Oak Ridge National Laboratory

Center for Molecular Biophysics

Bioenergy: the path to cellulosic bioethanol

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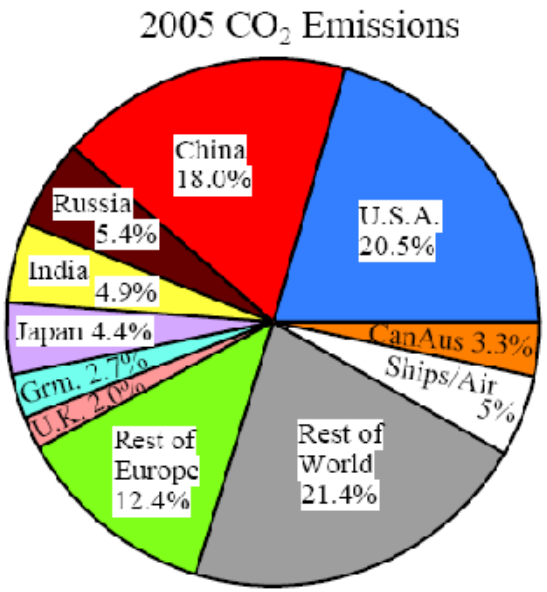
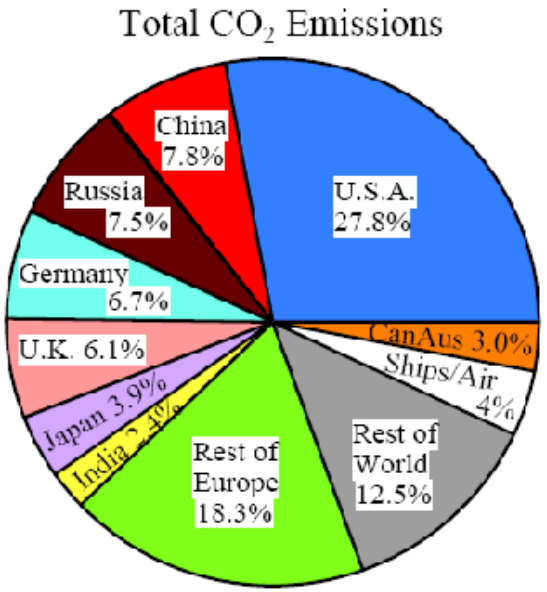
Outline

- Current Biofuels, why improve
- Cellulosic ethanol and Biomass Recalcitrance
  - cell wall structure
- BESC

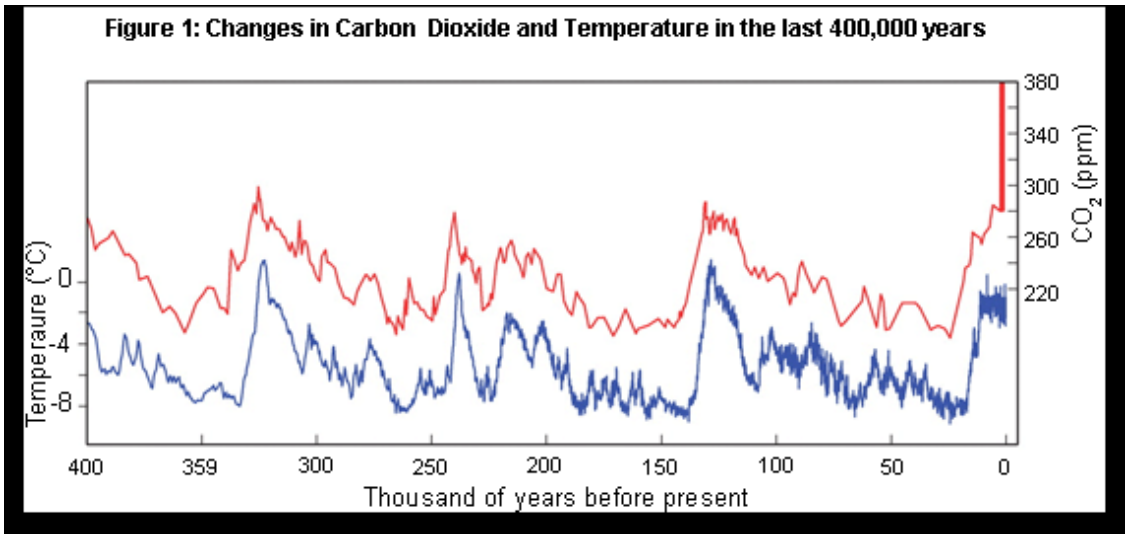
Need for Renewable and Green Energy

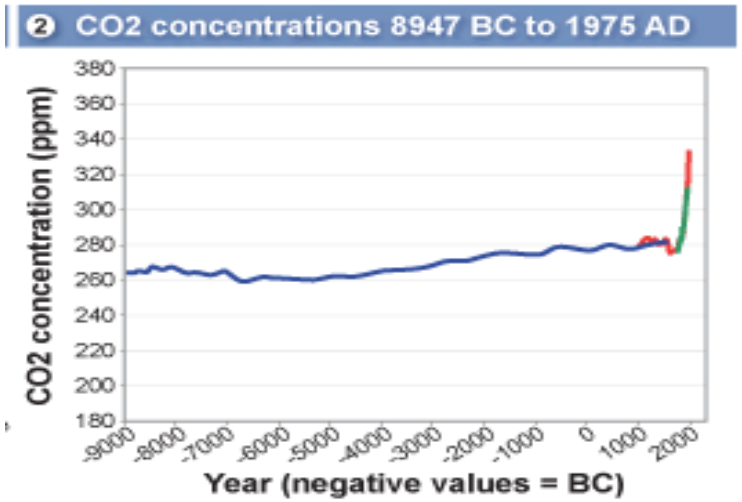
steep increase in oil demand
- geopolitics
- national security





current emissions lead to global warming





1st Generation Biofuels

produce ethanol using:

- starch from corn grain
- simple sugars from sugar cane

significant disadvantages:

- energy required to grow crops and process them into fuel
- emissions from 1st generation biofuels produce more greenhouse gas emissions than they save
- currently more expensive than petrol
- rising food prices

2nd Generation Biofuels: Ethanol from Biomass

biomass: material from plants

Switchgrass target biomass crop for TN

advantages:

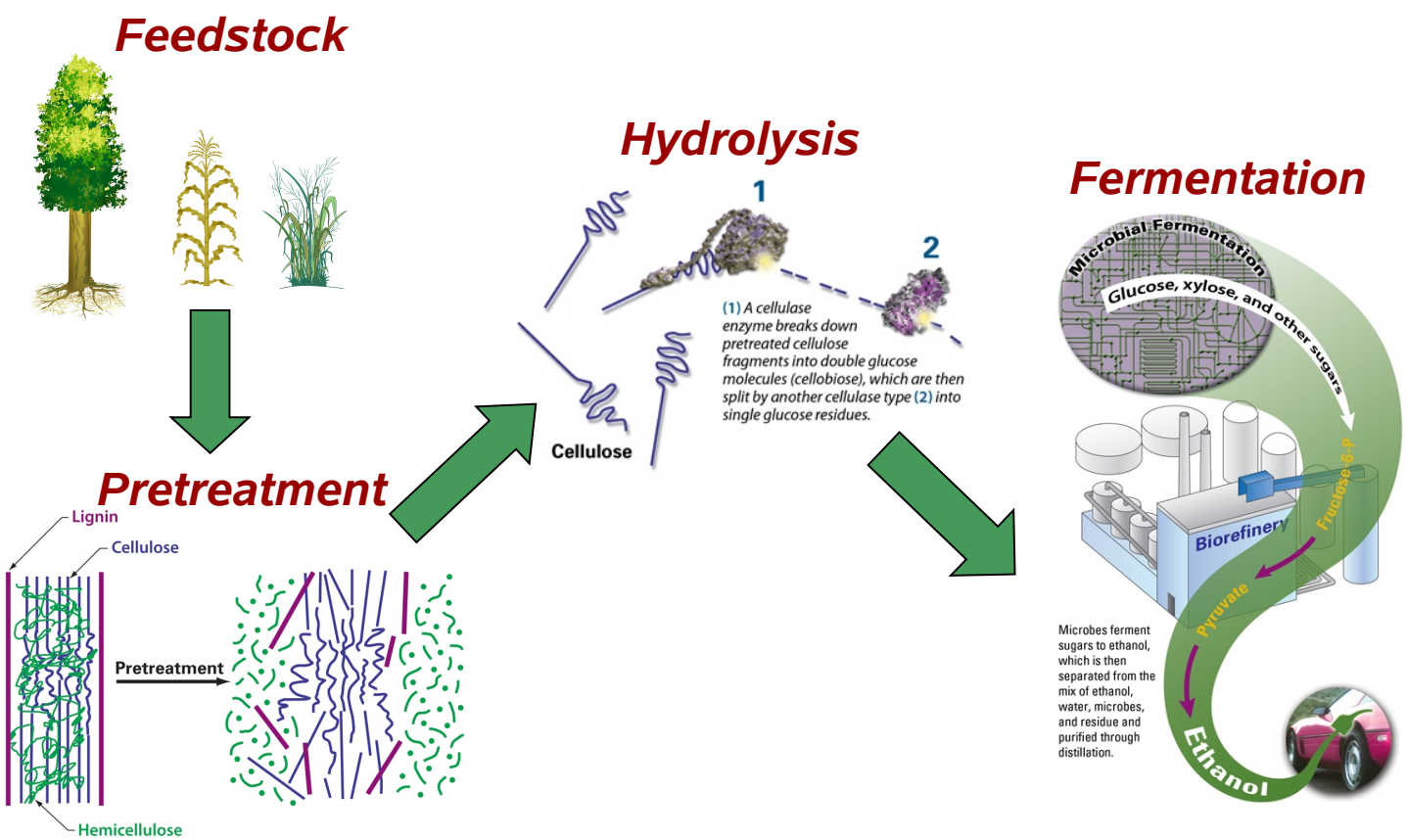
- lower cost
- higher availability
- no competition with food demand

➔ renewable energy aim to be *carbon neutral*
carbon released during the use of the fuel is reabsorbed and balanced by the carbon absorbed by new plant growth

➔ **aim: produce ethanol by enzymatic or microbial hydrolysis of plant carbohydrates**

difficult process, understand why by examining the main source of carbohydrates: plant cell wall

Biomass Recalcitrance



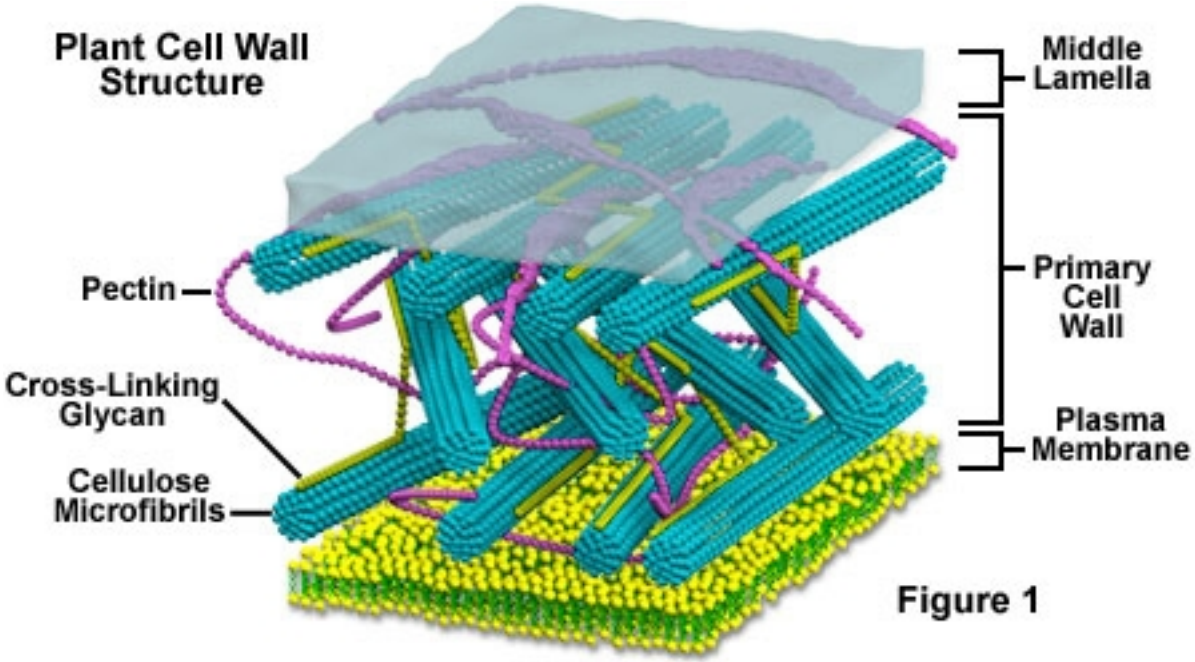
recalcitrance: natural resistance of plant to enzymatic microbial deconstruction complex phenotype

Pretreatment (heat, enzymes, acids)

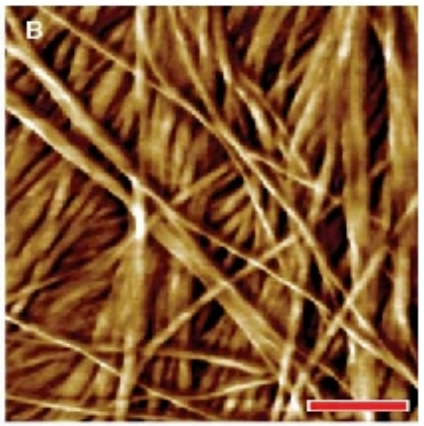
- removes polymers from cellulose before hydrolysis.
- one of the most expensive processing steps, has great potential for improvement

Plant Cell Wall

- cellulose
- lignin
- matrix polysaccharides
  - hemicellulose
  - pectin
- proteins



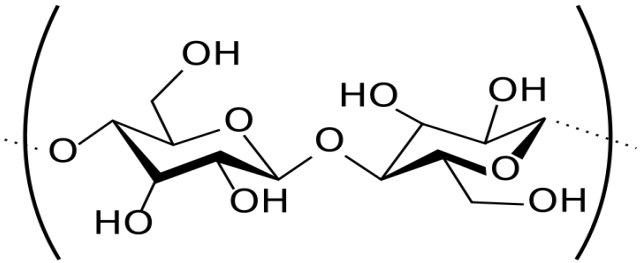
BioResource	**Lignin**	**:Hemicellulose :**	**Cellulose**
Corn Stover	0.47	0.58	1.00
Switch Grass	*0.58*	*0.64*	*1.00*



Cellulose

linear chain of β(1→4) linked D-glucose units

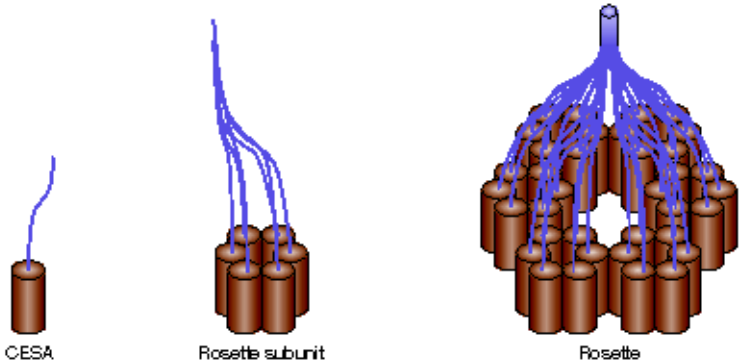
chain length: 2000 to 15000



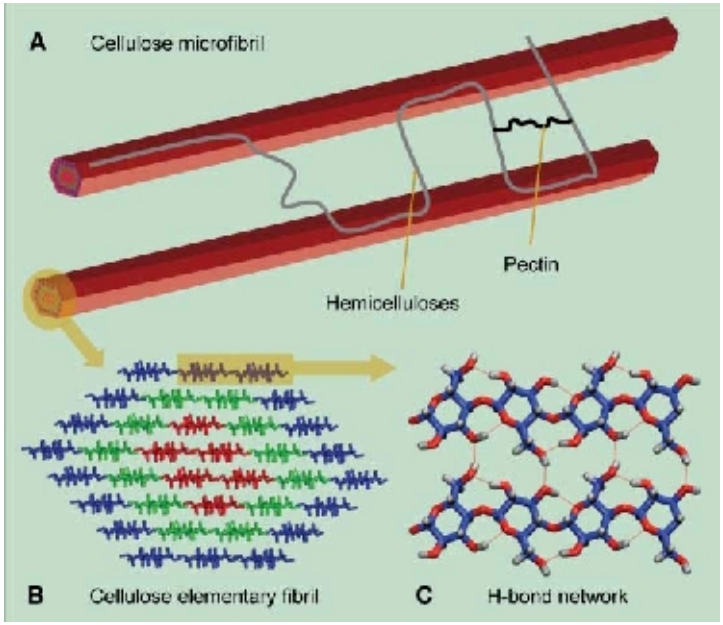
in plants:

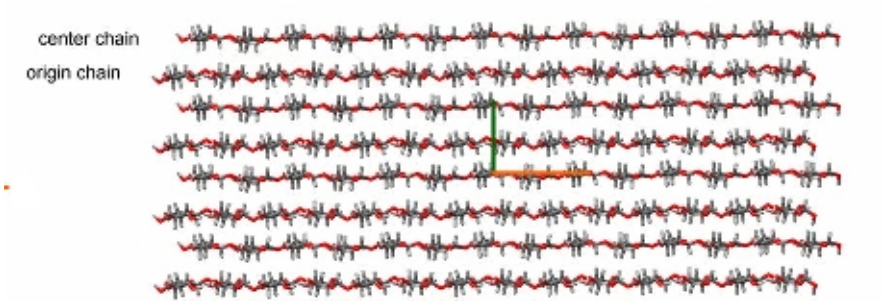
- microfibrils: many parallel, oriented chains, organized in highly crystalline structure
- many allomorphs (Cellulose I, II, III, IV), native Cellulose I
- Cellulose Iα and Iβ (higher plants)

- Cellulose Synthase proteins
- embedded in the plasma membrane
  - smallest subunits consist of six CESA
  - in hexameric arrays <u>rosettes</u>
  - elementary microfibril 36 chains
  - experiments: up to 1000 chains



Cellulose Structure and Recalcitrance



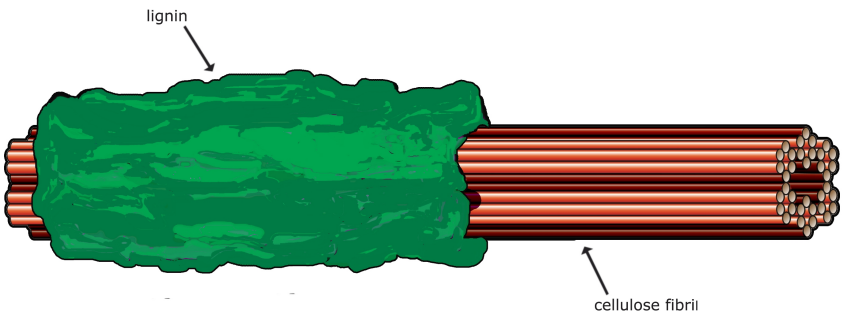


- chains precisely arranged
- OH groups in radial position, C in axial
- strong interchain h-bonds in same sheet
- weaker hydrophobic interaction between sheets (no h-bonds)
- amorphous part (surface?)

contribution to recalcitrance

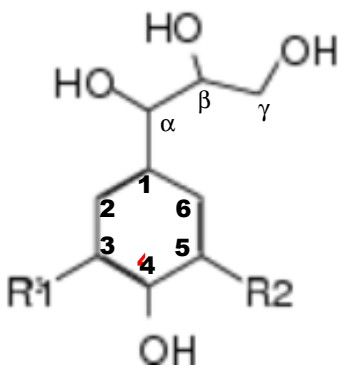
- the crystalline cellulose highly resistant to chemical and biological hydrolysis
- because of its compact structure and strong h-bonds
- hydrophobic face of sheets: formation of a dense layer of water near surface
- in comparison: amorphous cellulose are readily digestible

Lignin



- coats cellulose microfibrils
- heterogeneous amorphous biopolymer
- no unique chemical structure
- hydrophobic (phenyl groups)
- extremely difficult to digest

three types of units



Unit	R1	R2
H	H	H
G	MeO	H
S	MeO	MeO

common linkages

β-O-4' ⟶ linear lignin

5-5' ⟶ branched lignin

β-5'

α-O-4'

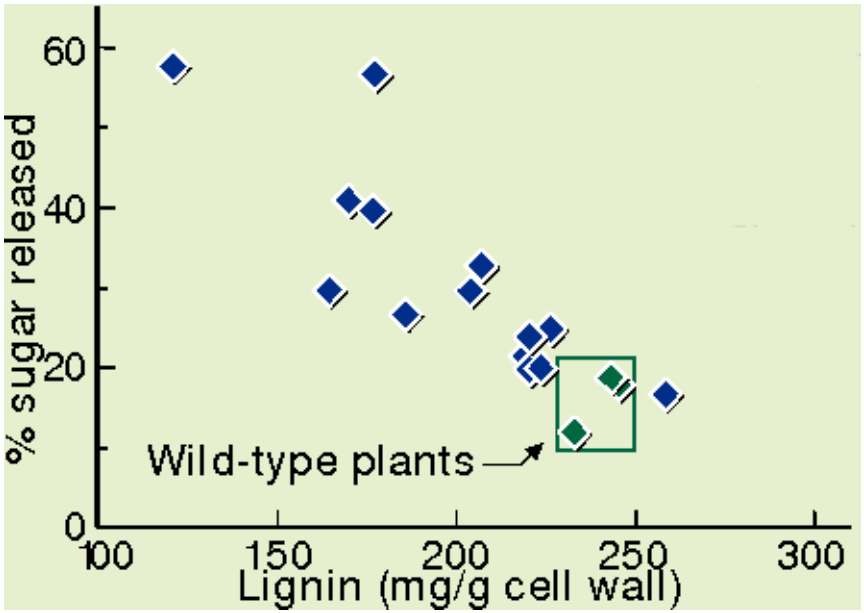
Specific model switchgrass

- Relative unit composition (S/G ratio)
- Linkage content

Lignin and Recalcitrance

together with cellulose crystallinity the most important factors

- physically impedes enzyme from reaching cellulose
- evidence of irreversible binding to cellulose-binding-domain of enzyme



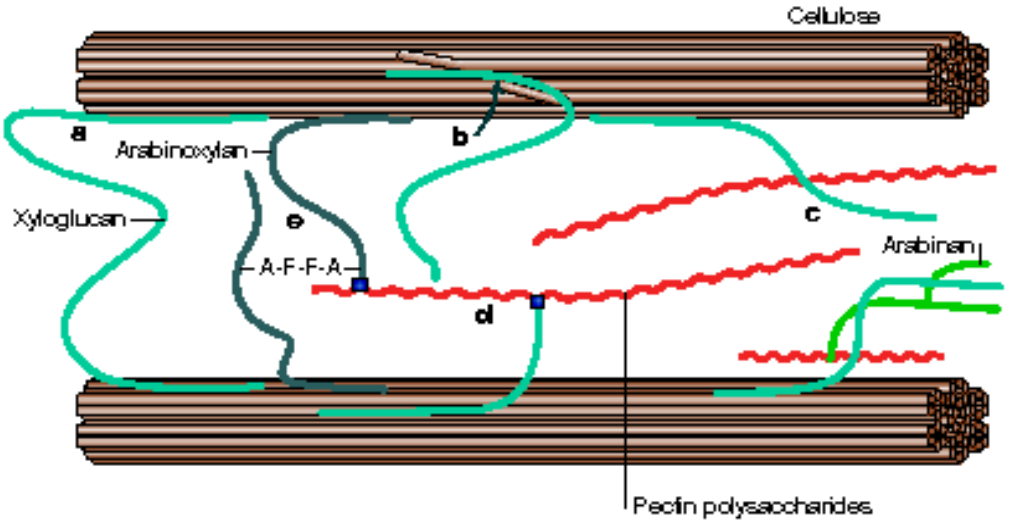
<u>factors that might influence to recalcitrance</u>:

eg hardwood less recalcitrant that softwood

- Average pore size of lignin
  - Linear (mostly β-O-4') vs branched (5-5')
- Cellulose-binding-domain and lignin interaction
  - G units (one methoxy group)
  - S units (two methoxy groups, steric hindrance?)

Hemicellulose and Pectin

matrix polysaccharides
form strong flexible network
bind to cellulose, lignin



hemicellulose
- backbone similar to cellulose
- branched and contains many different sugar monomers
- contributes to recalcitrance, but is easily hydrolysed

pectins
- heterogeneous
- helps formation of gels helps to bind cells together and regulates water in the plant
- readily digested

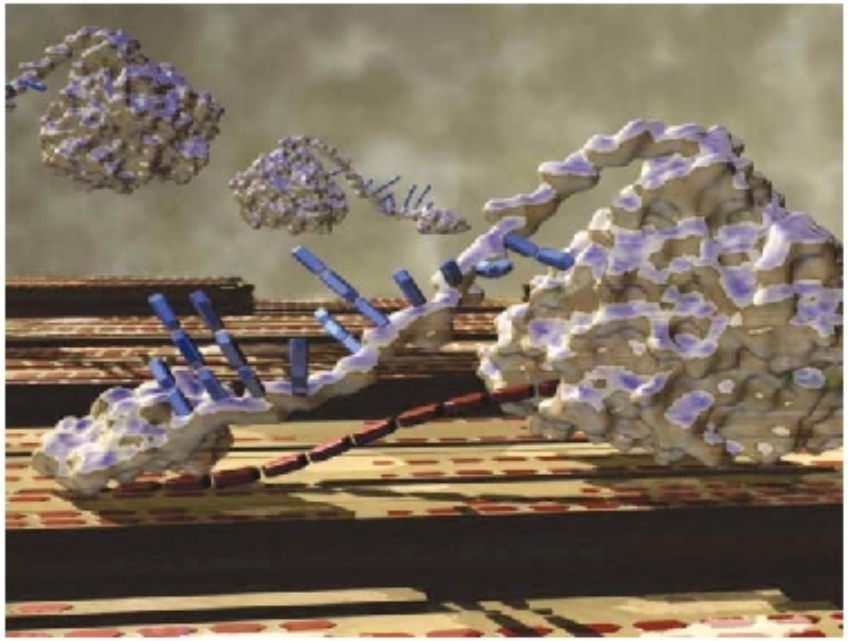
Cellulases

cellulose-binding domain
catalytic side

milder conditions (50^0C, pH 5.5)

function 2 order of magnitude slower than other polysacchridases

- cellulose crystallinity, lignin
- sugars binded to CBD



Endo-cellulase breaks internal bonds to disrupt the crystalline structure of cellulose and expose individual cellulose polysaccharide chains

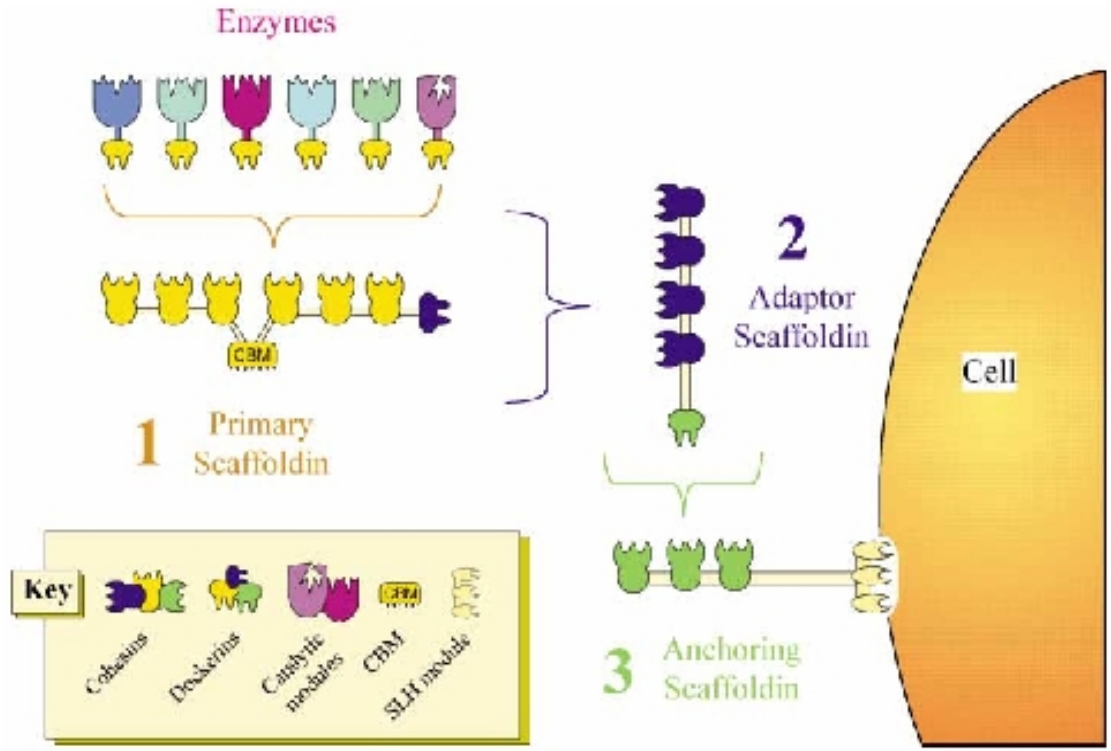
Exo-cellulase cleaves 2-4 units from the ends of the exposed chains to form disaccharides

enzymes act synergistically (cellulosome)

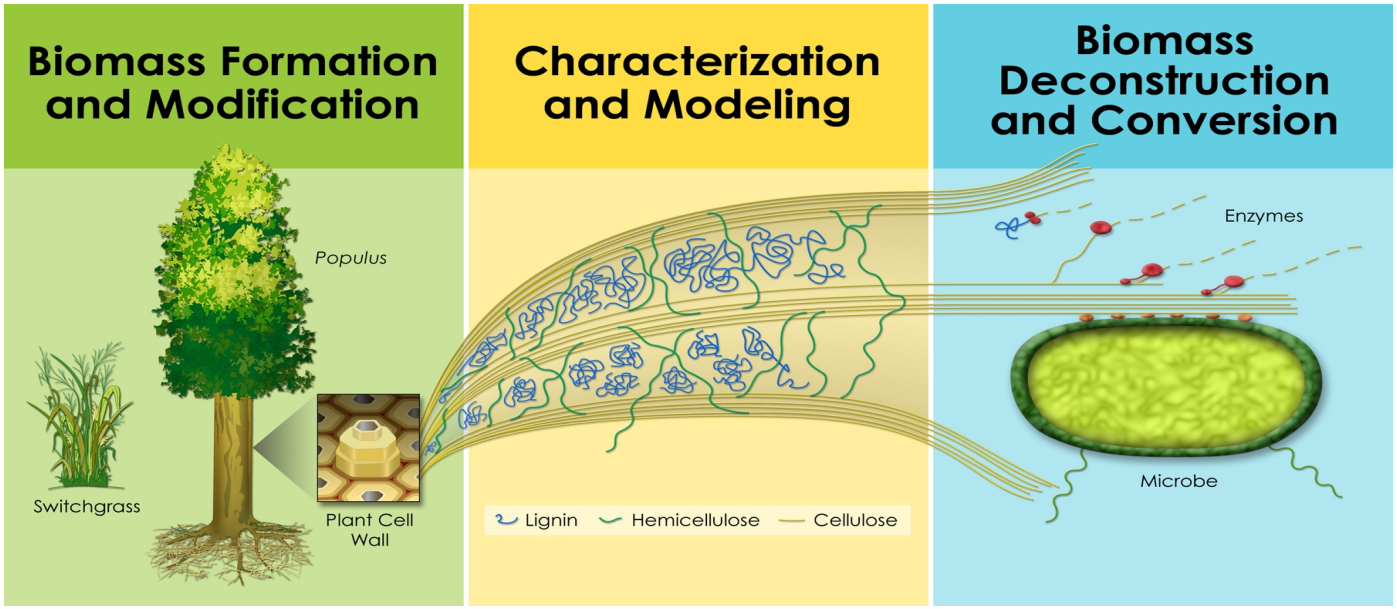
Cellulosome

Clostridium thermocellum is an anaerobic, thermophilic bacterium.

capable of directly converting a cellulosic substrate into ethanol.



BESC: understand and overcome biomass recalcitrance



plant → cell wall composition → pretreatment → sugar yield

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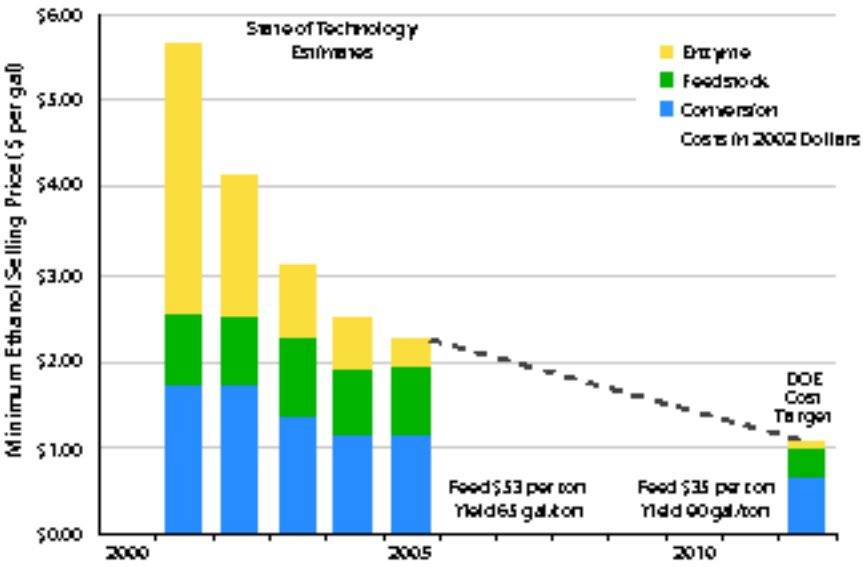
cell wall composition

plant + bacteria
identify important genes
modify them

Current Issues

ratio energy put : energy out = 1:6 (aim 1:10)
cost: 1.50/gallon (aim 1.33)
¼ pounds of enzymes / gallon of ethanol