

Lecture 2: Biomass

MCB 113

Biomass

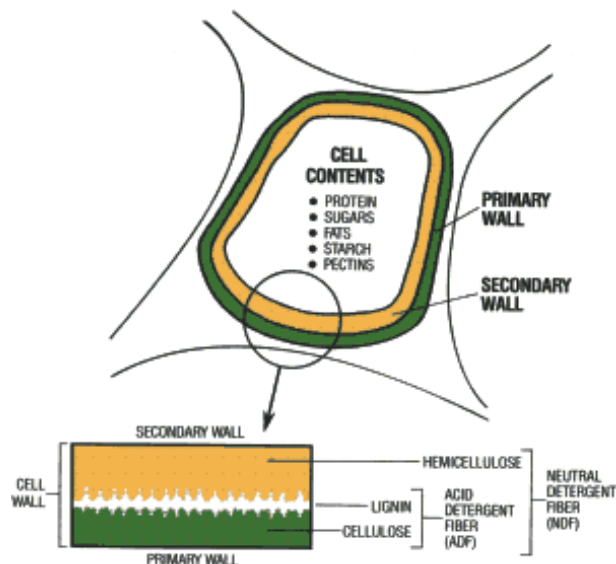
- All organic matter that grows by the photosynthetic conversion of solar energy.
- The total biomass produced annually by photosynthesis contains approx. 3×10^{21} J energy, 10 times that needed for human uses.
- About 43% of the total carbon fixed on earth is done in forests.

Lignocellulose

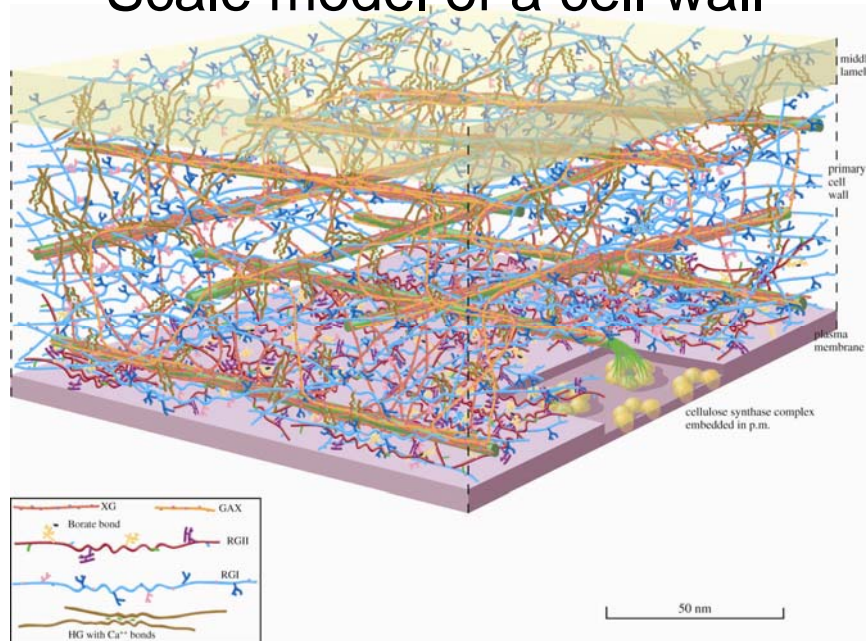
- Nearly universal component of biomass
- Consists of three types of polymers:
 - Cellulose
 - Hemicellulose
 - Lignin
- All three are degraded by bacteria and fungi

Component	Percent Dry Weight
Cellulose	40-60%
Hemicellulose	20-40%
Lignin	10-25%

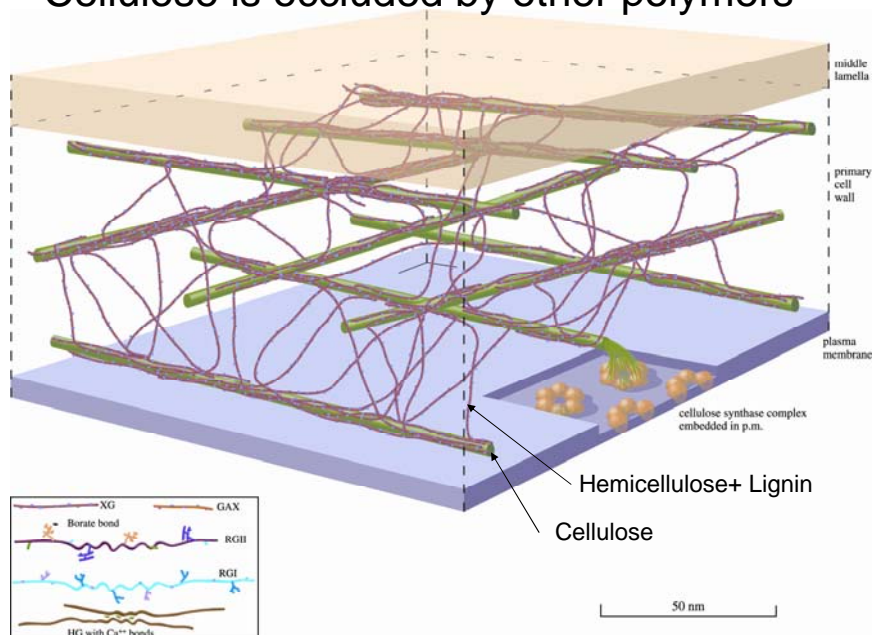
Plant cell components



Scale model of a cell wall



Cellulose is occluded by other polymers

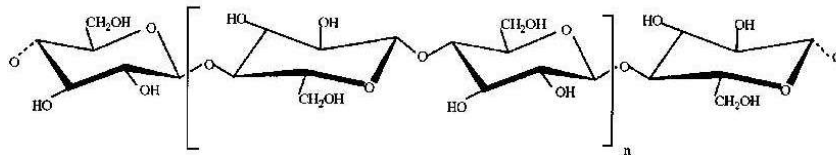


Structure and chemistry

	<i>Cellulose</i>	<i>Hemicellulose</i>	<i>Lignin</i>
Chain form	linear	linear, branched	complex x-link
Chain length	10,000 - 14,000	100 - 300	-----
Unit	β -D glyco- pyranose	β 1, 4 glycans	phenylpropane
Bond	β 1-4 glycosidic	β 1-4 : xylans (hw) glucomannans (sw)	5 types
Organization	microfibrils (H-bond)	H-bond to cellulose	H-bond to hemicellulose

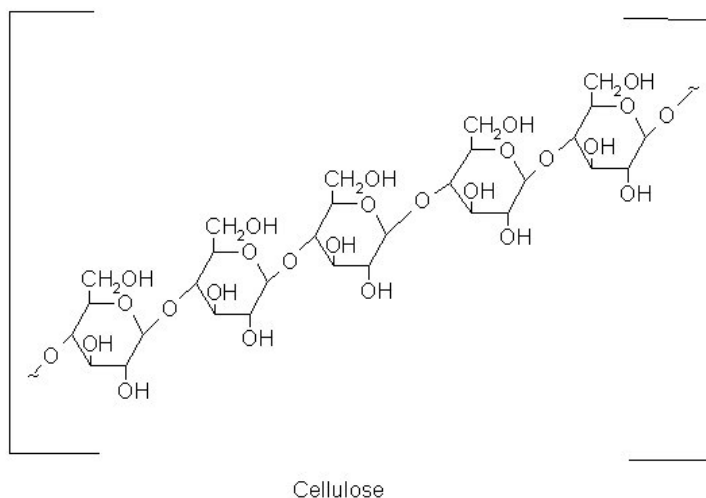
Cellulose

- Cellulose consists of unbranched polymers of - linked glucose residues arranged in linear chains, where every other glucose residue is rotated approximately 180



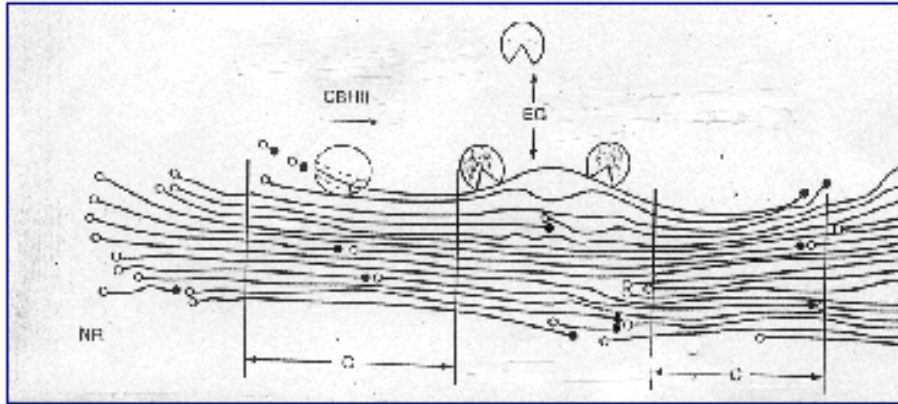
- As a result, cellobiose is the structural repeating unit of the glucan chains in cellulose.
- This is in contrast to other glucan polymers such as starch (α -1,4-glucan) and callose (β -1,3-glucan) where glucose is the repeating unit.

Cellulose

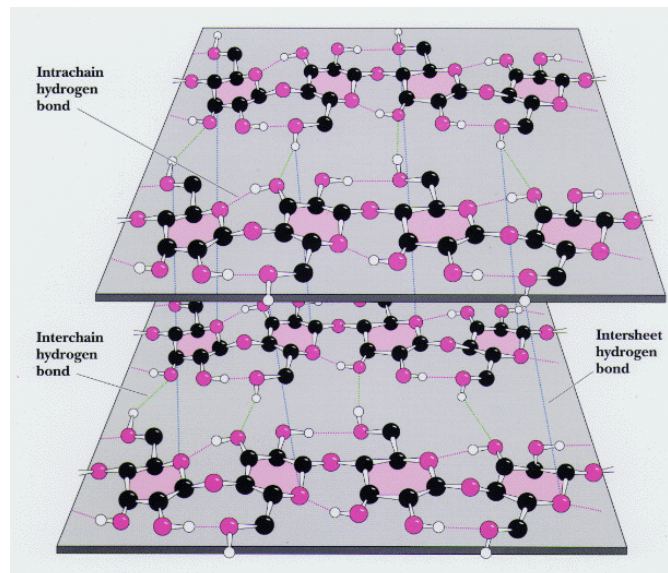


- Cellulose is the most abundant organic compound in the world.
- The greater part of wood is made of cellulose, and cotton is almost 100% cellulose.
- Thus people have been using cellulose since very ancient times for things such as clothing.
- Cellulose forms fibers which we can spin into thread and weave to make textiles.
- Most of our clothing are made of cellulose, from cotton to Rayon.
- Because cellulose is not water soluble certain forms of it can be used in other products such as shampoo and laxatives.

Cellulose structure

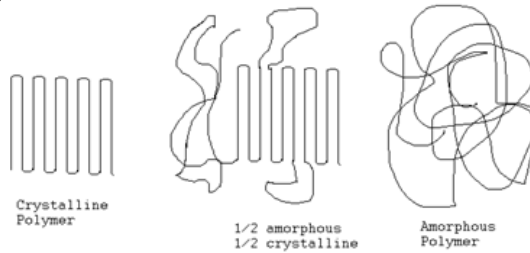


3-D Cellulose Structure



Crystalline vs. Amorphous:

- How rigid a polymer is depends on how the polymer is shaped



- When a polymer is disorganized or all tangled up, it is not rigid.
- When the polymer is organized and folded onto itself, the strength is increased.

- Polymers can also be part crystalline and part amorphous.
- Some parts of the long chain may be folded, while some parts may branch off without any particular order.
- This is good also, because materials that are completely crystalline may be too brittle.

Cellulose structure

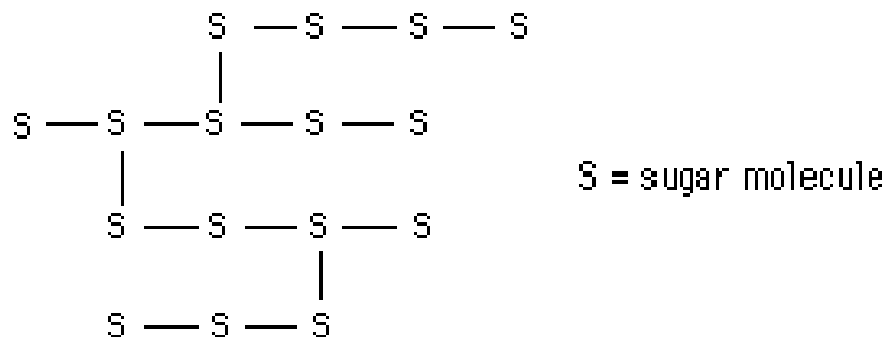
- In nature, cellulose never occurs as a single chain, but exists from the moment of its synthesis as a crystalline array of many parallel, oriented chains - microfibrils which are its fundamental structural units.
- The glucan chain length (degree of polymerization) varies from about 2000 to more than 25000 glucose residues.
- Microfibril size can vary from the so-called 'elementary fibril', which has approximately 36 chains, to the large microfibrils of cellulosic algae, which contain more than 1200 chains (and are so highly organized that they can diffract as a single crystal).

- The most common crystalline form (allomorph) of native cellulose is cellulose I, which is metastable (i.e. it is not the most thermodynamically favorable form) and can be irreversibly converted into another crystalline state, cellulose II, the most stable allomorph known.
- Cellulose I has parallel glucan chains and strong intramolecular hydrogen bonds.
- In nature, cellulose I exists as two crystalline suballomorphs, cellulose I- α ; and I β (Ref. 8).
- Cellulose II is seldom made in nature **9** - it is generally the product of re-precipitation after swelling and dissolution of cellulose I with various solvents and is more familiarly known as 'Rayon'.

Hemicellulose

- **Hemicellulose** is a polysaccharide composed of a variety of sugars including xylose, arabinose, mannose.
- Hemicellulose that is primarily xylose or arabinose are referred to as xyloglucans or arabinoglucans, respectively.
- Hemicellulose molecules are often branched.
- Hemicellulose molecules are very hydrophilic.
- They become highly hydrated and form gels.

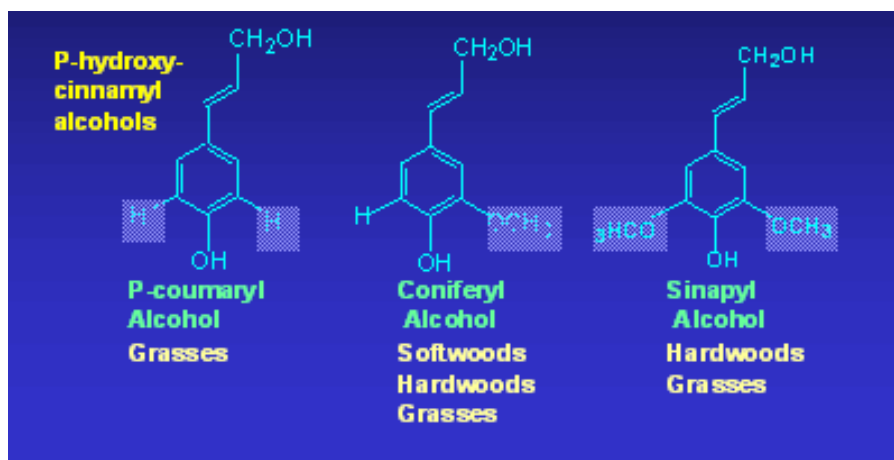
Hemicellulose structure

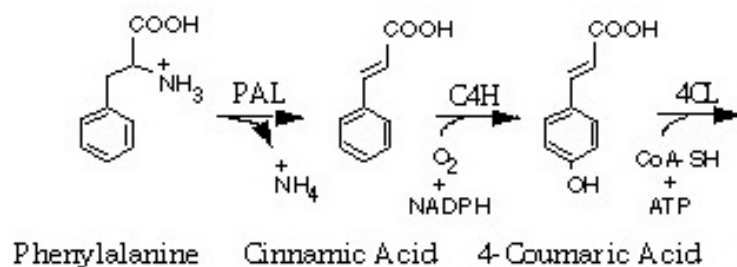


Lignin

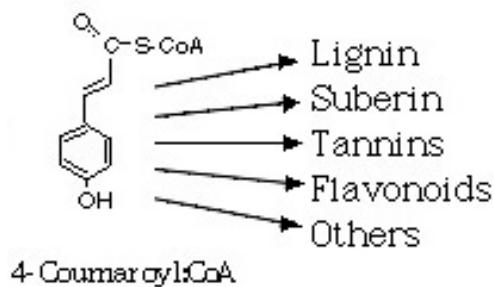
- A complex polymer built up of aromatic alcohol subunits
- Accounts for 20-30% of wood dry weight
- Provides rigidity and support to the tree: binds wood fibers together
- Must be removed to produce high quality paper (chemical pulping)

Basic building blocks of lignin

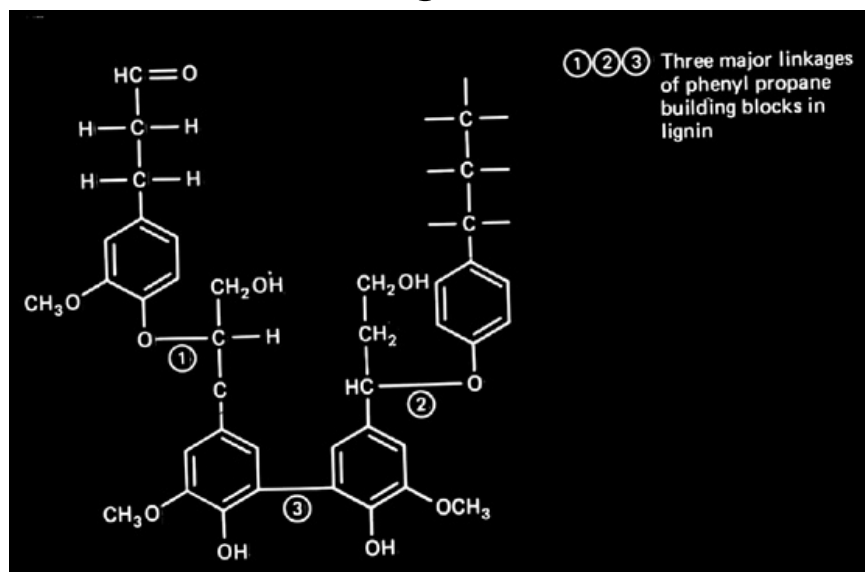




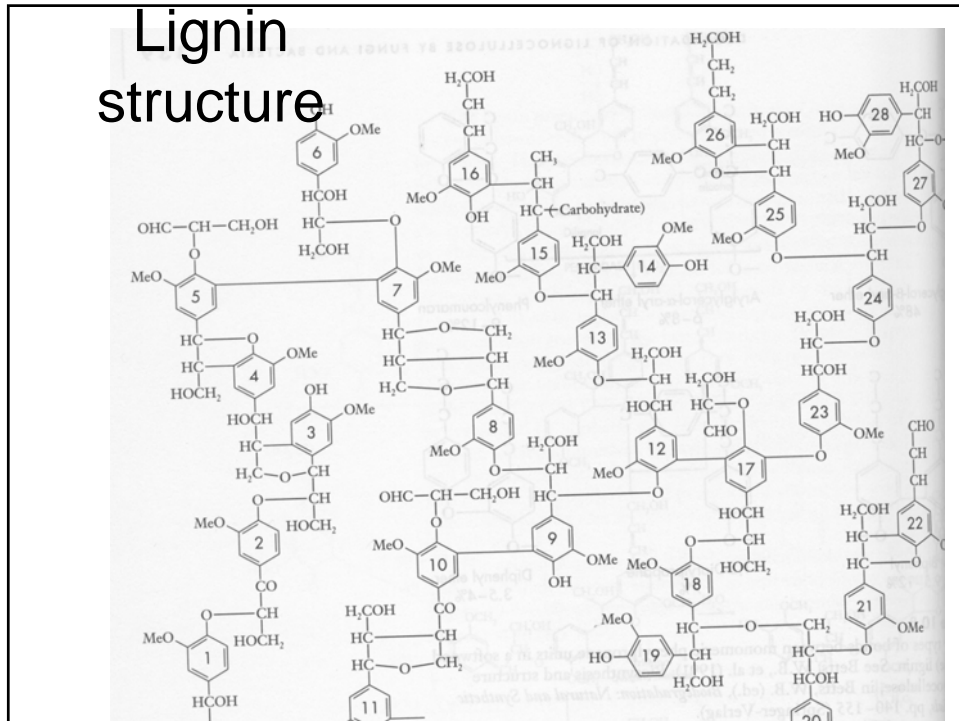
Pathways for lignin production



Lignin



Lignin structure



Lignocellulose degradation

- Degradation of wood by fungi
 - White-rot (> 90%)
 - Degrade lignin and cellulose at same rate
 - *Phanerochaete chrysosporium*
 - Brown-rot
 - Degrade cellulose and leave lignin
 - Soft-rot
 - Degrade lignin and cellulose at different rates
 - Work on moisture-exposed wood
- In general, bacteria do not degrade lignin

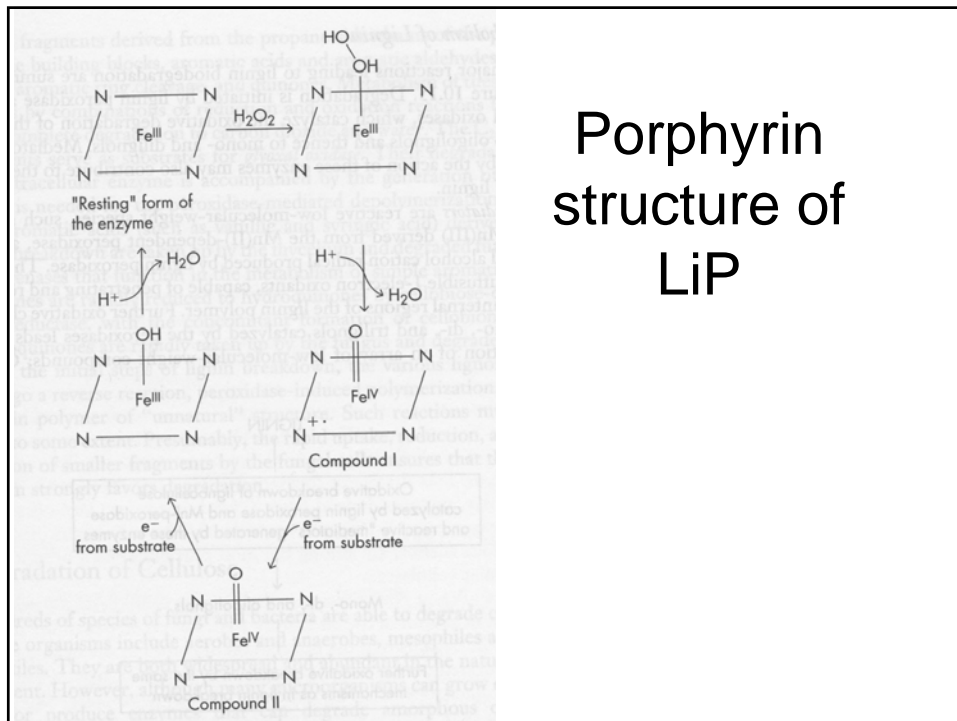
White-rot fungi



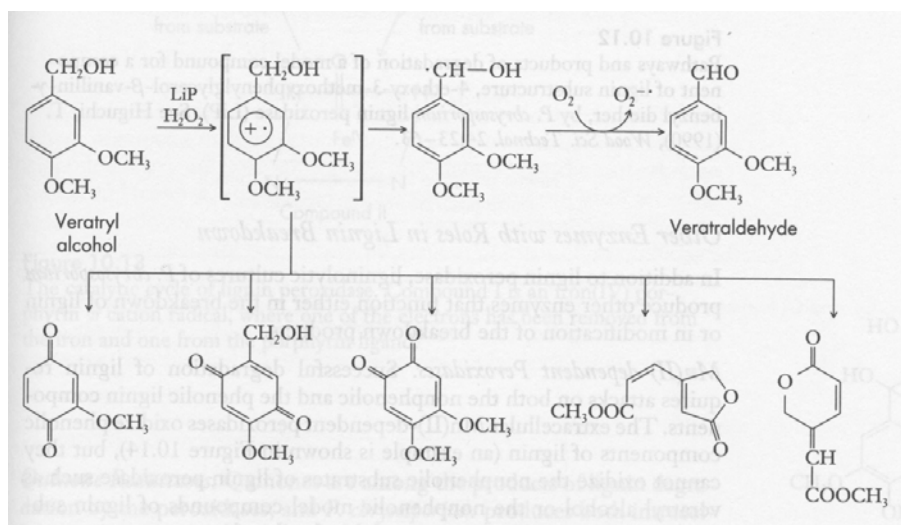
Lignin-degrading enzymes

- Lignin Peroxidase (LiP)
 - Extracellular H_2O_2 dependent enzyme
 - Produced during secondary growth phase
 - Extremely versatile
 - Substrates are non-phenolic aromatic rings and $\text{C}=\text{C}$ bonds
 - Reactive agent is hydroxyl radical ($\text{OH}\bullet$)
- Manganese Peroxidase (MnP)
 - Extracellular H_2O_2 dependent enzyme
 - Produced during primary growth phase ???
 - Less versatile than LiP
 - Substrates are phenolic
 - Reactive agent is Manganese ion (Mn^{3+})

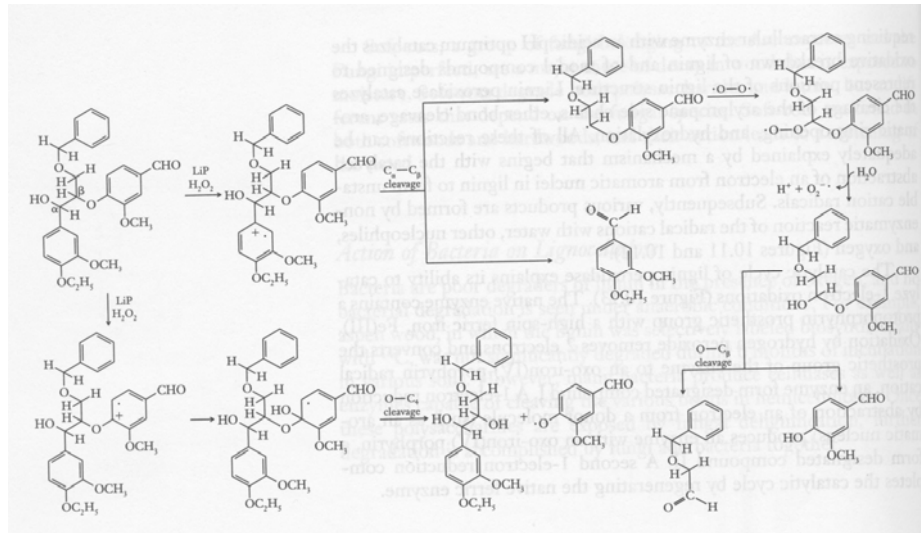
Porphyrin structure of LiP



Involvement of veratryl alcohol in LiP degradation of lignin



LiP degradation of lignin model



Lignin depolymerization products

- Products of lignin depolymerization include aromatic acids and aldehydes, quinones, hydroquinones, and C_1 , C_2 , and C_3 fragments.
- These products can be used by the fungus (i.e., *P. chrysosporium*) or by soil bacteria, such as *Pseudomonas* sp.

The role of cellulose degradation in the world carbon cycle

- Cellulose is the most abundant carbohydrate in the world and its recycling is dependent on microbial activity.
- It is estimated that 85 billion metric tons of CO₂ are produced per year from microbial degradation of cellulose.
- If such fermentation ceased with photosynthesis unabated, the earth would stagnate in 20 years from lack of CO₂

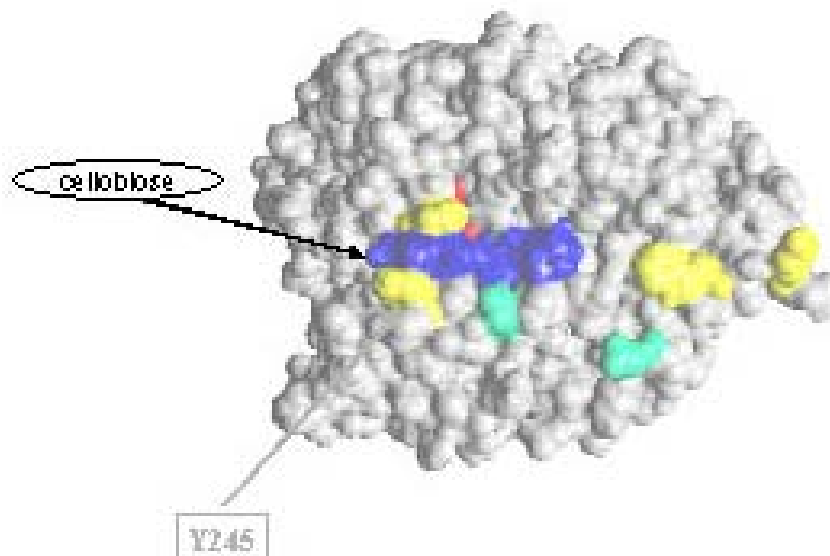
Organisms responsible for cellulose degradation

- Fungi
 - *Trichoderma viride*
 - *Phanerochaete*
- Bacteria
 - *Cellulomonas* (an anaerobe)
 - *Clostridium thermocellum* (an anaerobe)

Fungal cellulases

- Three types
 - Endoglucanases
 - Hydrolyze internal bonds in disordered regions along cellulose fibers
 - Cellobiohydrolases
 - Attack ends generated by endoglucanases and release the disaccharide cellobiose
 - β -glucosidases
 - Hydrolyze cellobiose to glucose

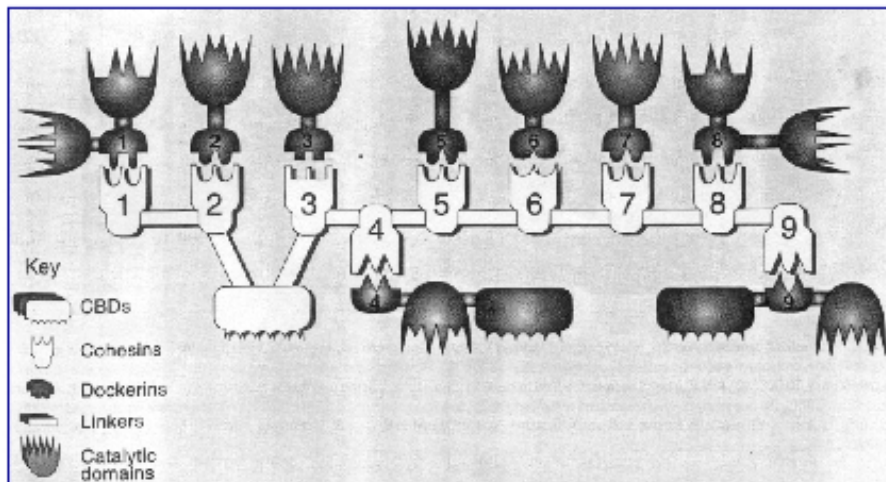
Endoglucanase



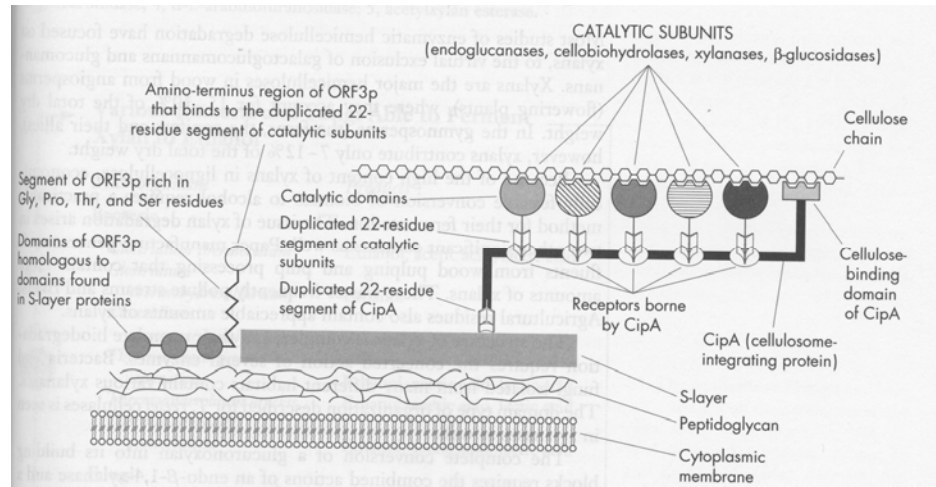
Bacterial cellulases

- **Cellulase** may exist in two main forms within the bacteria, either as the individual enzymes or as a collective unit called a **cellulosome**.
- The larger the surface area of the cellulose the easier it is for the cellulase to attack it.
- Experimentally the most rapid degradation is of cellulose powder.

Cellulosome structure



Cellulosome structure



Cellulosome structure

- Stable & flexible
- Many subunits, most being enzymes
- Organization promotes synergistic action
- Non-catalytic, multipurpose subunit which is the core of cellulosome structure
- Scaffoldin - 1800 amino acids; single Cellulose Binding Domain; Cohesins; anchors cellulosome to cell surface

Cellulosome structure

- All contain scaffoldin
- More active against crystalline than amorphous cellulose
- Differ in that have:
 - different types and arrangements of enzymes
 - additional domains
 - X-domains, with unknown function

Cellulosome – Cell interaction

- Form lengthened corridors between cell & substrate
- Cellulose degradation aided by noncellulosomal cellulases & cellulosomes released into environment

Biotechnological use of cellulosomes

- Use in industries?
- Studies to determine:
 - function of X-domain
 - how enzymes work together
 - which bacterium contain cellulosomes
- “Designer cellulosomes”
- Chemically incorporate different enzymes

Hemicellulose degradation

- Most studies have focused on xylan
- Fungi
 - *Neurospora crassa*
 - *Pichia stipitis*
- Bacteria
 - *Clostridium* sp.
 - *Thermoanaerobium brockii*
 - *Thermobacteroides acetoethylicus*
- Produce ethanol, acetic acid, and lactic acid from xylan