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Biomimetic pretreatment – a new approach to increase lignocellulose accessibility for enzymes

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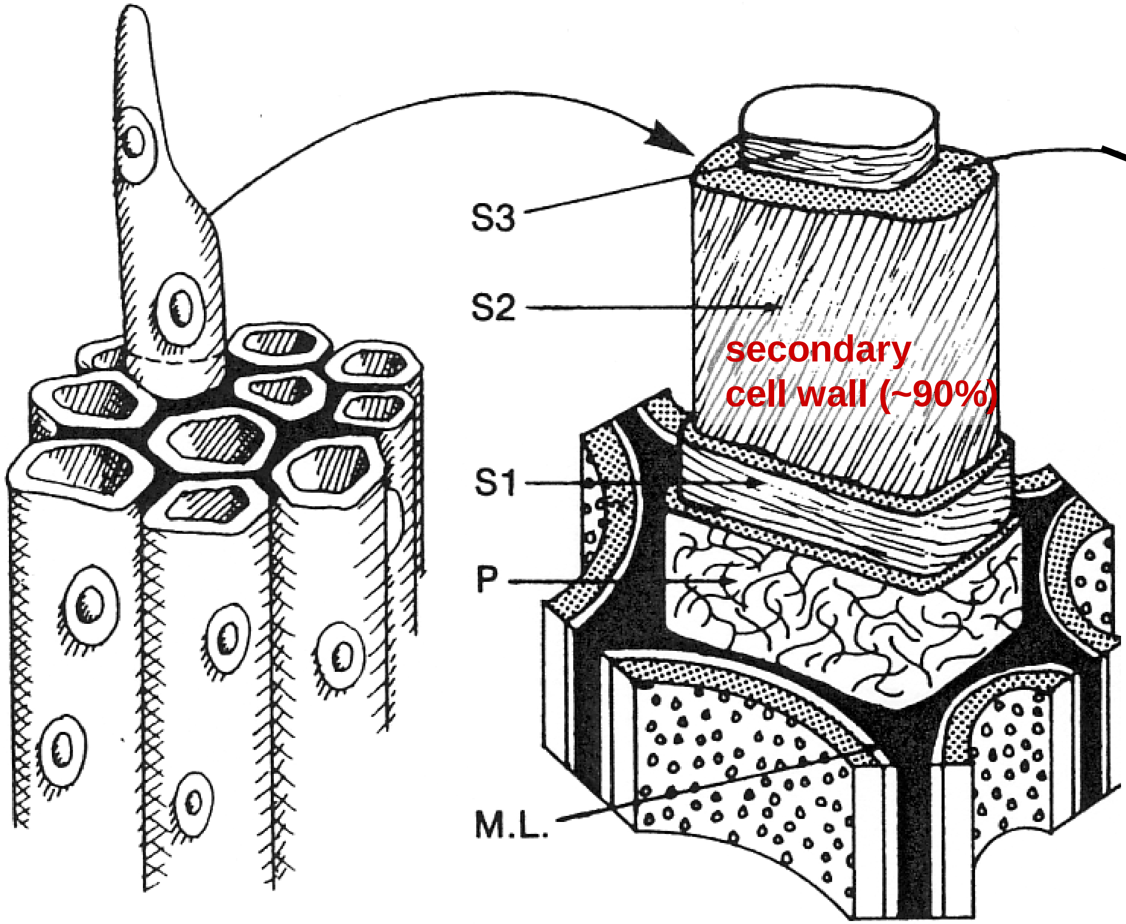
Biomimetics

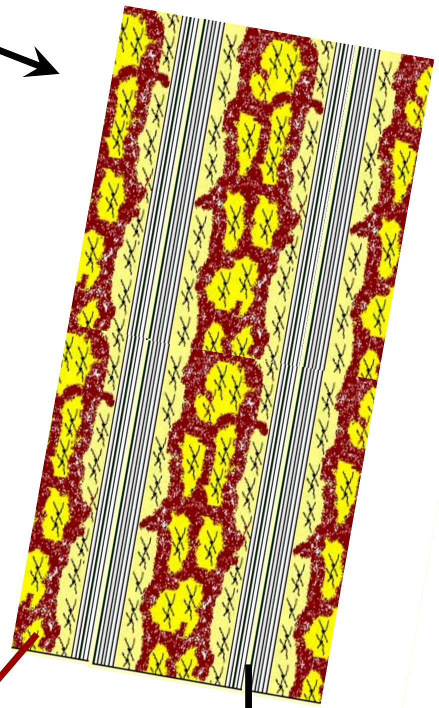
- ***Application of a basic principle of a biological system to technology***

- ***Application of the wood degrading molecular mechanisms of wood decay fungi to the technological pretreatment of lignocellulosic materials***

Wood: Ultrastructural prerequisites



S2 - wood cell wall
[Salmén; Olsson, 1998]



Hemicelluloses
(Glucomannan, Xylan)
+ Lignin

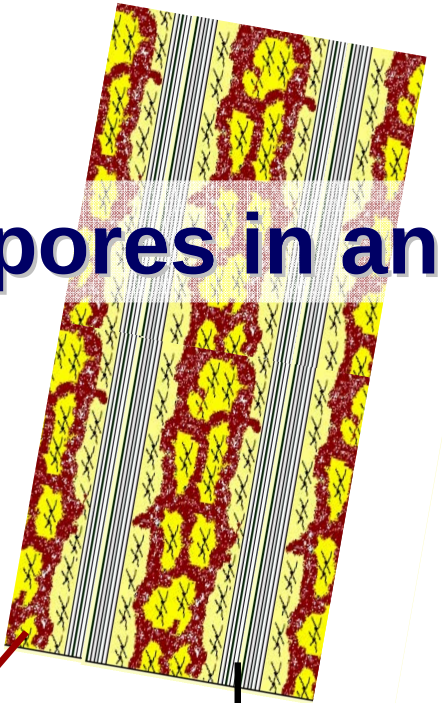
Cellulose fibril

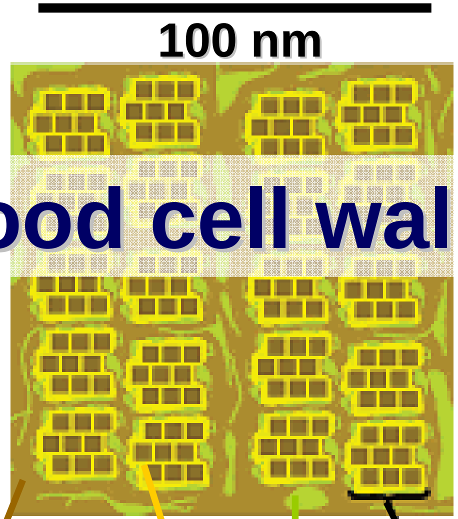
Wood: Ultrastructural prerequisites

S2 – longitudinal modelS2 – cross sectional model (AFM

[Salmén; Olsson, 1998]

[Fahlén, Salmén, 2005]





No pores in an intact S2 wood cell wall laye

Hemicelluloses (Glucomannan, Xylan) + Lignin

Cellulose fibrils

Lignin

Xylan

Cellulose fibril aggregate

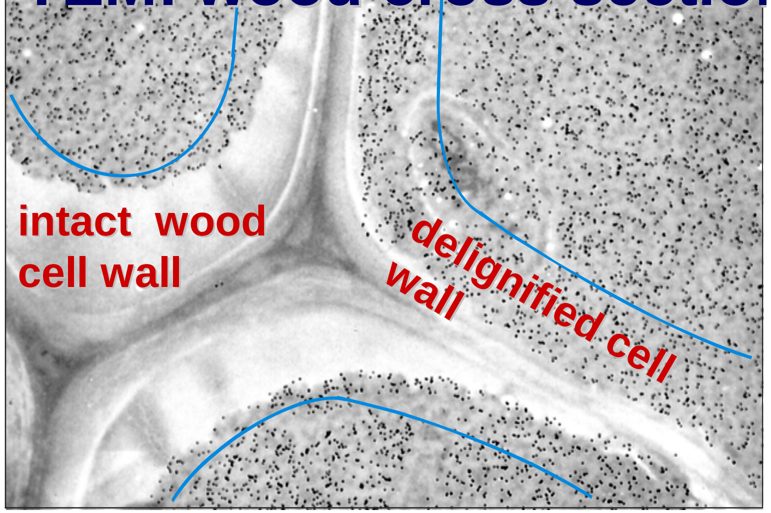
Glucomannan

Wood: Ultrastructural prerequisites

- Intact secondary wood cell walls are inaccessible to molecules larger than 5 kDa
- Lignin incrustation of wood polysaccharides is the main problem for enzymatic hydrolysis of sound wood by cellulase and hemicellulase enzymes

- Wood decay fungi (white rot) delignify wood cell walls, increase their porosity, and make wood polysaccharides more accessible to their cellulolytic enzyme system

TEM: wood cross section

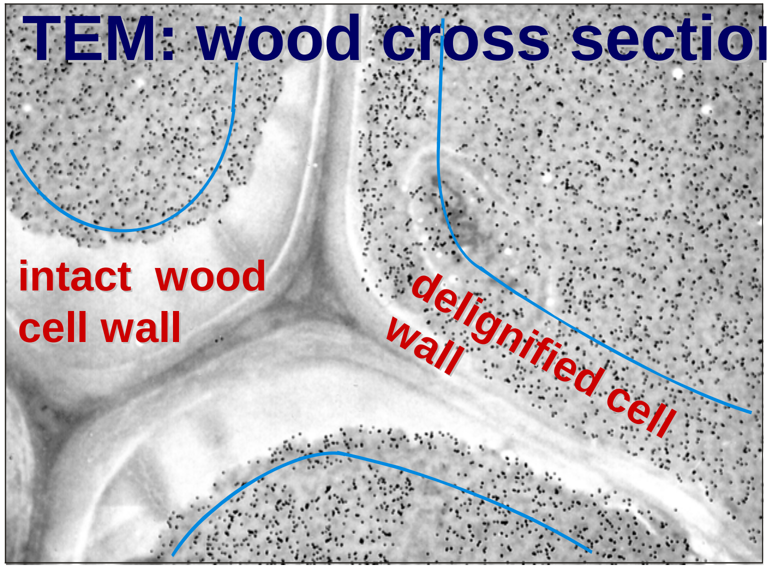


black dots: immunolabelled enzymes (40kDa)

Biomimetic Pretreatment

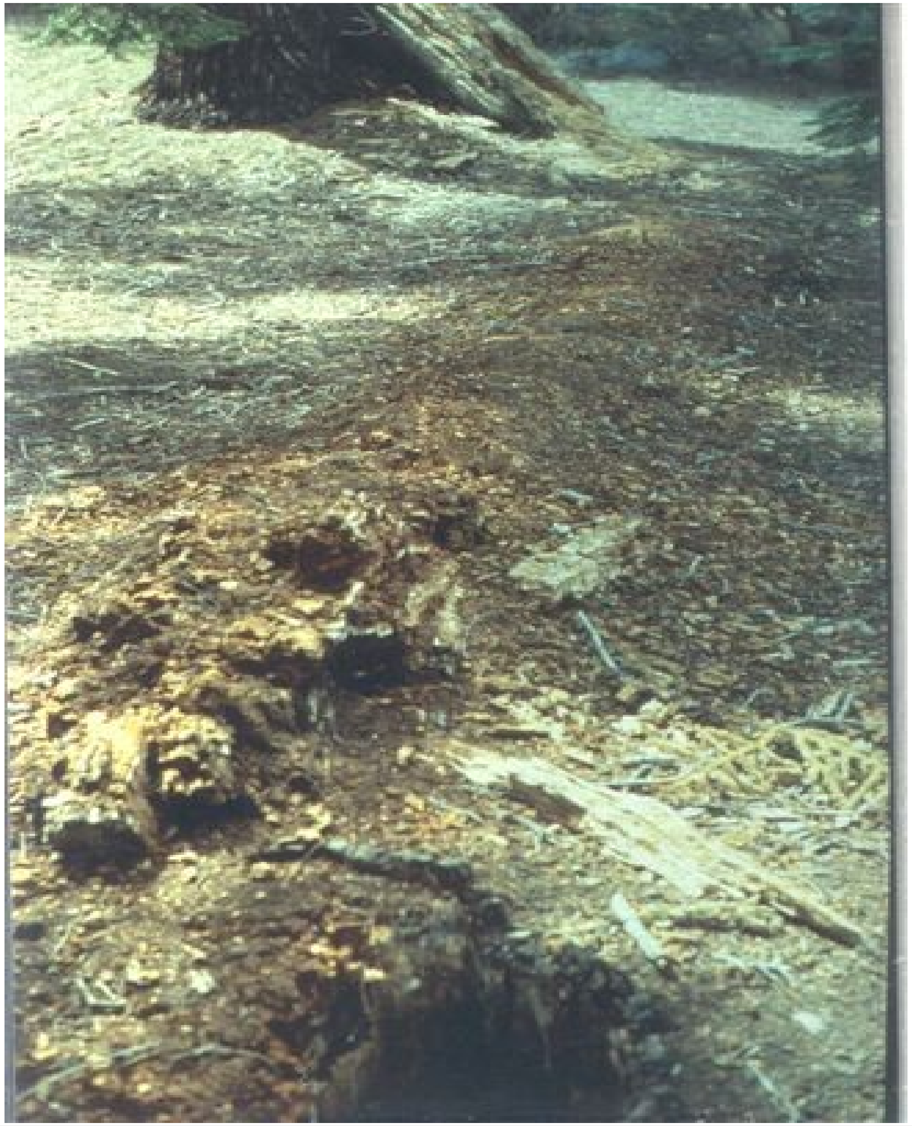
- **Increase the accessibility of wood polysaccharides by *biomimetic pretreatment***
- **Current pretreatment methods:**
  - **Harsh conditions: pressure, temperature, acids**
  - **Account for approx. 1/3 of the conversion costs (ethanol)**

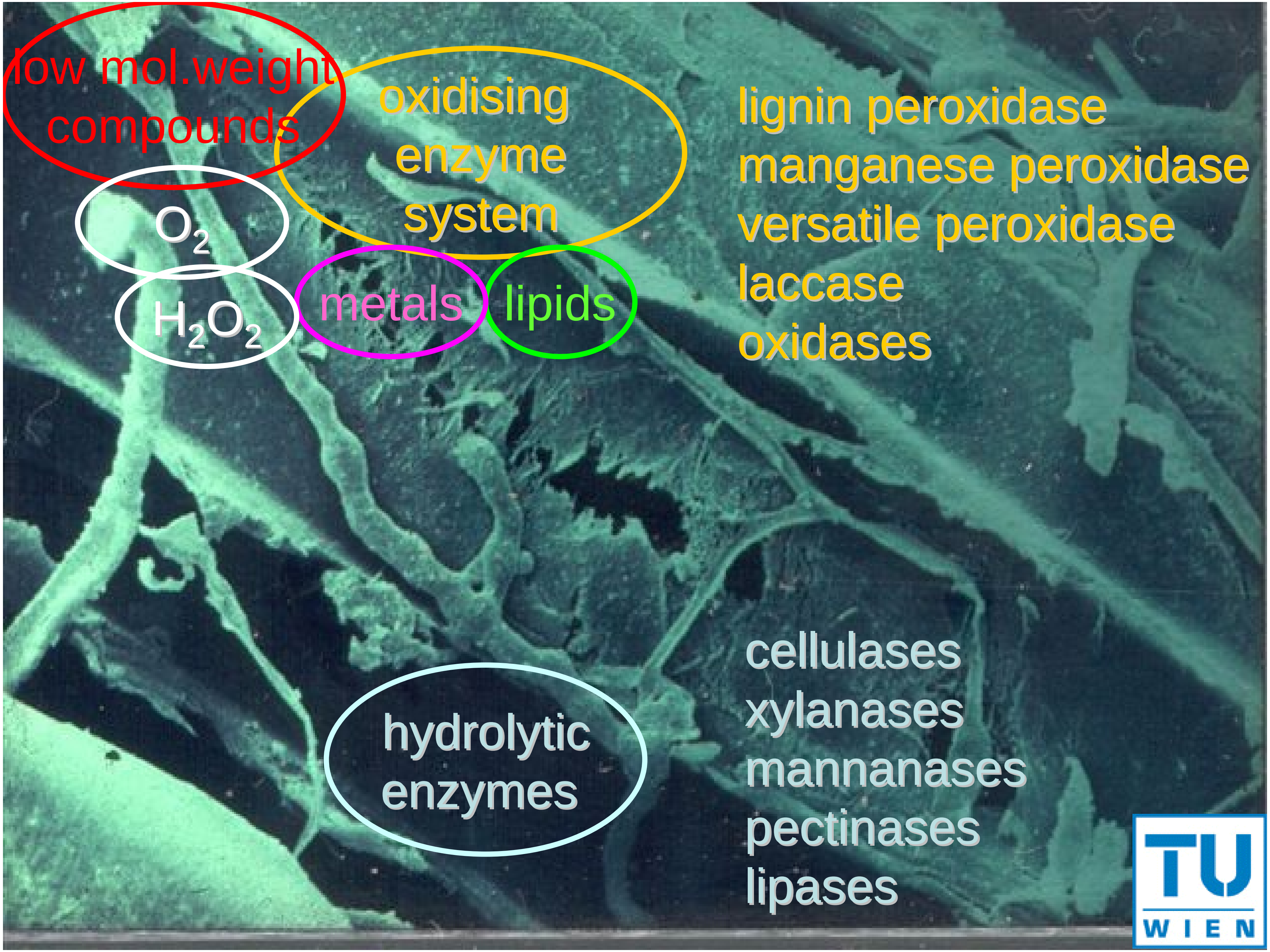
- **Wood decay fungi use mild conditions to break up the wood ultrastructure**
- **Wood decay fungi are models for pretreatment processes**



black dots: immunolabelled enzyme

Fungal wood biodegradation




low mol.weight compounds
oxidising enzyme system
lignin peroxidase
manganese peroxidase
versatile peroxidase
laccase
oxidases
O_2
H_2O_2
metals
lipids
hydrolytic enzymes
cellulases
xylanases
mannanases
pectinases
lipases

Low molecular weight compounds

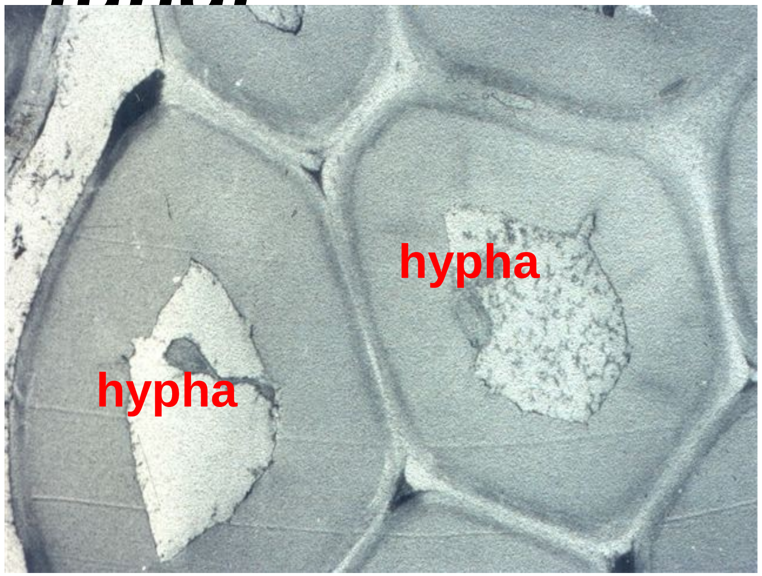
- **Fungal enzymes are too large to diffuse within intact wood cell walls**

- **The depolymersation system of wood degrading fungi must consist also of diffusible *low molecular weight compounds***

- **These systems cause radical formation and depolymerisation of wood polymers within the whole wood cell wall**

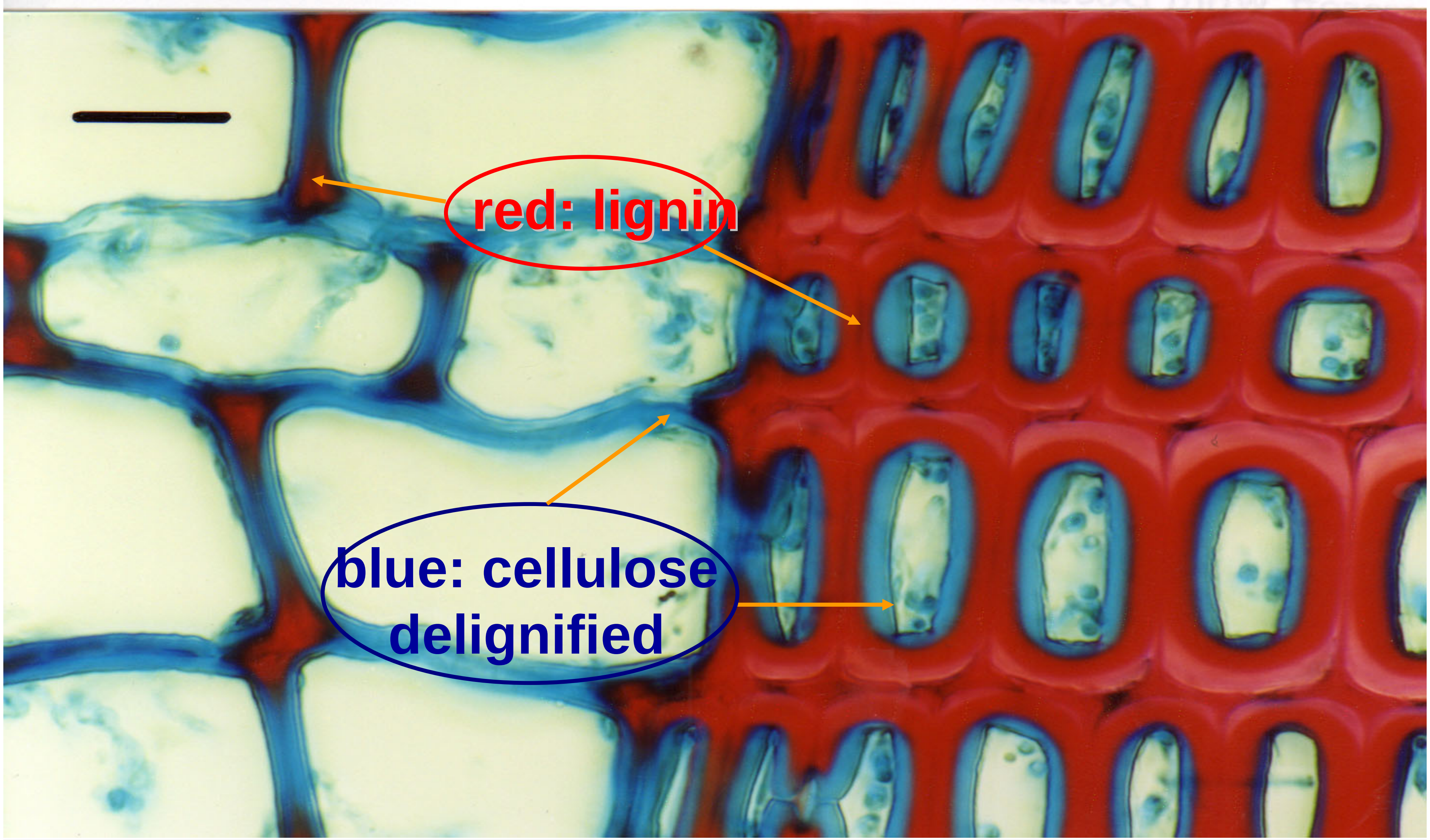
Decay features attractive for pretreatment

- *Selective white rot fungi*



Lignin intensively degraded **Cellulose preserved**

Selective staining by Safranin/ Astra Blue



Proposed diffusible low molecular weight systems

- ***Manganese Peroxidase/ H_2O_2/ Mn^{2+}/lipids***
  - **Lipid peroxidation via fatty acid radicals**
- ***Laccase-mediator-system***
  - **Laccase, HBT, ABTS, molecular oxygen**
- ***Fenton chemistry***
  - **Iron-ions, H_2O_2**
  - **Hydroxyl radicals**

How to apply the diffusible system of selective white rot fungi for delignifying pretreatment?

- ***Whole fungal approach***
  - **Disadvantages: slow, often unstable**
- ***Apply fungal redox systems***
  - **Disadvantages: Mn-peroxidase is not produced commercially, T < 40°C**
- ***Biomimetic approach***
  - **Copper based catalytic depolymerisation system**

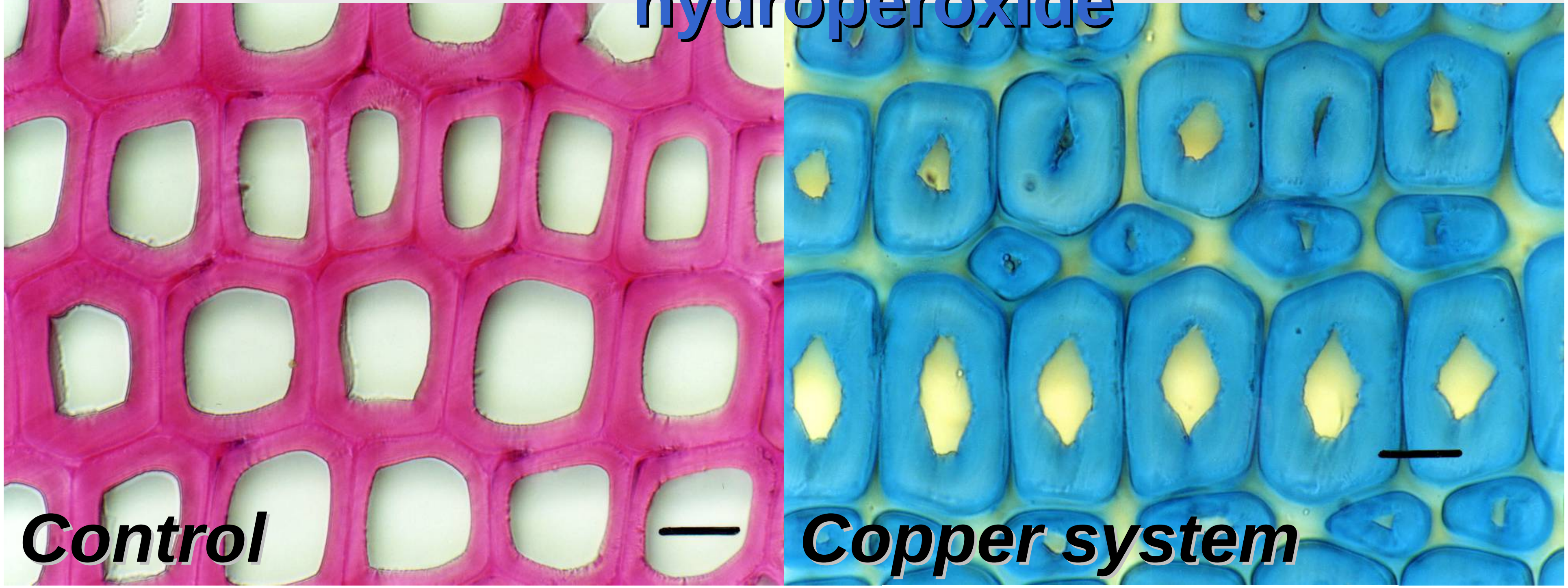
„ Copper system“

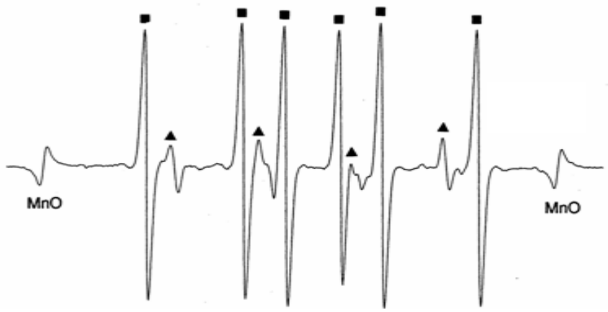
Biomimetic Copper System

- ***Copper complex***
  - **Copper(II)salt**
  - **Coordination compound (pyridine derivati**
  - ***Mimicks active centres of ligninolytic enzymes***
- ***Hydroperoxide***
  - **Hydrogen peroxide, t-Butylhydroperoxide**

- ***Catalyst*** **of radical mechanisms**
  - ***Mimicks oxidative mechanisms of wood biodegradation***
- ***Low molecular*** **(< 1kDa) → diffusible**
- ***Thermostable*** **(90°C)**
- ***Broad pH-range*** **(pH 6 -12)**

Delignification of spruce by biomimetic copper system with organic hydroperoxide

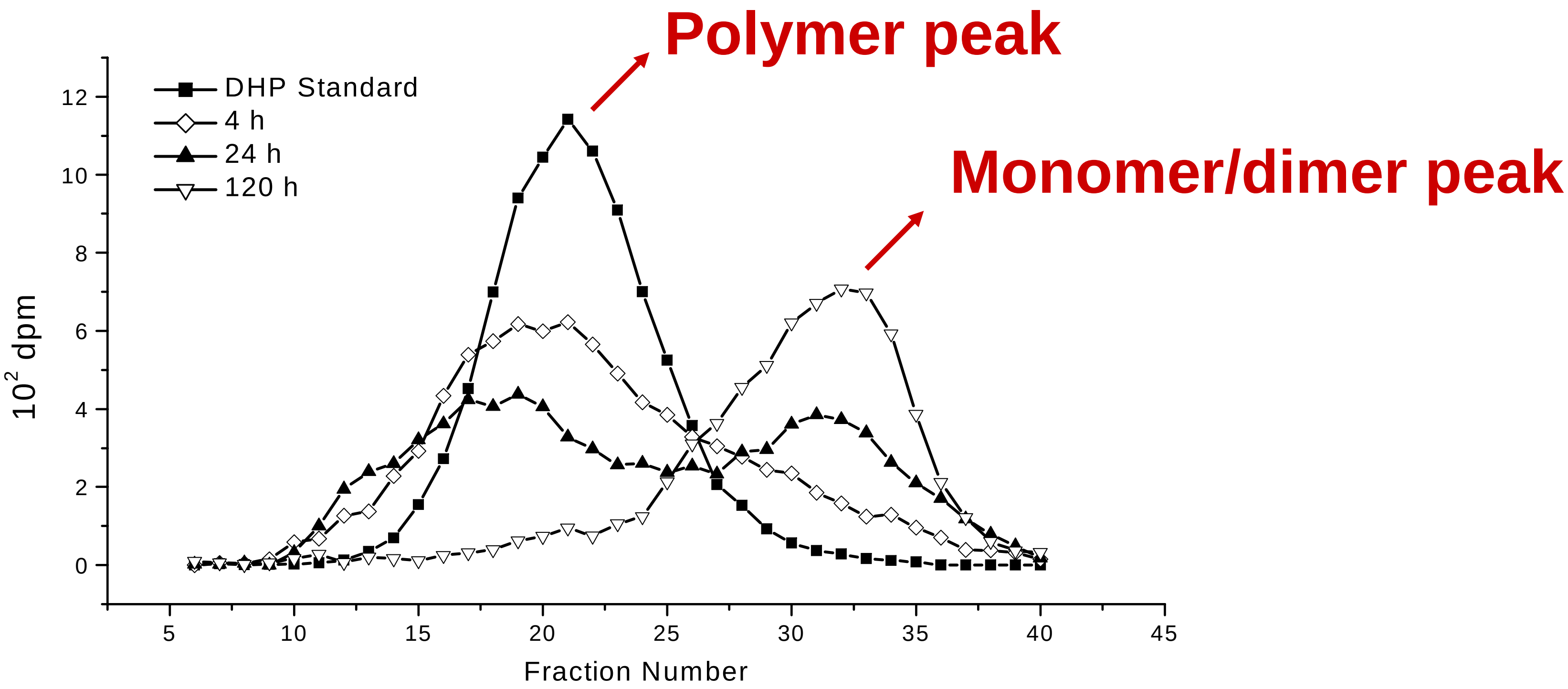




Oxidative free radicals monitored by

„Lipid peroxidation mechanism“

Depolymerisation of synthetic lignin with biomimetic copper system and H_2O_2



ɔothetic copper-peroxo-complex $[(Cu\text{-}O_2\text{-}Cu)py_n]^{2+}$ as active oxi

Peroxidase-like mechanism **via aromatic ring oxidation**

Aims

- **Development of catalytic biomimetic processes as pretreatment for lignocellulosic materials (wood, straw) to make them more accessible to hydrolytic enzymes (cellulases, hemicellulases)**

- **Development of catalytic biomimetic processes to convert the main components of lignocellulosic materials into value-added products (*biorefinery* concept)**

Main advantages of catalytic biomimetic systems

- **Temperature < 100°C**
  - Energy efficiency
  - Less toxic by-products expected
- **Atmospheric pressure**
  - Simple technology for local facilities
  - Low risk and time of process development
- **Near neutral pH**
  - Non-corrosive conditions
  - No recovery of bulk process chemicals (NaOH, $CaSO_4$)
- **High-value sulphur free lignin fraction**