# Project title

## Development of a process for the utilization both the carbohydrate and the lignin content from lignocellulosic materials of annual plants for the production of valuable products

Project acronym: Products from lignocellulose
Project no: EIB.10.013

European Research Area
Industrial Biotechnology

## Project aim

is the development of a process for the material utilization of both the carbohydrate and the lignin content from lignocellulosic materials of annual plants.

## General project approach

With this objective, the following main tasks have been studied:

(i) the **development of a pre-treatment process**, which allows the separation of both the lignin and the carbohydrate content of lignocellulosic raw materials,

(ii) the **development of a *Penicillium verruculosum* enzyme complex** optimized for the saccharification of the carbohydrate content of lignocellulose in a **S**imultaneous **S**accharification and **F**ermentation (**SSF**) process,

(iii) **investigations on the SSF-process**, using model yeast strains for the production of ethanol and platform chemicals,

(iv) the **modification of the separated lignin** for the production of fibre-reinforced biopolymers as well as for the production of fine chemicals.

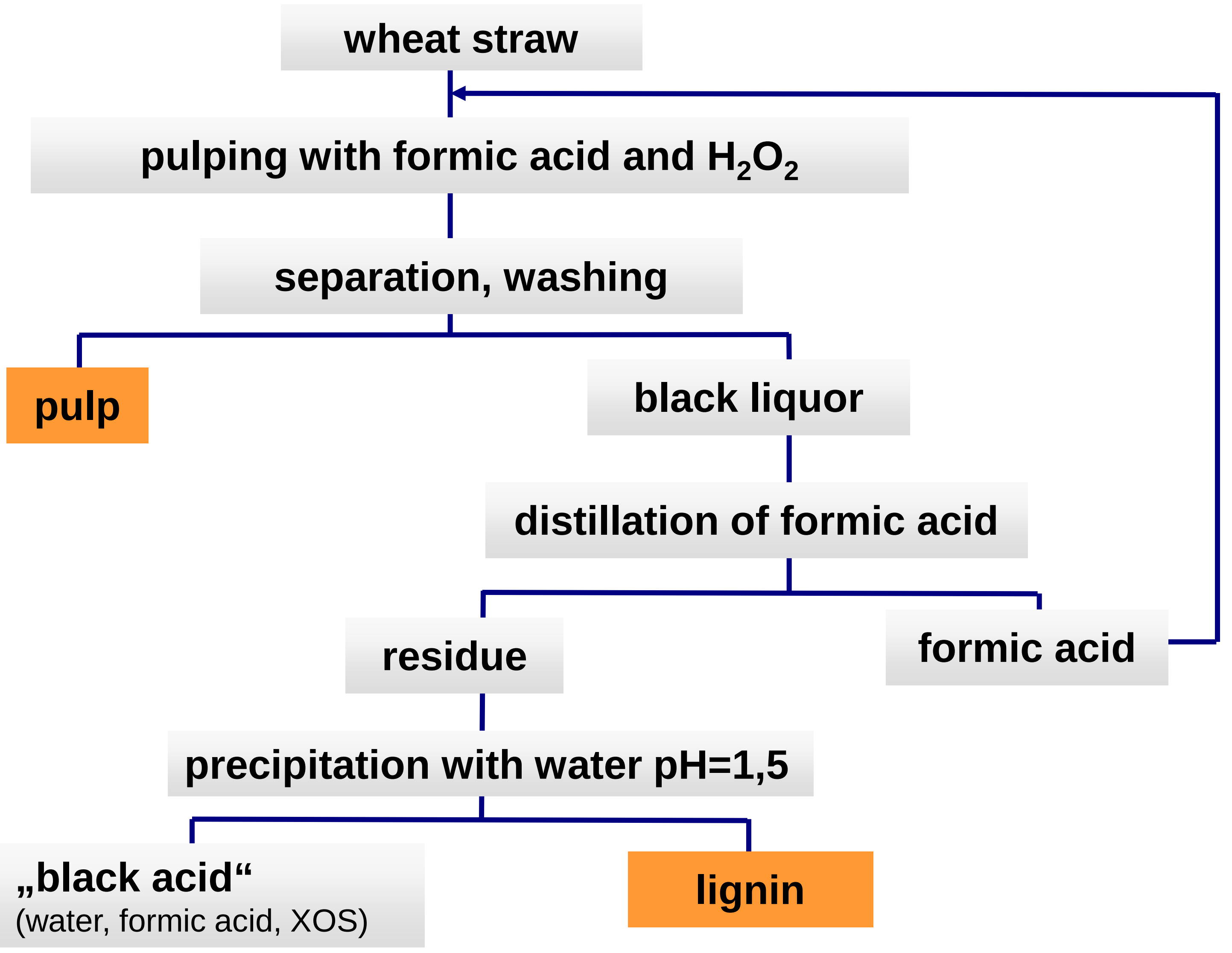
# Partner of the Consortium:

(www.era-ib-lignocellulose.eu)

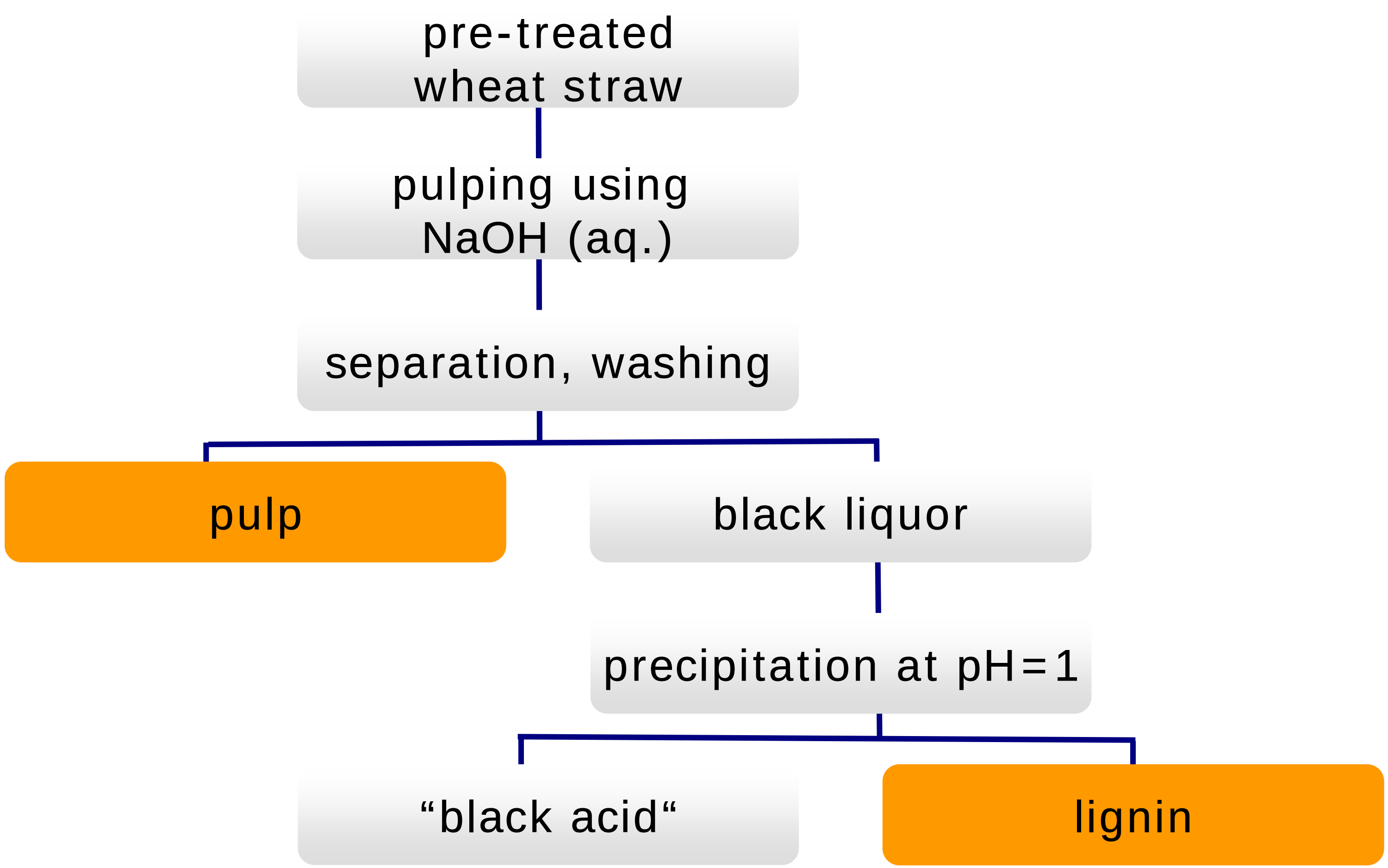
| | |
|---|---|
| **SIAB, UL** | SIAB and Leipzig University, Leipzig |
| **IPWC** | Technical University of Dresden |
| **IWPT** | Technical University of Dresden |
| **FUMT** | Freiberg University of Mining and Technology |
| **VTT** | VTT, Technical research centre of Finland |
| **LNEG** | Laboratório Nacional de Energia e Geologia, Lisbon |
| **WUR-FBR** | WUR-FBR, Wageningen, Netherlands |
| **Biotehnol** | Biotehnol, Bucharest, Romania |

# Pre-treatment of lignocellulose

# Natural pulping of wheat straw



# alkaline pulping of wheat straw



## Why the investigation of different methods for pre-treatment ?

- Investigation on different properties of the lignin depending on the method for pre-treatment
- Investigation on different properties of the pulp depending on the method for pre-treatment

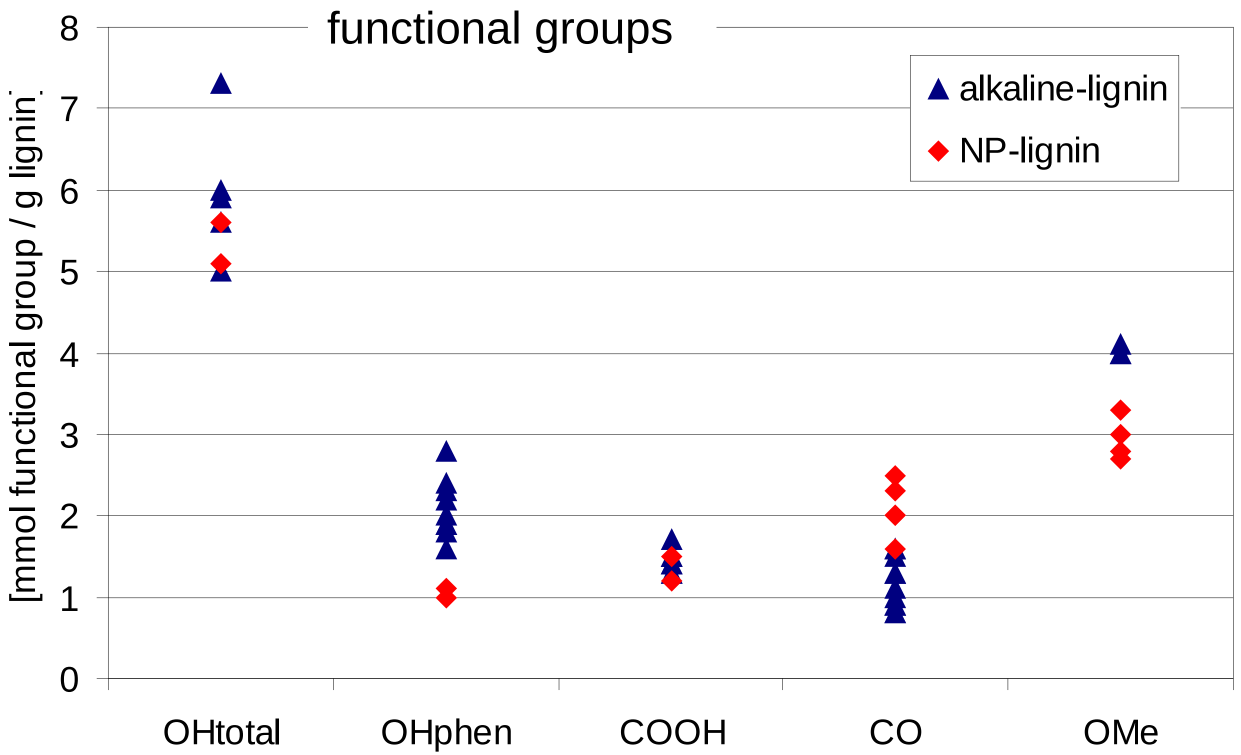
**<u>Scale-up of pre-treatment</u>**

- Investigation on changes of the lignin-properties and the pulp-properties by scale-up of the pre-treatment process
- Provision of adequate amounts of lignin and pulp for further investigations concerning their material utilization

# Influence of pre-treatment on properties of pulp and lignin

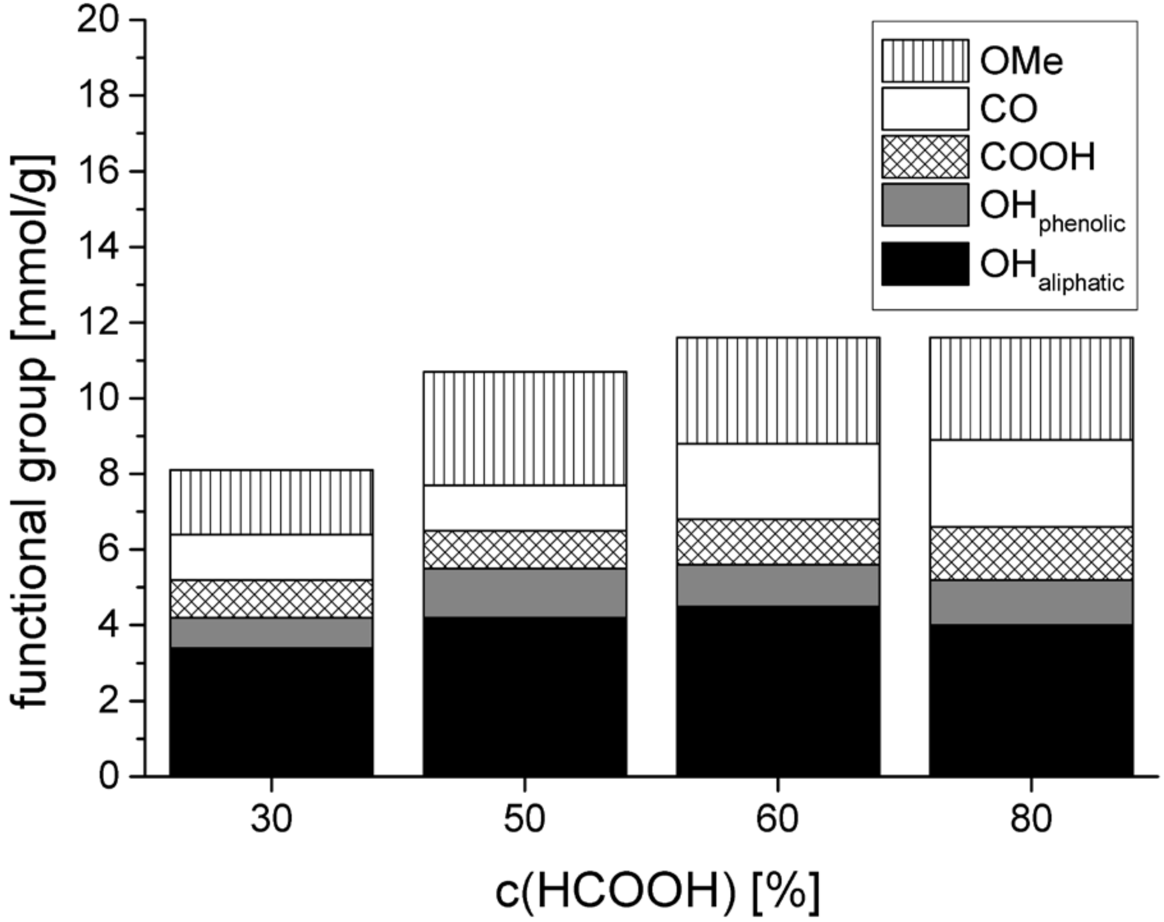
| | |
|---|---|
| | |
| Analytic of pulp: | ➢ Intrinsic viscosity<br>➢ Determination of crystallinity by X-ray diffractometry<br>➢ Scanning electron microscopy<br>➢ Composition of pulp; lignin, cellulose and holocellulose content<br>➢ Xylo-oligosaccharides at autohydrolysis |
| | |
| Analytic of lignin: | ➢ Influence of pulping duration / liquid ratio<br>➢ Influence of formic acid concentration (NP)<br>➢ Influence of NaOH-concentr. (alkaline p.)<br>➢ Functional groups<br>➢ IR-spectroscopy<br>➢ Molecular weight<br>➢ Klason lignin |

# Chemical composition of alkaline and NP-lignin




| | $M_w$ [g/mol] | PDI |
|---|---|---|
| Alkaline-pulping | 3000-4000 | 7-8 |
| Natural-pulping | 14500 | 9 |

# Influence of formic acid concentration on structure of NP-lignin



# Scale-up of alkaline pre-treatment

(400-L-scale in Fraunhofer Center for Chemical-Biotechnological Processes CBP, Leuna)

**1. Charging the digester**

1. Charging the digester

**2. Pulping procedure**

1. Charging the digester

2. Pulping procedure

**3. Separation of pulp**

1. Charging the digester

2. Pulping procedure

3. Separation of pulp

**4. Lignin precipitation**

1. Charging the digester

2. Pulping procedure

3. Separation of pulp

4. Lignin precipitation

**5. Lignin separation**

# Scale-up of alkaline pre-treatment in CBP Leuna

| Scale | Sample | Cellulose [%] | Hemi-cellulose [%] | KLASON-Lignin [%] | In/Output [kg atro] | Yield [%] |
|---|---|---|---|---|---|---|
| 350-L | Wheat straw | 41,9 | 31,7 | 21,7 | 25 | |
| | Pulp | 75,2 | 18,0 | 6,5 | 11,5 | **46,1** |
| | Lignin | - | - | **61,7** | 10,0 | 25,0 |
| 2-L | Pulp | 76,5 | 12,3 | 4,3 | 0,07 | **55,2** |
| | Lignin | - | - | **66,0** | 0,03 | 19,3 |

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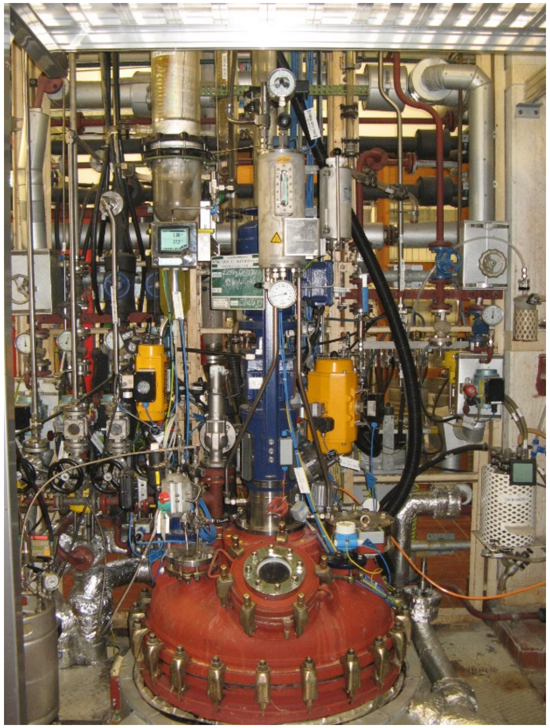
# Scale-up of Natural Pulping pre-treatment

- 600-L Reactor (enameled)
- Agitation: 1 impeller
- Including distillation-unit

Lab-scale

(SIAB)

Pilot-scale

(Lanxess Deutschland GmbH,
Group Function Innovation and Technology)

SIAB

# Scale-up of NP process, composition of pulp

| **Parameter** | **Lab-scale** | **Pilot-scale** |
|---|---|---|
| Vessel | 5 L (glass) | 600 L (enameled) |
| Wheat straw (DM) | 120g | 23 kg |
| *liquid ratio* | *14:1* | *14:1* |
| *Scale factor* | | *1:200* |
| *Yield of pulp* | *50-55%*<br>*related to straw-input* | *51,2%*<br>*related to straw-input* |
| | | |
| Content of cellulose | ~79% | |
| Hemicellulose | ~10% | *(still in process)* |
| Lignin | ~14% | |
| | | |

# Comparison of all pre-treatments

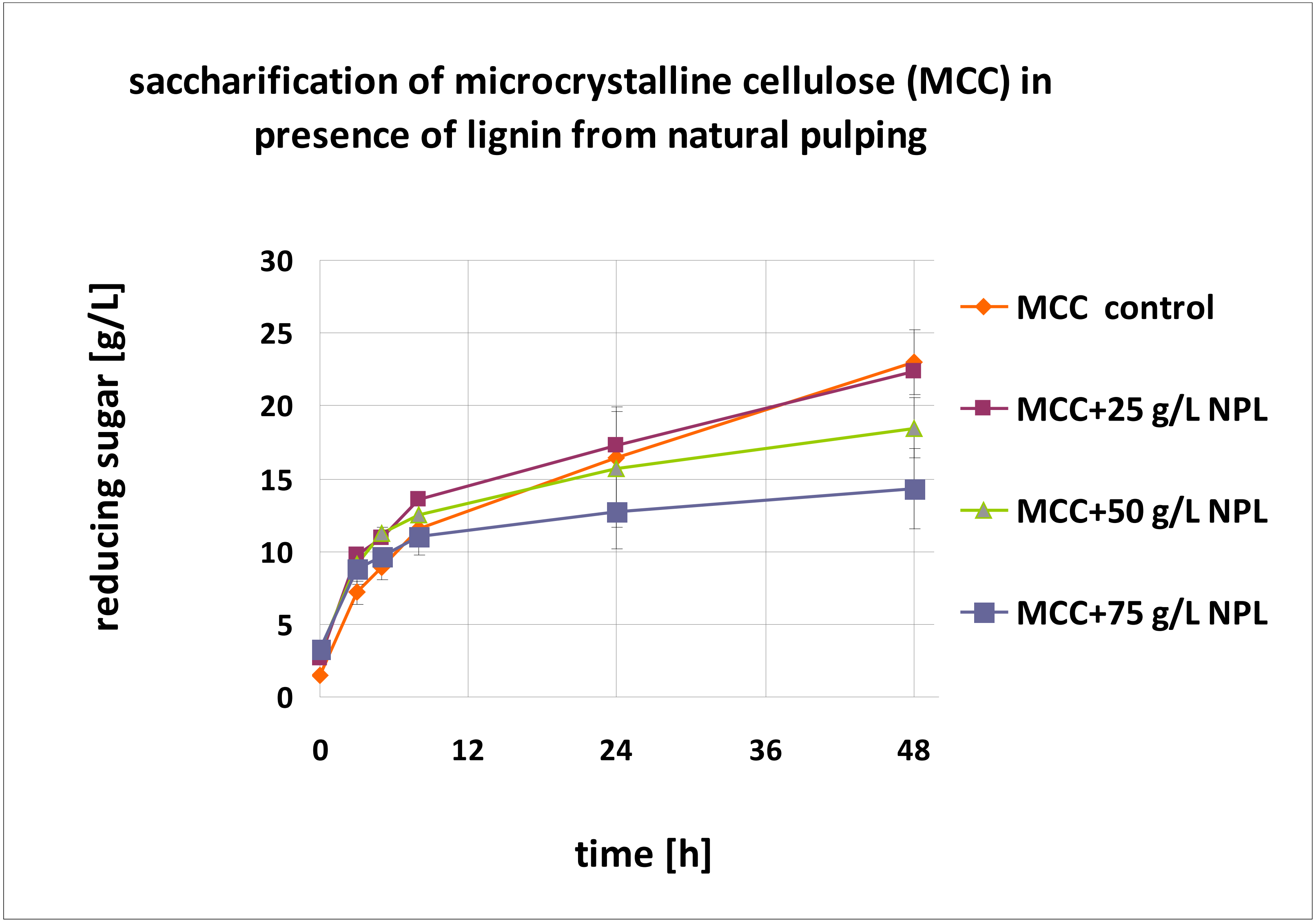
| Pre-treatment | Advantage | Disadvantage |
|---|---|---|
| **Natural Pulping**<br>Liquor ratio 1:14 | ▪ Recovery of formic acid<br>▪ High purity of lignin<br>▪ Non-pressurised process<br>▪ Pulp has a lower intrinsic viscosity | ▪ High content of lignin in pulp<br>▪ Low solid content (1:14!)<br>▪ Corrosion protection (e.g. enameled steel) |
| **Alkaline Pulping**<br>Liquor ratio 1:6 | ▪ Low lignin content in pulp<br>▪ High technological readiness | ▪ Recovery of sodium hydroxide<br>▪ Pressure of 6 bar |
| **Auto-hydrolysis**<br>Liquor ratio 1:8 | ▪ No chemicals needed<br>▪ Recovery of hemicellulose | ▪ High energy consumption<br>▪ Pressure of 20-25 bar |
| **Supercritical extraction** | ▪ Recovery of $CO_2$ | ▪ High energy consumption (pump and cooler)<br>▪ Pressure of 200-300 bar<br>▪ Low carbohydrate yield |

# Quality of lignin obtainable by the used processes

| Lignin | Advantage | Disadvantage |
|---|---|---|
| **IAT**<br>(kraft lignin)<br>95% lignin | ▪ High content of phenolic hydroxyl groups<br>▪ High Klason-lignin content | ▪ Contains sulfur |
| **Alkaline pulping**,<br>wheat straw<br>60%–70% lign. | ▪ High content of phenolic hydroxyl groups<br>▪ Sulfur-free | ▪ Low Klason-lignin content |
| **Natural pulping**,<br>wheat straw<br>80% lignin | ▪ High Klason-lignin content<br>▪ Sulfur-free | ▪ Low content of phenolic hydroxyl groups |

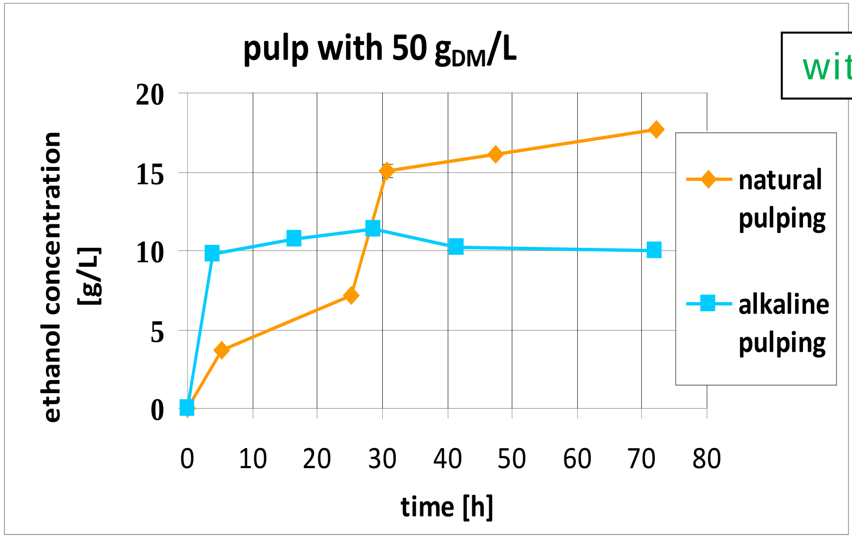
# *Penicillium verruculosum* enzyme complex

# Study on inhibition of *P. verruculosum* cellulase by NP-lignin

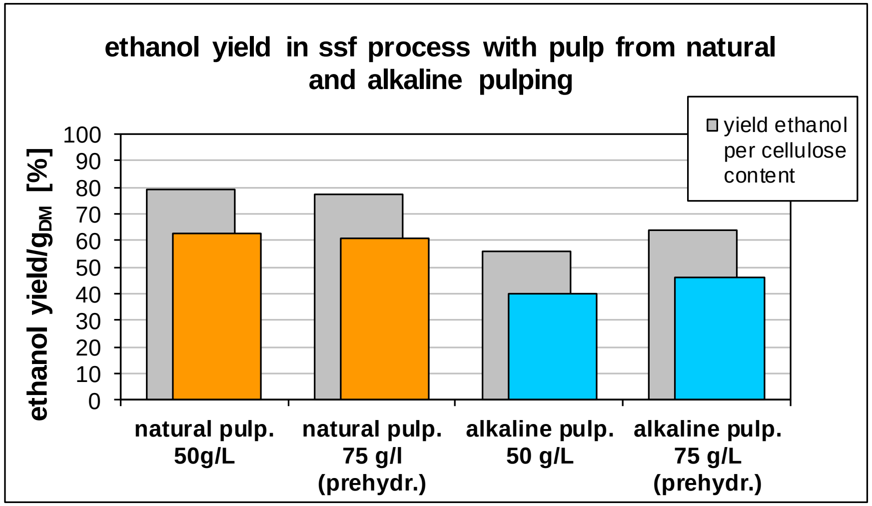


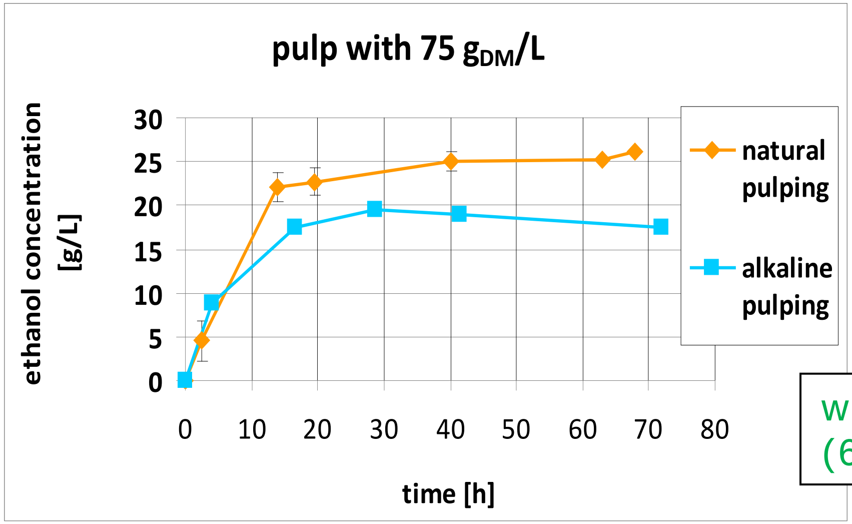
# Investigations on the SSF-process

# Comparison of SSF between pulp from natural and alkaline pulping




without pre-hydrolyses




with pre-hydrolyses (6h, 45°C)

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# SSF-Process in technical scale at CBP Leuna

- 220 L Reactor
- Agitation: anchor stirrer
- Medium composition:
  - 100 g/L alkaline pulp,
  - 50 FPU/g Cellulose (DM),
  - 7,5 g/L yeast, + inorganic compounds
- Pre-hydrolysis 45°C
- Fermentation 35°C; pH =4,5 – 5,5

**Results:**

- EtOH-max: 3,84 Vol.%
- EtOH-yield: 55,3% (related to pulp dry matter)
- EtOH-yield: 73,7% (related to cellulose-content in pulp)

# SSF-process

- economical ethanol production:

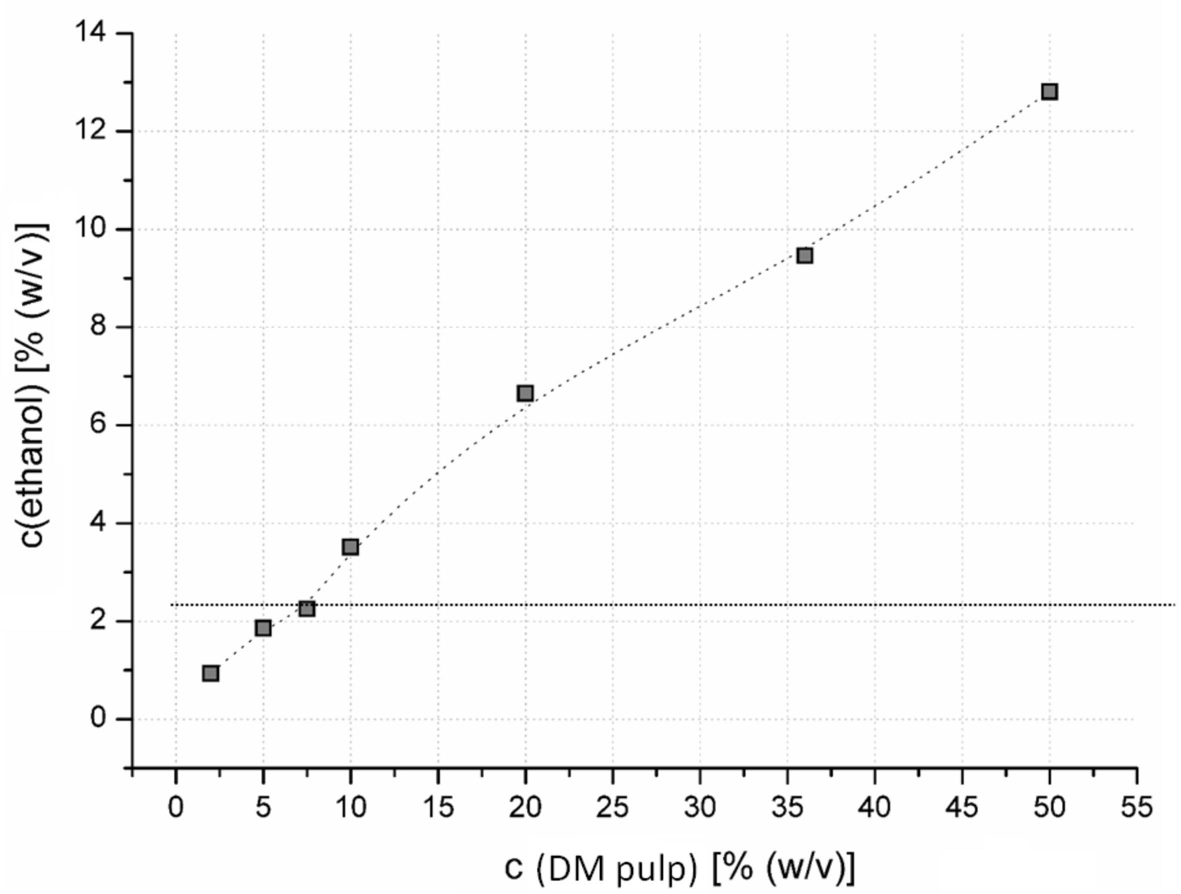
  ethanol conc. **>4 % (v/v)** required

  → **>10 % (w/v)** solid concentration necessary

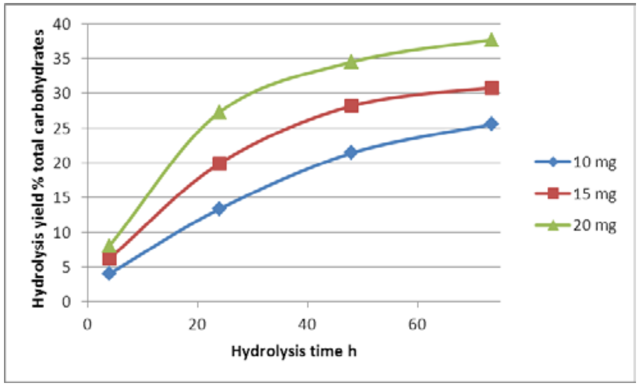
- High viscosity → “free-fall-mixing” required → 15 L solid state bioreactor

# SSF-process in solid state reactor

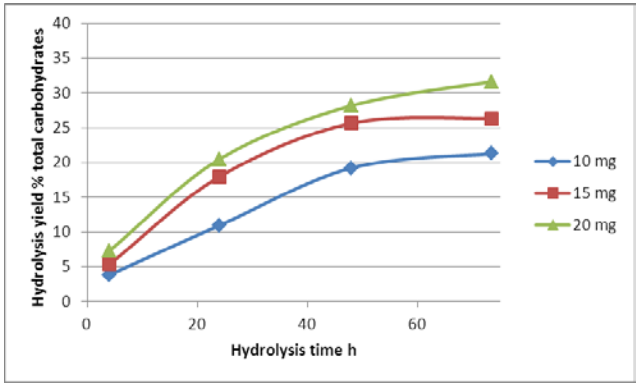
## High solid content



# Optimization of the SSF process




*Penicillium verruculosum* UK53

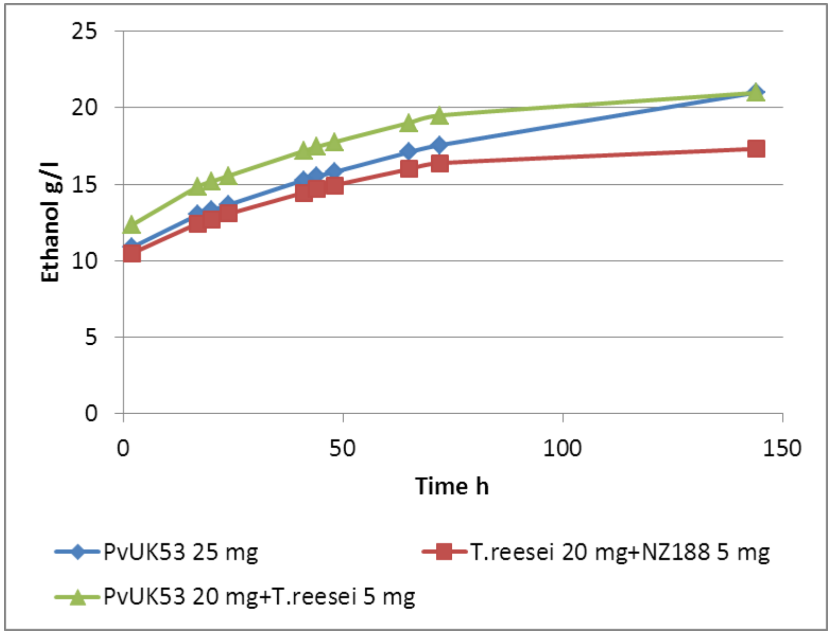



*Trichoderma reesei* +Novozym 188 4:1

## Comparison of *Penicillium verruculosum* and *Trichoderma reesei* enzyme mixtures in hydrolysis

- Penicillium verruculosum enzyme mixture was more efficient in hydrolysis of NP cellulose than *T. reesei*+Novozym 188 reference at enzyme dosages 10-20 mg/g substrate DM
- *Penicillium verruculosum* enzyme mixture has high β-glucosidase activity compared to *T.reesei*, which is seen as more efficient hydrolysis of Avicel-cellulose and hydrothermally pretreated wheat straw in absence of added β-glucosidase
- Partial replacement (at least 20 %) of *P.verruculosum* UK53 with *T. reesei* enzyme mixture slightly improved hydrolysis yield from NP wheat straw (ca 5%)
- No individual enzyme component (EGs, xylanases and β-xylosidases were tested) responsible for improvement could be identified (data not shown)
- Hydrolysis of NP cellulose required relatively high enzyme dosage
- Soluble compounds extracted from NP cellulose were not the cause of inhibition of hydrolysis.

Time-course hydrolysis of NP cellulose (10 % DM) with *Penicillium verruculosum* UK53 and *Trichoderma reesei* supplemented with β-glucosidase from Novozym 188 at different protein loads (10, 15 and 20 mg/g substrate (DM)). T=35°C, pH 5. Released sugars were quantified using PAHBAH assay and glucose standards and HPLC.




## Comparison of *Penicillium verruculosum* and *Trichoderma reesei* enzyme mixtures in SSF

- Ethanol yield from NP cellulose was 50.5-63.6 % from theoretical maximum when *P.verruculosum* UK53 enzyme mixture was used, whereas 45.5-51.5% yield was reached with *T. reesei*+Novozym 188 enzyme mixture (prehydrolysis 24 h at 35-45°C, enzyme load 20-25 mg/g substrate DM)
- Similar to hydrolysis results, addition of *T.reesei* enzymes to *P. verruculosum* mixture facilitated ethanol production in fermentation

Comparison of *Penicillium verruculosum* UK53, *P.verruculosum* UK53+*T.reese*i and *T.reesei*+Novozym 188 enzyme preparations in SSF ethanol production from NP cellulose. Conditions: 10 % substrate (DM), prehydrolysis at 45°C (24h), SSF at 35°C, pH 5, yeast RedStar 5g/l, enzyme load 25 mg/g substrate.

# Results to the SSF-process

## Hydrolysis

- Enzyme complex from *P. verruculosum* leads to higher hydrolysis rates in comparison with *T. reesei*
- No or only low inhibition of *P. verruculosum* cellulase by lignin

## SSF-Process

- Pulp from NP process generate more ethanol in SSF process than pulp from alkaline pulping
- Stirred bioreactors (CSTR) allow a maximum of about 7.5 %(w/v) pulp, therefore a partial pre-hydrolyses of pulp or feeding in fed-batch-technique must be performed to realize economic concentrations of ethanol
- Yield ethanol / g NP-cellulose > 75% (laboratory conditions)
- Pre-hydrolyses with *P. verruculosum* cellulase improves particularly strong the SSF-process with pulp from Natural Pulping pre-treatment
- Solid state fermenter: 50 %(w/v) pulp content → max. 12.8 %(w/v) ethanol
- The yeast needs to be optimized for the SSF process

## Dehydratisation of ethanol

- 100 % conversion of ethanol *(results shown in Appendix)*
- < 80 %(v/w) ethylene was produced directly from fermentation broth

# Compounding and press moulding of fibre reinforced composites

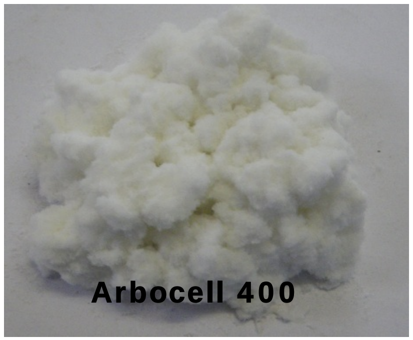
# Objektives (WP 6)

## Production of Lignin-based Bio-Composites

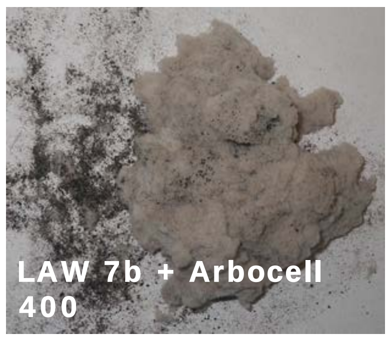
1. Examination of lignin processing:
   - Compounding,
   - Press moulding,
   - Moulding temperature,
   - Lignin acetylation (*Ghosh et al. 1999*),
2. Improvement of fibre to lignin bonding:
   - E-beam treatment of lignin and composites,
   - coupling agents,
3. Analyses of composite properties
   - Tensile strength, MOE (*EN ISO 527-4*)
   - 24h Water absorption (*EN ISO 62*)

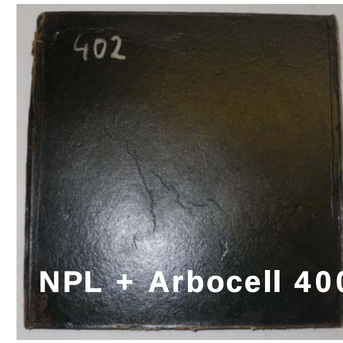
# Compounding and Pressing

**Raw material** → **Compounding** → **Hot Press** → **Composite**





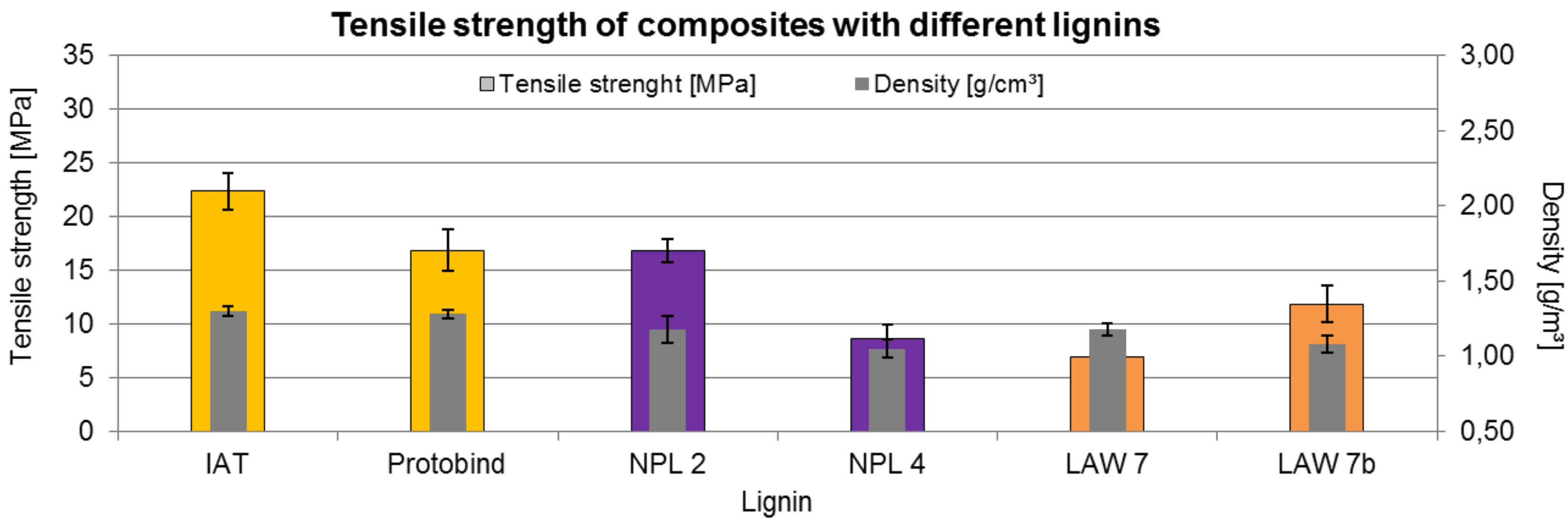



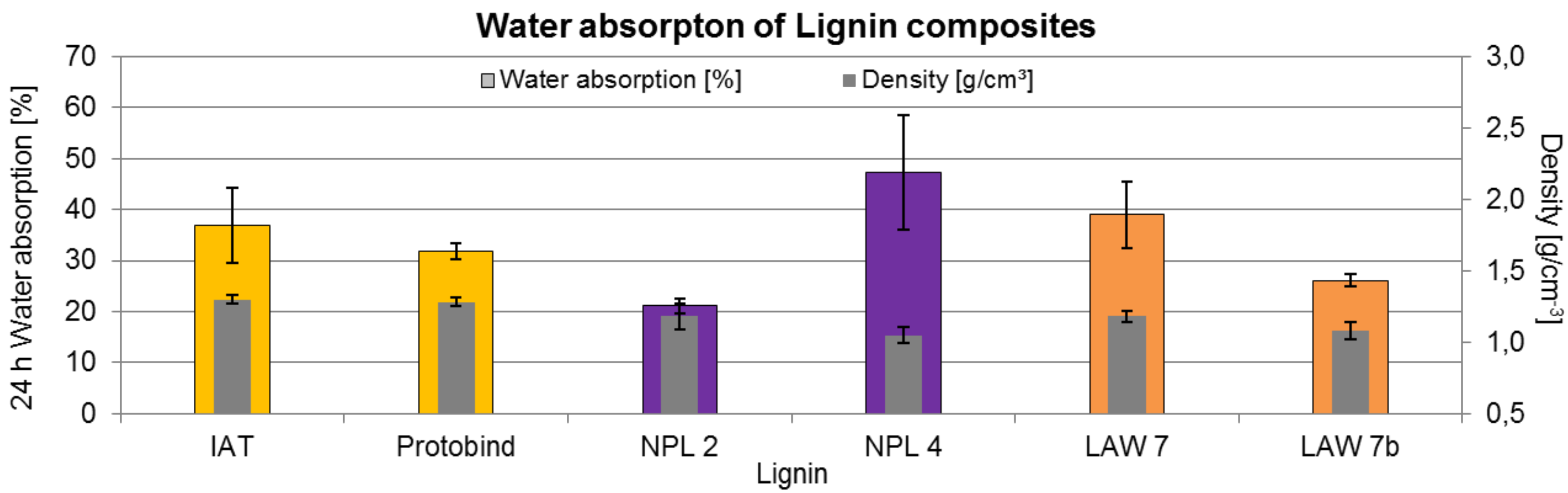


**Processing of Press moulding**

| | |
|---|---|
| **Bulk L/F** | : 55% / 45% (40 g (22 g Lignin + 18g fibre)), |
| **Pressure** | : 1,1 – 2 N/mm² |
| **Thickness** | : 3 mm |
| **Density** | : 1250 – 1300 kg/m³ |

# Different lignin



# Summary to composites

- Lignin particle size has strong influence on compounding,
- Lignin has a very small melting temperature range,
- Salt content influences the composite properties,
- TS of NPL- and LAW- composites are comparable to soda lignin,
- WA of NPL- and LAW- composites vs. com. Lignin is lower,
- Lignin acetylation improves melting but reduces TS,
- Coupling agents and e-beam treatment improve the composite properties

## Further work

- Processing of LAW 7 (alkaline pre-treatment) from CBP,
- Processing of NPL from Lanxess Pulping,

# Lignin modification

# Scope of the study

- Exploring the enzymatic modification of lignin with hydrophilic compounds to produce novel functional materials
  - functionalized carbohydrates
  - peptides & proteins

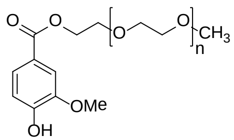
➢ lignin-chitosan conjugates:
  - improved optical response ⇒ pigmenting materials and fillers in paints and coatings
  - enhanced antimicrobial properties ⇒ packaging, antimicrobial hydrogels

➢ Characterization of native and modified lignin

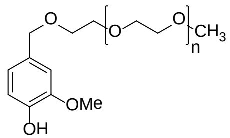
# Modification of lignin by chemo-enzymatic functionalization

**NP lignin**
**Indulin AT**

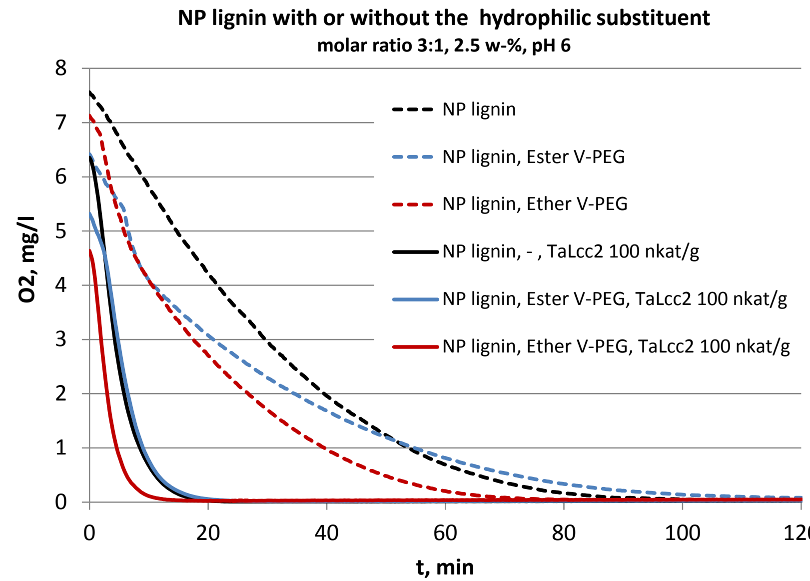
**Ester V-PEG**




**Ether V-PEG**




**Laccases**:
*Thielavia arenaria Lcc1* = TaLcc1
*Thielavia arenaria Lcc2* = TaLcc2
*Melanocarpus albomyces* = Mal / r-Mal




- Functionalization of NP lignin and Indulin AT with synthesized hydrophilic substituents *(Ester/Ether V-PEG)* was experimented using laccases in pH range 6-8 in order to soften lignin
- **Improved thermoplastic properties of lignin** are desirable in composite processing
  - Enhanced compatibility with reinforcement fibres
  - *Reduced VOC formation*

Monitoring of dissolved $O_2$ consumption in the system → Laccase/$O_2$ reactive towards lignins, hydrophilic substituents, and the 2-component system thereof

# Conclusions to lignin modification

- Selective extraction/fractionation in organic solvents produce LMW and HMW with defined properties
- FT-IR & chemometrics can be used to:
  - discriminate lignin based on processing conditions
  - predict molecular properties functional group composition ($^{31}P$-NMR) of lignin.
- S:G:H ratio controls the reactivity of lignins with laccase
- OHL and KSL lignin were successfully modified with glucosamine.
- OHL and AWL lignin were successfully modified with GYG and chitosan

# Open questions still to be worked:

## Pre-treatment of lignocellulose:

The different pre-treatment processes are economical to compare with respect to the particular application, e.g. pulp for ethanol, lignin for basic chemicals or for composites.

## SSF-process:

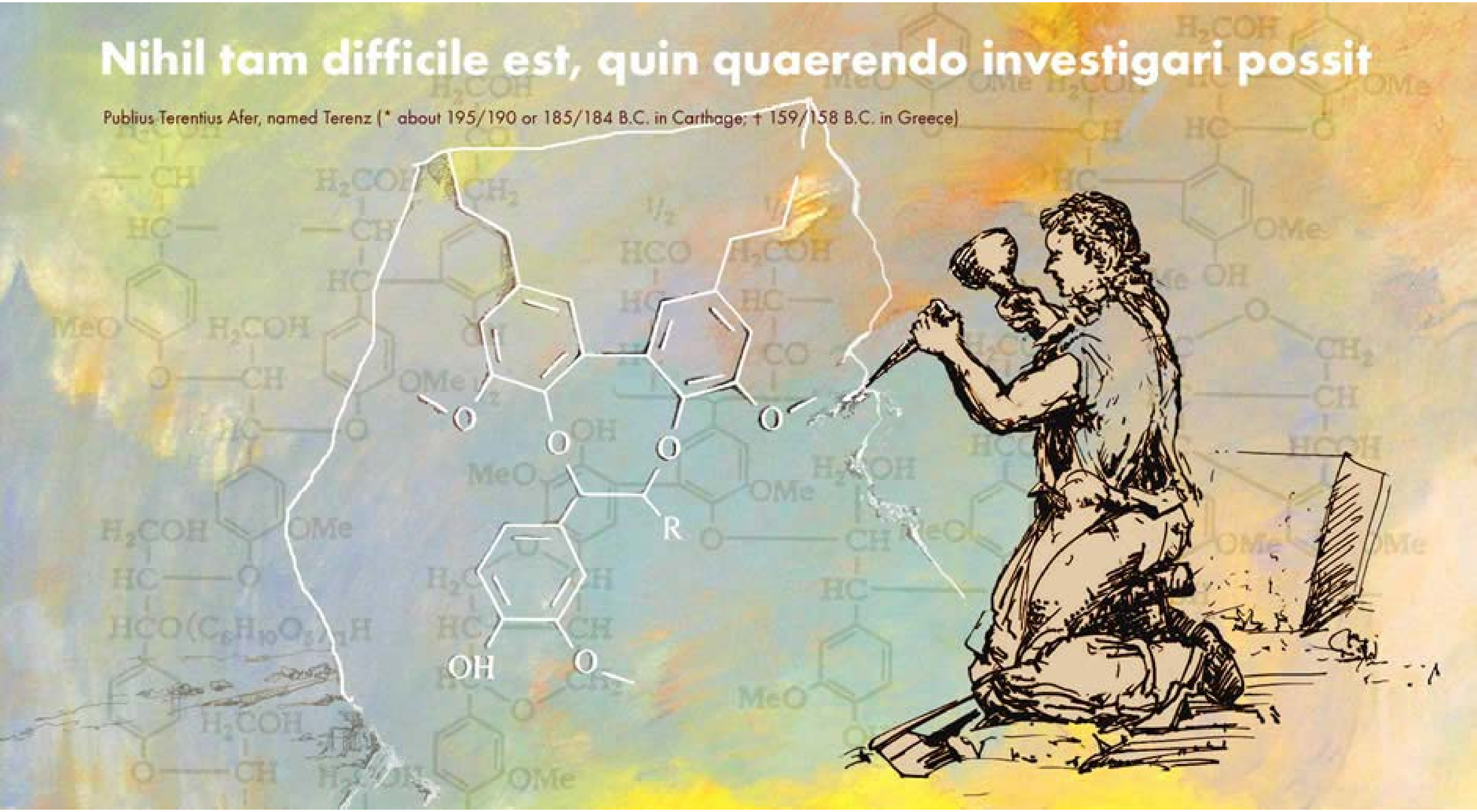
The provision of the necessary amount of pulp for > 10% ethanol in the SSF-process must be optimized. The possibilities for this are partial pre-hydrolysis of the pulp, feeding of pulp in fed-batch-technique or a solid-state-fermenter.

## *P. verruculosum* production strain:

The *P. verruculosum*-enzyme complex is favoured for the SSF-process in “second generation”. For industrial scale, the used *P. verruculosum* production strain must be improved, in particular to eliminate the carbon catabolite repression by classical genetic methods. This has the advantage that the strain can be produced in the ethanol plant without the requirements for GMOs.

## Composite material and lignin modification

The mechanical and hygroscopic properties of composites are to be investigated in relation to the lignin from different outstanding pre-treatment processes. This concerns e.g. phenolic OH-groups, Mol. weight or Klason lignin.



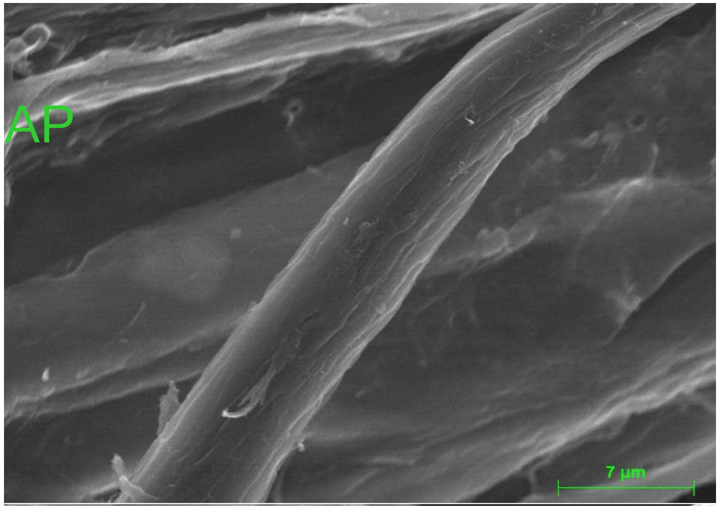

**Thank you for your attention!**

## Consortium at project meeting in Bucharest, 2012

# Notes
# to the presentation

# To pre-treatment of lignocellulose

# Scanning electron microscopy

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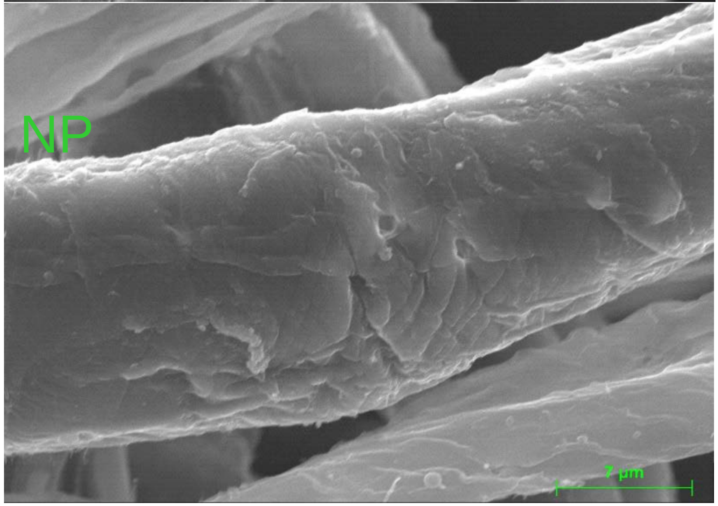
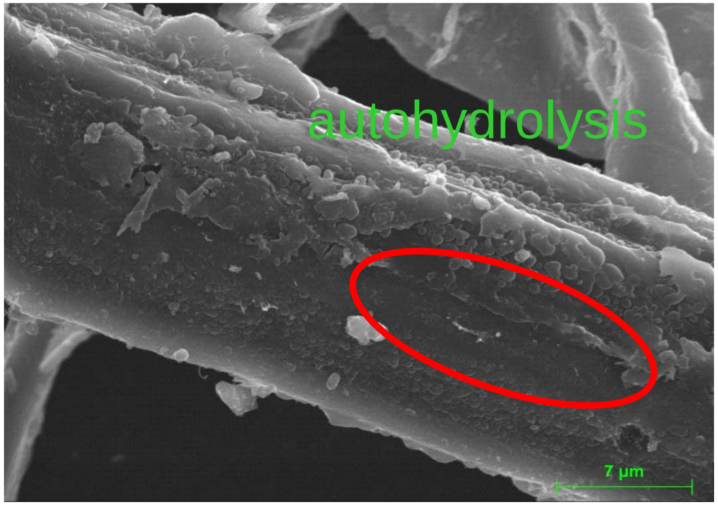


# Comparison of the pre-treatment:

| | Alkaline pulping | Natural Pulping |
|---|---|---|
| **optimal pulping conditions** | $c_{(NaOH)}$ = 3 wt-%<br>T=160 °C<br>t= 30 min | $c_{(HCOOH)}$ = 60 %<br>T=103-105°C<br>t=40 min after $H_2O_2$ addition (30 %) |
| **yield of pulp*** | ca. 55 %<br>Contains:<br>2 % Klason-lignin<br>79 % cellulose<br>19 % hemicellulose | ca. 45-50 %<br>Contains:<br>11 % Klason-lignin<br>83 % cellulose<br>6 % hemicellulose |
| **yield of lignin precipitation product*** | ca. 20 %<br>➢ contains 70 % Klason-lignin<br>➢ ca. 60 % of original lignin is obtained | ca. 10 %<br>➢ contains 80 % Klason-lignin<br>➢ ca. 40 % of original lignin is obtained |

* in relation to wheat straw

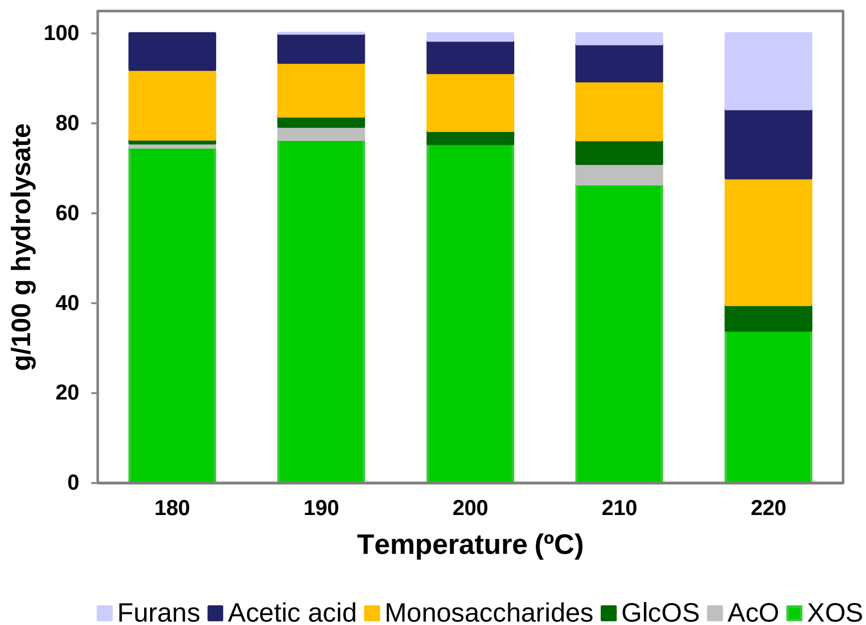
# Determination of crystallinity

| pulp | grinding | hydrolysis rate [%] | $C_r$ [%] |
|---|---|---|---|
| alkaline pulping c(NaOH)= 3 % | milled | 83.4 | 72.2 |
| alkaline pulping c(NaOH)= 3 % | TMP | 82.9 | 69.2 |
| alkaline pulping c(NaOH)= 9 % | TMP | 94.0 | 61.3 |
| natural pulping optimum conditions | milled | 77.0 | 58.0 |
| autohydrolysis optimum conditions | milled | 100.0 | 63.3 |

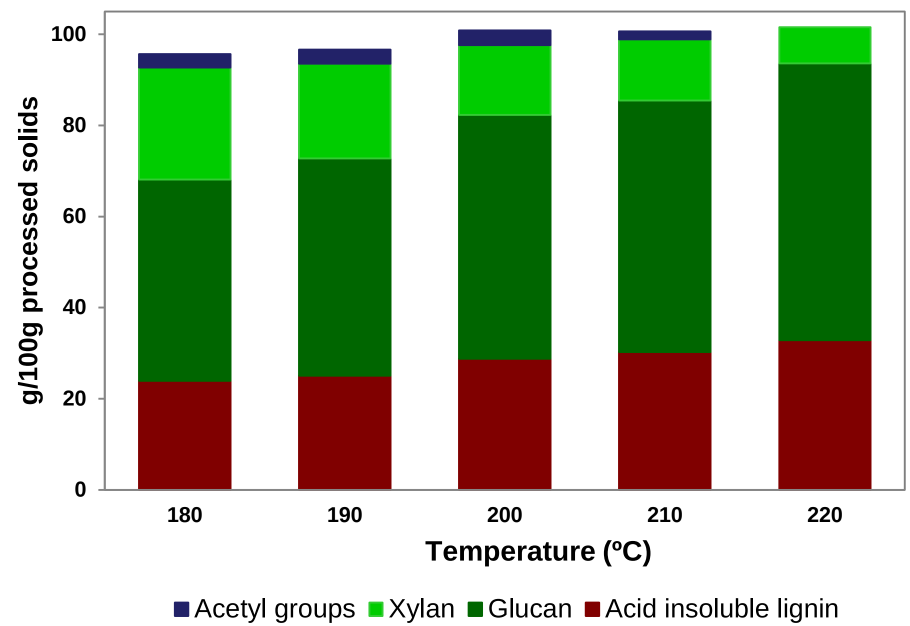
# Hydrothermal pre-treatment (autohydrolysis)

## ➢ Optimisation of xylo-oligosaccharides production

➢ Liquid phase




➢ Solid phase

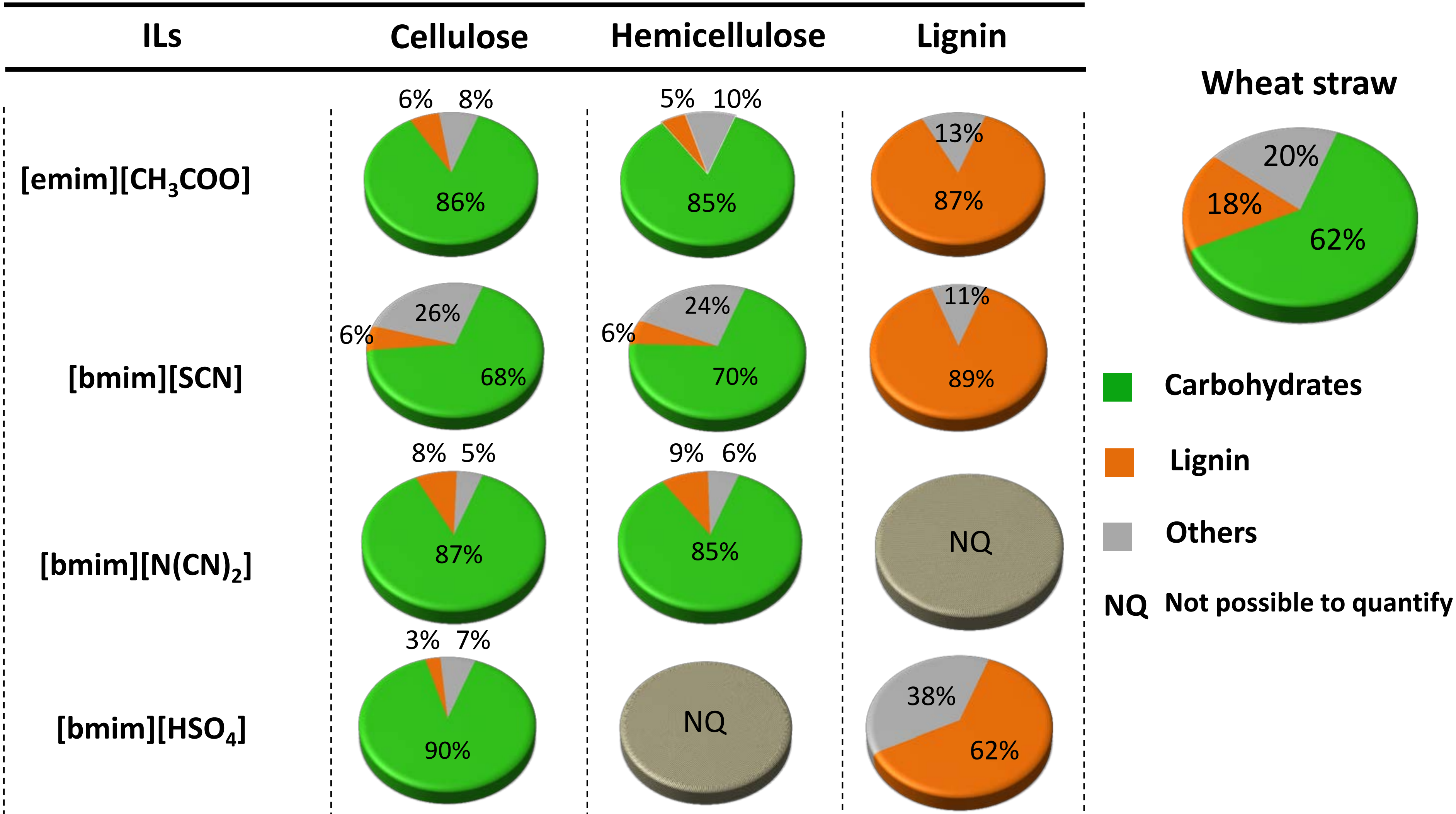



- ✓ **Autohydrolysis is highly selective towards hemicellulose enabling a high recovery of xylo-oligosaccharides (XOS)**
- ✓ **An important glucan and lignin enrichment of the solid phase was possible making the solids very attractive for further processing (*i.e.* enzymatic saccharification)**

# Conclusions to autohydrolysis:

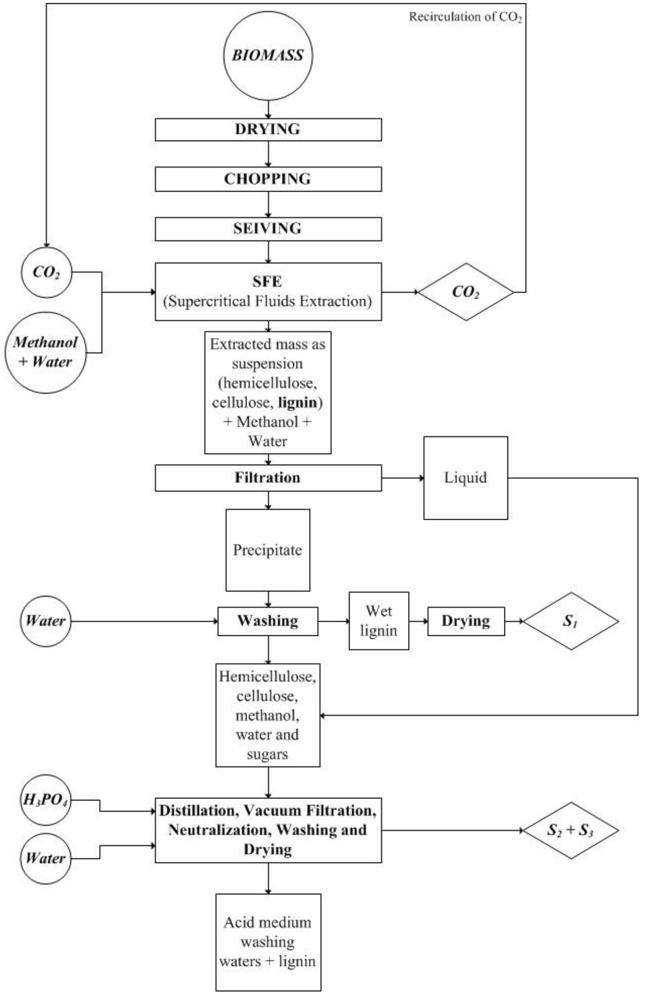
- ✓ Autohydrolysis is highly selective towards **hemicellulose** enabling a high recovery of **XOS (~50% of initial xylan)**
- ✓ An important **glucan** and **lignin enrichment** of the solid phase was possible making the solids very attractive for **further processing**
- ✓ **Ionic liquid** is a good solvent for wheat straw and can be **recovered** (up to **95%**$_{\textbf{initial}}$) and reused
- ✓ **Carbohydrate** rich-samples demonstrate **elevated purities**
- ✓ **High purity lignin** could be extracted

# Ionic Liquids Pre-treatment




✓**All ILs** tested are good solvents for wheat straw
✓Can be **recovered** and reused
✓Enable a **selective fractionation** of the main macromolecular components
✓**High purity lignin** and **cellulose** can be extracted

# Pilot scale supercritical fluids extraction installation



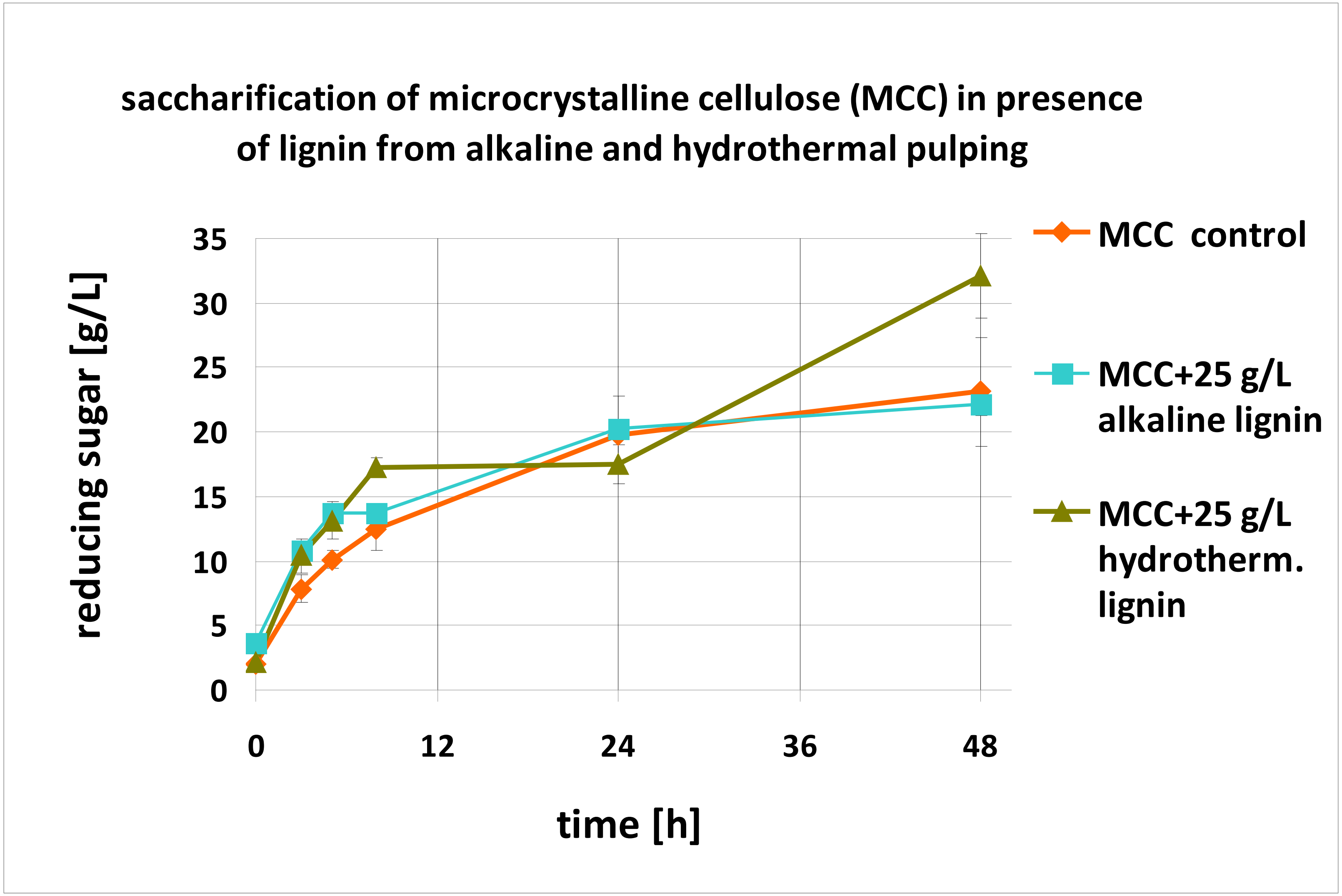

Technology of lignin extraction in laboratory pilot plant, 100 ml - BIOTEHNOL

$CO_2$ extraction unit: SFE Model 100
Producer - Thar

- Batch weight — 20-30 g
- $CO_2$ flow — 4 g/min
- Co-Solvent flow — 4 g/min
- ( MeOH/water mixture) — 70%
- Pressure — 200 bars
- Temperature — 113-118 $^oC$
- (pseudo) steady state regime — 60 min
- Initial transitory regime — 20 min
- Co-solvent removal — 30 min

# To the SSF-process

# Study on inhibition of *P. verruculosum* cellulase by lignin from alkaline and hydrothermal treatments

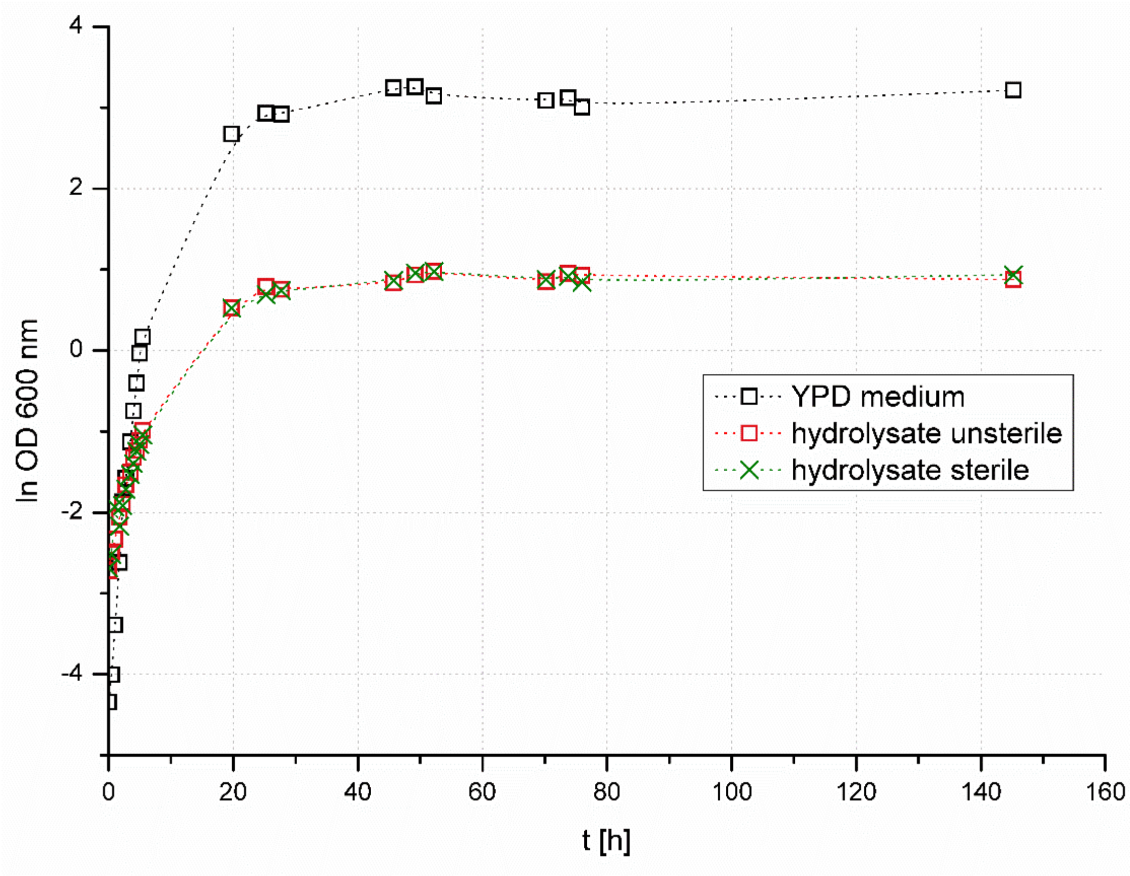


# Preliminary tests for SSF-process

## Fermentation

- 37 °C
- pH 7.0, tap water
- 200 mg nitrogen
  - ✓ → 1:1 $(NH_4)_2SO_4$ : thin stillage
- 3 %(w/v) yeast

  *(S. cerevisiae)*




Conclusion:

- Yeast grows on Hydrolysate,
- no sterilization necessary

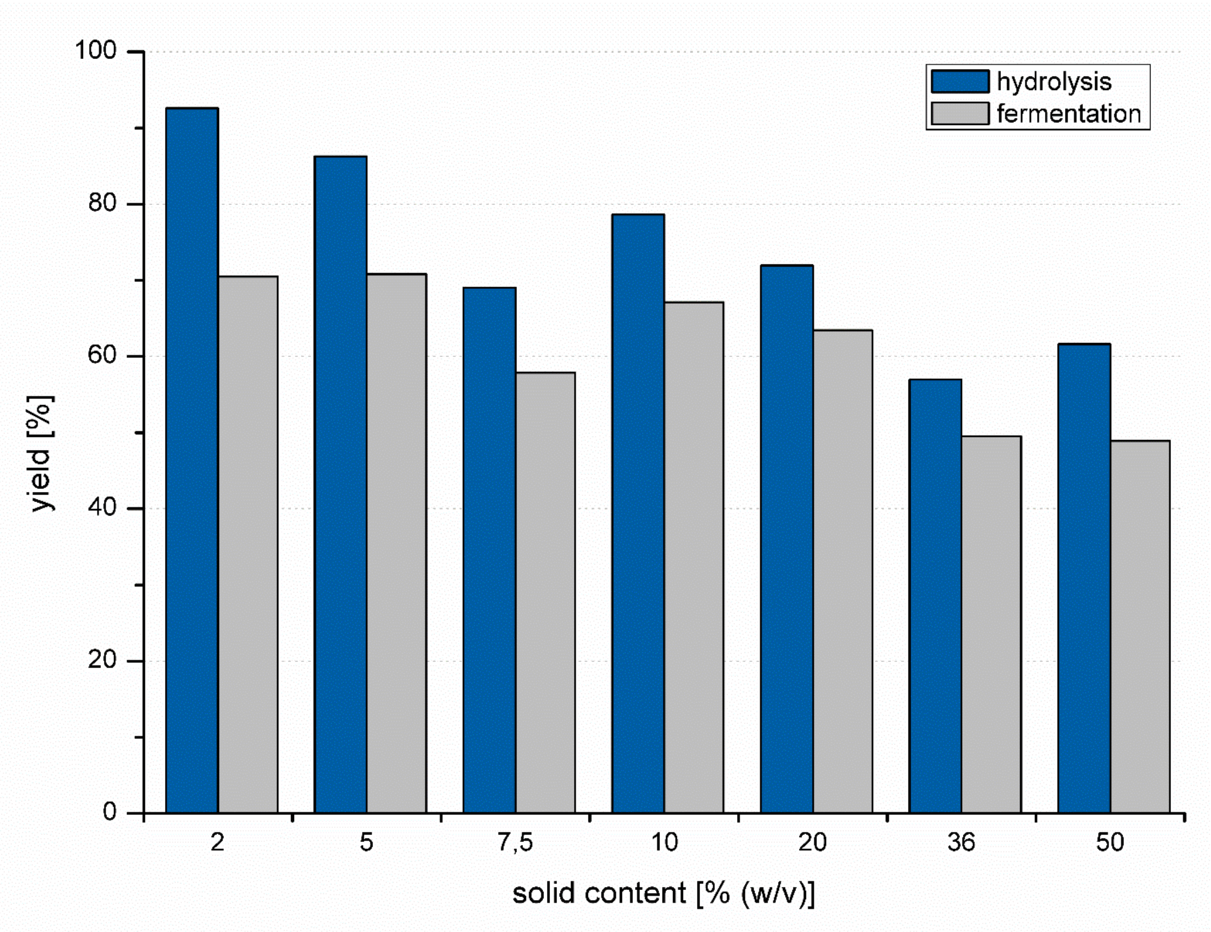
# Preliminary tests for SSF-process

- **Enzymatic hydrolysis with *P. verruculosum***
- Influence of solid concentration

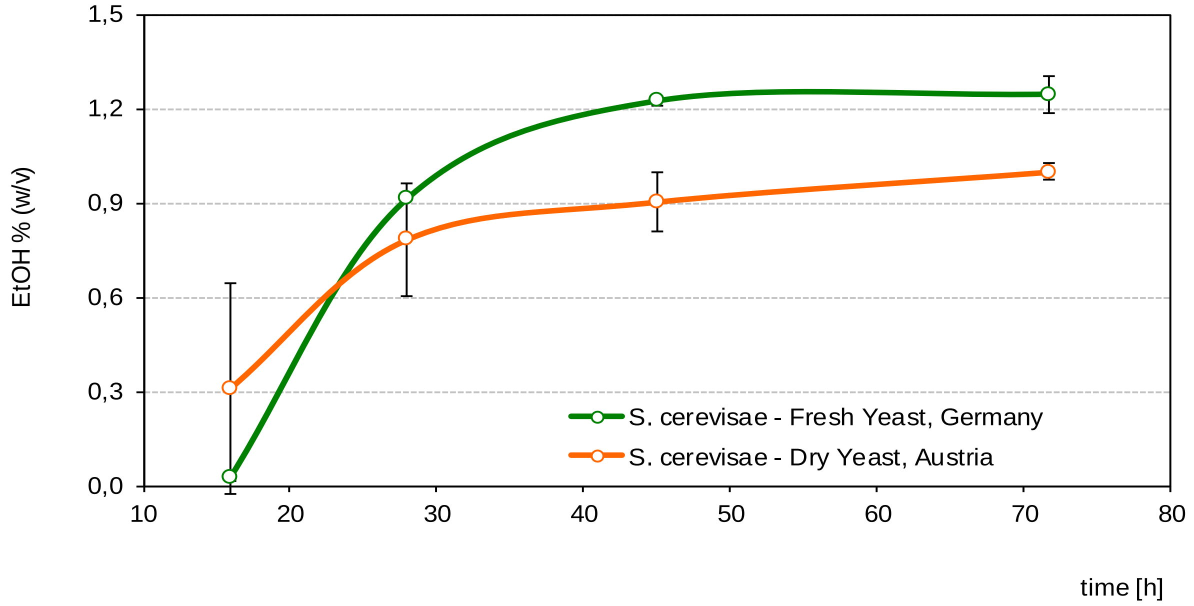
**2 %(w/v)** **5 %(w/v)** **10 %(w/v)**

→ In stirred bioreactor max. 7.5 %(w/v) solid concentration

# SSF-process at different solid content



# Comparison of different yeast strains



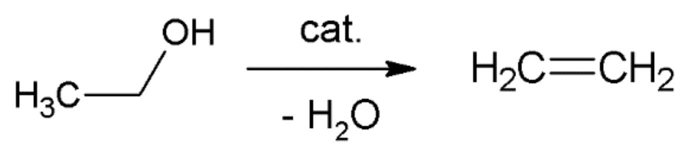

Shake flask culture: 50 g/L MCC, 30 FPU/gDM

initial $OD_{600}$=12, Temp.=35°C

# • Dehydratisation of ethanol

SSF-process

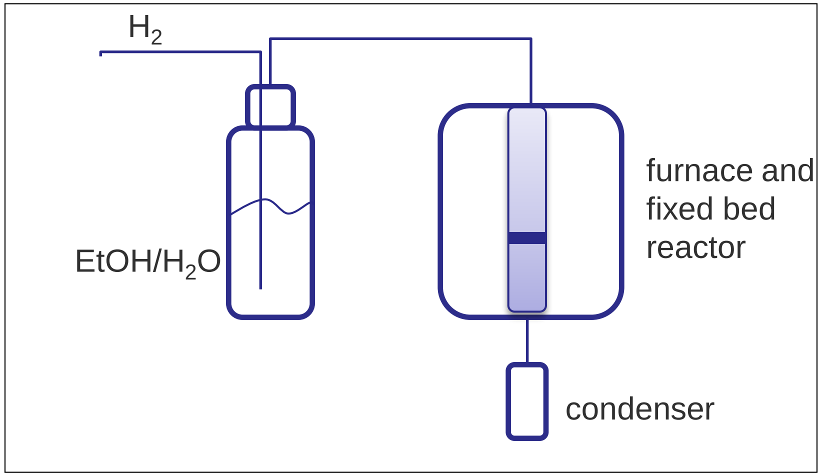
Cellulose → Glucose → Ethanol → Ethylene



# WP 8: Conversion of saccharides into alkenes

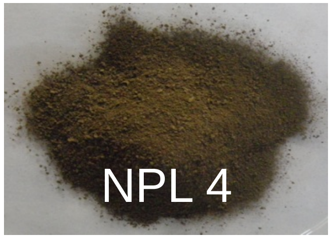
## Dehydratisation of ethanol

- catalytic conversion of ethanol to ethylene over a modified HZSM-5 catalyst
- Optimisation with model solutions:
  - T(furnace) = 350 °C → 100 % conversion but low selectivity
  - Yields up to 33 %
- Conversion of fermentation residue: **80 % (v / w) ethylene**
- By-products: diethyl ether, methane, ethane, propene, isobutene, pentane



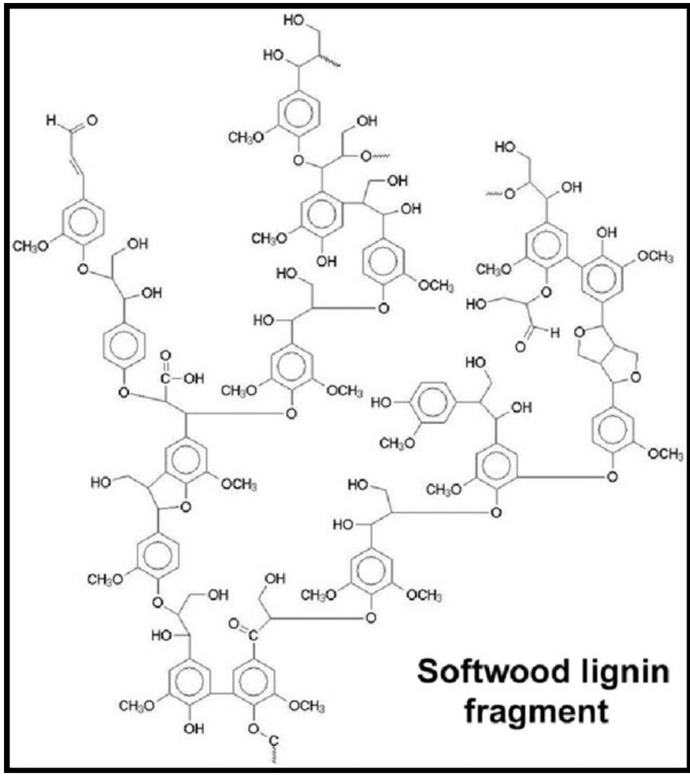
| Acronym | Name, Source |
|---|---|
| IAT | Indulin AT, Mead Westvaco (US) |
| Protobind 1000 | Protobind 1000, ALM (India) |
| NPL 1 | 120214_Fedbatch, Univ. Leipzig |
| NPL 2 | 111028-NPL, Univ. Leipzig |
| NPL 3 | 110929-NPL, Univ. Leipzig |
| NPL 4 | 130212-NPL, Univ. Leipzig |
| LAW 1 | 1206LAW$_1$19-25, TUD |
| LAW 7 | 1308-LAW$_7$ 40-41, TUD (CBP) |
| LAW 7b | 1308-LAW$_7$ 40-41 washed, TUD (CBP) |
| | |
| OS_Acetate | Organosolve_Lignin_Acetate, TUD |
| IAT_Acetate | Indulin AT-Acetate, TUD |






# To lignin modification

# Lignin application



Softwood lignin fragment

- Commercial applications:
  - dispersant, binder, emulsifier and sequestrant
  - Fuel for energy
- Emerging applications
  - Phenolic monomers and oligomers, aromatics
  - Functional materials

# Results Highlights

1. Characterization of wheat straw lignin samples
   - Mw (SEC), FT-IR, functional groups ($^{31}P$-NMR), reactivity vs. laccase, solubility organic solvents
   - 50 samples (TU Dresden), 4 commercial, 56 lignin fractions (selective fractionation)

➢ Models for fast determination of lignin properties
   - Mw prediction based on IR spectral properties
   - Discrimination of lignins obtained by different processes (natural pulping vs. alkaline)

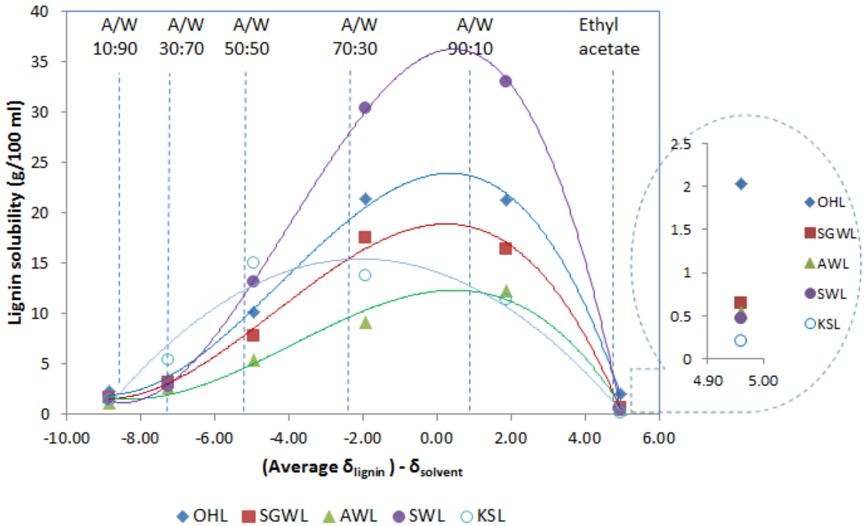
Collaboration TUD, Manuscript in preparation

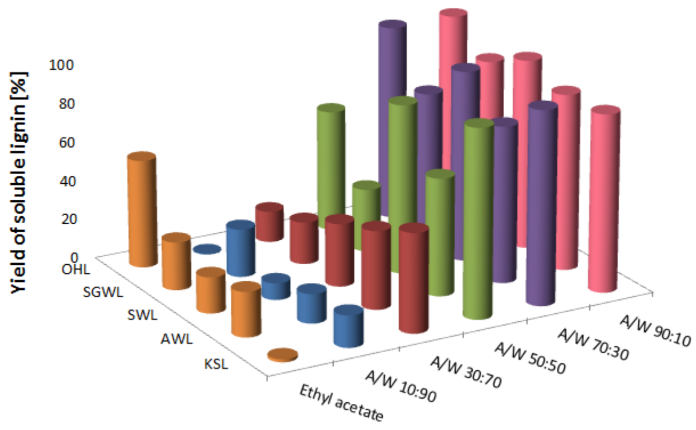
   - Functional group (NMR) prediction based on IR data

Manuscript submitted

# Results Highlights

## 2. Solubility in organic solvents and selective fractionation




- Solubility depends on composition and degree of condensation
- LMW (low condensed) and HMW (high condensed) lignin fractions can be obtained by selective extraction
- Full characterization of isolated lignins

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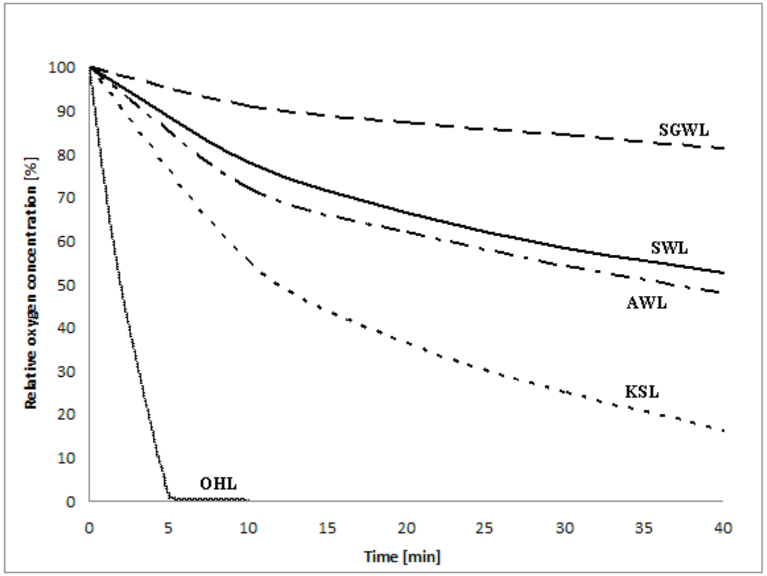
Manuscript submitted

# Results Highlights

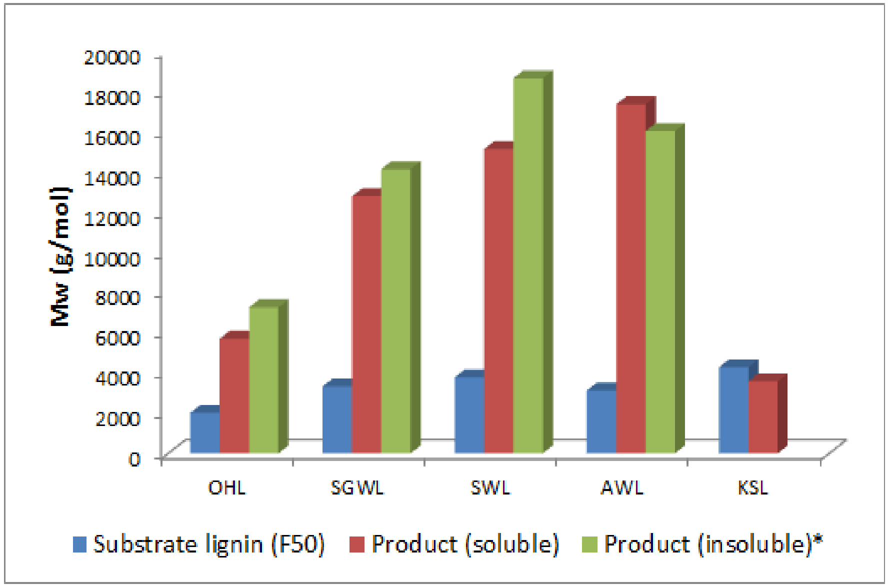
## 3. Enzymatic modification of lignins in water/acetone

### a) Lignin polymerization

Kinetics




Products




- ➤ DP = f(H)

Article In Press

# Highlights – Enzymatic modification

b) Reactions with glucosamine (GlcN):

- In solution
- Only wood lignin reacted
  OHL, KSL
- Grafting of GlcN proved by NMR and FT-IR
- Inhibition of laccase by GlcN observed

| | Mw | $OH_{aliph}/OH_{Ar}$ |
|---|---|---|
| OWL-50 | 2346 | 0.35 |
| OWL-50/laccase/GlcN | 2450 | 0.43 |
| SGWL-50 | 3913 | 0.51 |
| SGWL-50/laccase/GlcN | 4031 | 0.56 |
| SWL | 4300 | 0.63 |
| SWL-50/laccase/GlcN | 4398 | 0.66 |
| AWL-50 | 3123 | 0.39 |
| AWL-50/laccase/GlcN | 3137 | 0.31 |
| KSL-50 | 7980 | 0.63 |
| KSL-50/laccase/GlcN | 12493 | 1.01 |

# Highlights: Enzymatic modification

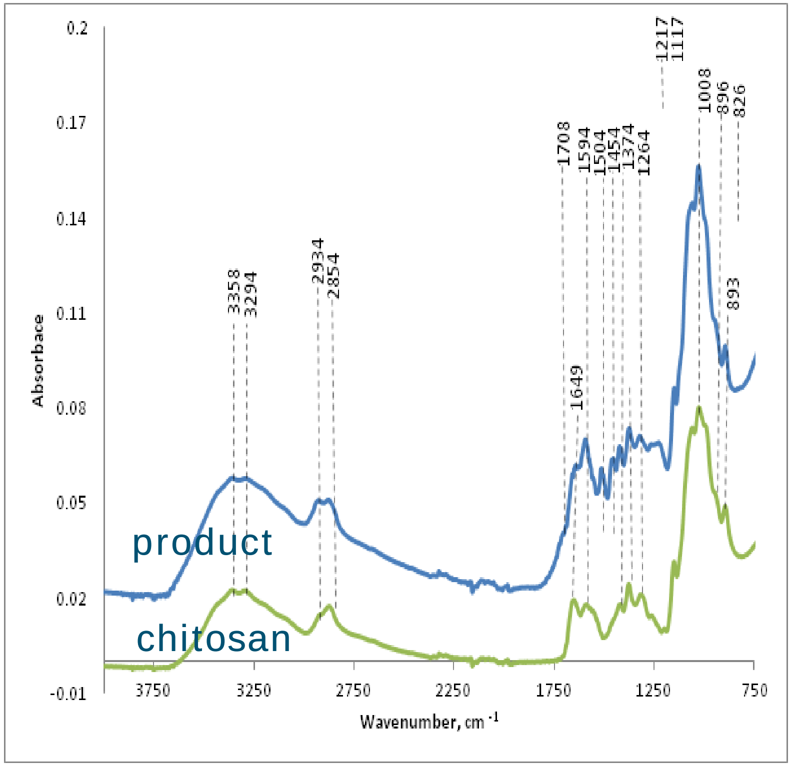
## c) Coupling lignin + chitosan

- Reaction in suspension
- Product – light brown
- Characterization
    - FT-IR
    - GPC

Soluble product

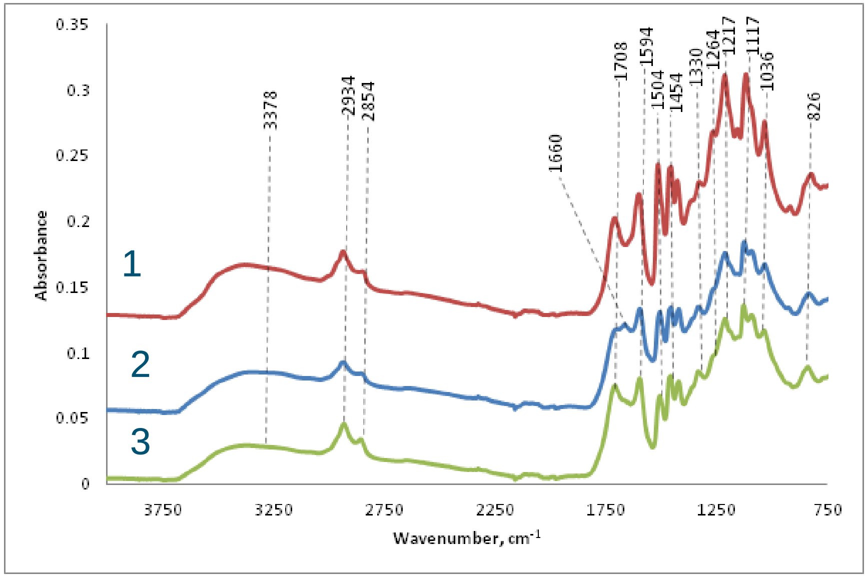
| | Mw | Mn | Mw/Mn |
|---|---|---|---|
| OHL-50 | 2346 | 264 | 8.9 |
| OHL-50/Chitosan | 2330 | 282 | 8.3 |
| OHL-50/Laccase | 5708 | 714 | 8.0 |
| OHL-50/Laccase/Chitosan | 10091 | 858 | 11.8 |

Inoluble product



# Highlights: Enzymatic modification

## d) Coupling lignin + Gly-Tyr-Gly (GYG)




1: control alkali wheat straw lignin-GYG, no enzyme
2: reaction alkali wheat straw lignin-GYG-laccase
3: control alkali wheat straw lignin-laccase

| | Mw | Amide/G (IR) | $OH_{Ar}/G$ |
|---|---|---|---|
| OHL-50 | 2346 | 0.35 | 3.85 (NMR) |
| OHL-50/GYG | 2423 | 0.36 | 3.85(NMR) |
| OHL-50/laccase | 18587 | 0.8 | 4.00 (NMR) |
| OHL-50/laccase/GYG | 5708 | 0.4 | 4.4 (NMR) |
| | | | |
| AWL-50 | 3123 | 0.6 | 0.3 (IR) |
| AWL-50/GYG | 3759 | 0.5 | 0.27 (IR) |
| AWL-50/laccase | 17359 | 0.8 | 0.28 (IR) |
| AWL-50/laccase/GYG | 13214 | 1.0 | 0.4 (IR) |

- New adsorption – 1660 $cm^{-1}$
- Change in Mw

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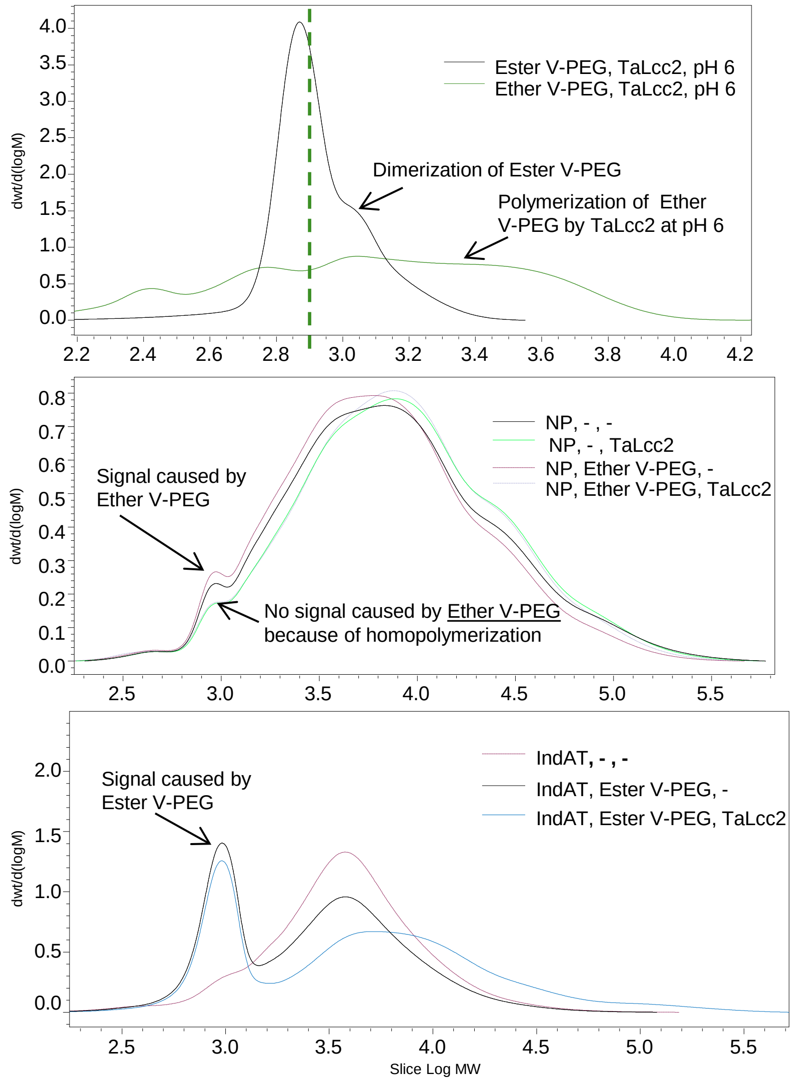
- Laccase catalysed oxidation forms phenoxyl radicals in substrates; Radical coupling in the 2-component system leads to homo- or hetero-polymerization (i.e. functionalization)
- According to $O_2$ consumption and MMD (SEC) determination, the tested laccases were active towards all the substrates and homo-polymerization took place
    - Ether V-PEG clearly more preferable substrate for laccase than Ester V-PEG
- Yet, functionalization of Indulin AT or NP lignin by the substituent could not be verified – no or only minor hetero coupling reactions of radicals generated by laccase

- Functionalization of Indulin AT with Ester V-PEG by $O_2$ oxidation under alkaline conditions was also tested
    - No functionalization of Indulin AT based on dialysis yield of the reaction solution, MMD and Tg determinations
    - Efficient condensation of Indulin AT especially under conditions of high lignin concentration (25 w-%)



# What we have achieved:

| | |
|---|---|
| **SIAB**<br>**UL** | • Optimization of the Natural Pulping Process for pre-treatment of wheat straw up to pilot scale<br>• Production of *P. verruculosum* cellulase and adaption for the SSF process up to pilot scale |
| **IPWC** | • Optimization of the alkaline pulping for wheat straw up to pilot scale<br>• Characterization of lignin from NP- and alkaline pre-treatment, analysis of functional groups in lignin preparations |
| **IWPT** | • Lab-scale production of polymer test samples from NP- and Kraft-lignin in combination with different fibres and additives<br>• Preparation of the samples by press moulding and tests of the mechanical and hygroscopic properties |
| **FUMT** | • Kinetic studies of enzymatic hydrolysis (pulp and *P. v.*-cellulase)<br>• Studies on SSF process in CSTR and Solid state fermenter<br>• Methods for conversion of saccharides into alkenes |

| | |
|---|---|
| **VTT** | • Modification of lignin by chemo-enzymatic functionalization<br>• Comparison between *P.verruculosum* and *T.reesei* enzymes<br>• SSF with the enzyme mixtures using NP cellulose as substrate |
| **LNEG** | • Hydrothermal treatment of wheat straw (autohydrolysis)<br>• Optimisation of xylo-oligosaccharides (XOS) production<br>• Ionic liquids for fractionation of lignocellulose, high purity lignin |
| **WUR-FBR** | • Characterization of lignin samples from wheat straw<br>• Enzymatic modification of lignin and characterisation of lignin derivatives (coupling with carbohydrates and proteins)<br>(FBR works as subcontractor of FUMT) |
| **Biotehnol** | • Lignin separation with critical / supercritical fluids and characterization by spectrocolorimetry<br>• Purification and characterization of extracted lignocellulosic raw materials and composites |