New concepts and approaches in forest bioeconomy: extractives, innovative materials, biorefineries

Florian Zikeli, PhD
UNITUS Viterbo, DIBAF

Outline:

Energy sustanability politics

Biorefineries

Wood – ultrastructure and components

Lignin

Lignin nanotechnology


Decarbonizing the world economy will require renewable energy generation from vast solar farms, such as this one in Nevada.

Three years to safeguard our climate

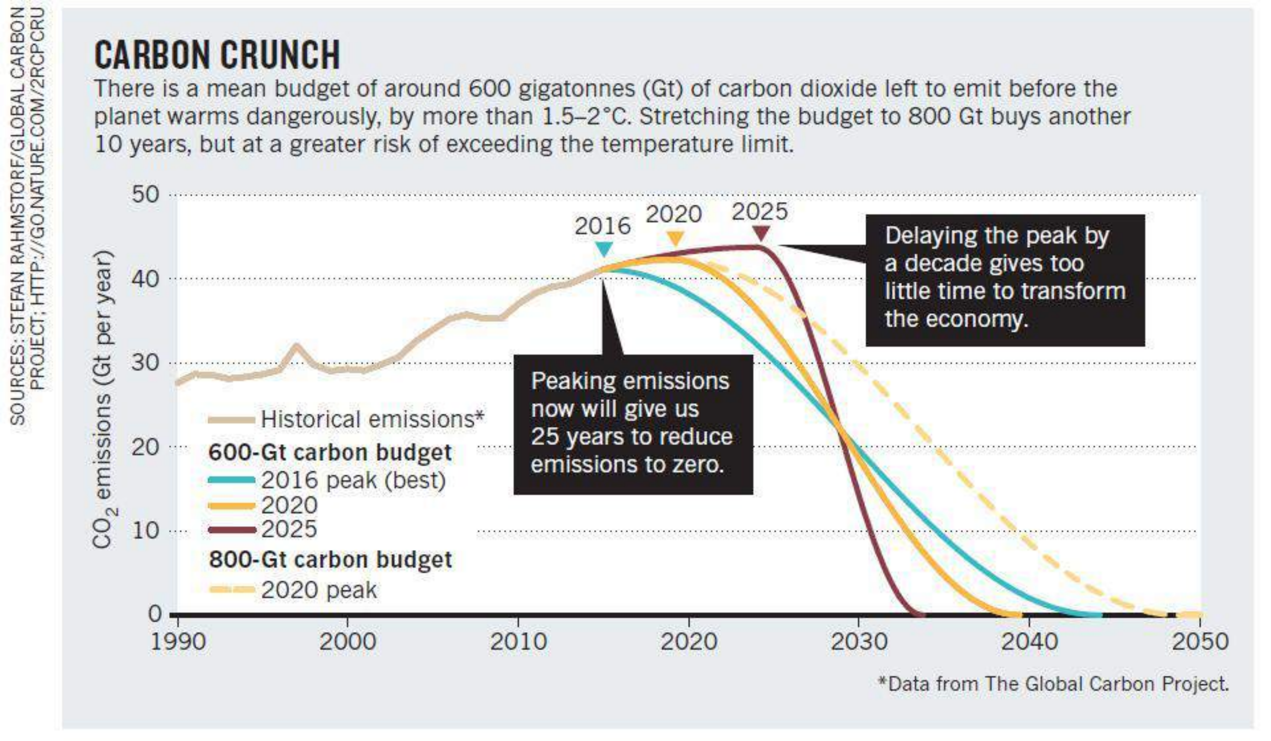
Christiana Figueres and colleagues set out a six-point plan for turning the tide of the world's carbon dioxide by 2020.

The year **2020** is crucially important:

if carbon dioxide emissions continue to rise after 2020 or remain at the same rate, the temperature goals of the 2015 Paris climate agreement become nearly unreachable!

meaning a **dangerous temperature rise** of the planet by more than **1.5-2%!**

Figueres et al. 2017. Nature 546, 593–595, doi:10.1038/546593a



For the past three years, worldwide CO_2 emissions from fossil fuels have stayed flat, while the global economy and GDP of major developed countries have grown!

US: emissions decrease by 3%. GDP growth 1.6%
China: emissions decrease by 1%, GDP growth by 6.7%

Signs of a **change in the feedstock selection** for energy generation towards **carbon free renewables!**

Figueres et al. 2017. Nature 546, 593–595, doi:10.1038/546593a

Examples of investments in the renewables energy sector:

EU:
- **Wind** and **solar** made up more than **3/4** of **new energy capacity** installed
- **coal** demand was **reduced by 10%**

China:
- 2/3 of the 5.4% extra demand for electricity in 2016 were supplied by carbon-free energy resources, mostly by **hydropower** and **wind**

US:
- almost 2/3 of the **electricity-generating capacity** installed was based on **renewables**

Figueres et al. 2017. Nature 546, 593–595, doi:10.1038/546593a

www.mission2020.global

New bioinfrastructures for the bioeconomy

Pulp Mill **Today**

Pulp, Energy;

e.g. UPM Fray Bentos, Uruguay: 8% of the Uruguayan thermal energy supply

340 M€/year

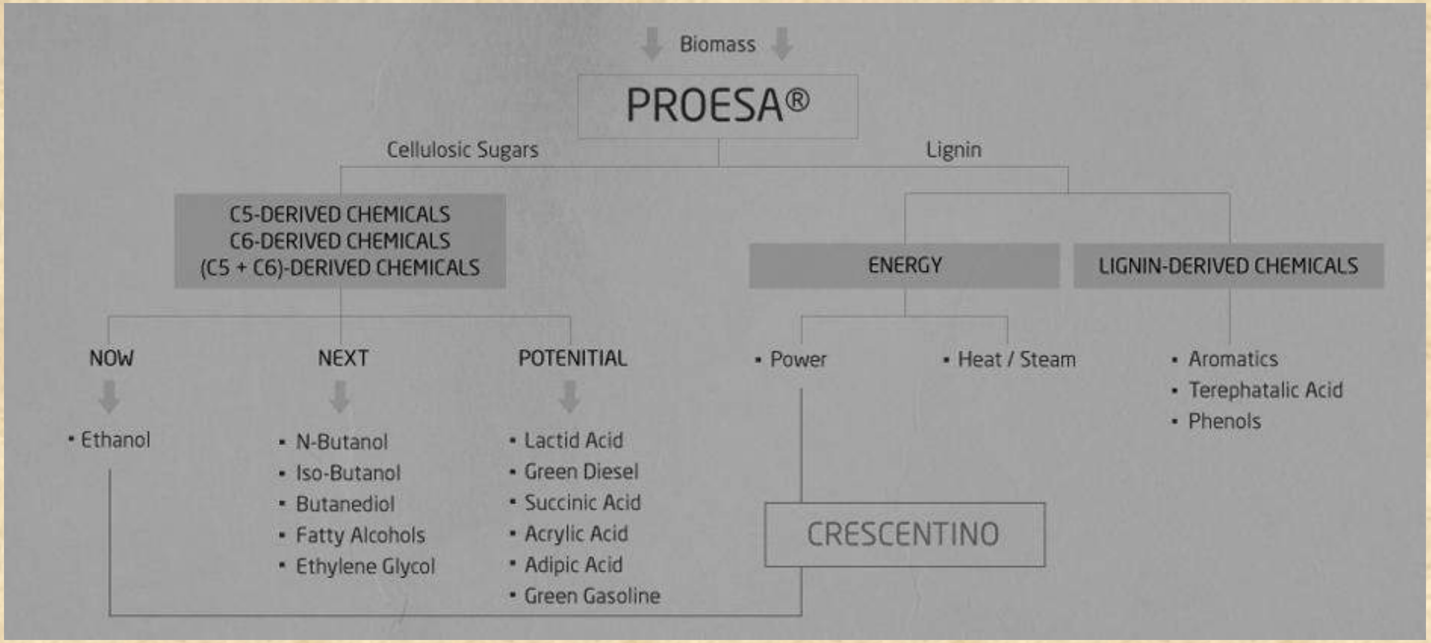
Bioproduct Mill **Tomorrow**

Pulp, chemicals, energy, textiles, food, ≈ 2030

1 bn€/year

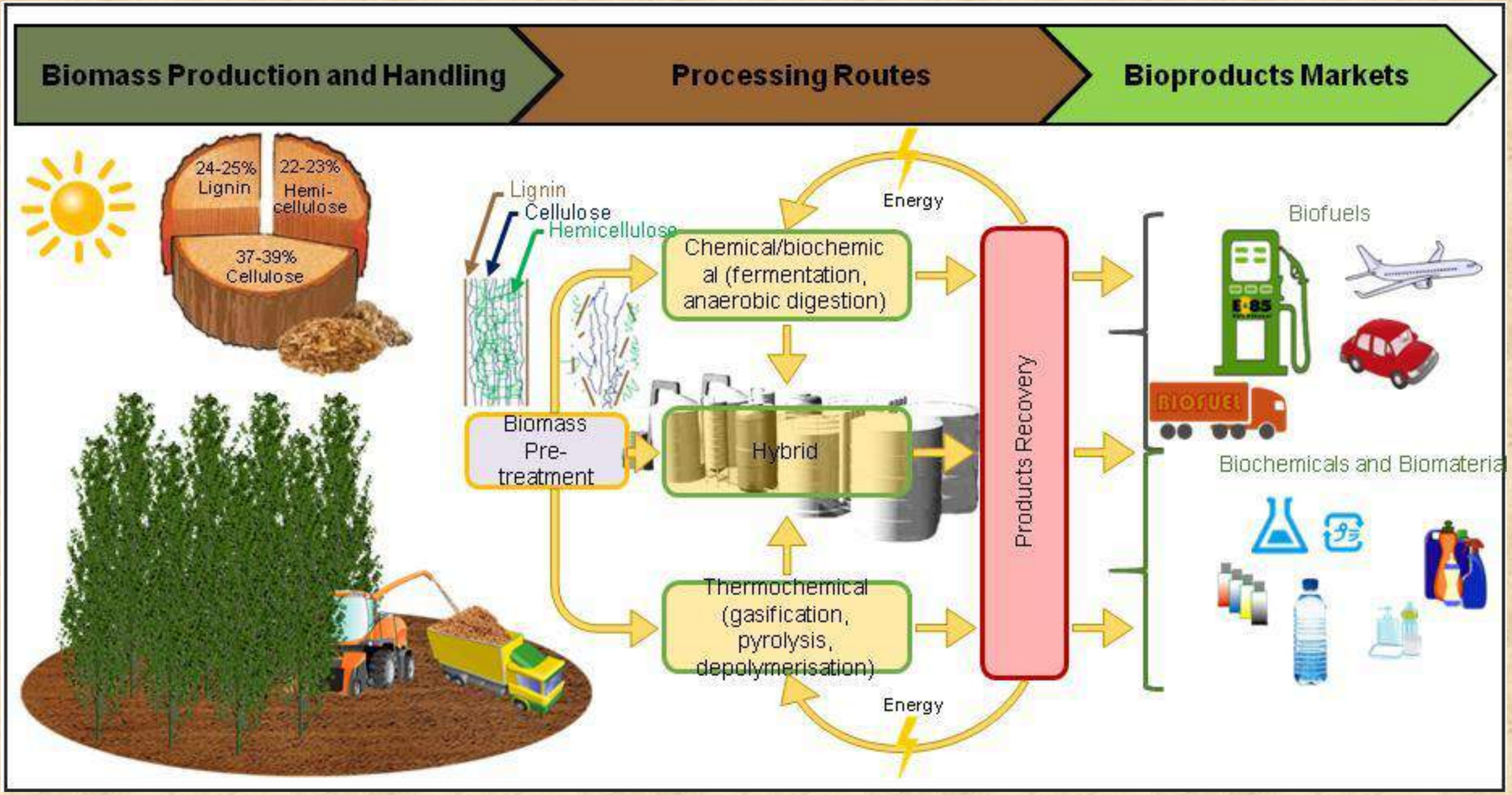
New bioinfrastructures for the bioeconomy

PROESA® Crescentino, Italy; first 2nd generation Biorefinery plant



http://www.betarenewables.com/en/proesa/biorefinery

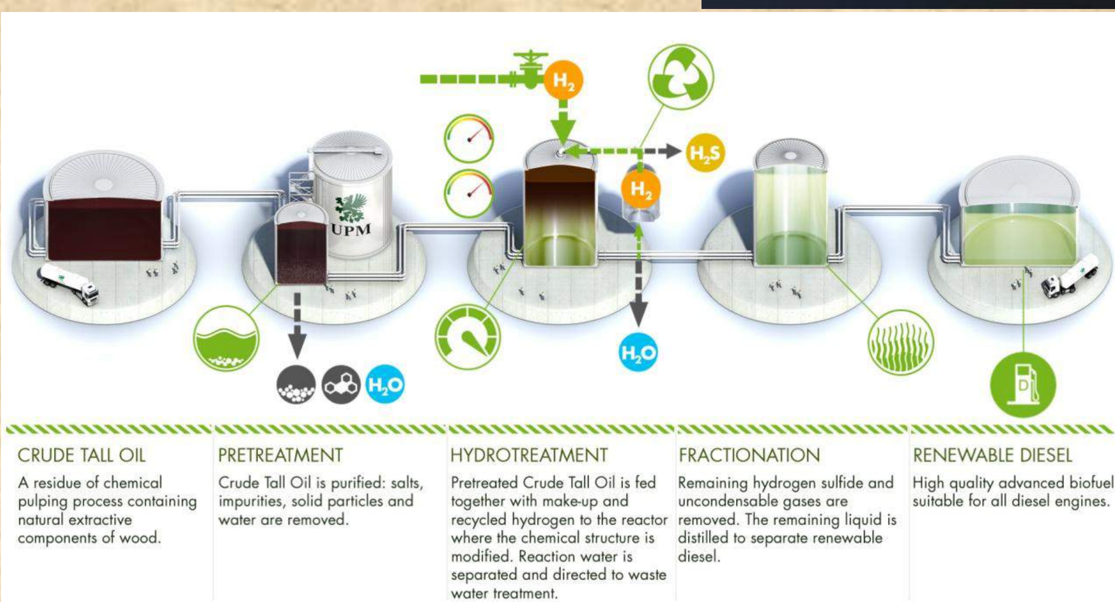
2nd Generation Biofuels and bio-based materials that do not compete with food crops:
Coupling forest and industrial biotechnology!



Harfouche et al. (2014)

First biorefinery producing wood-based diesel

- UPM 's biorefinery: 100,000 tonnes of **second generation biodiesel** for transport per year



http://www.upmbiofuels.com/Pages/default.aspx

- Decreasing **transport emissions up to 80%** compare to fossil fuels
- 25% of Finland's biofuel target

First biorefinery producing wood-based diesel

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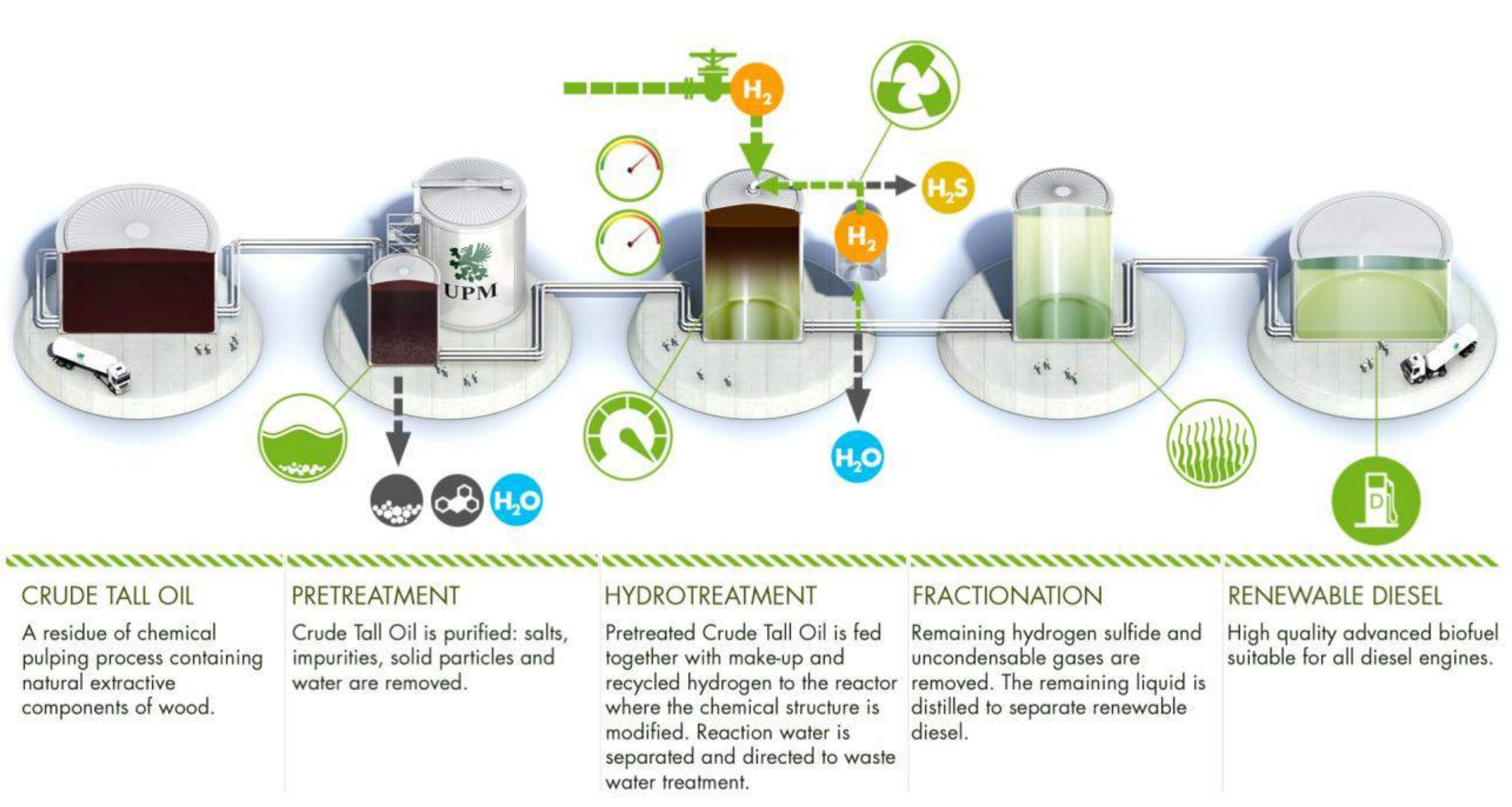


http://www.upmbiofuels.com/Pages/default.aspx

Biorefineries in nature

PNAS

Lignocellulose pretreatment in a fungus-cultivating termite

Hongjie Li[a,b,c,1], Daniel J. Yelle[d,1], Chang Li[e], Mengyi Yang[f], Jing Ke[g], Ruijuan Zhang[h], Yu Liu[h], Na Zhu[a], Shiyou Liang[a], Xiaochang Mo[a], John Ralph[b,i,2], Cameron R. Currie[b,c,2], and Jianchu Mo[a,2]

[a]Ministry of Agriculture Key Laboratory of Agricultural Entomology, Institute of Insect Sciences, Zhejiang University, 310058 Hangzhou, China; [b]Department of Energy Great Lakes Bioenergy Research Center, Wisconsin Energy Institute, University of Wisconsin–Madison, Madison, WI 53726; [c]Department of Bacteriology, University of Wisconsin–Madison, Madison WI 53706; [d]US Forest Products Laboratory, Madison, WI 53726; [e]Department of Chemistry, Zhejiang University, 310058 Hangzhou, China; [f]Xiaoshan Management Center of Termite Control, 311200 Hangzhou, China; [g]Department of Energy Joint Genome Institute, Walnut Creek, CA 94598; [h]State Key Laboratory of Plant Physiology and Biochemistry, College of Life Sciences, Zhejiang University, 310058 Hangzhou, China; and [i]Department of Biochemistry, University of Wisconsin–Madison, Madison WI 53706

Edited by Ronald R. Sederoff, North Carolina State University, Raleigh, NC, and approved March 24, 2017 (received for review November 4, 2016)

"Fungus-cultivating termites are icons of ecologically successful herbivores in (sub)tropical ecosystems, **cultivating *Termitomyces* fungi for overcoming the rigid lignin barrier of wood resources**. …. We found **young worker termites rapidly depolymerize and degrade even the most recalcitrant wood lignin structures,** facilitating polysaccharide cleavage by symbiotic fungi. These results suggest that the **natural systems for lignin degradation/pretreatment** are far beyond the systems currently recognized and are potential sources of novel ligninolytic agents, enabling more efficient plant cell wall utilization."

Li et al. (2017): www.pnas.org/cgi/doi/10.1073/pnas.1618360114

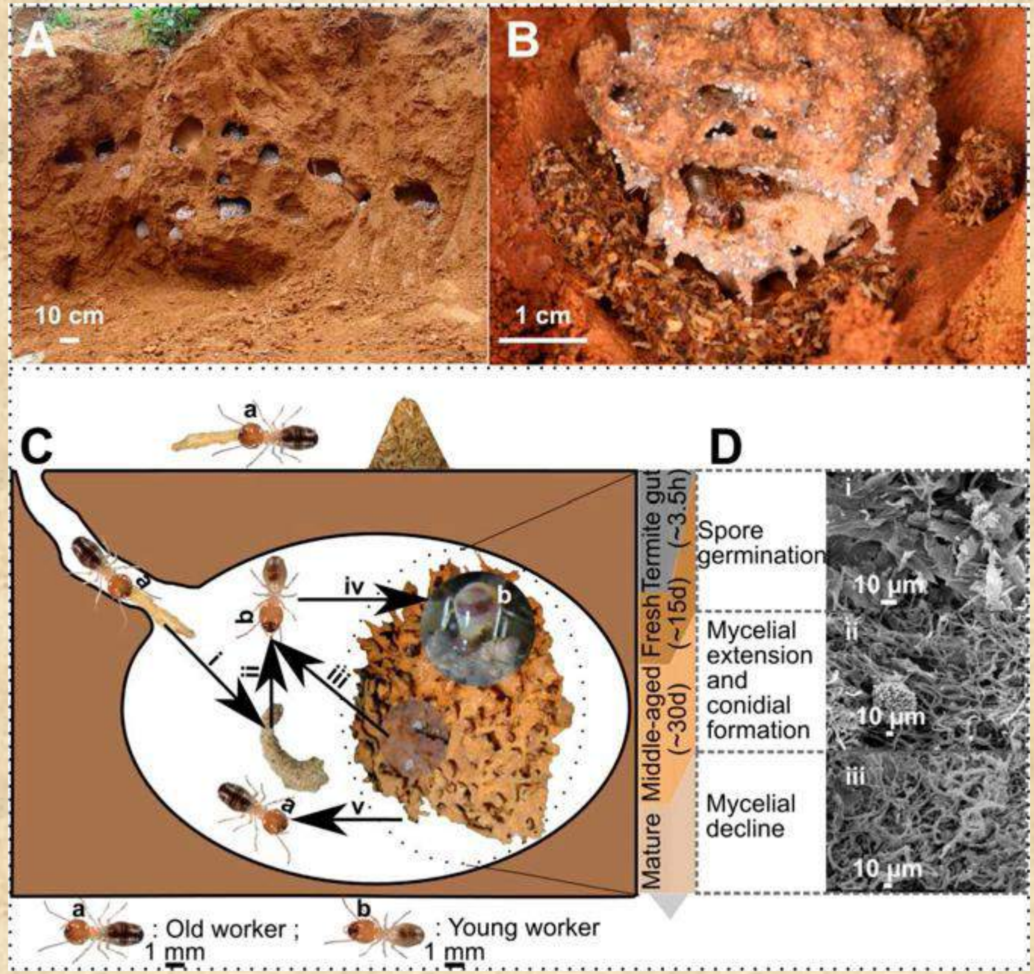
Biorefineries in nature

Termite colony in subtropical China:

Forms massive fungus combs underground (A), made of various plant materials foraged by worker termites (B).

Age-related labor division in food processing of fungus-cultivating termites (C):

Old workers forage **outside** and **transport** plant materials back to nest and form a **food store (i)**; **young** workers ingest the food store **(ii)**, impregnate them with the fungal nodules **(iii)**, and use their feces to build **fresh fungus combs (iv)**; **old workers** gain their nutrition by consuming the **mature fungus comb (v)**.



Li et al. (2017): www.pnas.org/cgi/doi/10.1073/pnas.1618360114

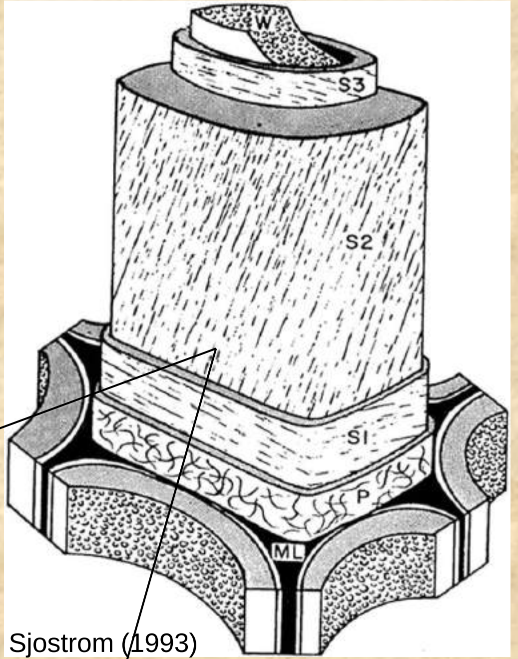
WOOD:

Wood **cell wall compartments:**

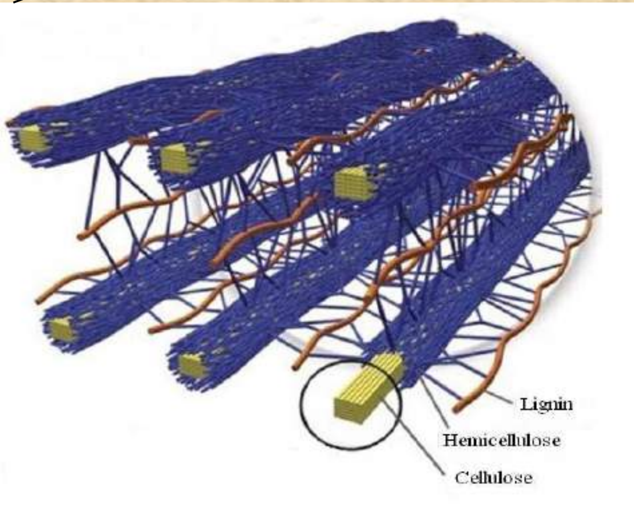
- middle lamella (ML)
- primary cell wall (P)
- layers of the secondary cell wall (S1, S2, S3)
- warty layer (W)

Ultrastructure:

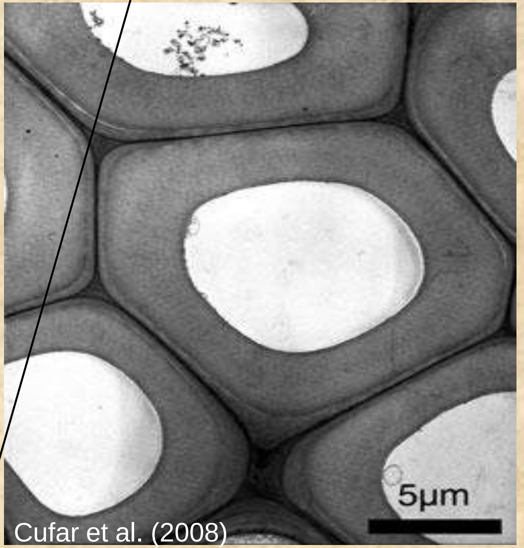
Cellulose microfibrills coated by hemicelluloses and inter-connected by lignin



Sjostrom (1993)

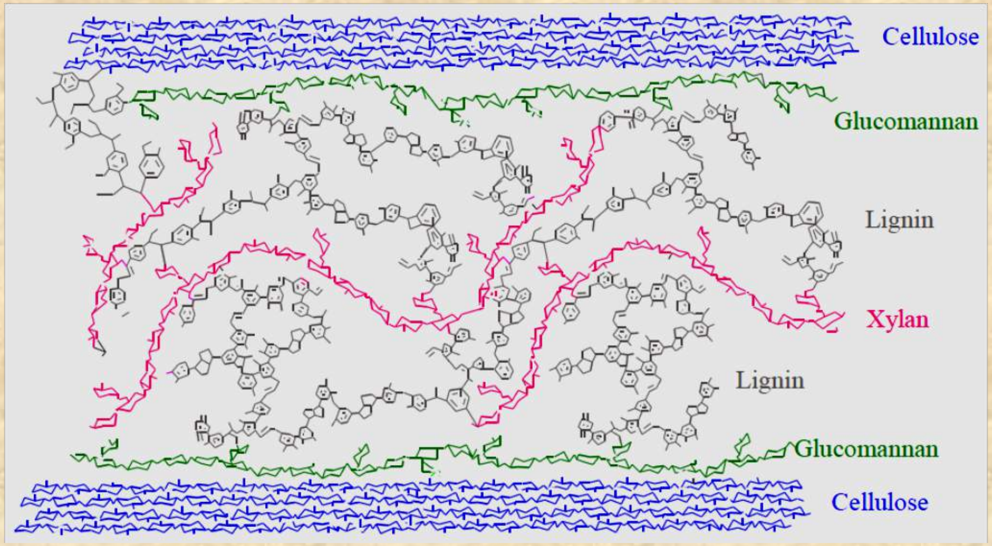


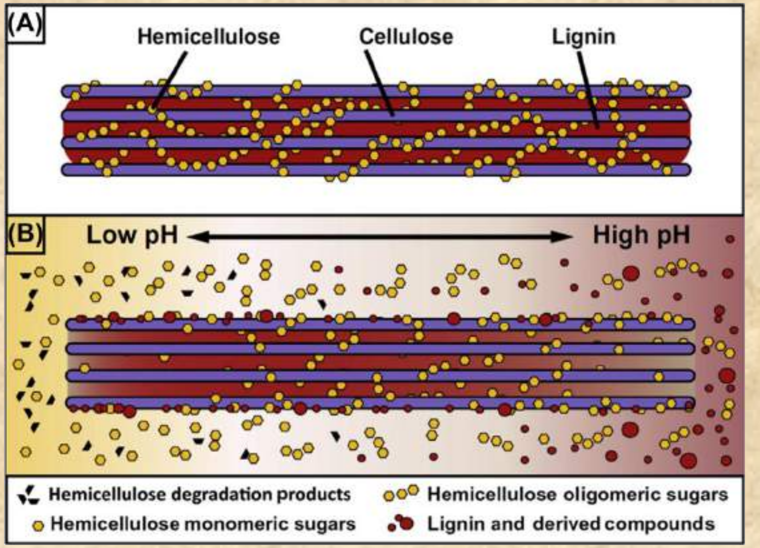
Doherty et al. (2011)



Cufar et al. (2008)

Wood ultrastructure:





Wood is a very well integrated biocomposite of different biopolymers!

Disintegration of the lignified cell wall is essential in Biorefinery processes!

Henriksson (2009)

Garlock et al. (2011)

Wood extractives: Tannin

Fruits
Leaves
Heartwood
Bark

Leather, Textile
Pharmacology
Food, beverage
Cement
Wood adhesives
Waste water treat.
Foams

Pichelin F.: *Innovation in Wood Technologie* (2014)

Tannin sources: European wood species

Silver fir (*Abies alba*)

Norway spruce (*Picea abies*)

Scots pine (*Pinus sylvestris*)

European Larch (*Larix decidua*)

Douglas fir (*Pseudotsuga menziesii*)

Pinus laricio (*Pinus Nigra*)

Chestnut

Pichelin F.: *Innovation in Wood Technologie* (2014)

Tannin production: Extraction

- Autoclaves
- Temperature: 60 – 80 °C
- Additives:
  - Carbonates ($CaCO_3$)
  - Alkaline (NaOH)
  - Sulfites ($NaSO_3$)
  - Urea

Pichelin F.: *Innovation in Wood Technologie* (2014)

Tannin glues:
Low formaldehyde emission

Tannin can be easily extracted from several local wood species

Tannin can be used as natural adhesive for the production of wood-based panels and other glued products

Tannin-based adhesives are formaldehyde free and can replace synthetics adhesives

Pichelin F.: *Innovation in Wood Technologie* (2014)

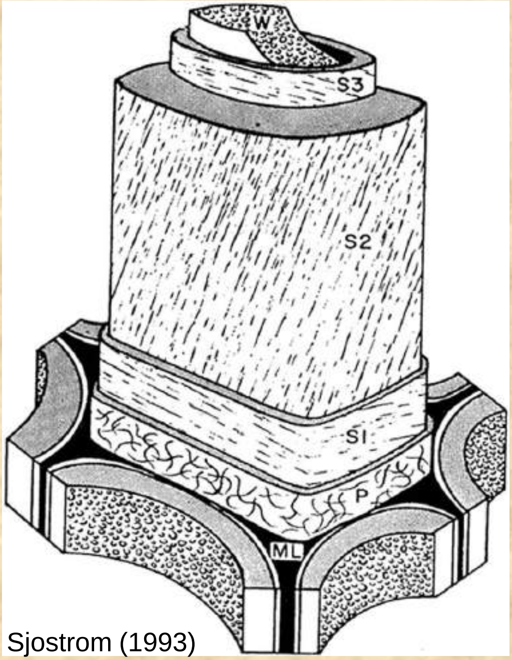
LIGNIN:

- lat.: ***lignum*** … wood
- **second most abundant natural organic polymer** (after cellulose)
- **fills out** the **space between** the plant **polysaccharides** in the cell wall

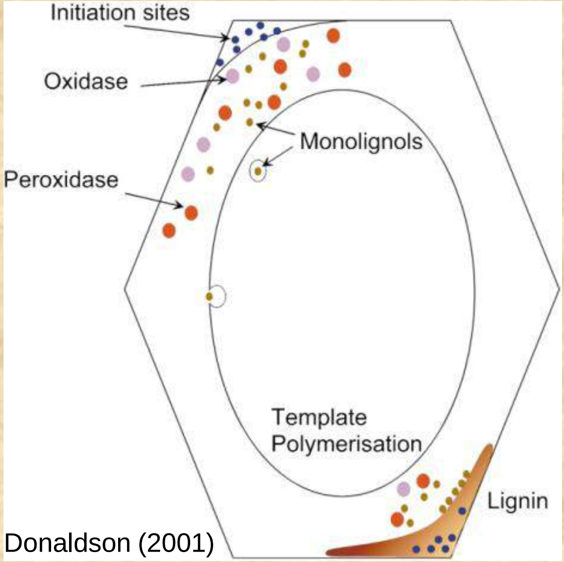
  → **lignification of the wall**

  → ensures the **pressure resistence** of the plant tissue
- **highly hydrophobic!**

  → allows **transport** of **nutrients** and **water**



Sjostrom (1993)



Donaldson (2001)

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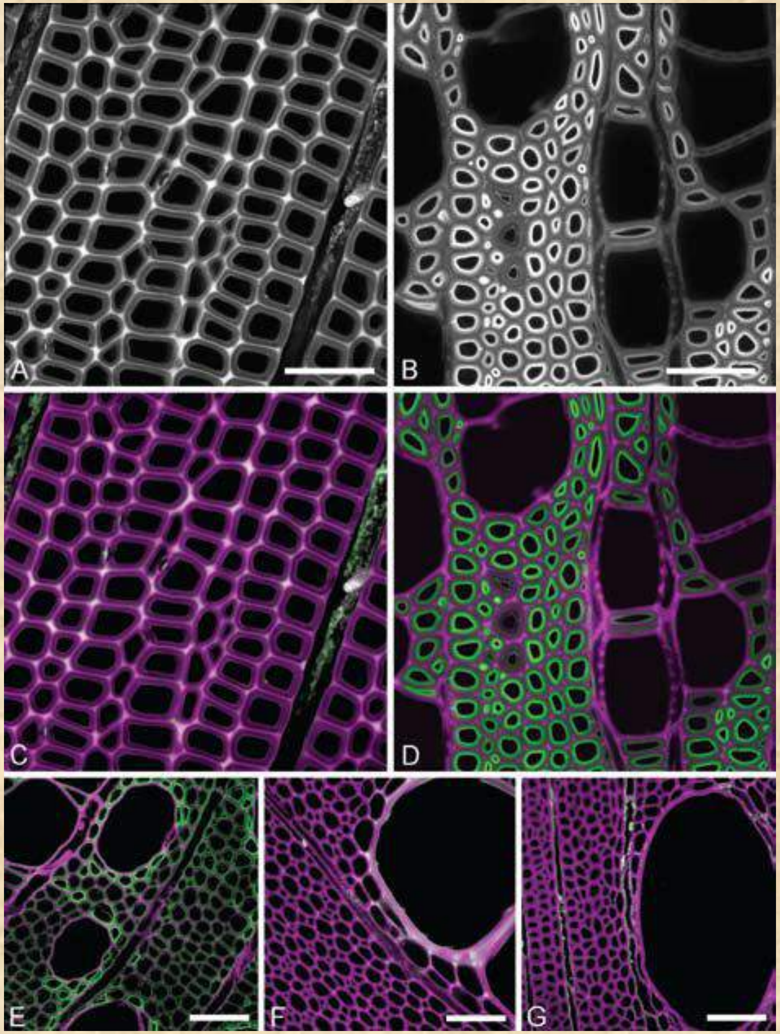
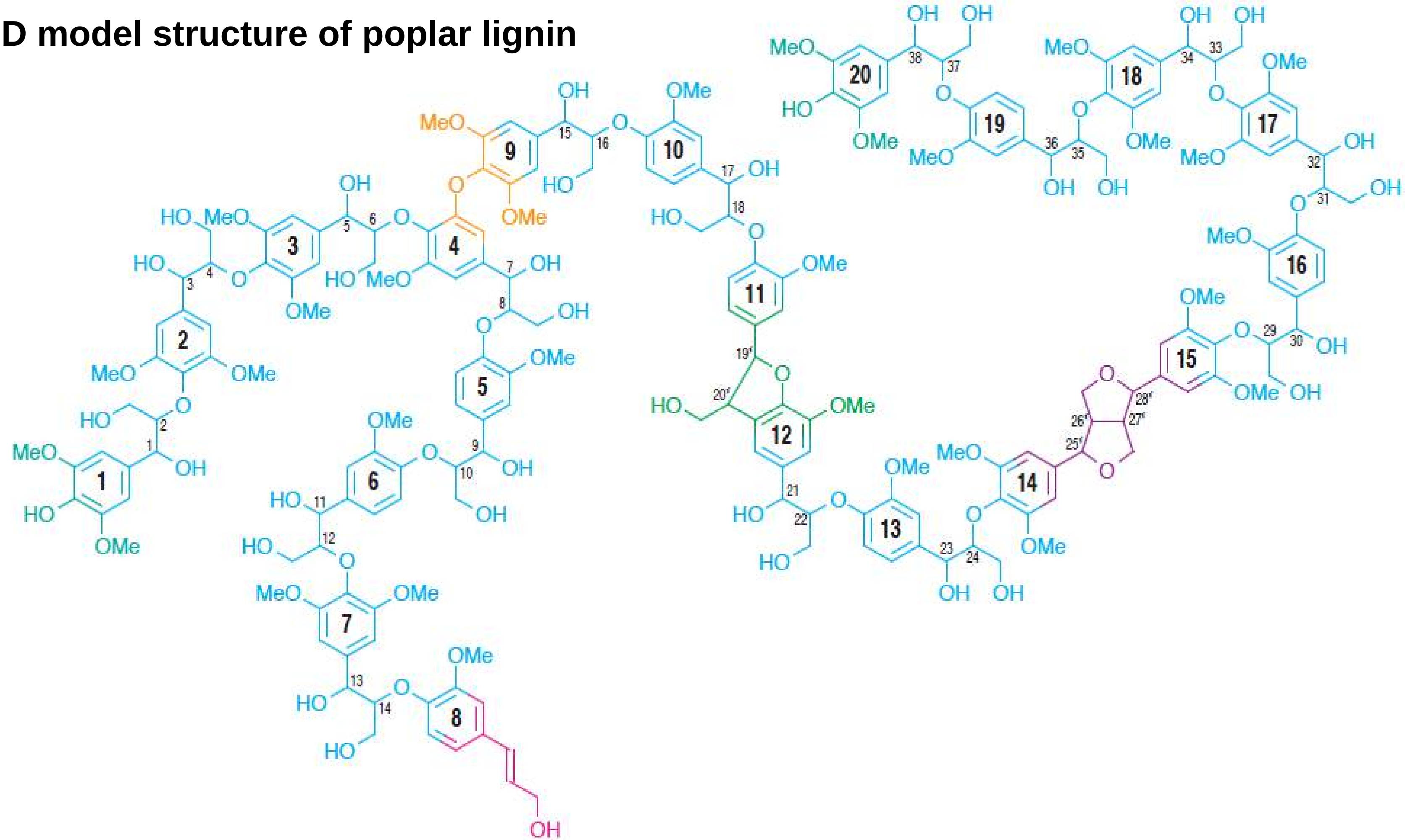


Donaldson (2013)

Typical lignin structures – lignin macromolecule models

2D model structure of poplar lignin



Boerjan, W., Ralph, J., Baucher, M., 2003. Lignin Biosynthesis. Annu. Rev. Plant Biol. 54, 519-546.

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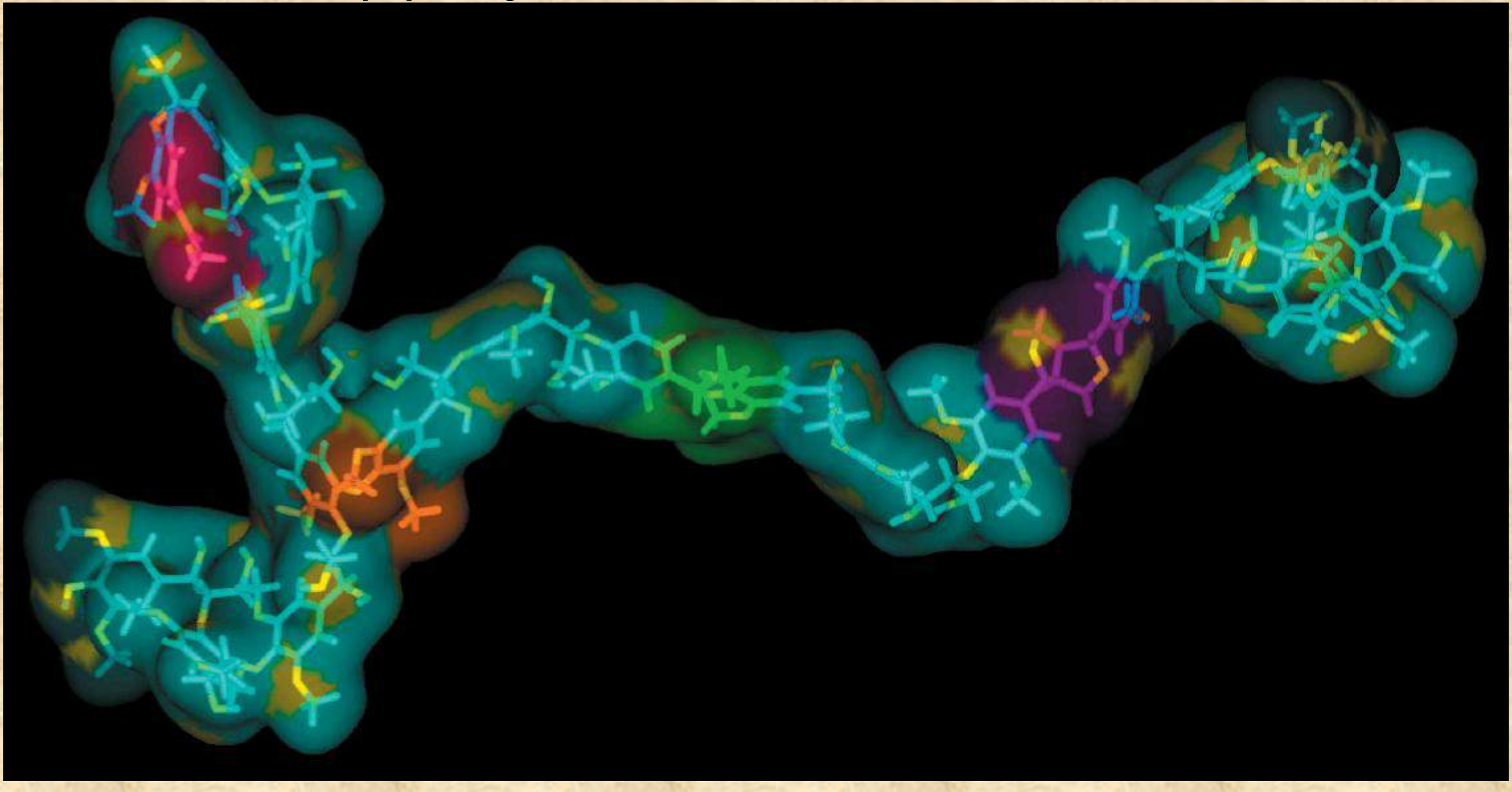


Boerjan, W., Ralph, J., Baucher, M., 2003. Lignin Biosynthesis. Annu. Rev. Plant Biol. 54, 519-546.

DIBAF
DIPARTIMENTO PER LA INNOVAZIONE NEI SISTEMI BIOLOGICI, AGROALIMENTARI E FORESTALI

TECHNISCHE UNIVERSITÄT WIEN
Vienna University of Technology

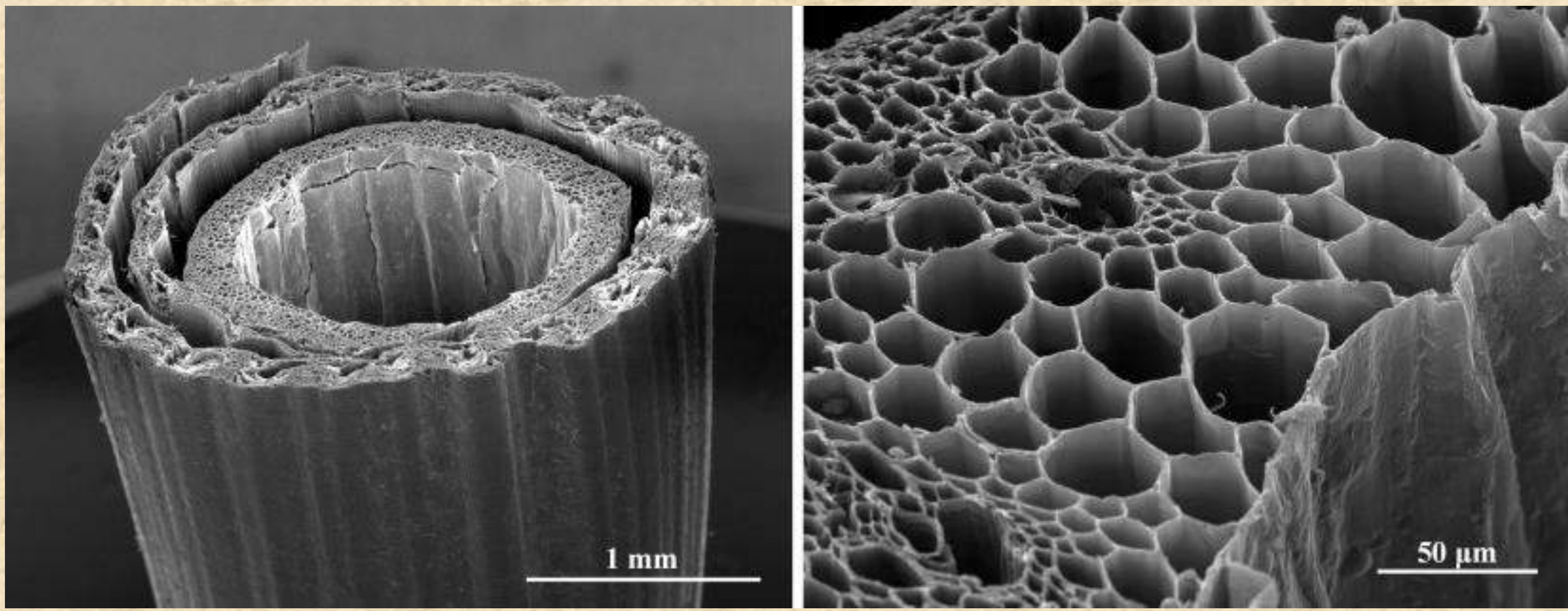
Wheat straw lignin –
Isolation and characterization of a plant cell wall networking polymer

KTH - Royal Institute of Technology Stockholm

Florian Zikeli

Cumulative dissertation

Vienna University of Technology, 04.08.2016



SEM images of wheat straw: the straw itself surrounded by a sheath leaf around (left) and individual cells off the straw wall on the right (Kristensen et al. 2008)

Wheat straw lignin –
Isolation and characterization of a plant cell wall networking polymer

Florian Zikeli
Cumulative dissertation

Publication 1:

ELSEVIER
Industrial Crops and Products
Volume 61, November 2014, Pages 249–257

Successive and quantitative fractionation and extensive structural characterization of lignin from wheat straw

Florian Zikeli[a, b], Thomas Ters[a, 1], Karin Fackler[a, 1], Ewald Srebotnik[a, 1], Jiebing Li[b, 2]

Publication 2:

ELSEVIER
Industrial Crops and Products
Volume 85, July 2016, Pages 309–317

Wheat straw lignin fractionation and characterization as lignin-carbohydrate complexes

Florian Zikeli[a, b], Thomas Ters[a], Karin Fackler[a], Ewald Srebotnik[a], Jiebing Li[b]

Publication 3:

ELSEVIER
Industrial Crops and Products
Volume 91, 30 November 2016, Pages 186–193

Fractionation of wheat straw Dioxane lignin reveals molar mass dependent structural differences

Florian Zikeli[a, b], Thomas Ters[a], Karin Fackler[a], Ewald Srebotnik[a], Jiebing Li[b]

Wheat *(Triticum aestivum, T. durum, T. monococcum)*

- **world** annual production: **650 Mio. tons** (A: 1.5 Mio t)
- **3rd most-produced** cereal after maize and rice

(2016, FAOSTAT)

Wheat straw

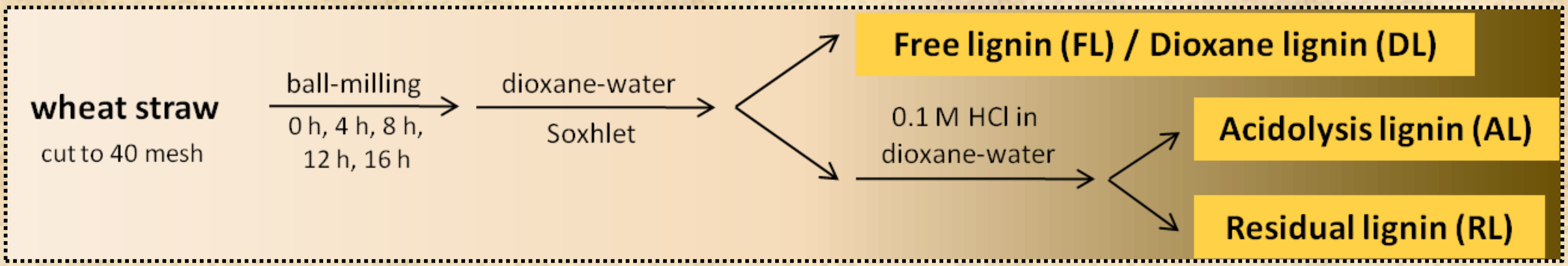
- **A:** ca. **720.000 tons** avail.
- ca. **180.000** tons for **industrial utilization** (theoretically)

Polysaccharides

Lignin

Publication 1: Successive and quantitative fractionation and extensive structural characterization of lignin from wheat straw

Methodology



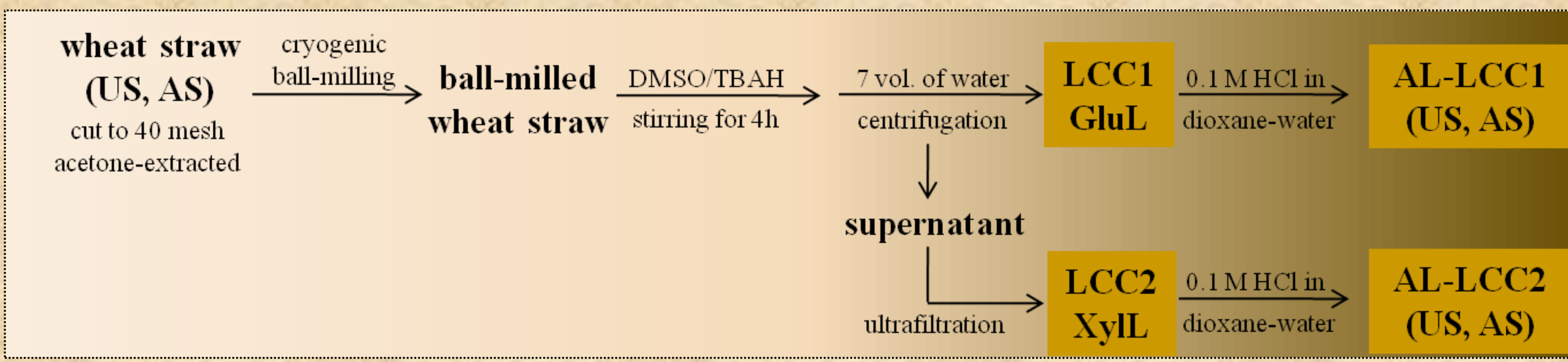
Fractions:	- ***Dioxane lignin (DL):***	***Free lignin (FL)***	*0h BM*
		Dioxane lignin (DL)	*8h BM*
		NetDL	***deducted:*** *DL(8h) - FL*
	- ***Acidolysis lignin (AL)***		*8h BM*
	- ***Residual lignin (RL)***		

Analytical parameters:	- yields	gravimetry
	- composition	KL - CHOs analysis
	- molar mass	alkaline HP-SEC
	- monomeric ratio S:G:H	2D HSQC NMR
	- interunit linkages ratio	2D HSQC NMR
	- estimated *p*HCAs, tricin contents	2D HSQC NMR
	- functional groups	^{31}P NMR

Publication 2: Wheat straw lignin fractionation and characterization as lignin-carbohydrate complexes

Methodology

Isolation protocol for the LCC fractions and the containing Acidolysis lignin fractions (AL-LCC1, AL-LCC2) from untreated and alkali-treated wheat straw:



Analytical parameters:	- yields	gravimetry
	- composition	KL - CHOs analysis
	- molar mass	HP-SEC (alkaline)
	- monomeric ratio S:G:H	2D HSQC NMR
	- interunit linkages ratio	2D HSQC NMR
	- estimated *p*HCAs, tricin contents	2D HSQC NMR
	- functional groups	^{31}P NMR

Publication 3: Fractionation of wheat straw Dioxane lignin reveals molar mass dependent structural differences

Methodology

BÜCHI Sepacore® flash system X50

Starting material: *Dioxane lignin* from acetone-extracted wheat straw ball-milled for 16h

Products: 6 separate molar mass fractions of wheat straw *Dioxane lignin*

→ **subjected to the identical analysis protocols used for the lignin fractions in publications 1 and 2**

OUTCOME OF THE THESIS:

- **Several wheat straw lignin fractions** were isolated and their **compositions** and **structures** were thoroughly investigated. Their **isolation schemes** are well **documented** in order to be able to access in a **straight-forward** and **reproducible** way **specific** lignin fractions of **desired structural characteristics** that exhibit **less heterogeneous structures than bulk** wheat straw lignin.

- The fractions were well described regarding **structural characteristics** like **molar mass distribution, monomeric composition, inter-unit linkages, functional groups and polysaccharide content.** These parameters provide **essential information and orientation** for the **design of future lignin application strategies.**

LIGNIN VALUE-ADDED APPLICATIONS:

Lignin nanoparticles

Ago (2017): *"…In materials science, nano- and colloidal particles (are used to achieve properties that are otherwise difficult to realize (i.e., to impart color, thermal and light stability, or to improve material strength). Typically,* ***nanoparticle isolation is less energy demanding than chemical-synthesis****…"*


Contents lists available at ScienceDirect
Industrial Crops and Products
journal homepage: www.elsevier.com/locate/indcrop

Fabrication of uniform lignin colloidal spheres for developing natural broad-spectrum sunscreens with high sun protection factor

Yong Qian, Xiaowen Zhong, Ying Li, Xueqing Qiu*
School of Chemistry and Chemical Engineering, State Key Lab of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, PR China


Contents lists available at ScienceDirect
Industrial Crops and Products
journal homepage: www.elsevier.com/locate/indcrop

Novel method for the preparation of lignin-rich nanoparticles from lignocellulosic fibers

Anupama Rangan[a,*], Manjula V. Manchiganti[b], Rajendran M. Thilaividankan[c], Satyanarayana G. Kestur[d], Reghu Menon[e]
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[b] Scientific Assistant, Department of Physics, Indian Institute of Science, CV Raman Road, Bengaluru, 560012, India
[c] Consultant: Industrial Biotechnology, Formerly with Epygen Biotech Pvt Ltd, No 14, Raheja Arcade, CBD Belapur, Navi Mumbai, India
[d] Honorary Professor, Poornaprajna Institute of Scientific Research (PPISR),Sy. No. 167, Poornaprajnapura, Bidalur Post, Devanahalli, Bengaluru, 562 110, Karnataka, India
[e] Professor, Department of Physics, Indian Institute of Science, CV Raman Road, Bengaluru, 560012, India

Quian et al. (2017)

Rangan et al. (2017)

LIGNIN VALUE-ADDED APPLICATIONS

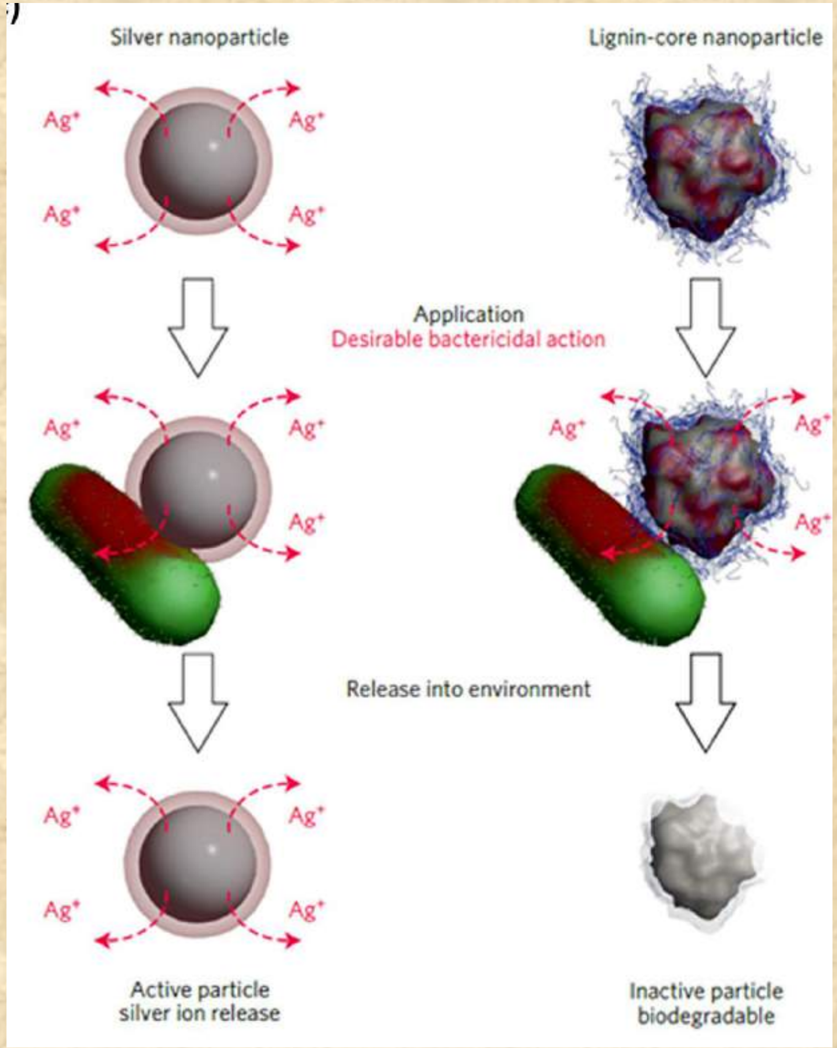
LNPs – tuned silver nanoparticles:

Silver nanoparticles efficient against *E. coli* and *Pseudomonas species*

BUT:

Unused silver particles remain in the environment and could have adverse effects on non-pathogenic soil and water MOs

- **LNPs core infused with silver nanoparticles outperformed the controls in their antibacterial effect!!**
- **12-20x less silver needed!!**

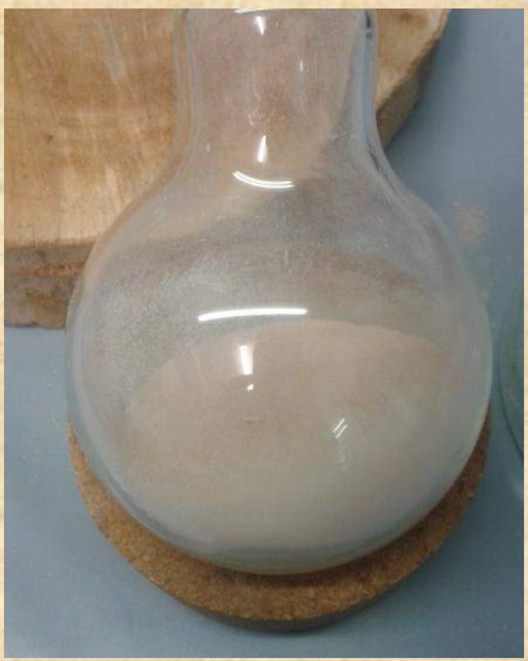


A.P. Richter, et al. (2015)

Lignin nanoparticles (LNPs):

Lignin isolated from: Beech
Chestnut (sawdust)

⟶ value-added use for woody feedstock nowadays designated for thermal utilization in e.g. pellets (sale price ca. 25€/ton)



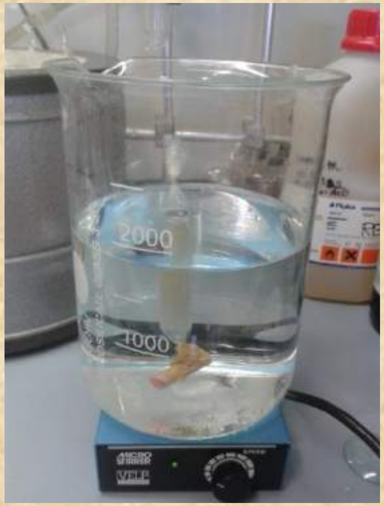


Lignin nanoparticles (LNPs):

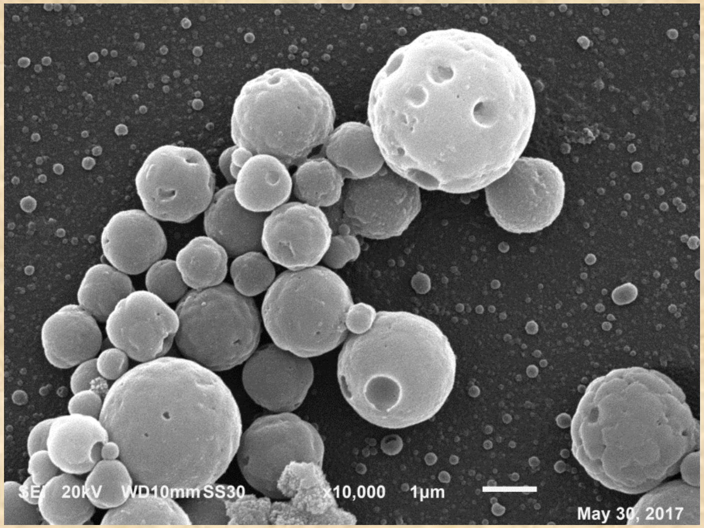
“Anti-solvent” method:

Gradual introduction of an anti-solvent by dialysis:

- Lignin dissolved in THF inside the dialysis tube in an excess of water
- Dialysis for 48h.



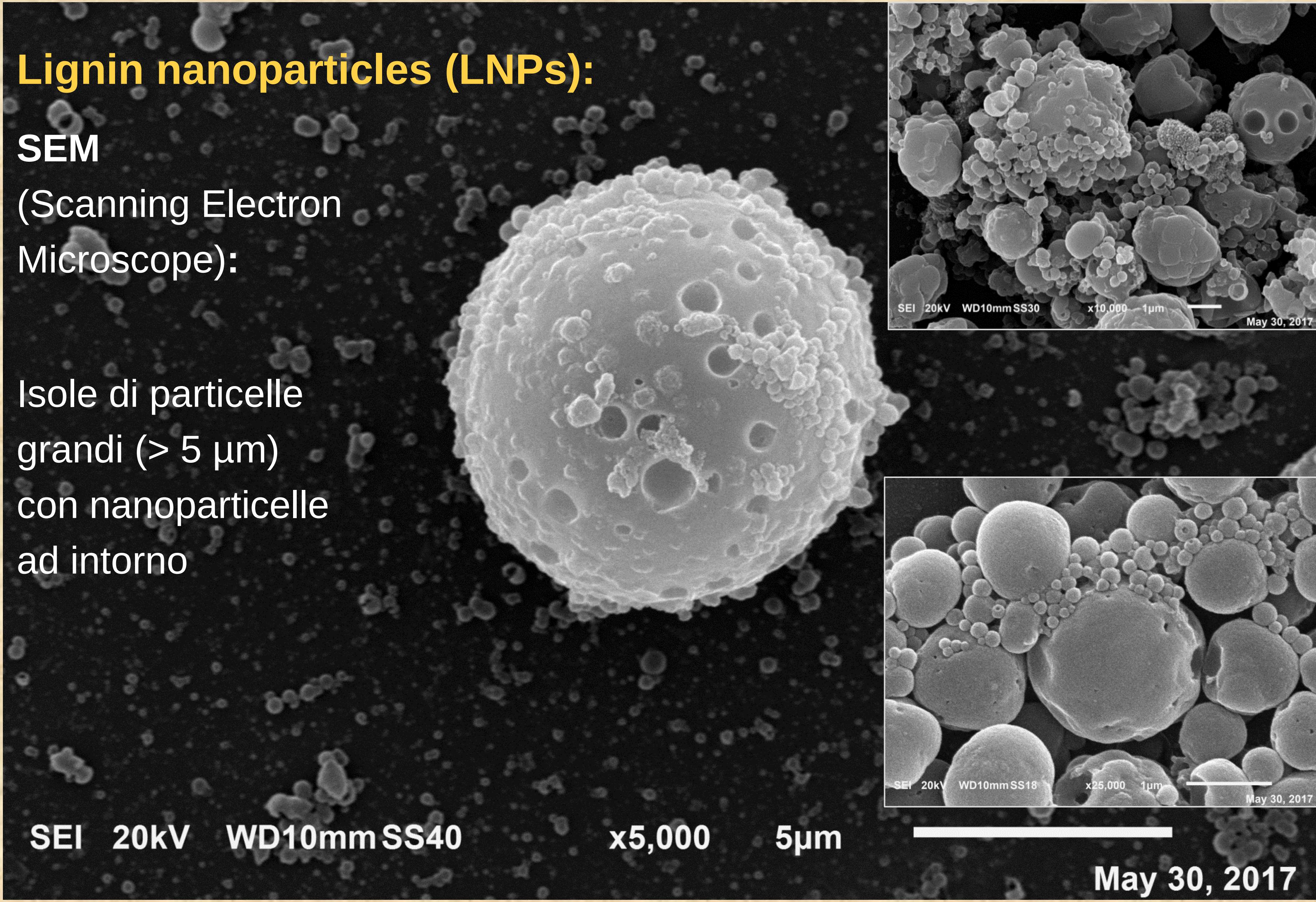
⟶ Lignin precipitates forming **particles in micro- and nano-scale!**



Lignin nanoparticles (LNPs):

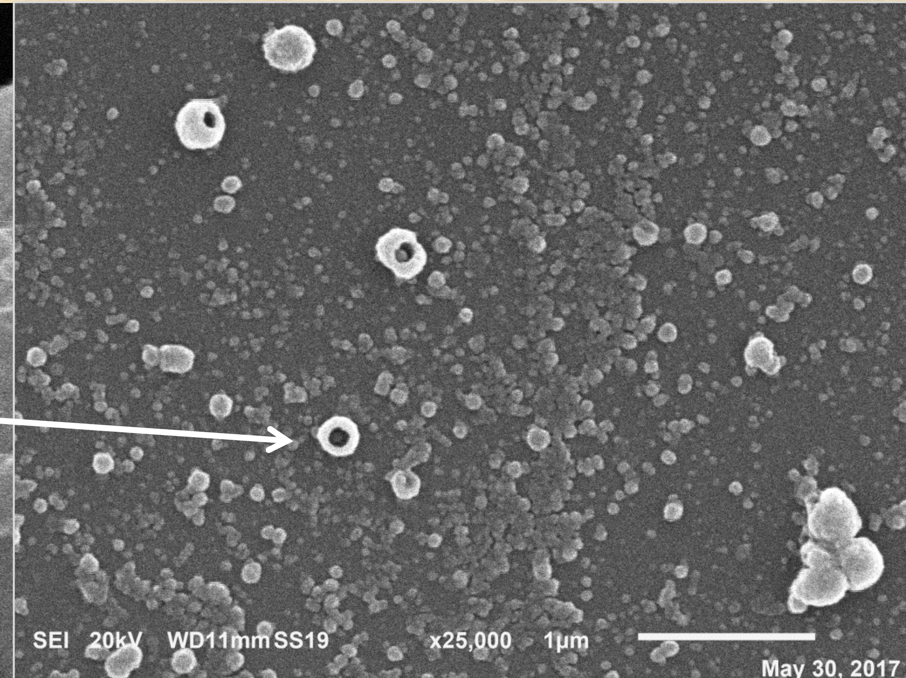
SEM
(Scanning Electron Microscope)**:**

Isole di particelle grandi (> 5 µm) con nanoparticelle ad intorno



Lignin nanoparticles (LNPs):

SEM:



Presence of holes inside the nanoparticles;

→ **possibility to load them with bioative molecules!**

→ **for controlled release applications (drugs. pesticides, …) or**

→ **for adsorption applications (heavy metal ions, pesticides, …)**

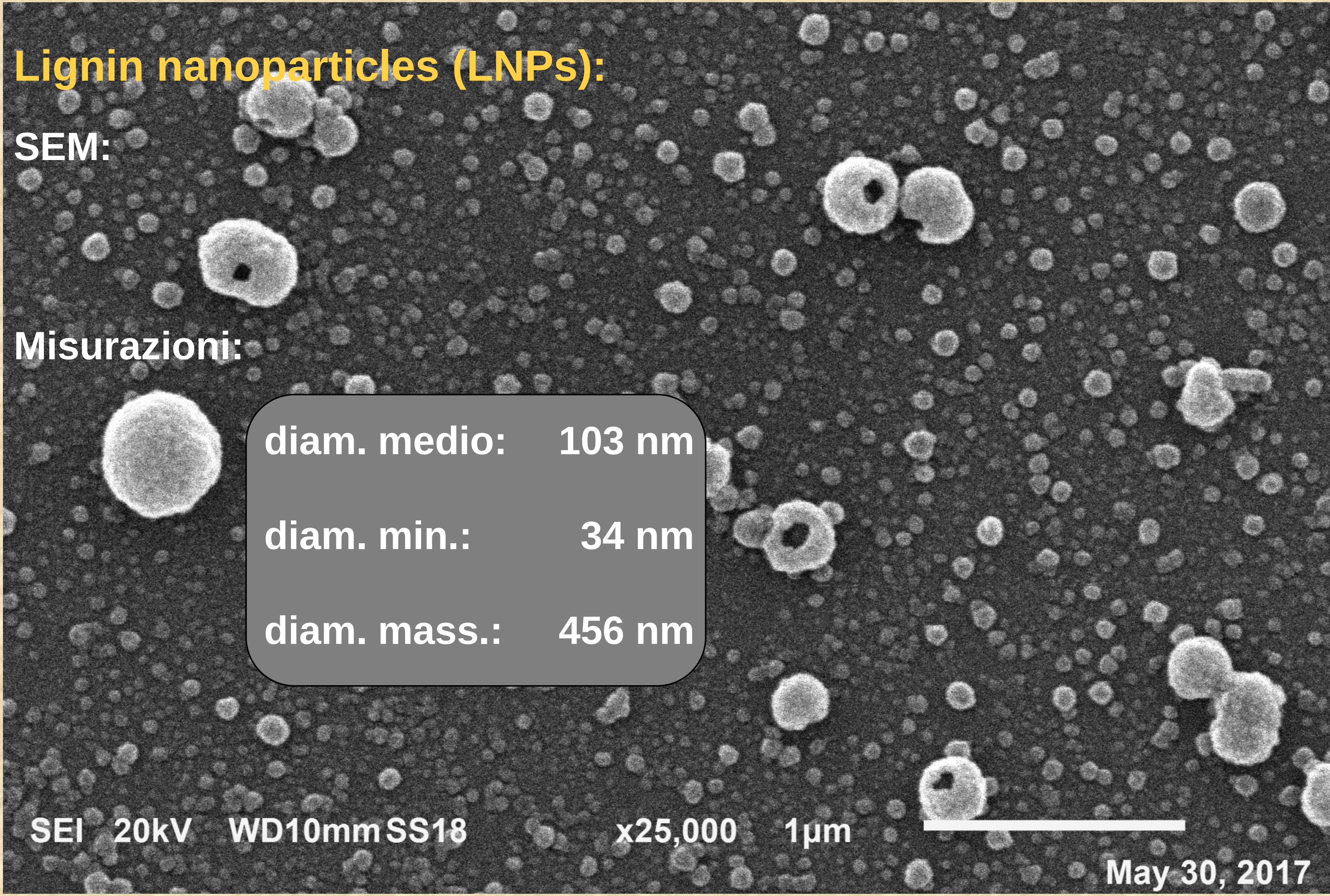
SEI 20kV WD10mm SS20 x18,000 1μm
May 30, 2017

Lignin nanoparticles (LNPs):

SEM:

Misurazioni:

diam. medio:	103 nm
diam. min.:	34 nm
diam. mass.:	456 nm



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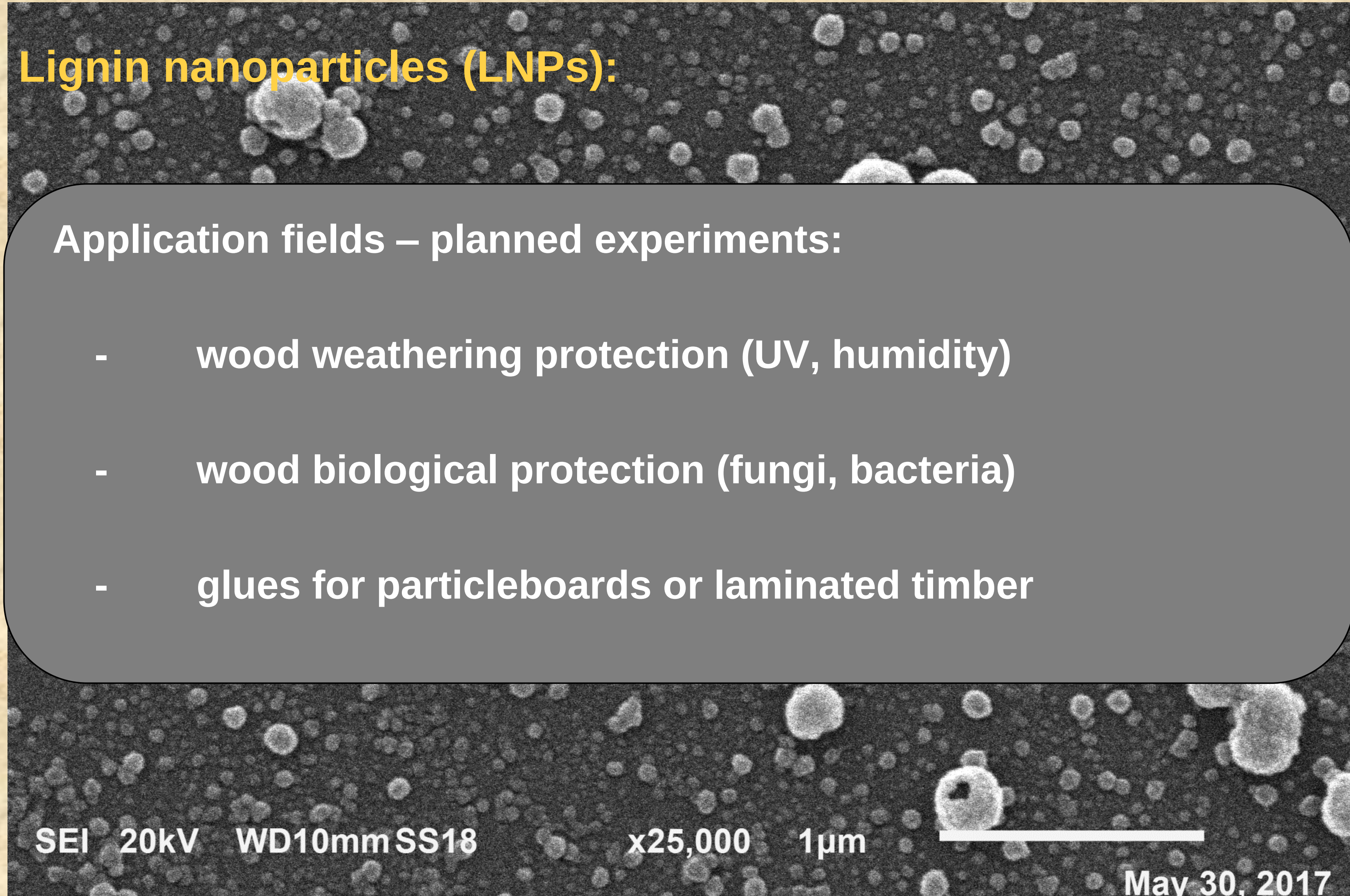

Application fields – planned experiments:

- **wood weathering protection (UV, humidity)**
- **wood biological protection (fungi, bacteria)**
- **glues for particleboards or laminated timber**


Thanks for your attention!

Literature:

Figueres et al., 2017. *Three years to safeguard our climate*. Nature 546, 593–595. doi:10.1038/546593a

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