

wth
growth

The biochemistry of wood degradation

Kari Steffen



Overview

- Degradation of dead wood focuses on fungal activity
 - Enzymatic attack of natural biopolymers
- The main organic components of dead wood
 - Cellulose
 - Hemicellulose
 - Lignin
 - Others



Facts

- Wood exposed to any environment will degrade over time
- In conducive conditions decomposition may be swift and extensive
- With limiting factors decay will be slow and wood survives for long periods
- Wood is more recalcitrant than many other organic material
 - Complex structural components: lignin, extractives



Conditions dictating decay

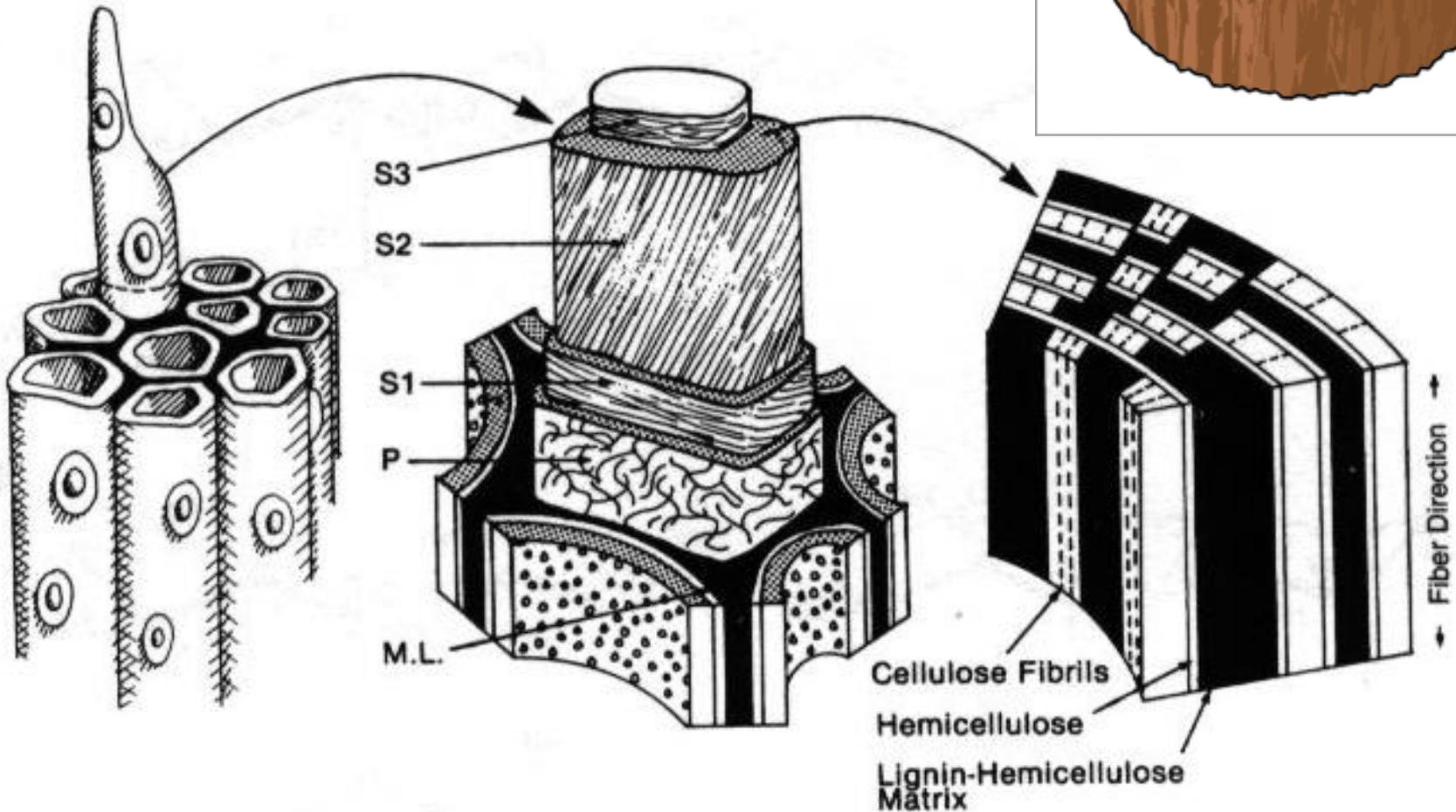
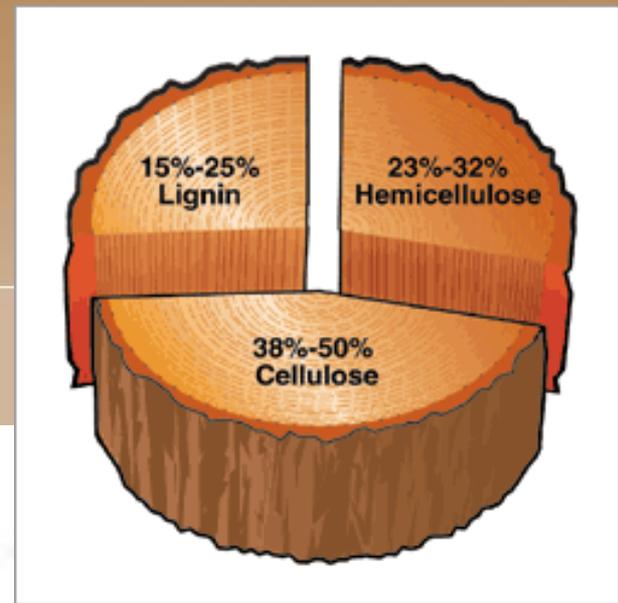
- Water
- Oxygen
- Temperature
- Other nutrients for microbes e.g. nitrogen
- Type of wood (soft and hard wood)
- Content of lignin and extractives
- Cell structure



Types of Wood Degradation

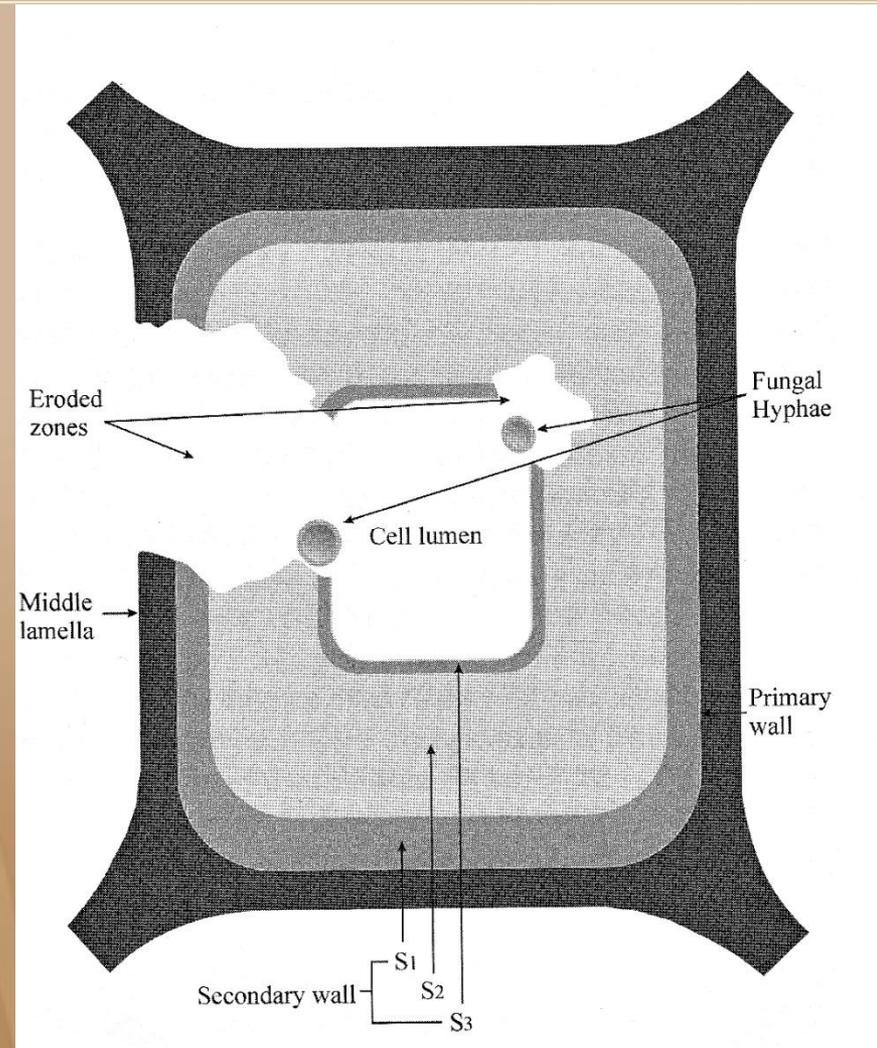
- Fungal degradation
 - White-rot
 - Brown-rot
 - Soft-rot
- Bacterial degradation
 - Erosion
 - Tunneling
 - Cavitation
 - Scavengers

Structure of lignocellulose



Fungal decay

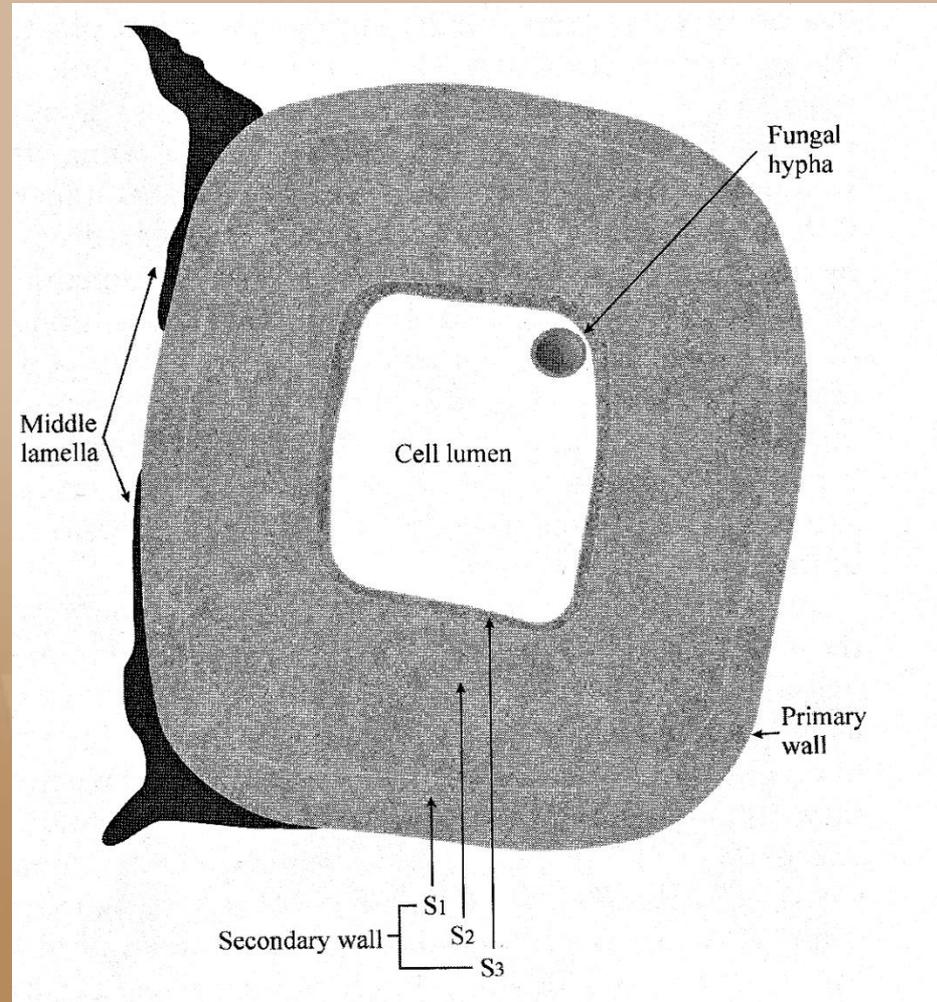
- As wood decays, color of substrate changes
- White-rot fungi can degrade all cell wall components
- Use of nonspecific extracellular enzymes



Picture: Jurgens

Selective white-rot

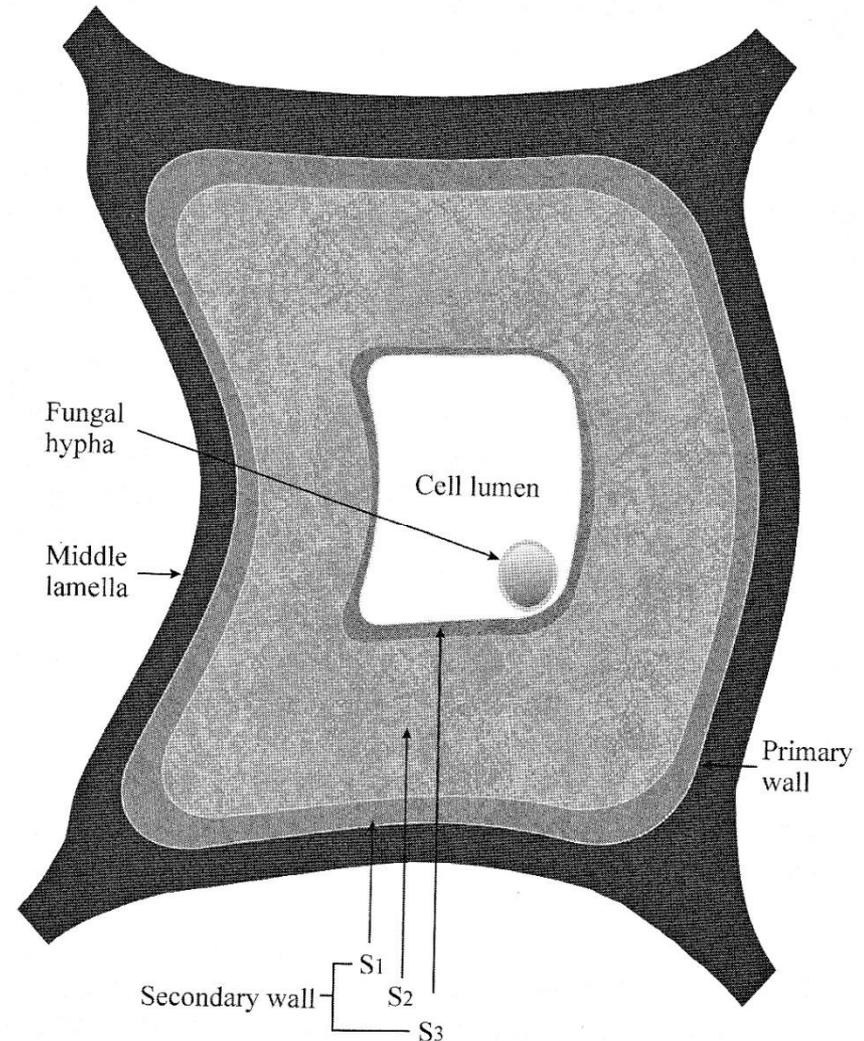
- Lignin removal predominant
- Utilization of hemicellulose as C
- Cellulose remains
- Whitish appearance



Picture: Jurgens

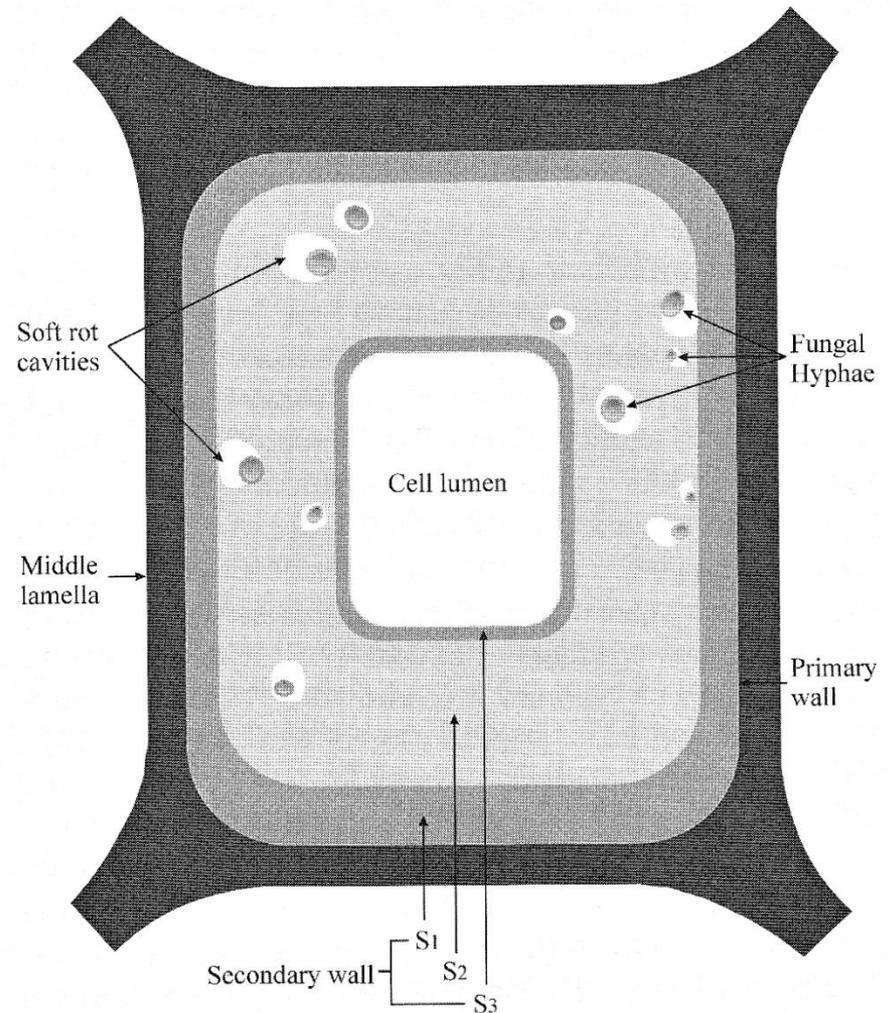
Brown-rot

- Common wood destroyers
- Problems of buildings and wood in service
- Degrade hemicellulose and cellulose
- Alter lignin
- Brownish appearance

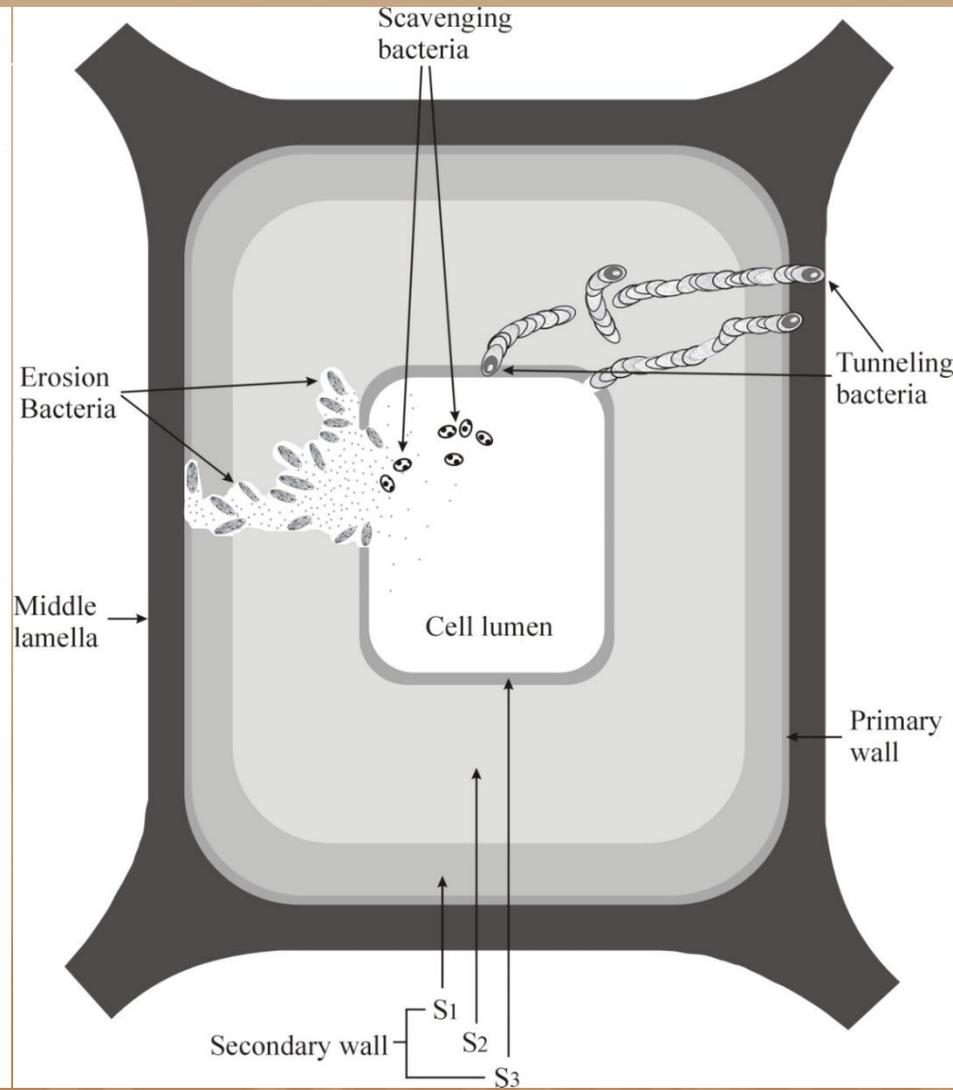
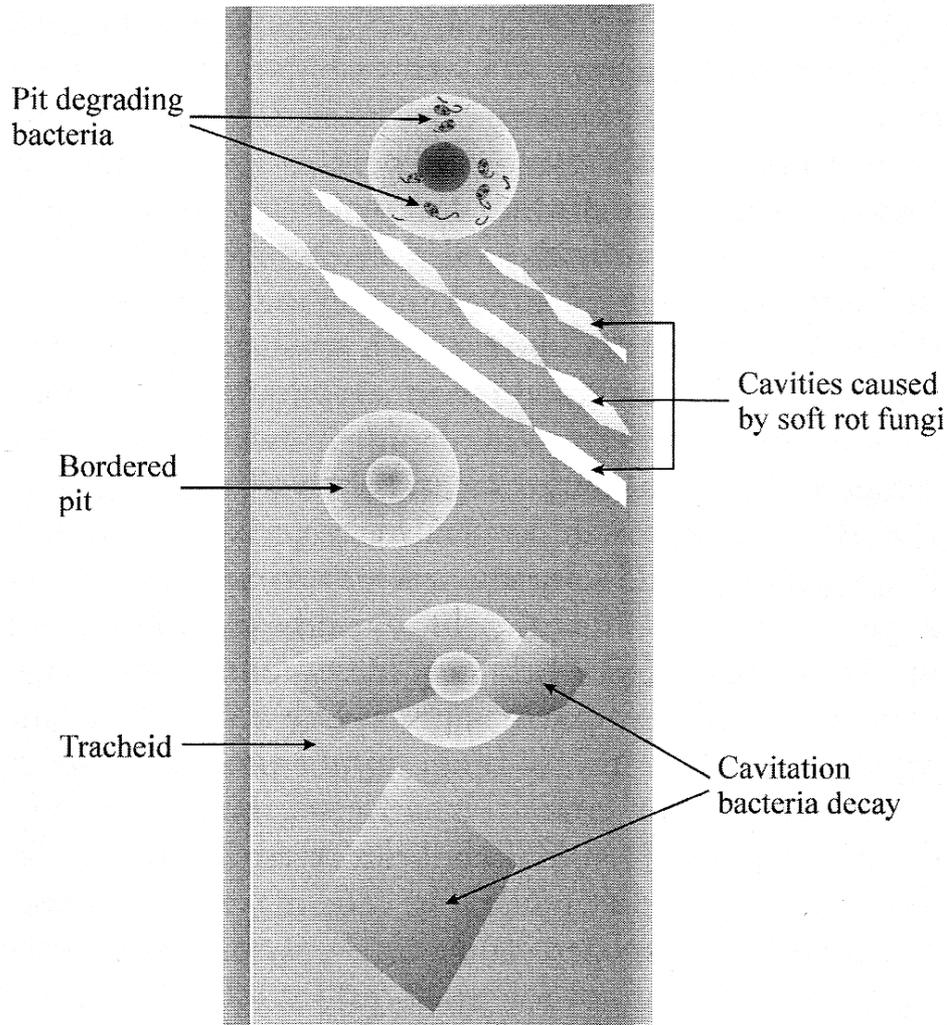


Soft-rot fungi

- Found in terrestrial and aquatic environments
- Like extreme environmental conditions
- Appears brown and soft
- Progressive from the edges to the center



Bacteria





Extracellular enzymes

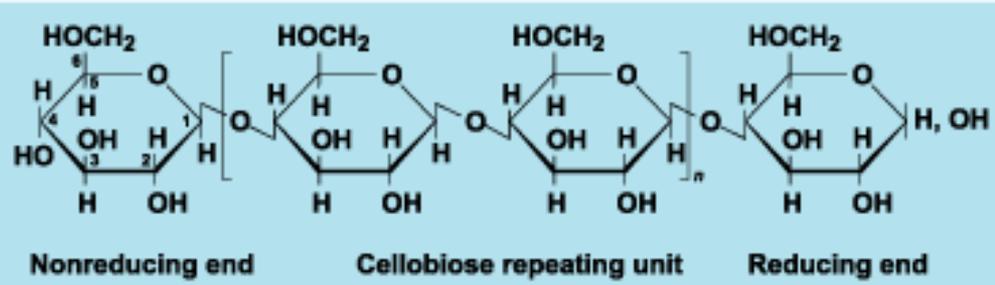
- Mycelium secretes extracellular enzymes and organic acids that break down lignin and cellulose.
 - Hydrolytic and oxidative enzymes
 - H_2O_2 producing oxidases
- Breakdown products (sugars) are taken up.
 - Note!: The breakdown of lignin does not produce utilizable carbon for the fungus.
The C-source are cellulose and hemicelluloses.



Hydrolytic enzymes (hydrolases)

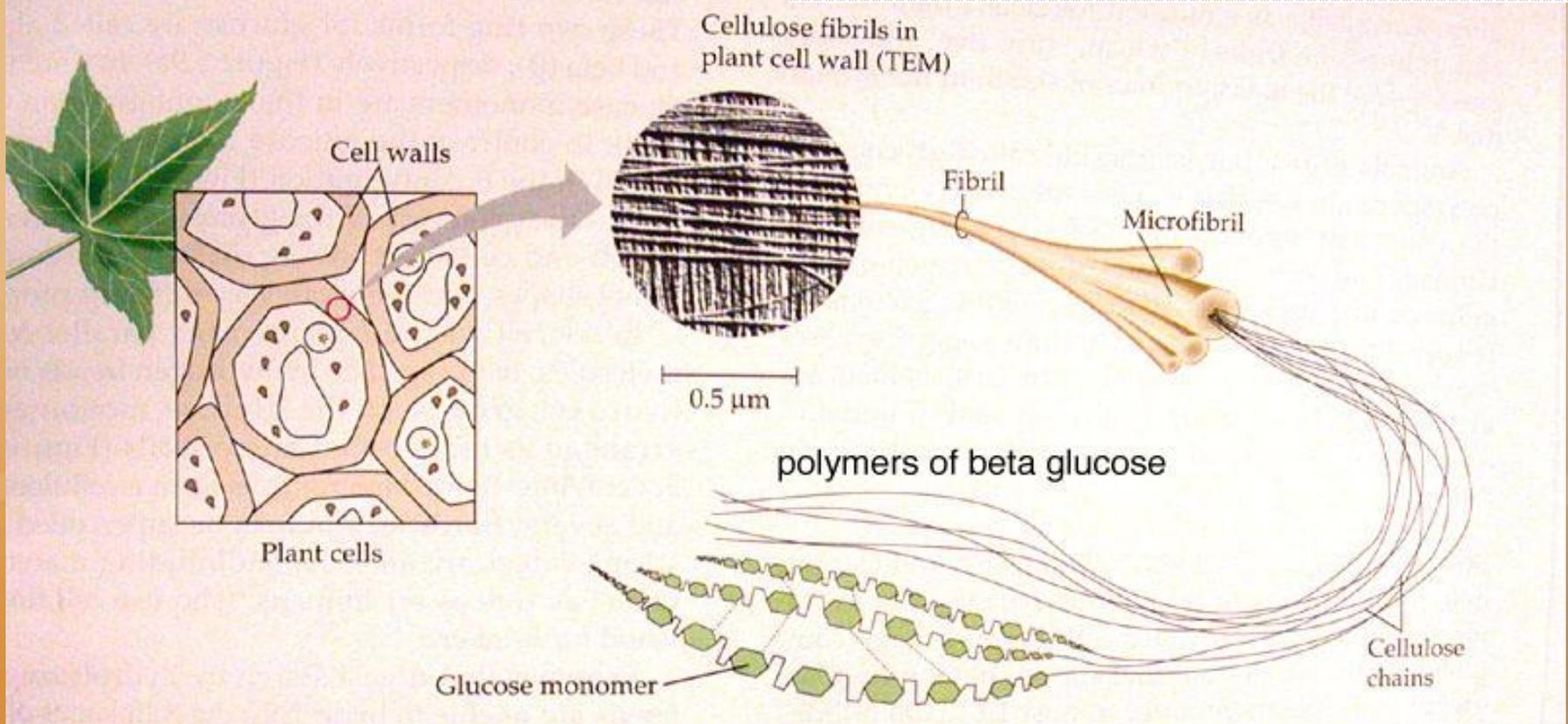
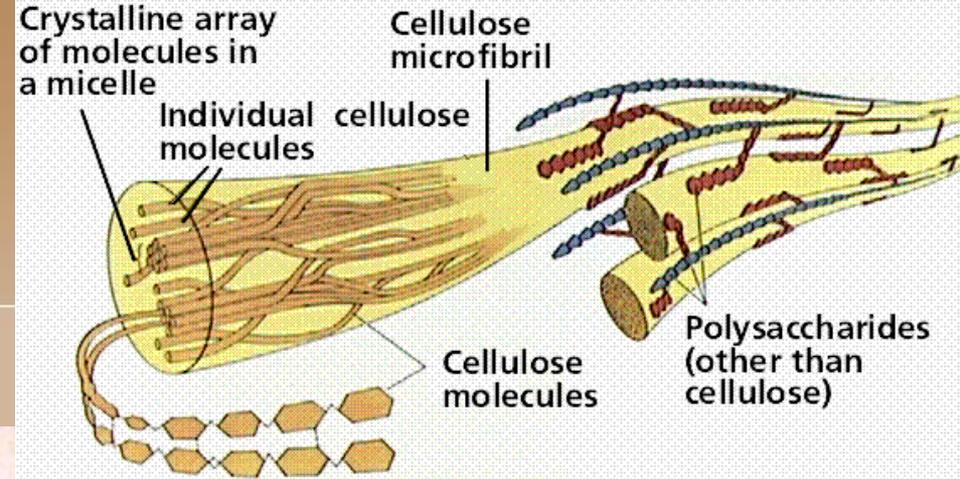
- Catalyze the hydrolysis of a variety of compounds like, proteins, nucleic acids, starch, fats, phosphate esters, and other macromolecular substances.
 - **Cellulase** is an enzyme complex which breaks down cellulose to beta-glucose.
 - **Hemicellulases** are enzymes that hydrolyzes hemicelluloses to smaller mono- or disaccharides.
 - Others: lipases, esterases, peptidases etc.

Cellulose



- A linear polymer of (beta 1-4)-linked beta-D-glucose units
- Cellulose is a straight chain polymer
- Most abundant of all naturally occurring substances.
- Cellulose constitutes approximately a third of all vegetable matter and thus it exists in far greater quantity than any other polysaccharide.

Cellulose



growth

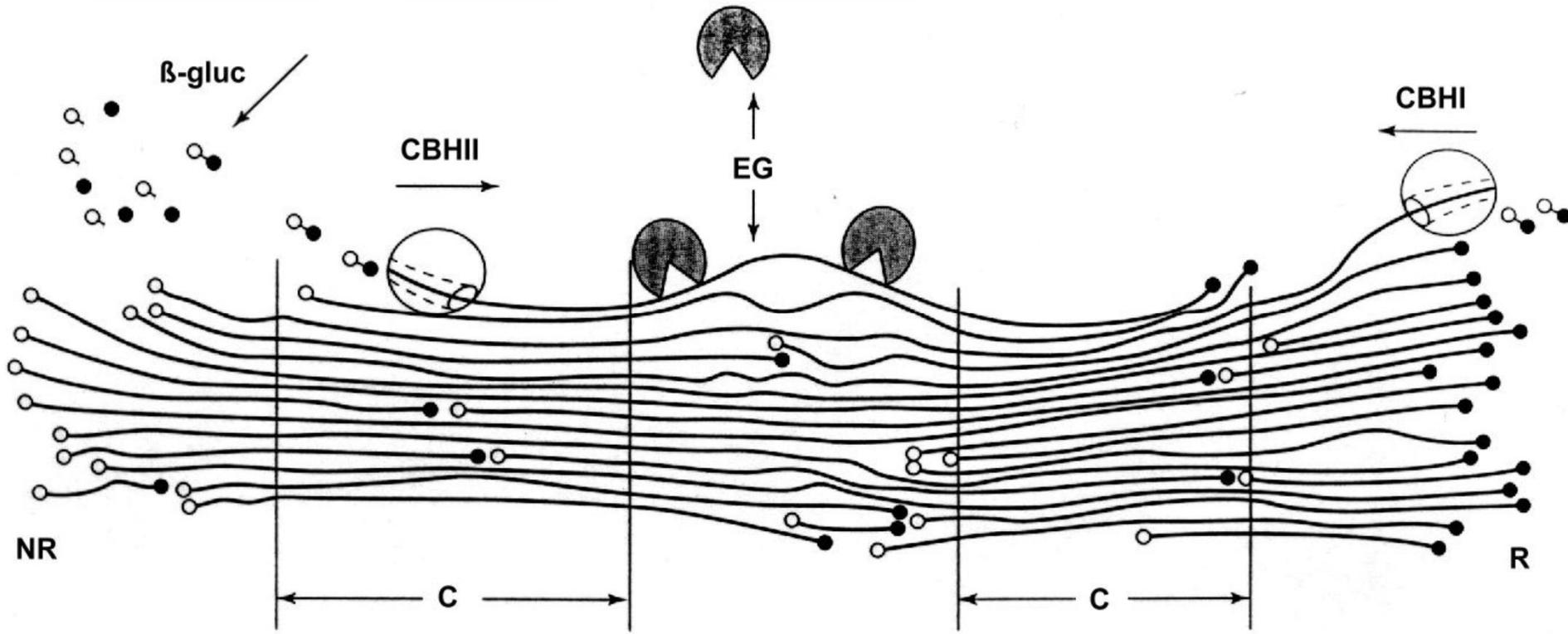
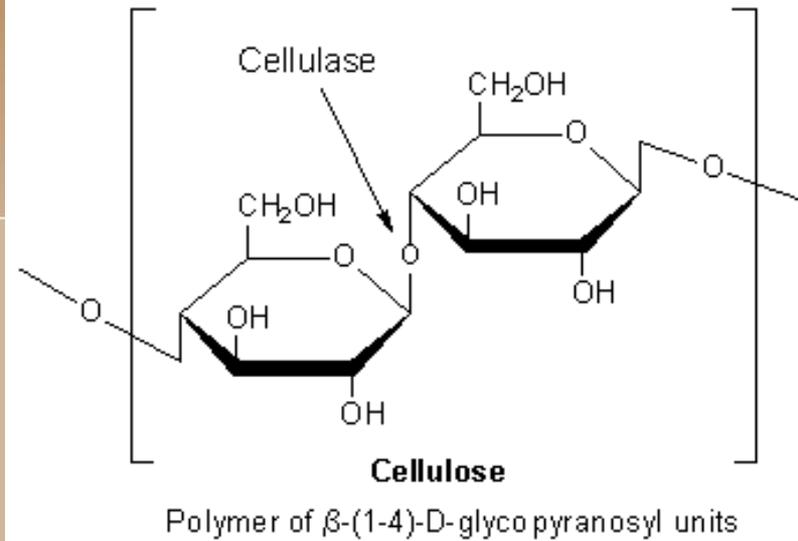
15% of cellulose has an amorphous structure



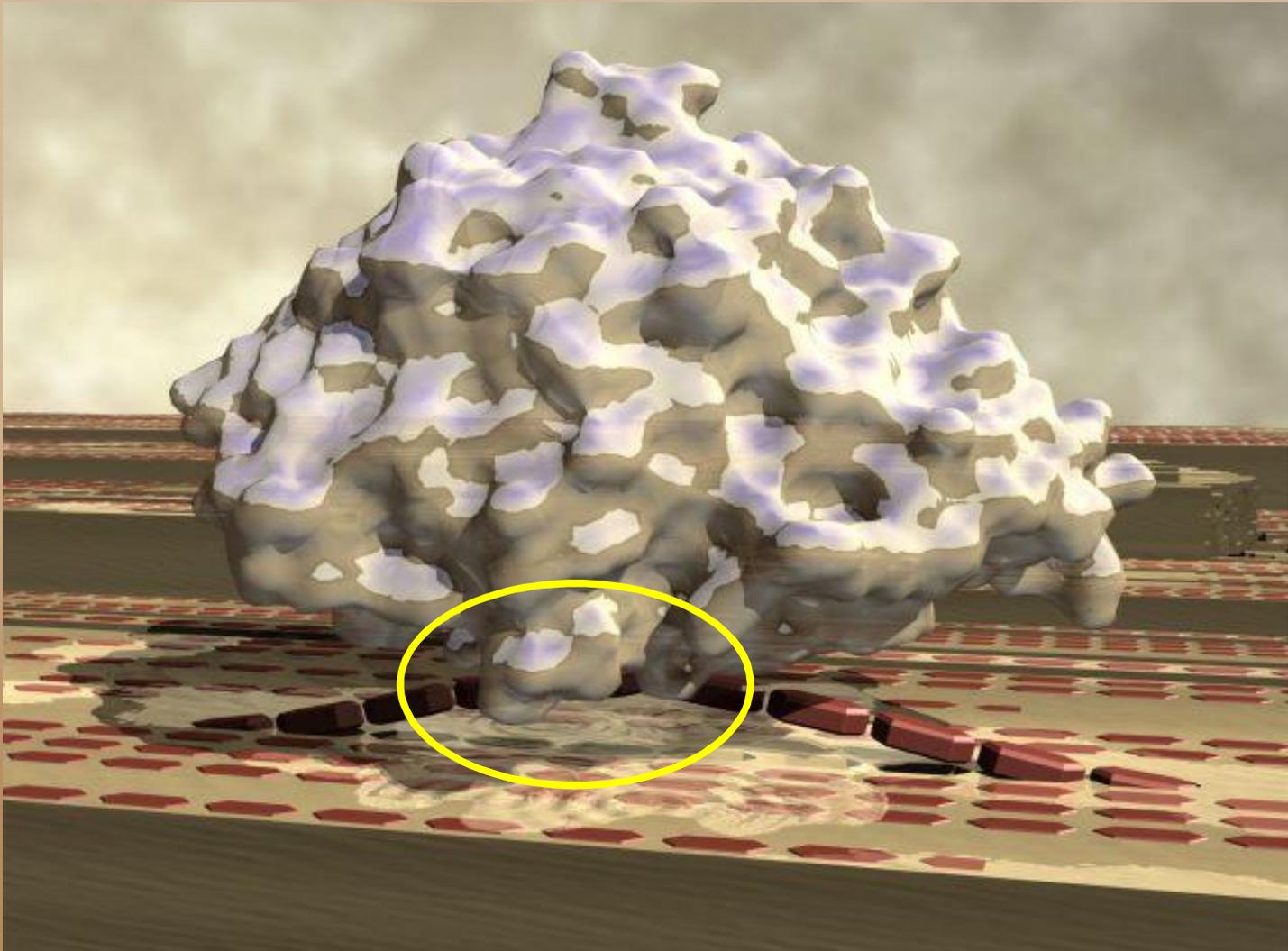
Cellulases (a complex)

- Cellobiohydrolase
- Endoglucanase
- Beta-glucosidase

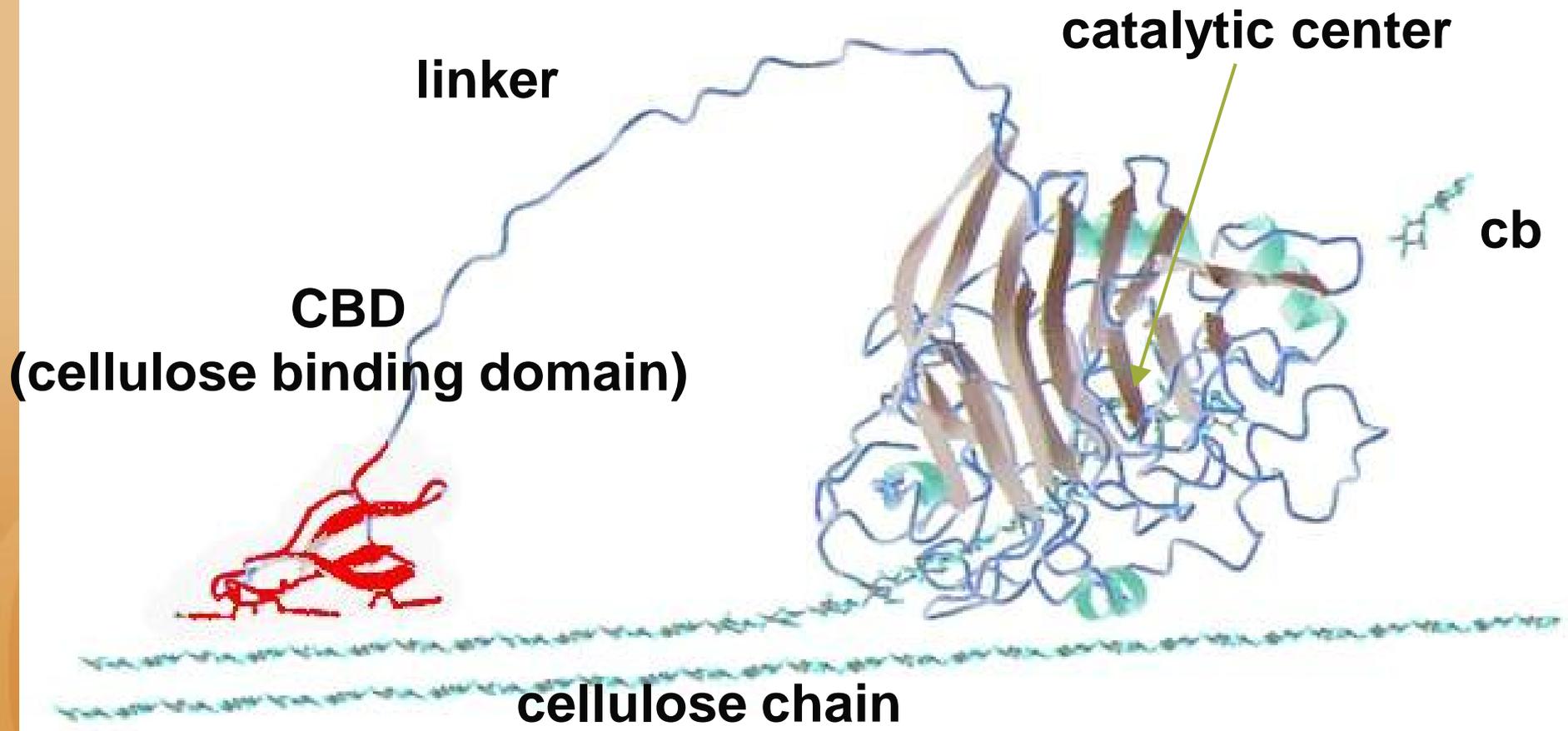
Cellulase Specificity



3D model of an endoglucanase



Cellobiohydrolase I acting on cellulose





Hemicellulose

- Any of several hetero-polymers (matrix polysaccharides) present in almost all cell walls along with cellulose.
- While cellulose is crystalline, strong, and resistant to hydrolysis, hemicellulose has a random, amorphous structure with little strength.
- Contains different sugar monomers, in contrast to cellulose which contains only glucose.
- Contain most of the D-pentose sugars and occasionally small amounts of L- sugars as well.
- Xylose is always the sugar monomer present in the largest amount, but mannose (mannuronic acid) and galactose (galacturonic acid) also tend to be present.

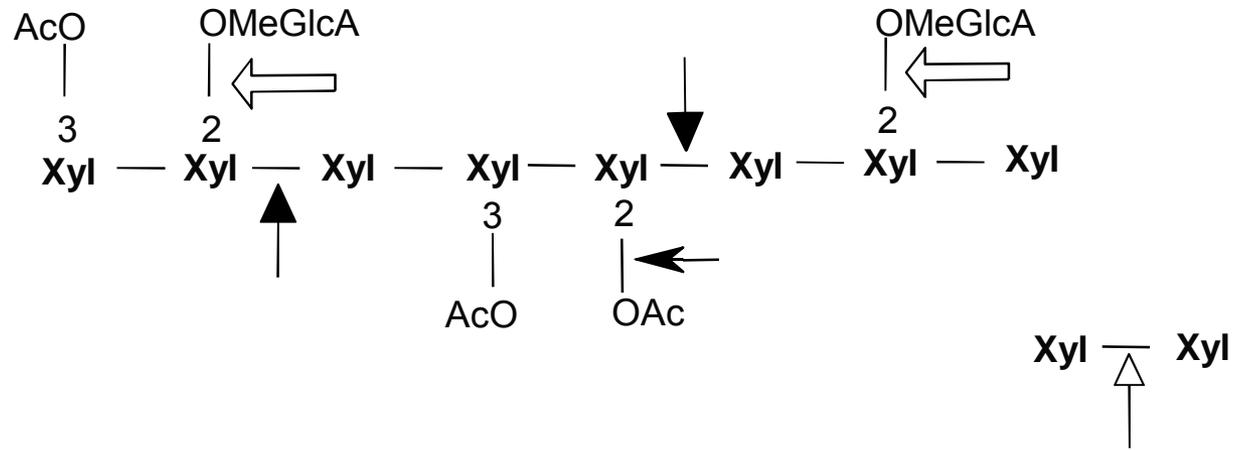


Hemicellulases

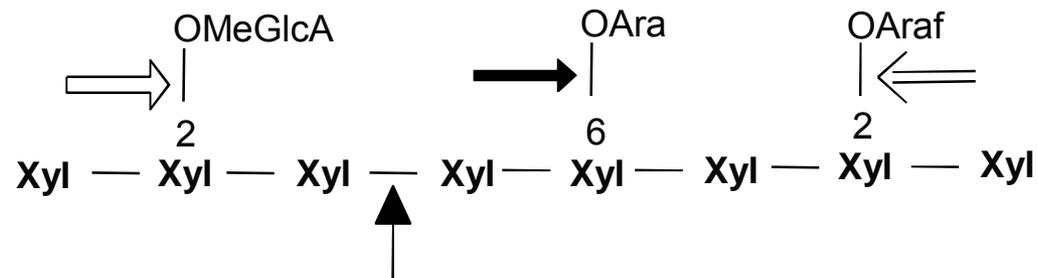
- Xylanase, mannanase, galactosidase and more
- Names are according to the sugars they release.
- There are more enzymes needed for the degradation of a hemicellulose than for a cellulose.

Fungal xylan-degrading system

Hardwood xylan



Softwood xylan



- | | | | | | |
|--|---------------------|--|-------------------------|--|-------------------------------|
| | β-xylanase | | α -glucuronidase | | α -arabinofuranosidase |
| | β-xylosidase | | α -arabinosidase | | acetylxylanesterase |

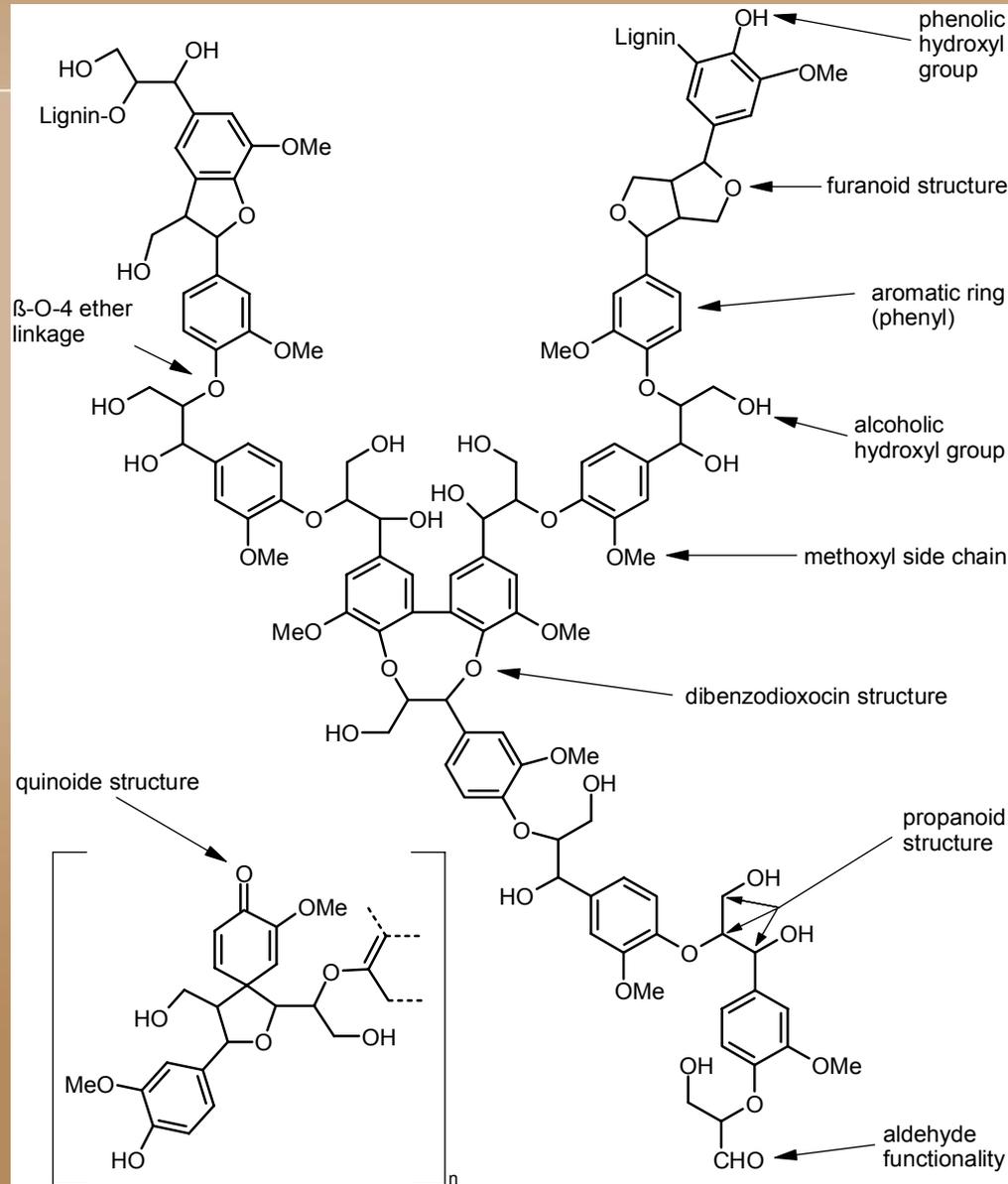


Lignin

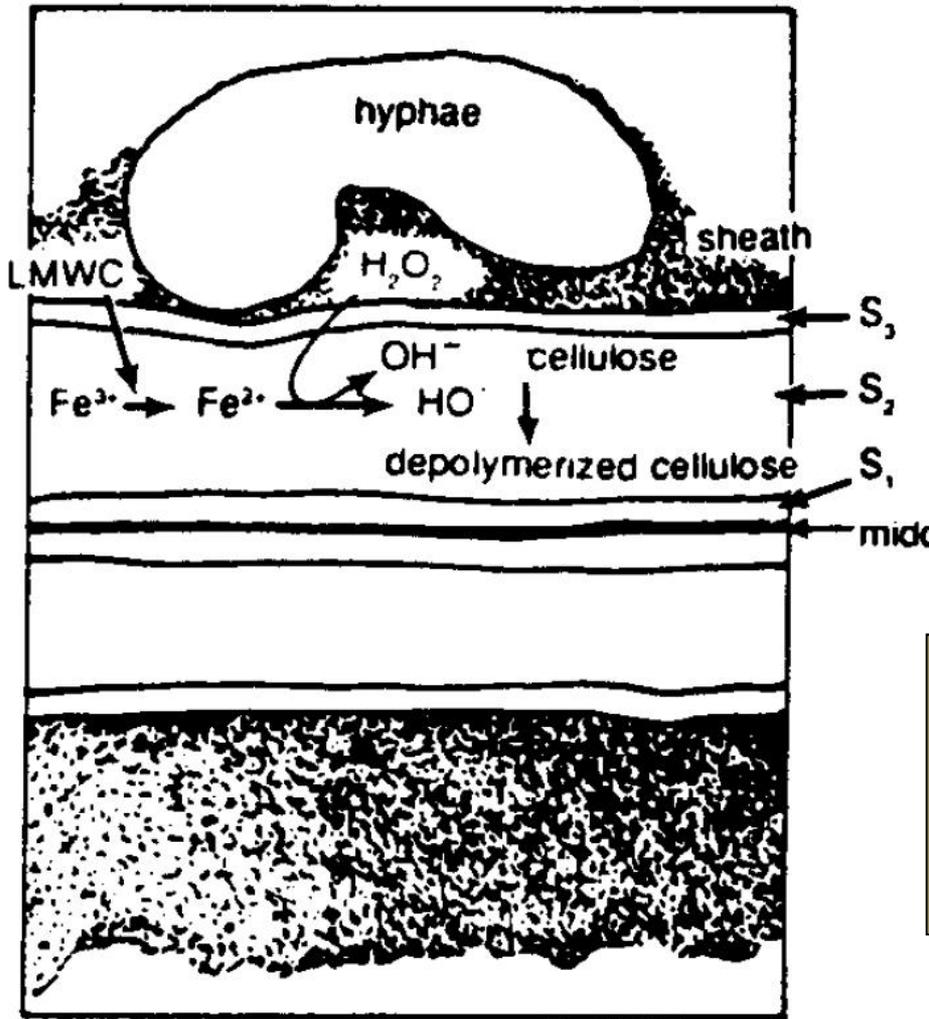
- A complex polymer, binds to cellulose fibers and hardens and strengthens the cell walls of plants.
- Lignin is a large, cross-linked macromolecule with molecular mass in excess of 10,000 Da.
- It is largely hydrophobic and aromatic in nature.
- The molecule consists of various types of substructures which appear to repeat in random manner.
- After cellulose, it is the most abundant organic material on Earth, making up one-fourth to one-third of the dry weight of wood.

The structure of lignin

- Amorphous 3-D polymer
- Precursors are coniferyl, sinapyl and p-coumaryl alcohol
- Guaiacyl, syringyl and p-OH-phenyl substructures
- Lignin contains a 8-ring dibenzodioxocin structure



The Fenton reaction



Fenton chemistry



Haber-Weiss reaction



This free radical would cleave long chain cellulose into smaller fragments.

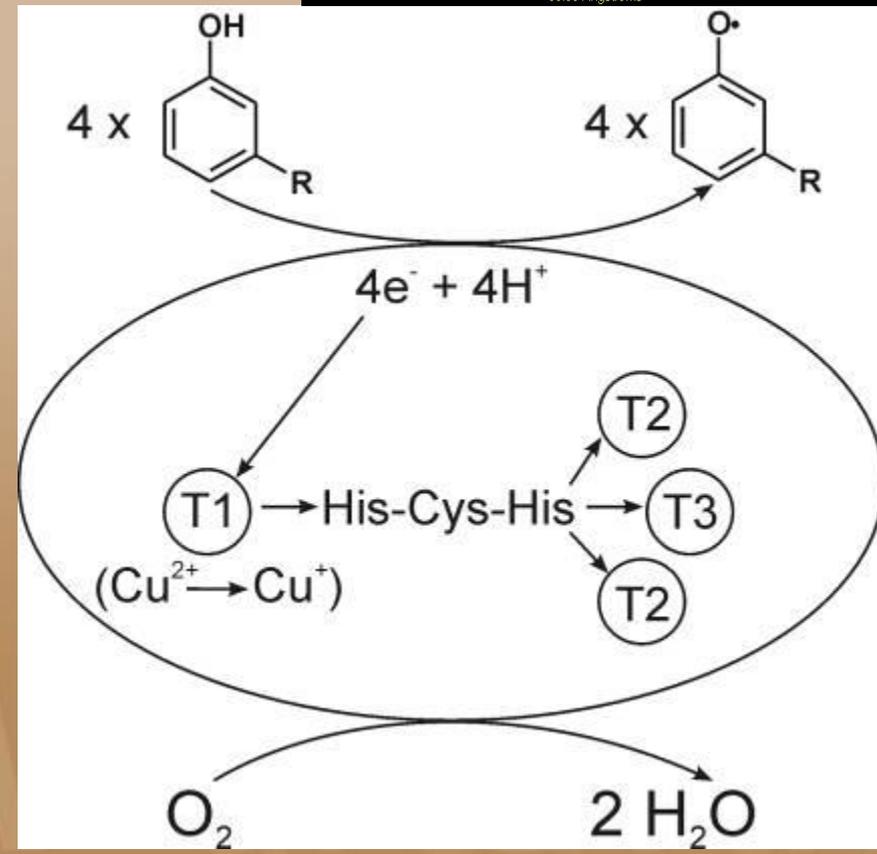
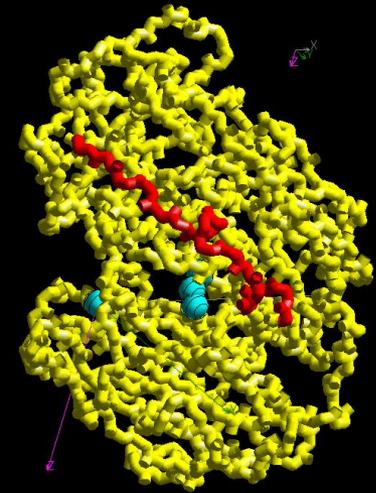


Oxidoreductases

- Phenol oxidase
 - Laccase
- Peroxidases
 - Manganese peroxidase
 - Lignin peroxidase
 - Independent peroxidase
 - Other peroxidases
 - Haloperoxidase
 - *Mycena* peroxidase

Laccase

- Copper containing oxidase found in many plants, fungi and microorganisms.
- Copper is usually bound in several sites, Type 1, Type 2, and Type 3.
- When types 2 and 3 are together, they form a trinuclear cluster.
- Enzymatically active on phenols and similar molecules and perform a one electron oxidation.





Manganese peroxidase (MnP)

- Described 1984 (Gold), 3 D structure 1990 's
- Resembles lignin peroxidase (LiP), protoporphyrin IX, MW 46.000 D
- Requires H_2O_2 , MnII, organic acids, e.g. malonate, lactate, oxalate (stabilizes Mn(III)-complex)
- White-rot fungi produce organic acids
- MnP oxidizes phenol red, o-dianisidine, polymeric dyes and more

Manganese binding site of MnP

MnP
blue
residues

LiP red
residues

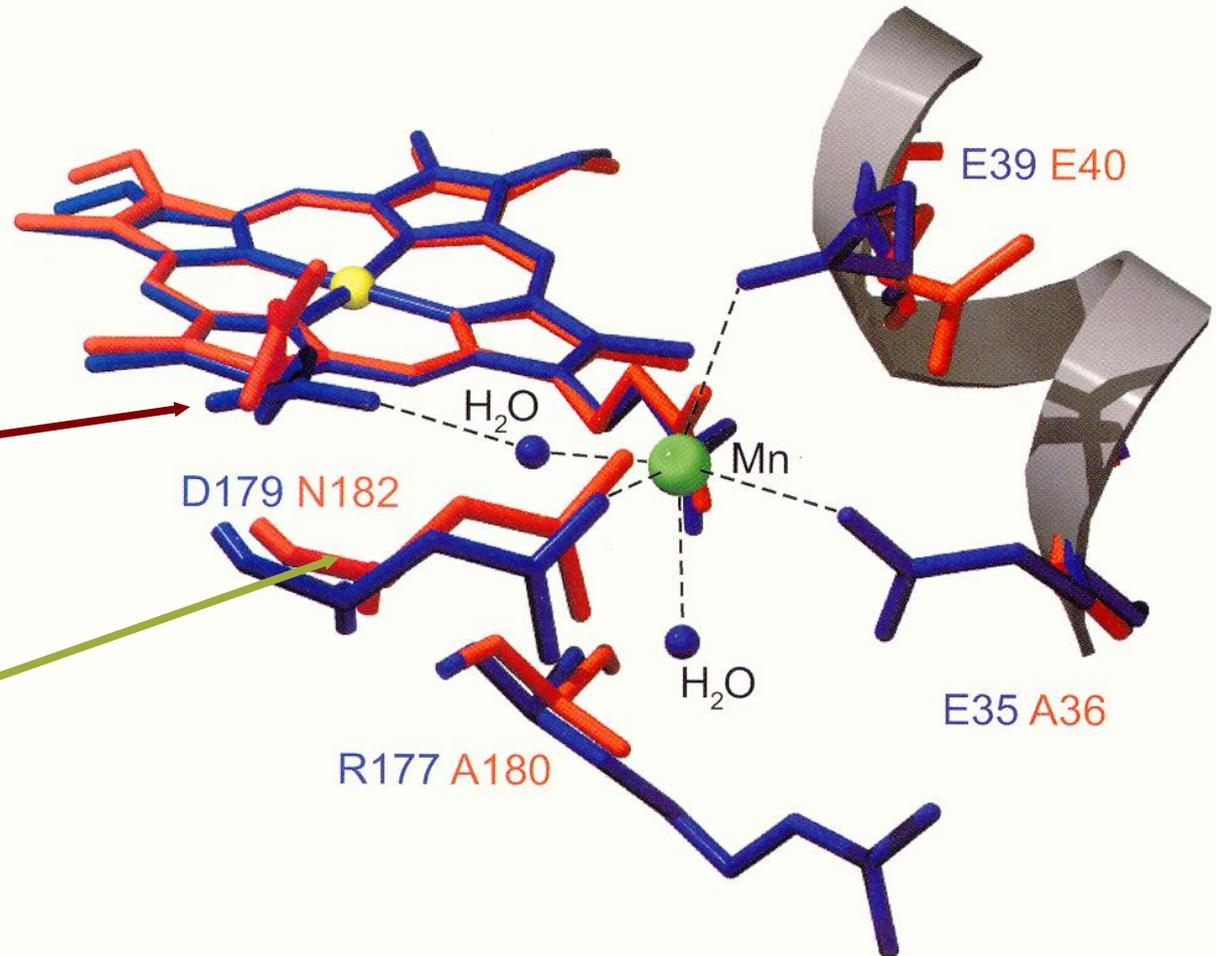
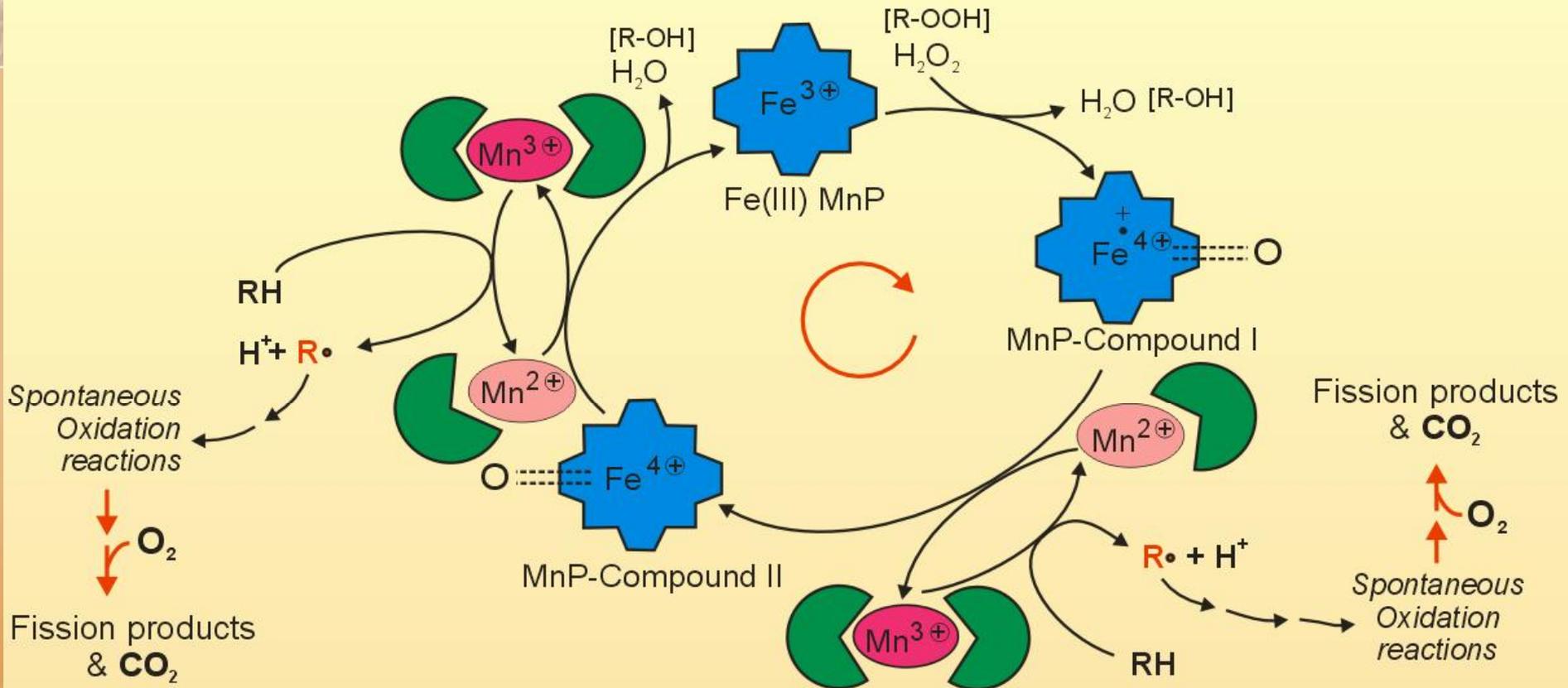


FIG. 5. The Mn^{II} binding site from the crystal structure of MnP (6) superimposed on the same region of the crystal structure of LiPH8 (7). The number and identity of MnP amino acid ligands are shown in blue, and the number and identity of LiPH8 residues are shown in red. Sequence comparison shows that these residues in LiPH8 are conserved in LiPH2. The heme, Mn atom, and two water ligands are also shown.

(Sollewijn Gelpke et al. (2000)
ABB 381:16-24)

Catalytic Cycle of Manganese Peroxidase (EC 1.11.1.13)

(GLENN & GOLD 1984; GOLD et al. 1989; WARIISHI et al. 1992; KISHI et al. 1994; HOFRIKTER et al. 2001)



RH - aromatic or aliphatic substrates (e.g. Lignin, humic substances)

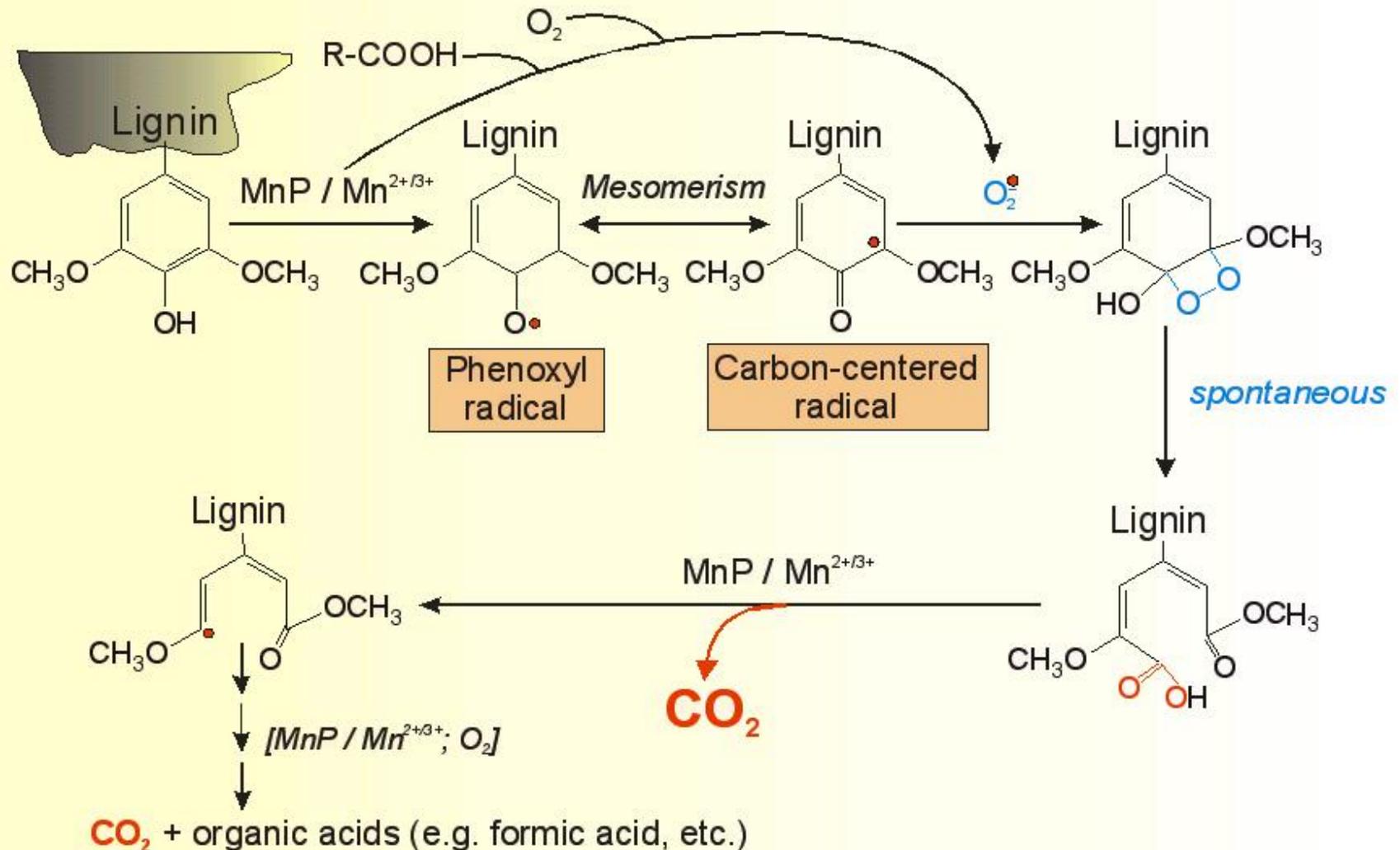


Chelators
(e.g. oxalate, malonate, malate)



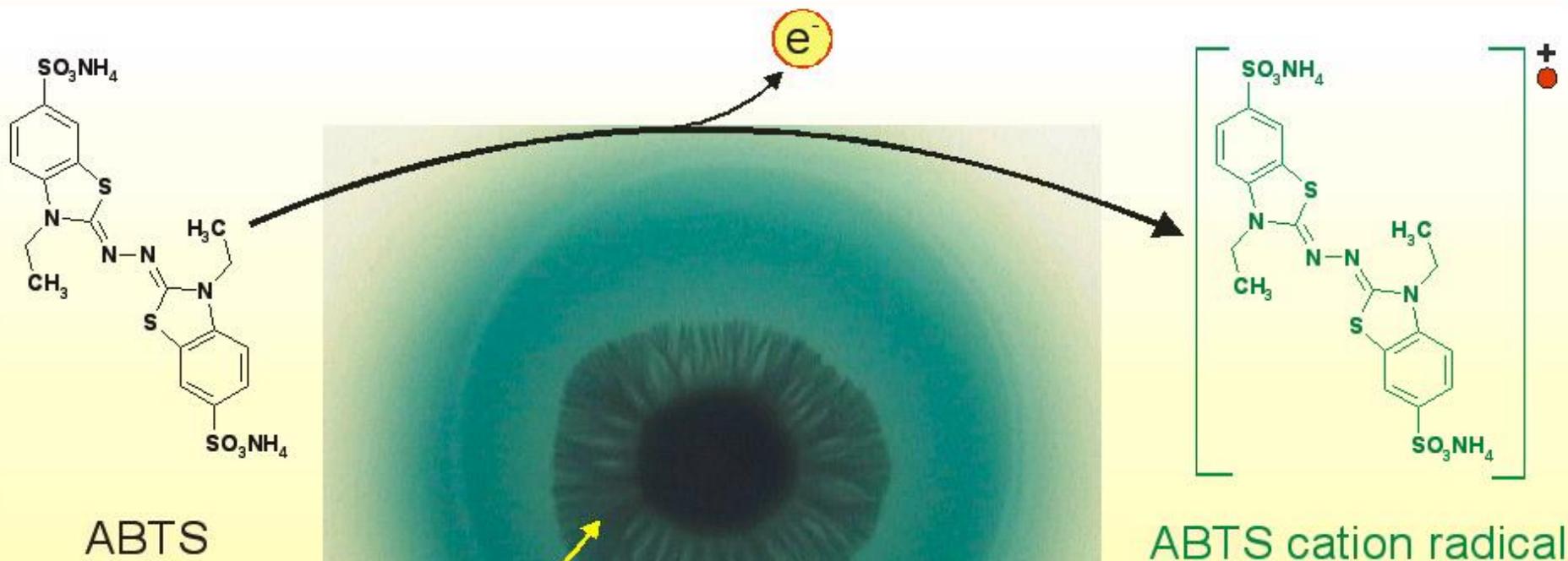
Heme in the catalytic center of Mn-Peroxidase

Hypothetical mechanism of manganese peroxidase-catalyzed ring-cleavage and mineralization of aromatic lignin structures



Oxidation of ABTS by extracellular fungal oxidoreductases

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt



Magnified ABTS-agar plate inoculated with the litter decomposer *Agrocybe praecox*

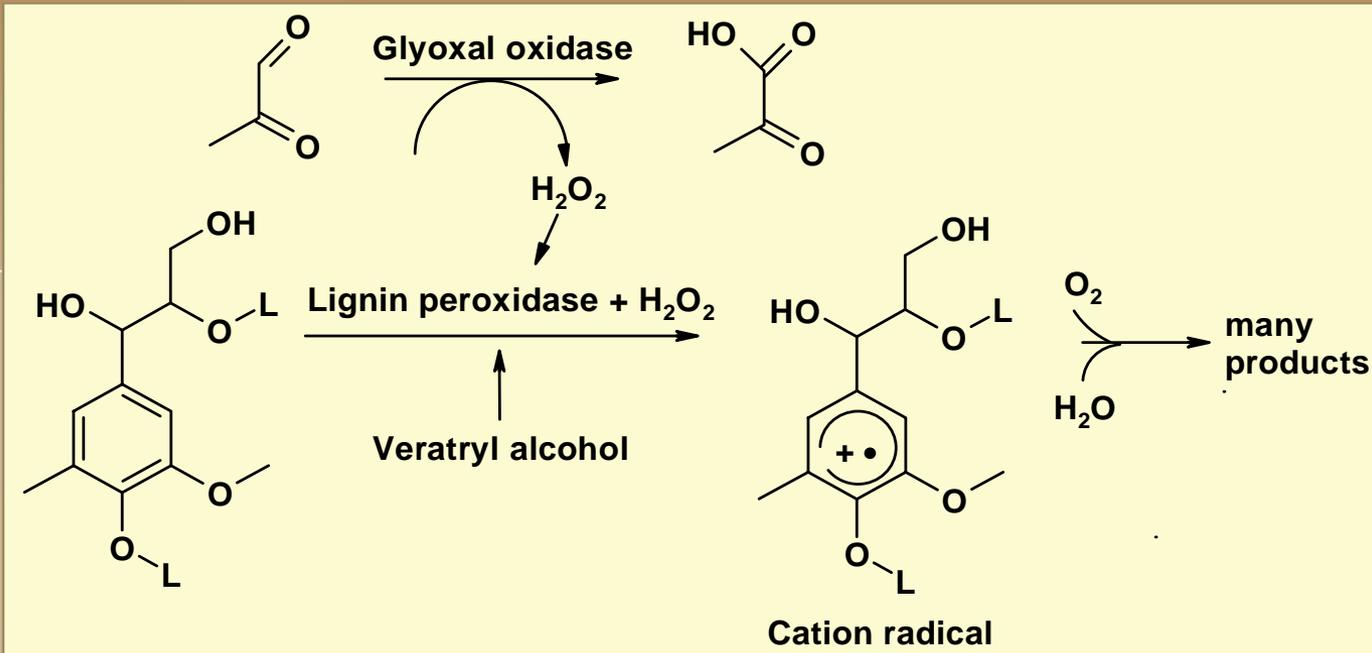


Lignin peroxidase (LiP)

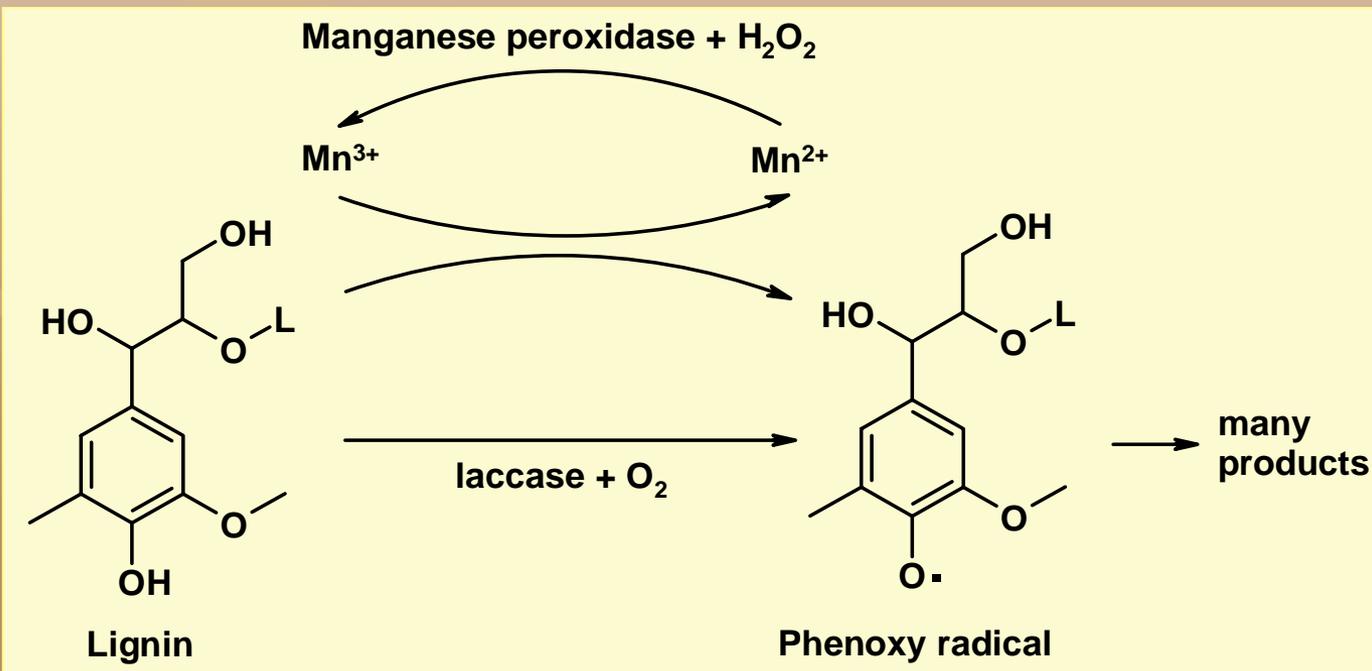
- Discovered 1982, published 1993 (Kirk, Gold), *Phanerochaete chrysosporium*
- Hemeprotein (protoporphyrin IX), MW 40.000 d
- Glycoprotein
- 3 D structure is known
- Optimum-pH 2.5-3, pI 3.3-4.7
- *P. chrysosporium* produces LiP during secondary metabolism
- Several isoforms known (6-15) *P. chrysosporium*
- Described from several fungi: *Phlebia radiata*, *Trametes versicolor*, *Agrocybe praecox*



Reactions of LiP



Reactions of MnP and laccase





Other peroxidases

- Mn-independent peroxidase
 - *Bjerkandera adusta*, *Pleurotus spp.*
- Haloperoxidase
 - *Agrocybe aegerita*
- *Mycena* peroxidase
 - Possible hybrid of LiP and horseradish peroxidase

wood-rotting types

white-rot



- **Basidiomycetes**
- Lignin- ja hemicellulose degradation
- Manganeseperoxidase
- *Trametes, Ceriporiopsis, Phanerochaete, Fomes*



brown-rot



- **Basidiomycetes**
- Cellulose degradation
- Hydroxyl radicals!
- *Serpula, Gloeophyllum, Laetiporus*

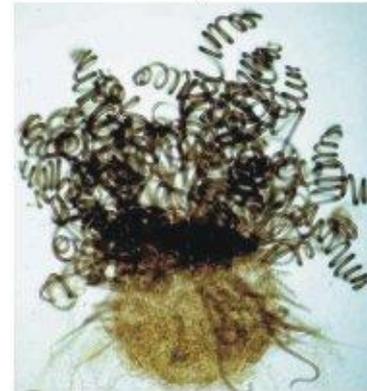


soft-rot



type 1

- **Deutero/Ascomycetes**
- Cellulose- and hemicellulose degradation
- Hydrolases
- *Trichoderma, Chaetomium*



type 2

- **Ascomycetes**
- Cellulose-, hemicellulose and lignin degradation
- Hydrolases, ox. enz.?
- *Xylaria, Hypoxylon, Daldinia*

