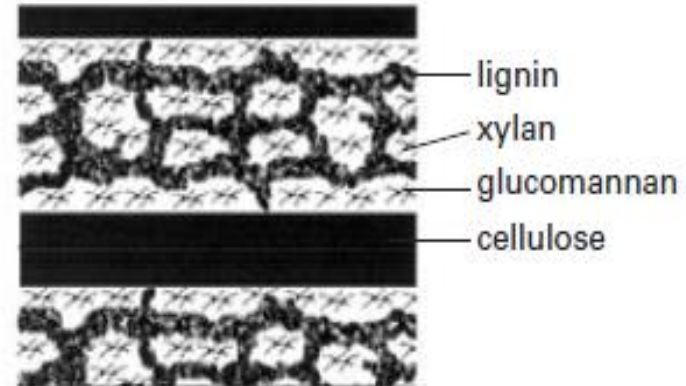


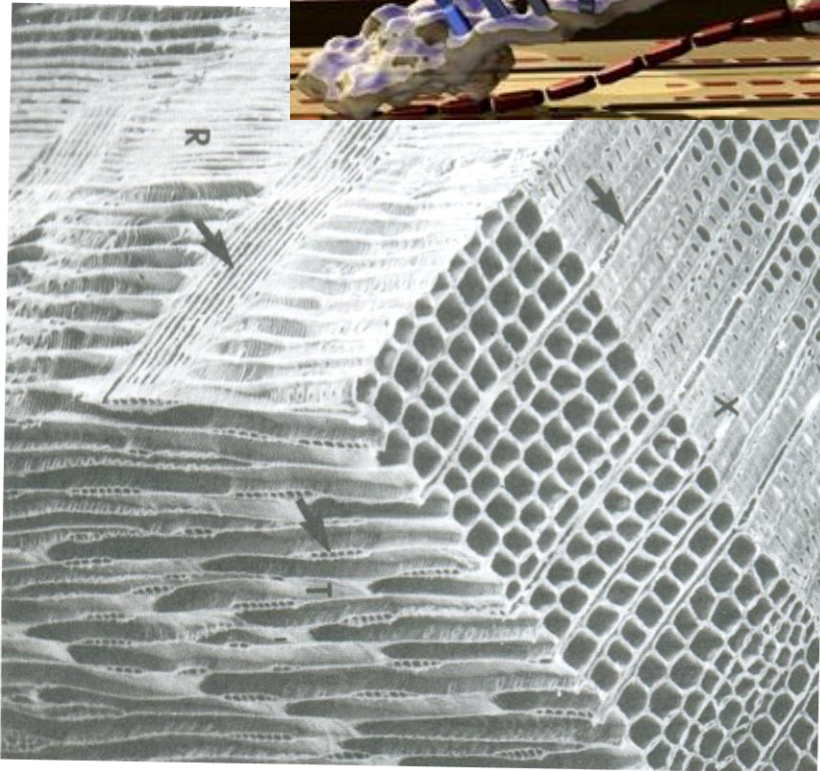
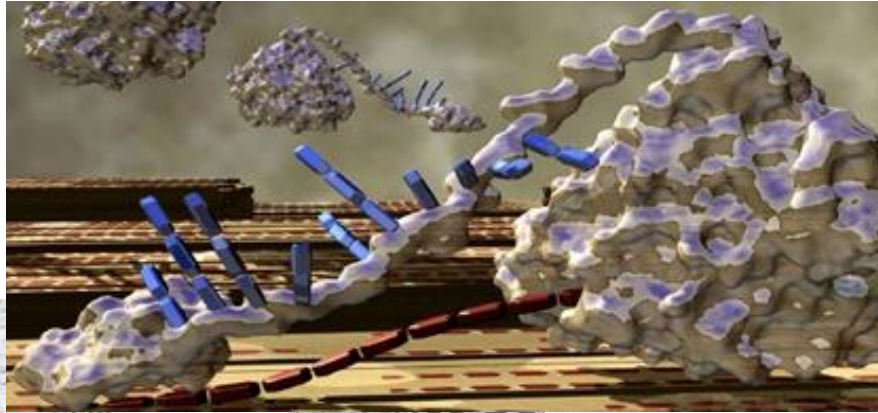
Cell wall SEM

Cell wall models



□ cellulose fibril    ● polyoses    ▲ lignin

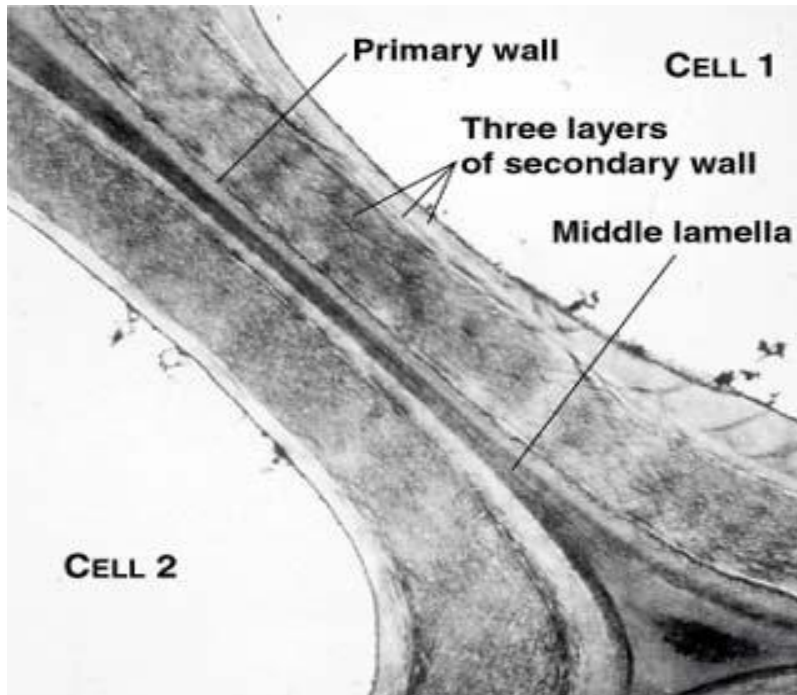




Lignified cells are arranged in a complex matrix!

*Softwood*

The polysaccharide are inside cell walls with low porosity

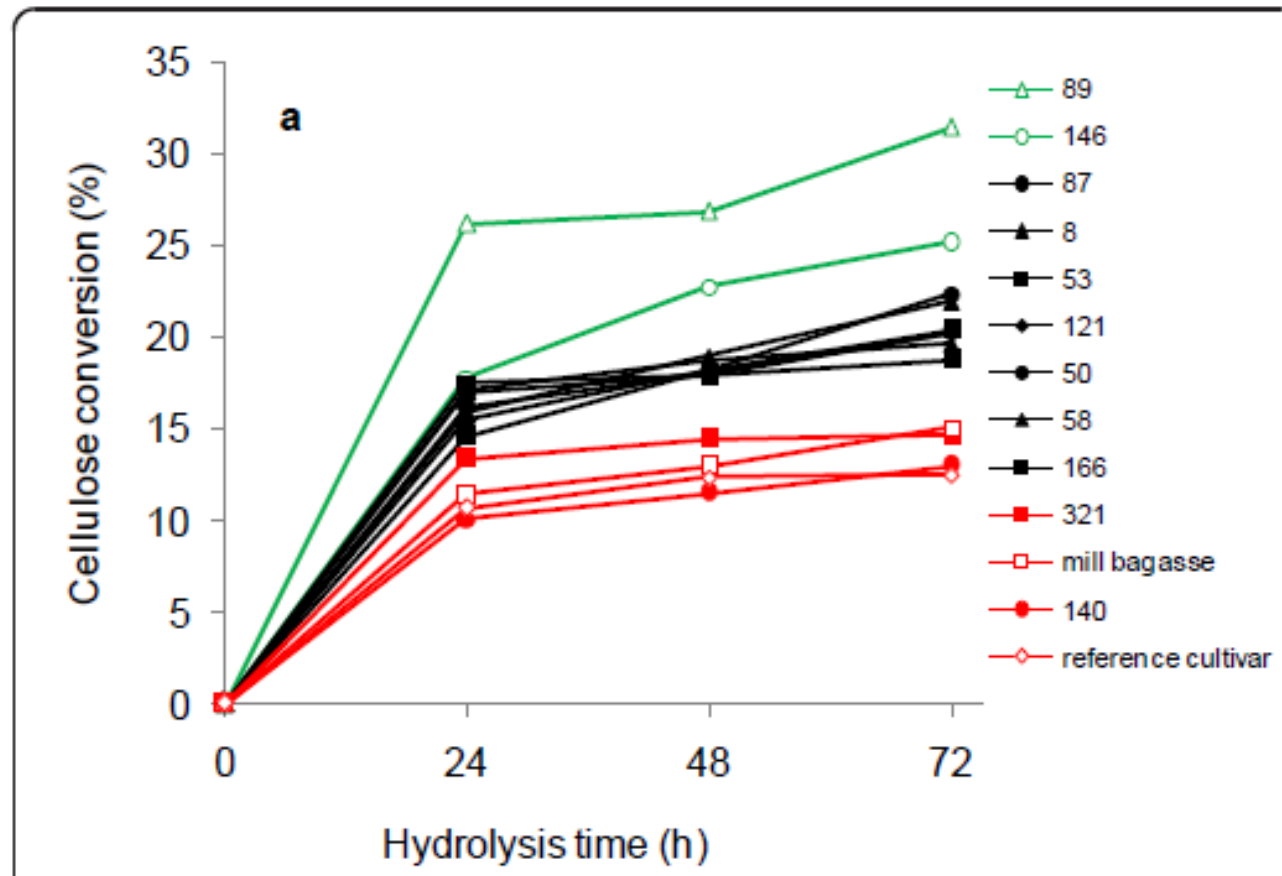


Mesmo proteínas de baixa massa molar não infiltram na parede celular intacta

insulina 5,7 kDa	mioglobina 17,6 kDa	ovoalbumina 44,3 kDa
não infiltra	não infiltra	não infiltra

Massa molar das celulasas e hemicelulasas é maior do que 20 kDa. A maioria é maior do que 40 kDa

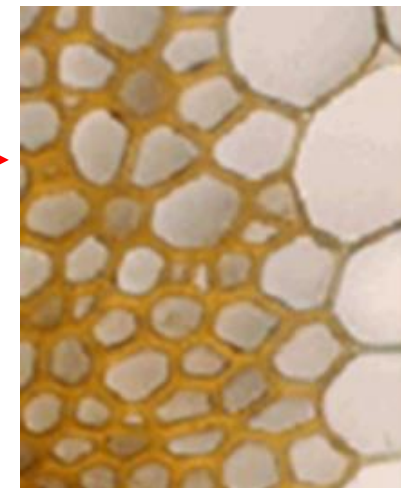
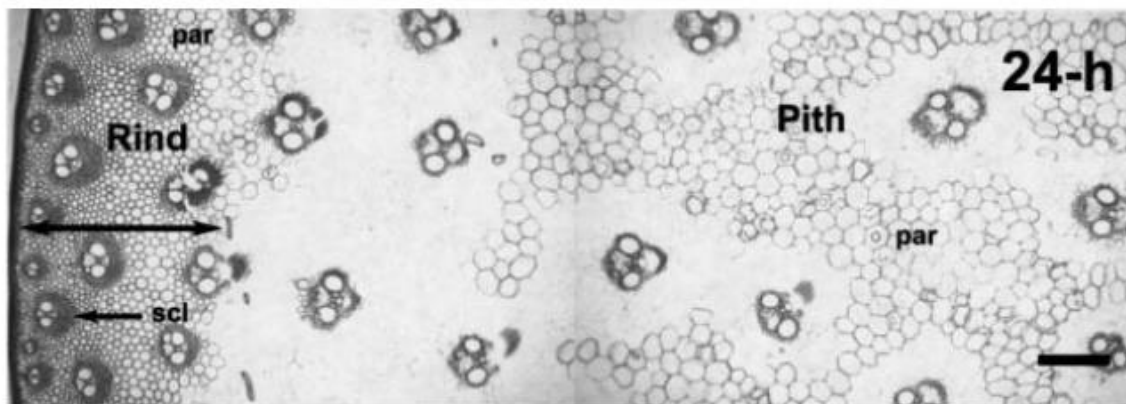
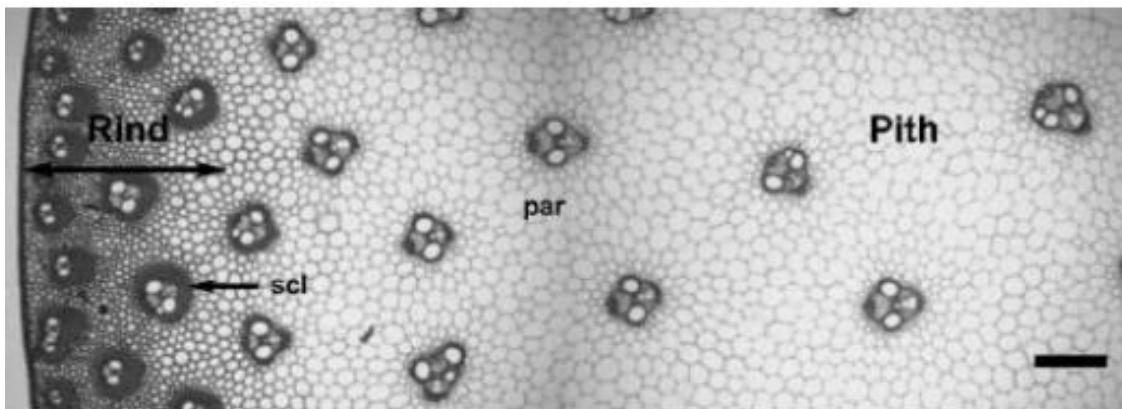
A hidrólise da cellulose contida no material *in natura* (sem pré-tratamento) é limitada à cerca de 30%



# Ação das enzimas em **biomassa lignocelulósica**

Observações experimentais relevantes:

*A recalcitrância difere nos diferentes tipos celulares*



Entrenós maduros de caule de milho submetidos à digestão por um fluído do rumem bovino

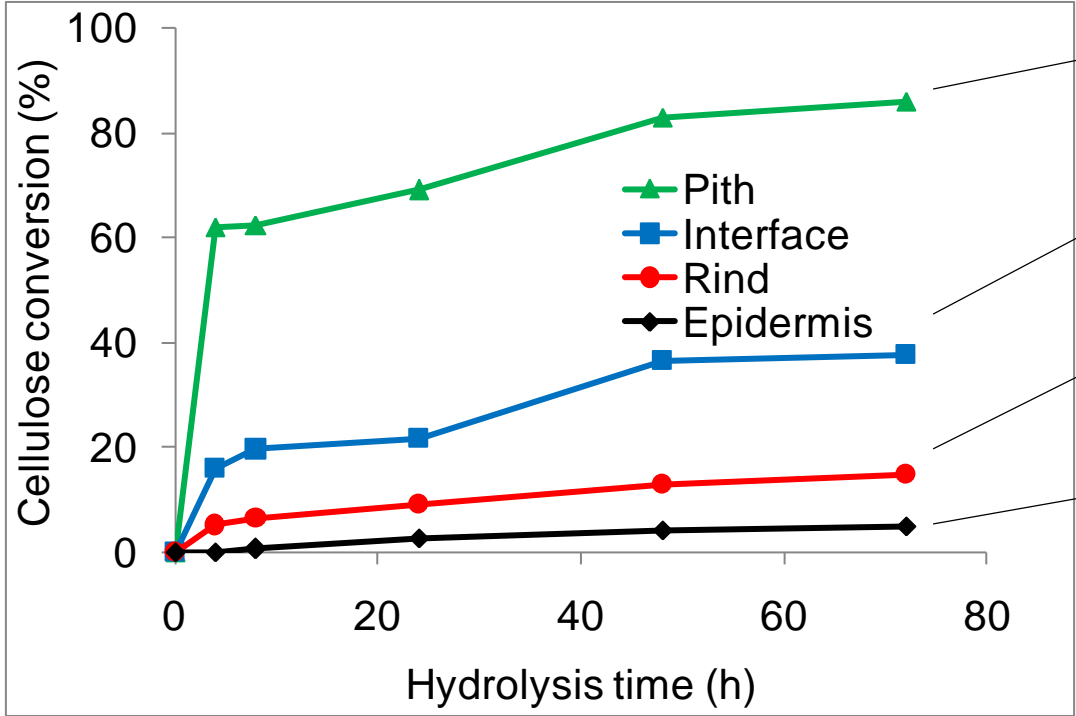
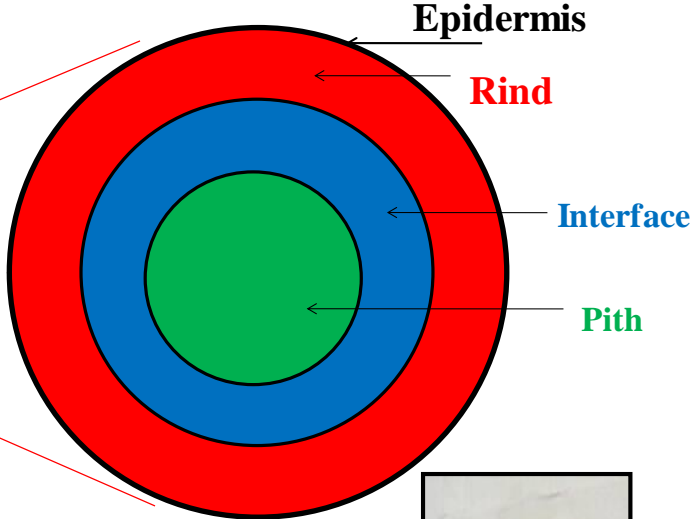
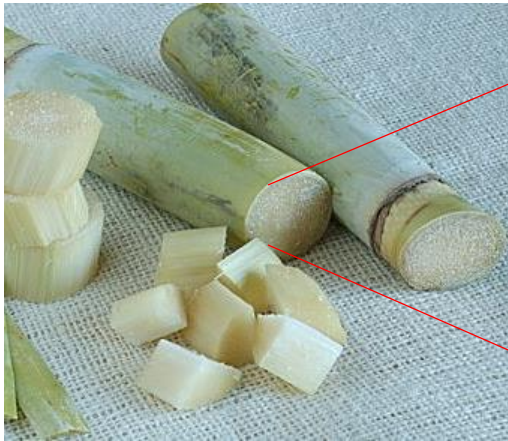
*Jung e Casler, Crop Sci. 46:1801-1809, 2006.*

*celulases agindo em entrenó de milho (video)*



How Does Plant Cell Wall Nanoscale Architecture Correlate with Enzymatic Digestibility?  
Shi-You Ding *et al.*  
*Science* 338, 1055 (2012);  
DOI: 10.1126/science.1227491

# Enzymatic hydrolysis of varied sugar cane regions in an internode

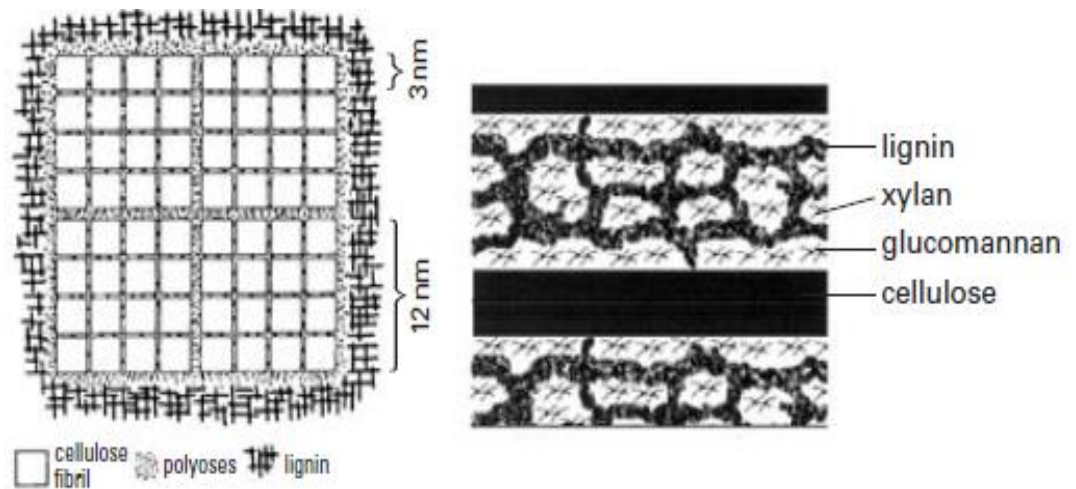


**Sugar cane as a model substrate**

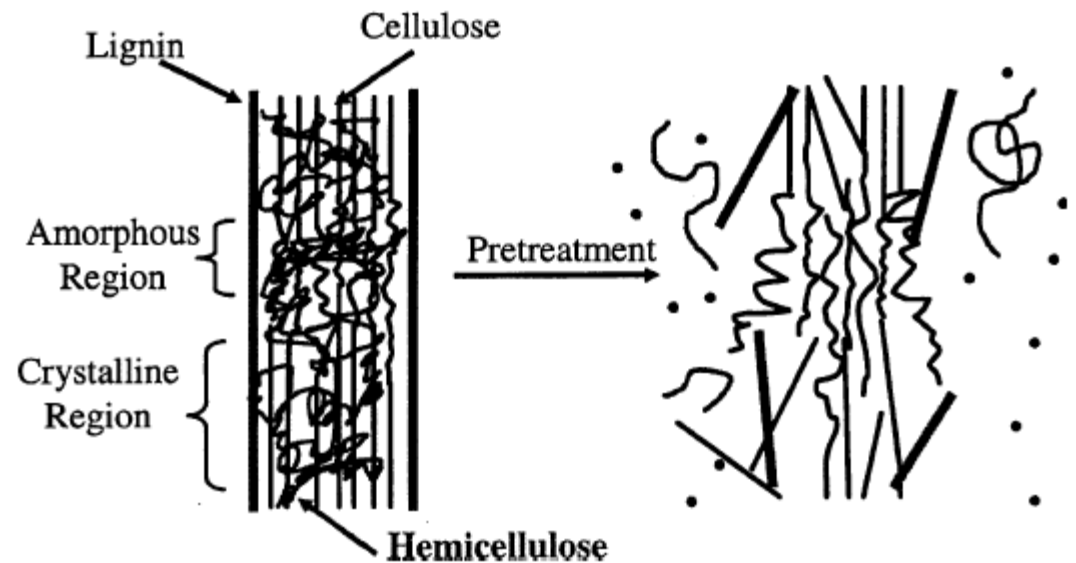


As células mais recalcitrantes à digestão enzimática requerem algum tipo de desestruturação da parede celular a fim de permitir, minimamente, a infiltração de enzimas.

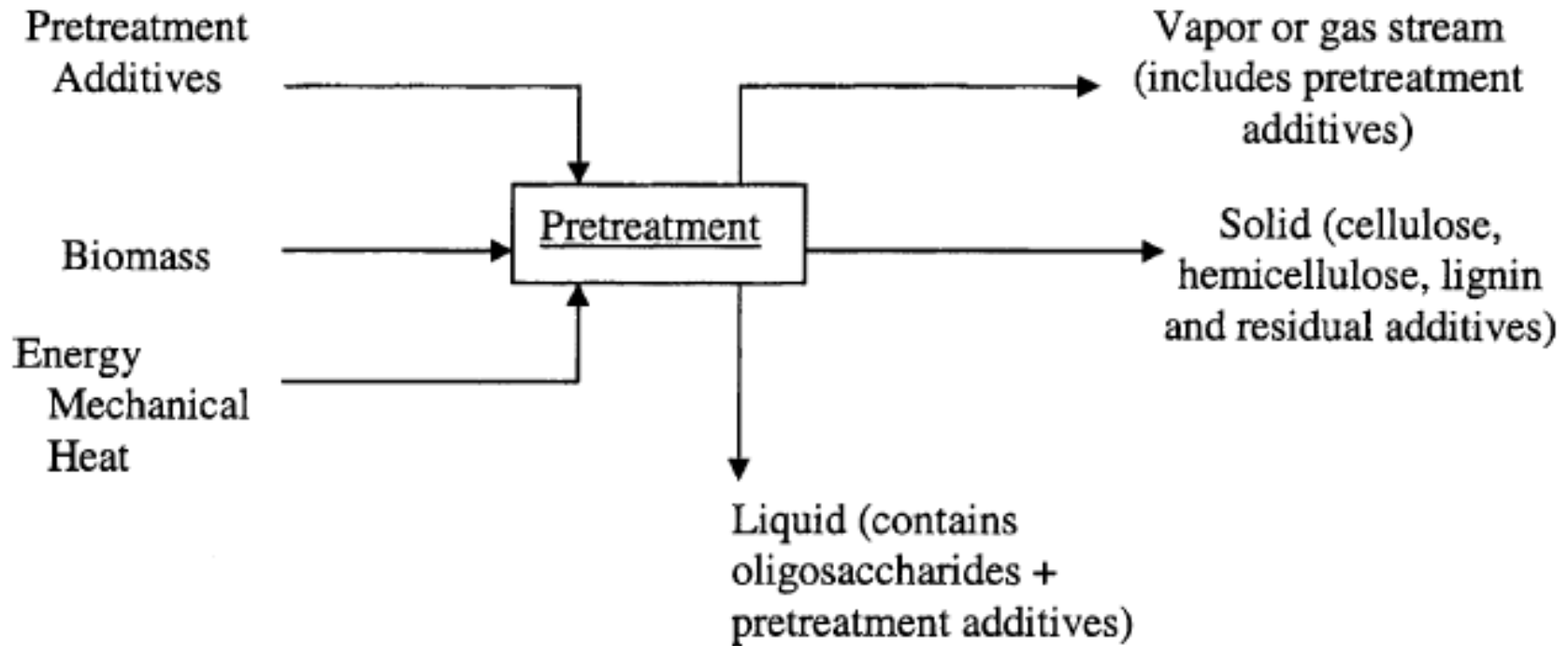
Além de permitir a infiltração de enzimas, o pré-tratamento requer a exposição dos polissacarídeos



### Effect of Pretreatment



## Diagrama básico de processo para o pré-tratamento



As características composicionais de cada fração dependem do meio reacional. Meios ácidos geram frações consideravelmente diferentes daquelas geradas em meio alcalino

# Tipos de pré-tratamento e os efeitos principais sobre a ultra-estrutura da parede celular e sobre os seus componentes

Table 2  
Effect of various pretreatment methods on the chemical composition and chemical/physical structure of lignocellulosic biomass

	Increases accessible surface area	Decrystalizes cellulose	Removes hemicellulose	Removes lignin	Alters lignin structure
Uncatalyzed steam explosion	■		■		■
Liquid hot water	■	ND	■		■
pH controlled hot water	■	ND	■		ND
Flow-through liquid hot water	■	ND	■	■	■
Dilute acid	■		■		■
Flow-through acid	■		■	■	■
AFEX ammonia fibre expansion	■	■	■	■	■
ARP ammonia recycled	■	■	■	■	■
Lime percolation	■	ND	■	■	■

■: Major effect.

■: Minor effect.

ND: Not determined.

## Pretreatment

### Hydrothermal/ Chemical

- **Low pH**
  - Sulfur dioxide
  - Sulfuric acid
  - Hydrogen chloride
  - Phosphoric acid
- **Neutral pH**
  - Hot compressed water
  - Steam explosion
- **High pH**
  - Ammonia
    - Ammonia fiber expansion (AFEX)
    - Ammonia recycled percolation
    - Aqueous ammonia soaking
    - Sodium hydroxide
  - Alkaline wet oxidation
  - Lime
- **Others**
  - Organosolv
  - Liquid ionic solutions
  - Co-solvent enhanced lignocellulosic fractionation

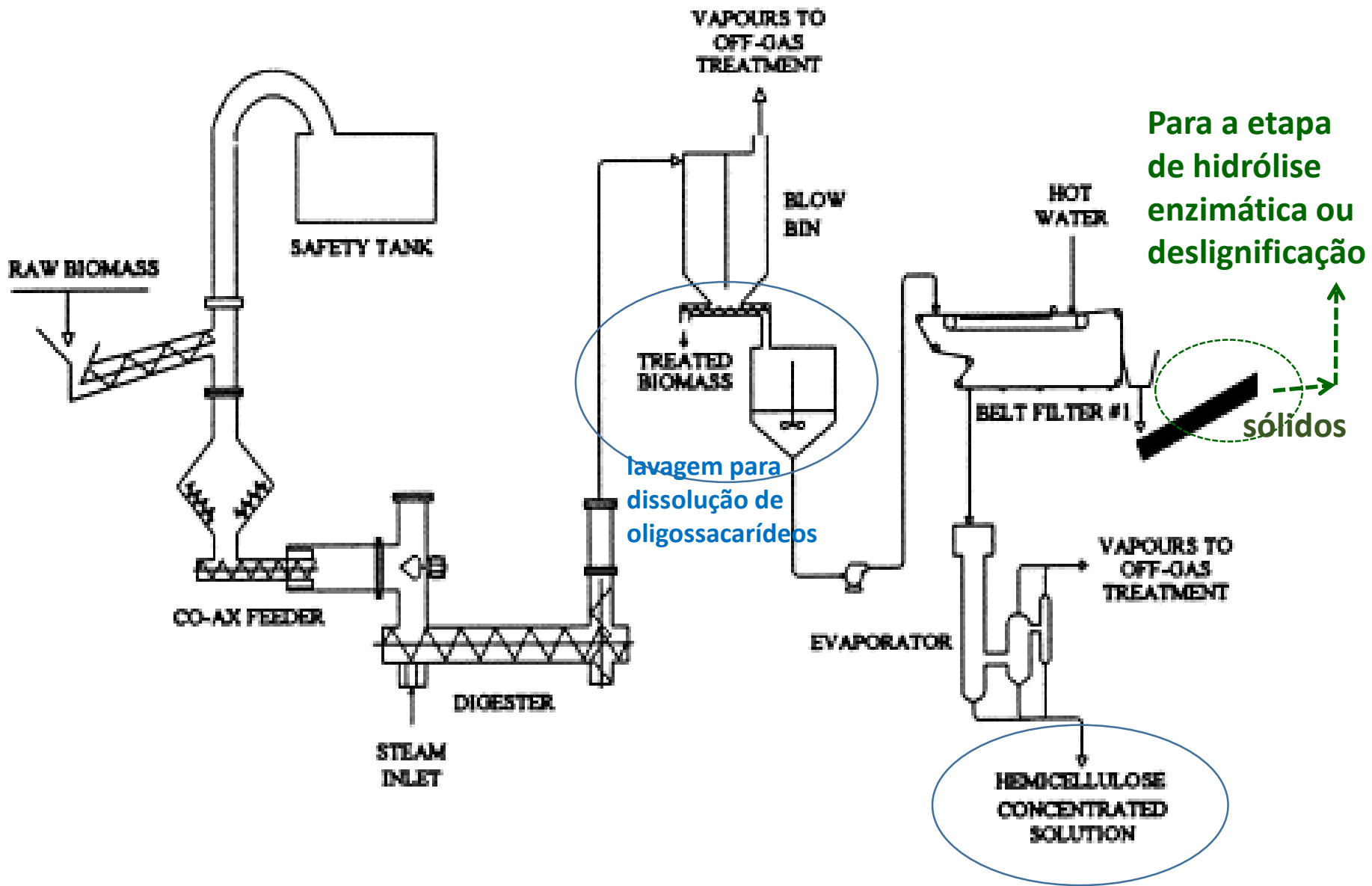
### Physical

- **Mechanical refining**
  - Shredding
  - Grinding
  - Milling
    - Hammer mill
    - Disk mill
    - Ball mill
    - PFI mill
  - Extrusion
- **Irradiation**
  - Gamma rays
  - Electron beam
  - Microwave radiation

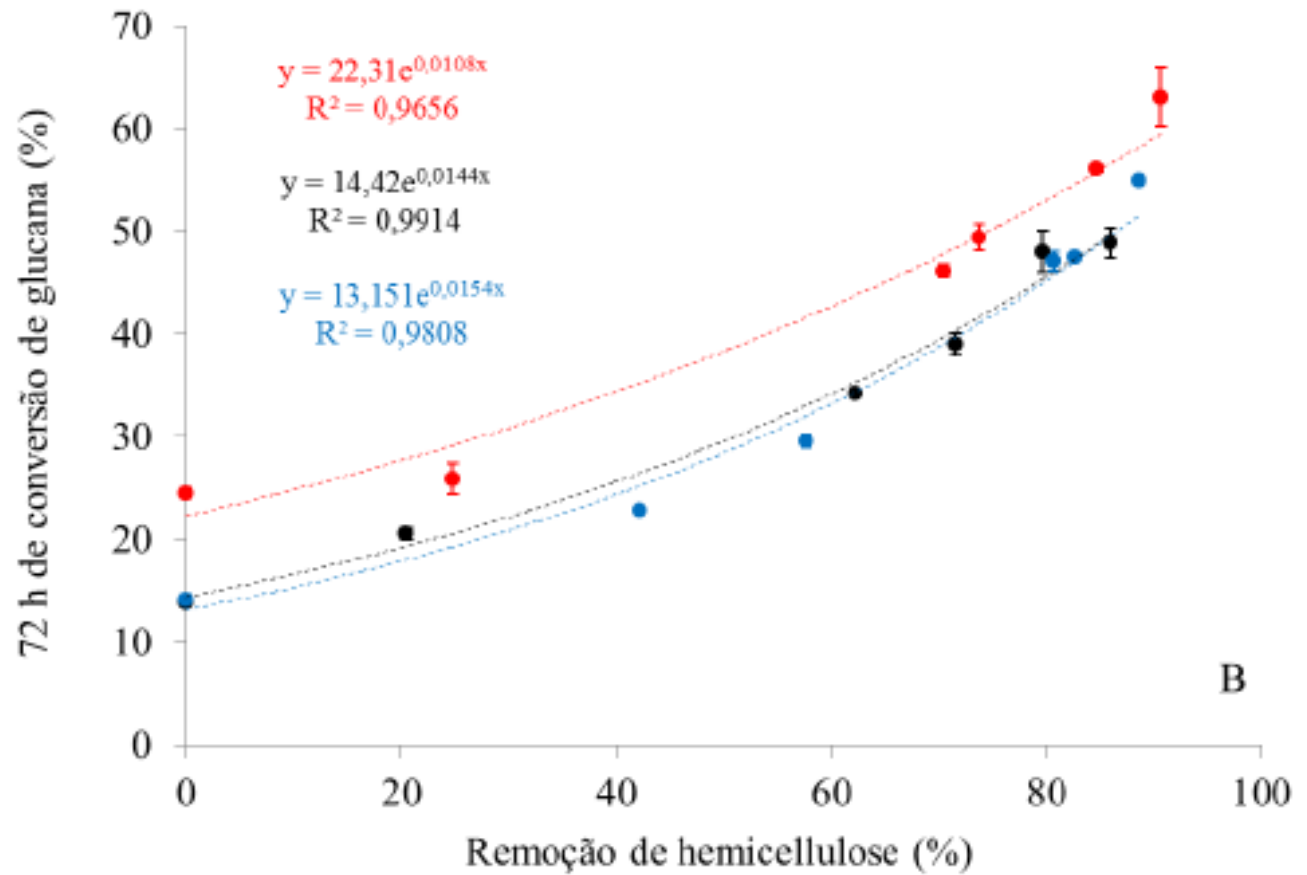
### Biological

- **White-rot fungi**
  - *Phanerochaete chrysosporium*
  - *Trametes versicolor*
  - *Ceriporiopsis subvermispora*
  - *Heterobasidium annosum*
  - *Pleurotus ostreatus*
- **Brown-rot fungi**
  - *Coniophora puteana*
  - *Gloeophyllum trabeum*
  - *Laetiporus sulphureus*
  - *Piptoporus betulinus*
  - *Postia placenta*
  - *Serpula lacrimans*
- **Soft-rot fungi**
  - *Chaetomium globosum*
  - *Ustilina deusta*

# Ilustração de uma planta de pré-tratamento por explosão à vapor (Stake Technologies – Canadá)



# Efeito da remoção de componentes na digestibilidade do material pré-tratado



Remoção de hemicelulose em meio ácido

Efeito da remoção de componentes na digestibilidade do material pré-tratado

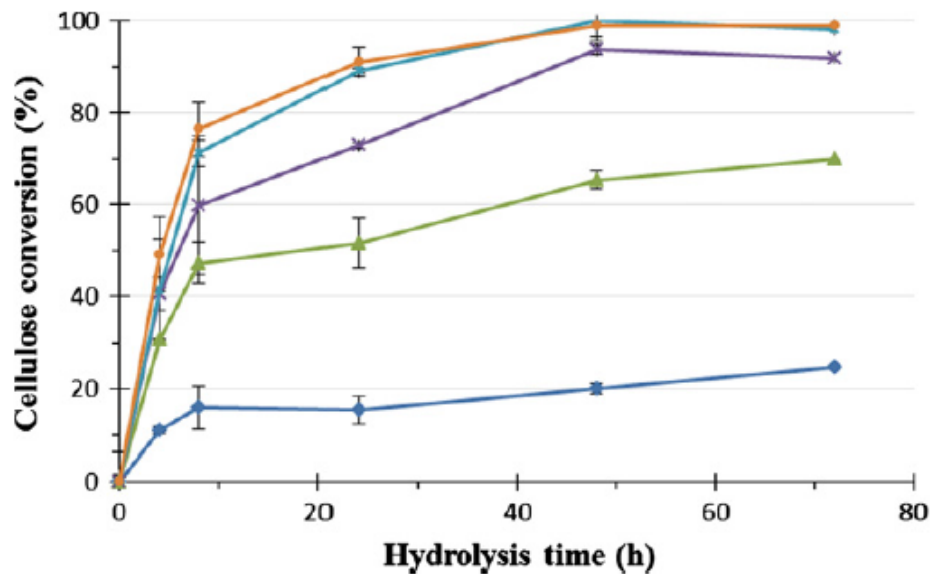


Fig. 2. Effect of lignin content on the kinetics of cellulose hydrolysis by cellulases. Samples containing 2% solids were supplemented with  $\beta$ -glucosidase and treated with cellulases for the indicated time (◆ Untreated sample; ▲ 1 h treatment; × 2 h treatment; \* 3 h treatment; ● 4 h treatment).

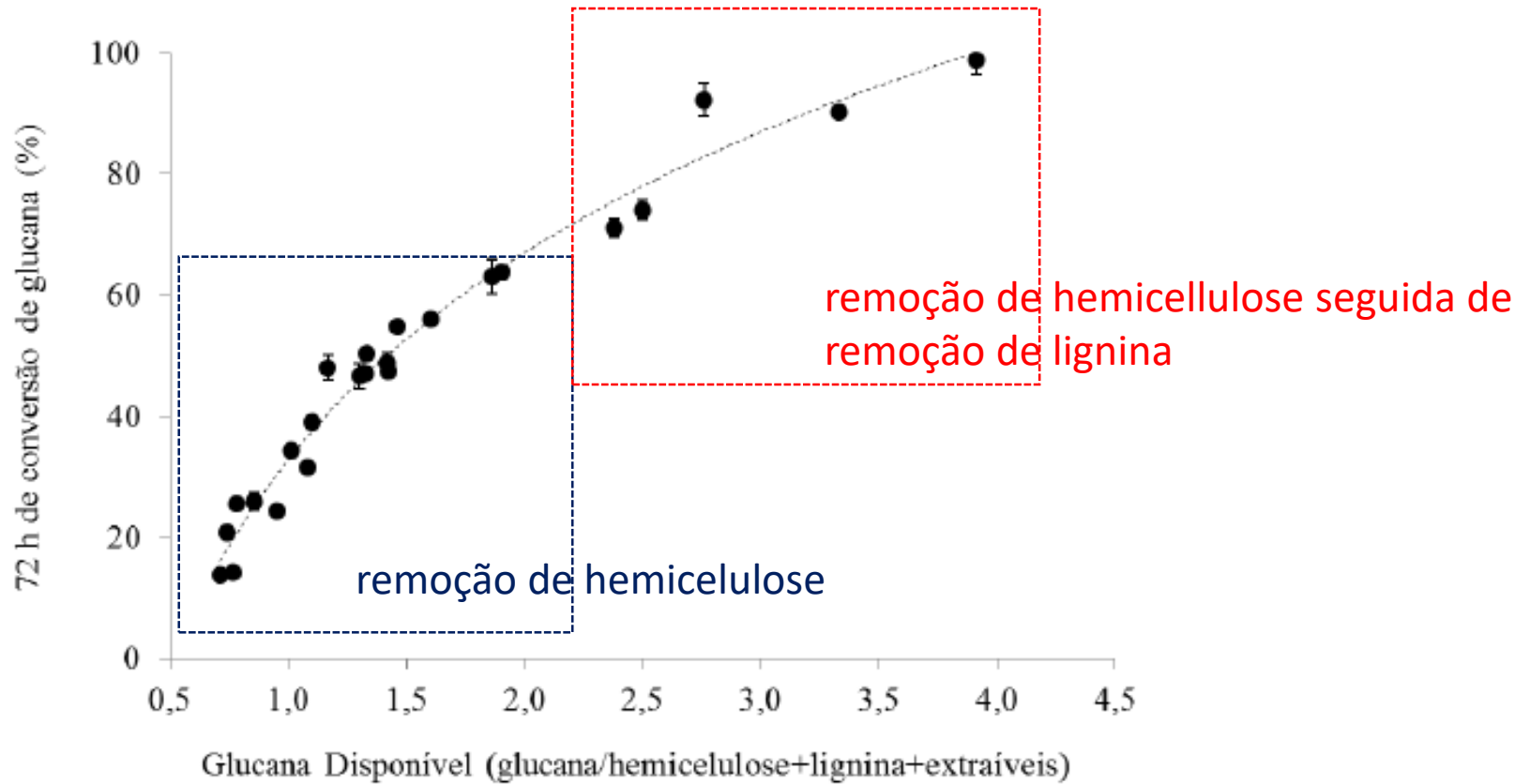
Remoção seletiva de lignina (clorito de sódio)

Table 2

Initial (4 h) rate of enzymatic hydrolysis of cellulose and extracted components from the sugarcane bagasses.

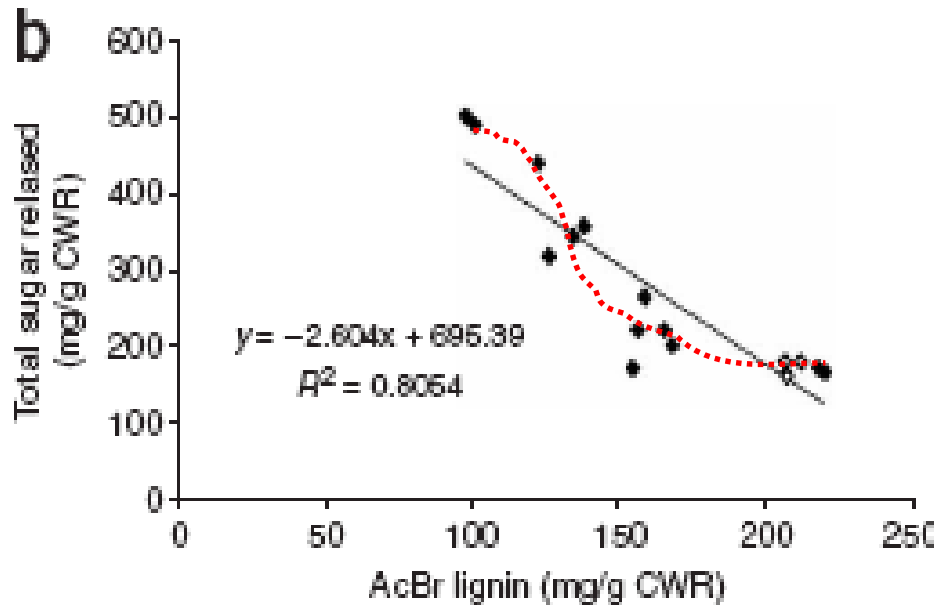
Treatment time with sodium chlorite (h)	Extracted lignin (%)	Extracted hemicellulose (%)	Extracted cellulose (%)	Initial hydrolysis rate (% h <sup>-1</sup> )
0	0	0	0	2.75
1	41.0	0	0	7.70
2	63.2	0	0	10.18
3	72.2	9.2	0	10.38
4	76.2	10.3	0	12.30

# Efeito combinado da remoção de componentes



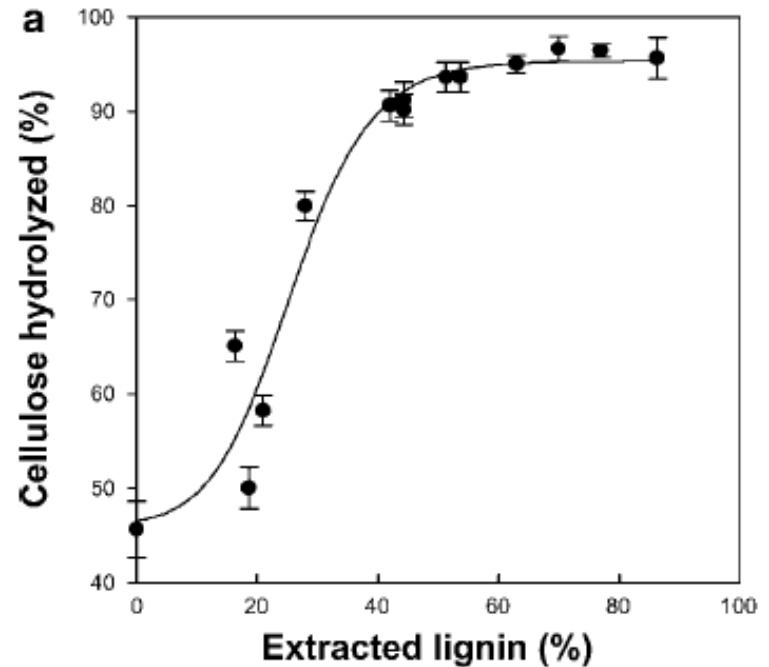


# Lignin-depleted plants



*Chen and Dixon, Nature Biotechnology, 2007*

Transgenic alfalfa

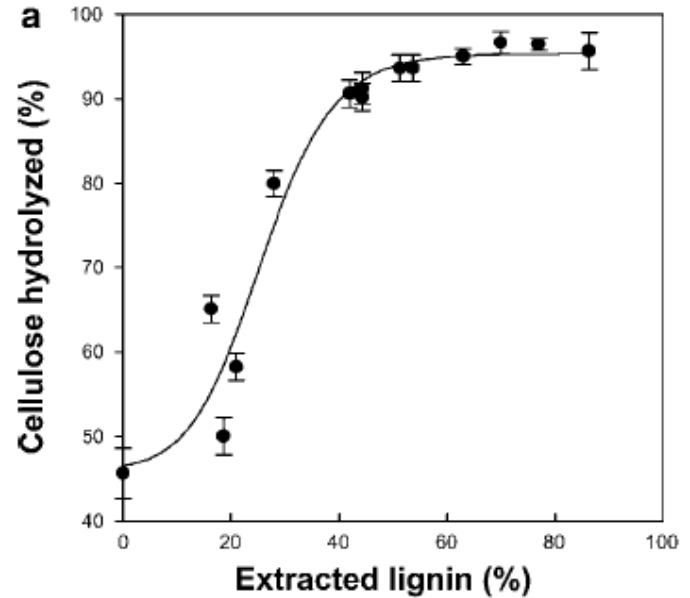
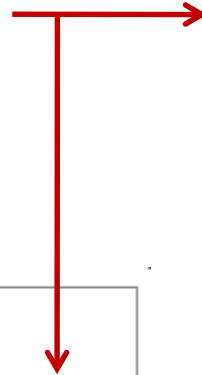


*Lee et al., Biotechnol Bioeng, 2009*

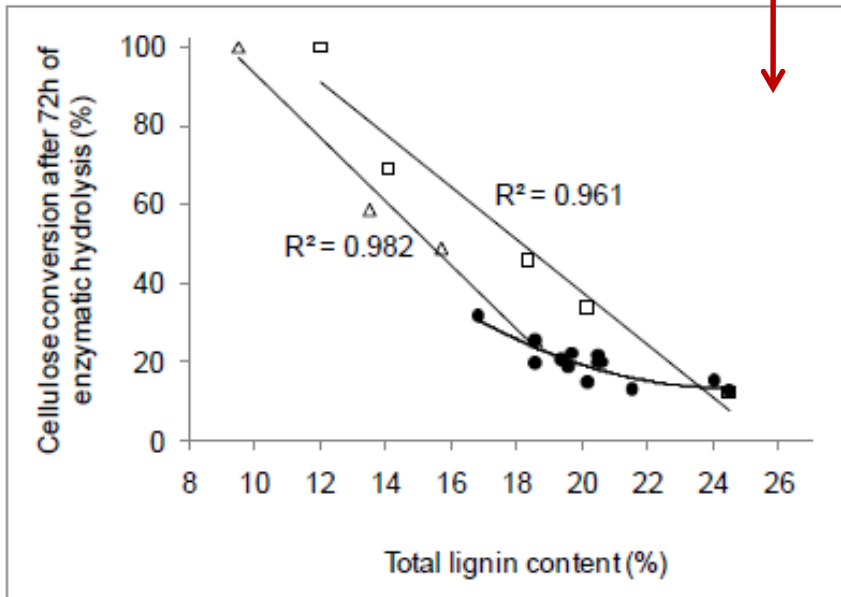
Selectively delignified Maple using ionic liquids

Partial delignification is enough, but wood chips are not disrupted at this delignification level

>> However, for wood chips, a mechanical fibrillation is necessary



Lee et al., *Biotechnol Bioeng*, 2009  
Selectively delignified Maple using ionic liquids



Masarin et al., *Biotechnol Biofuels*, 2011  
Sugarcane hybrids with varied lignin contents

Figure 3 Cellulose conversion as a function of lignin.

# Tratamento mecânico associado ao pré-tratamento químico



**Fig. 6** Size reduction of dilute acid-pretreated corn stover by disk milling. **a** Dilute acid-pretreated sample; **b** dilute acid-pretreated and disk-milled sample