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The SSF process of ethanol production from pulp from wheat straw

ERA-IB-project EIB.10.013:

“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”

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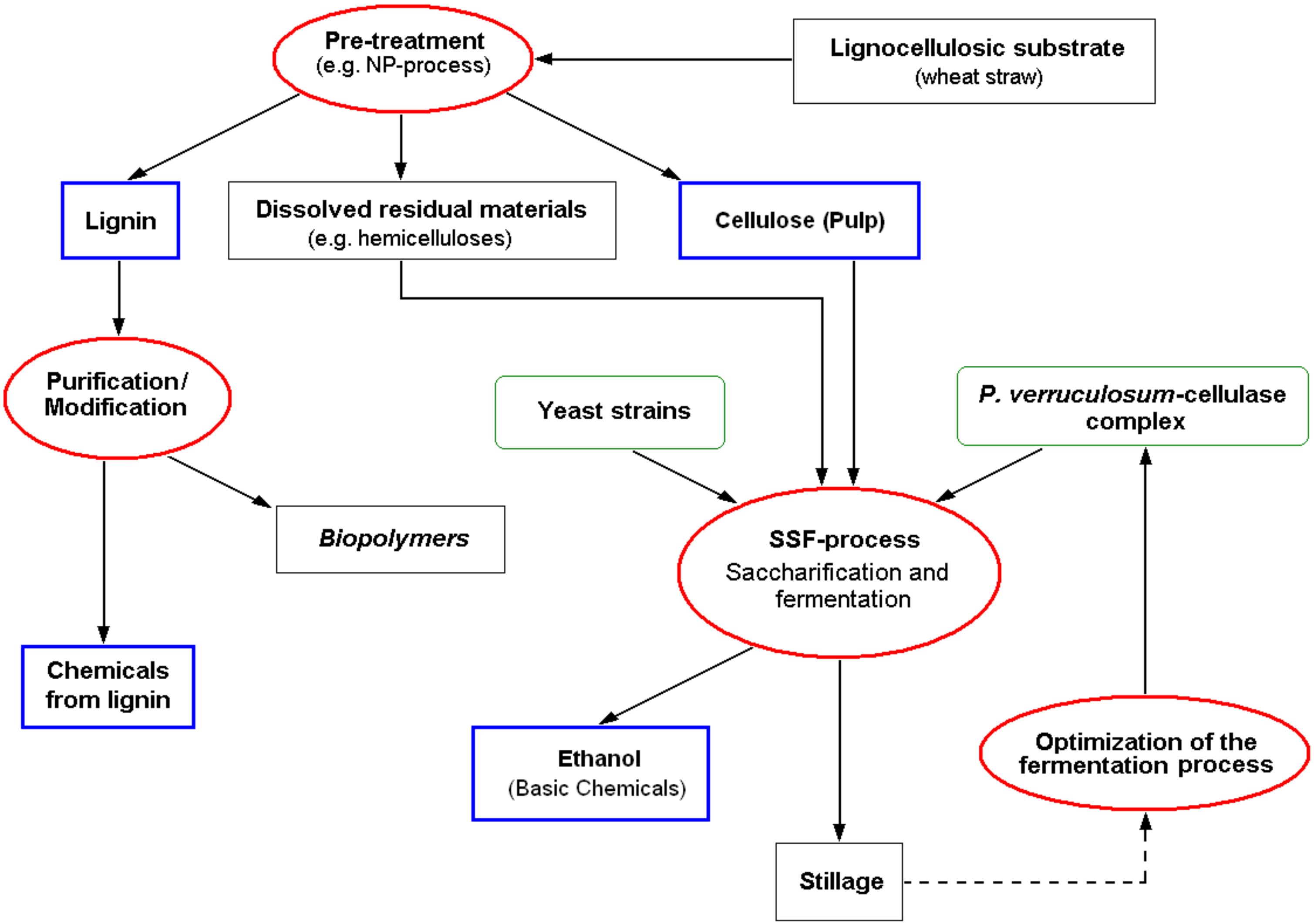
Overall process for the utilization of wheat straw



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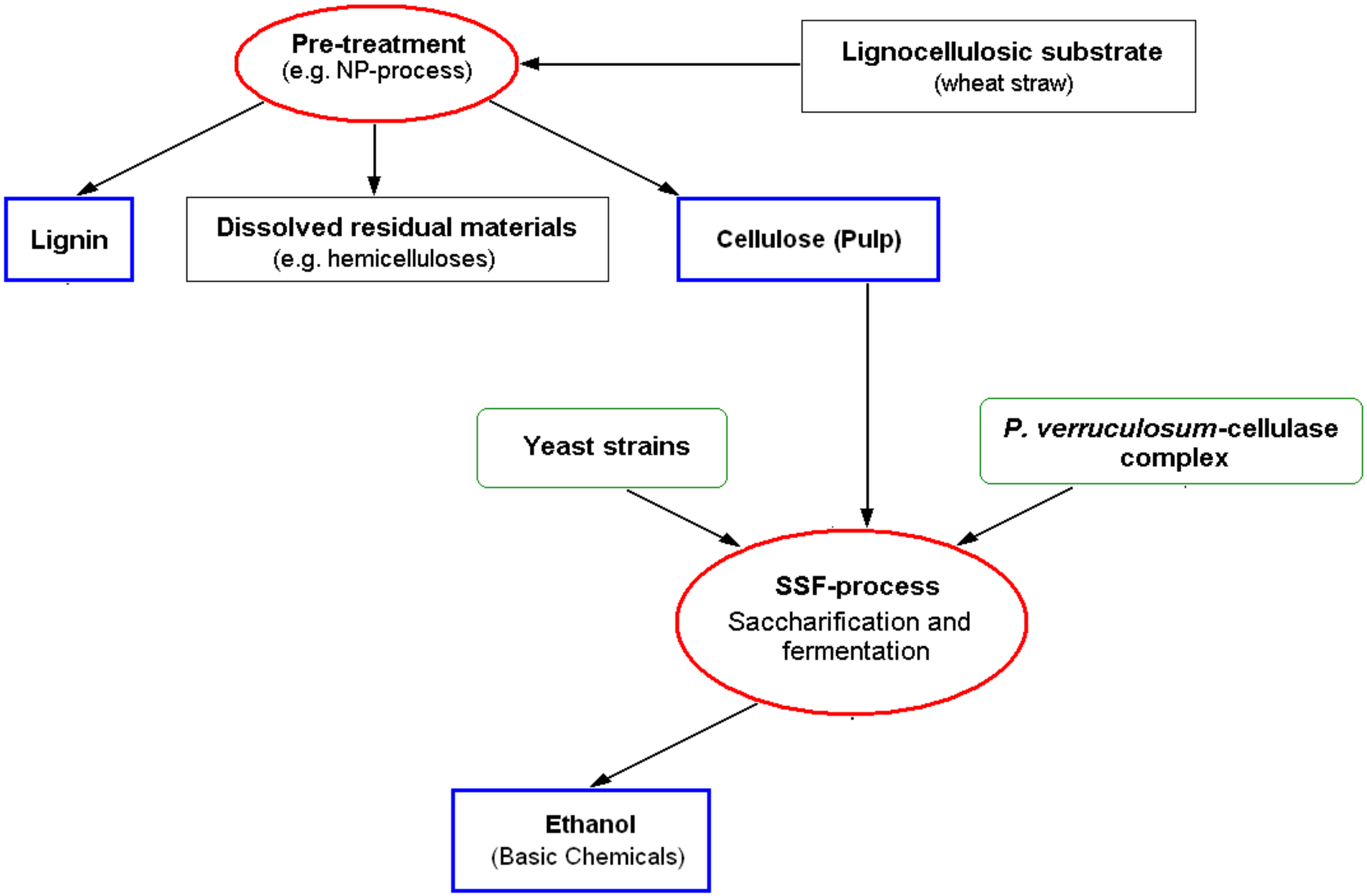
SSF-process of ethanol production from pulp



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SSF-process from the technical point of view

Advantage of SSF-process	Requirements
Reduction and simplification of the process steps • Lower investment costs	• Stability of the cellulase complex in the SSF process during the entire fermentation period
• Overall simplified process execution when the cellulase complex is produced on the basis of the lignocellulosic substrates in the ethanol plant	• Less inhibition of the cellulase complex by ethanol and by-products of the lignocellulose-pre-treatment such as lignin
	• Optimal supply of the required amount of pulp in the fermentation process taking into account the high intrinsic viscosity of pulp suspension
	• Yeast strains, stable to by-products of the pre-treatment process

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The following main tasks are studied:

Pre-treatment of wheat straw

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

Penicillium verruculosum cellulase-complex

- Production of the P. v.-cellulase using substrates of the pre-treatment process
- Saccharification of pulp
- Inhibition by lignin and ethanol

SSF-process

- Influence of pre-treatment on yield of ethanol
- Stability of the cellulase in the SSF-process
- Supply of the required amount of pulp by pre-hydrolysis, fed-batch feeding and SSF-process in solid-state-fermentation

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Pre-treatment of lignocellulose

- Alkaline pre-treatment with NaOH
- Natural Pulping pre-treatment with formic acid / H_2O_2
- Autohydrolysis

Scale-up of **alkaline pre-treatment** at Fraunhofer Center for Chemical-Biotechnological Processes CBP, Leuna

1. Charging the digester
2. Pulping procedure
3. **Separation of pulp**
4. Lignin precipitation
5. Lignin separation

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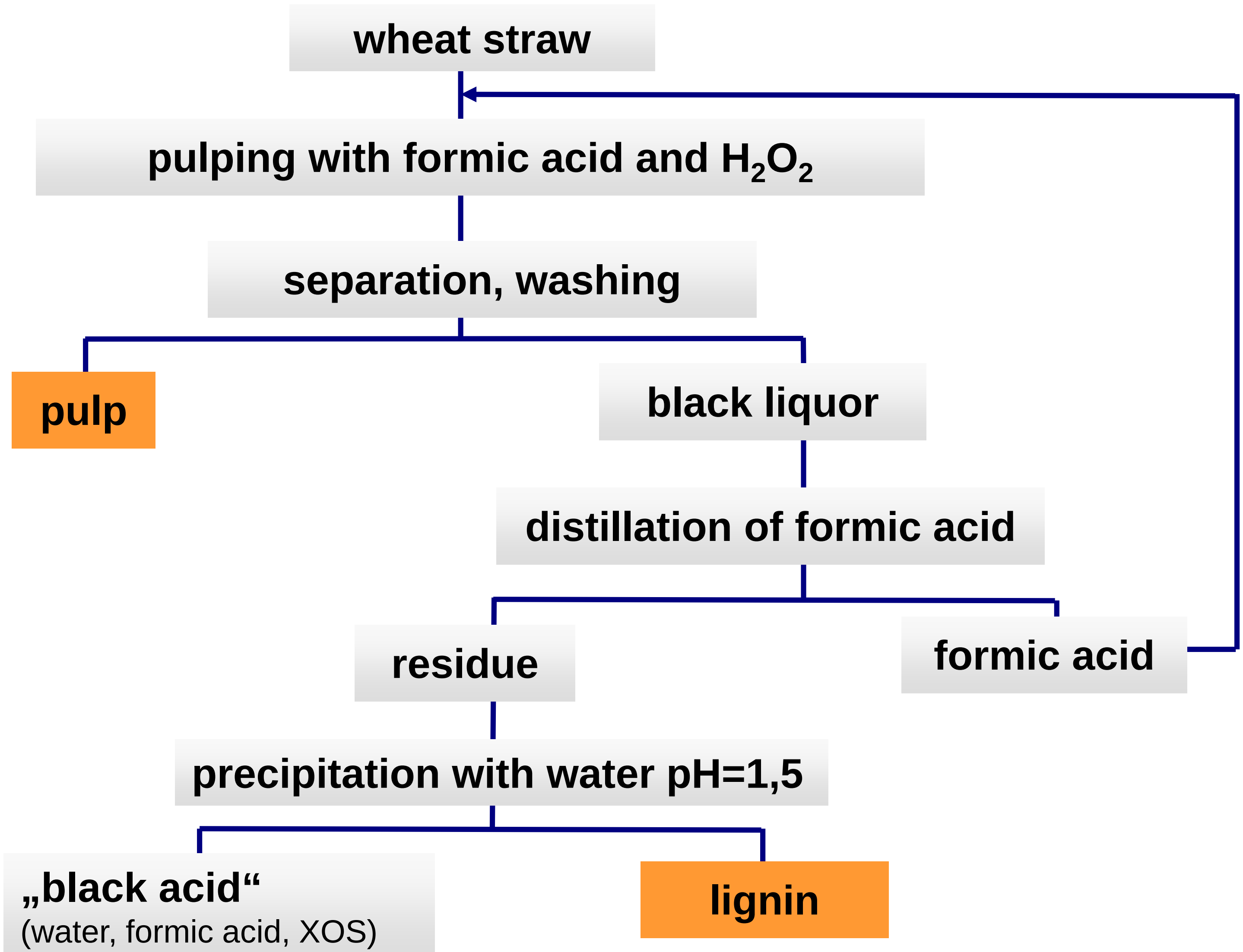
Natural pulping of wheat straw



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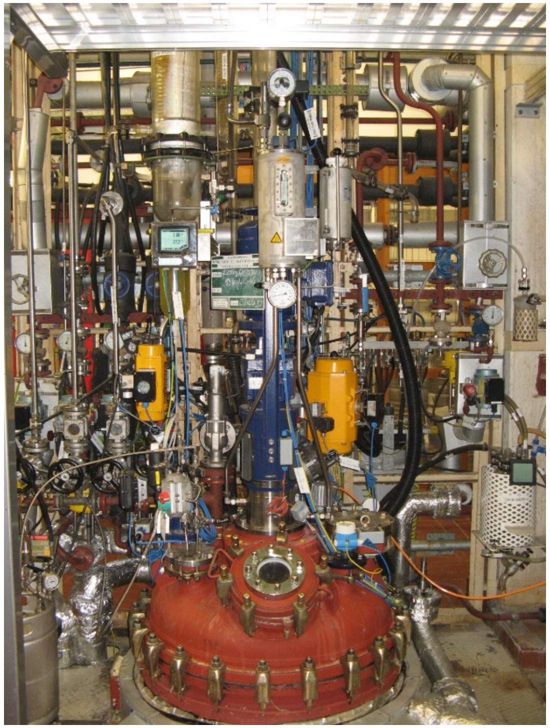
Scale-up of Natural Pulping pre-treatment



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

Lab-scale

(SIAB)

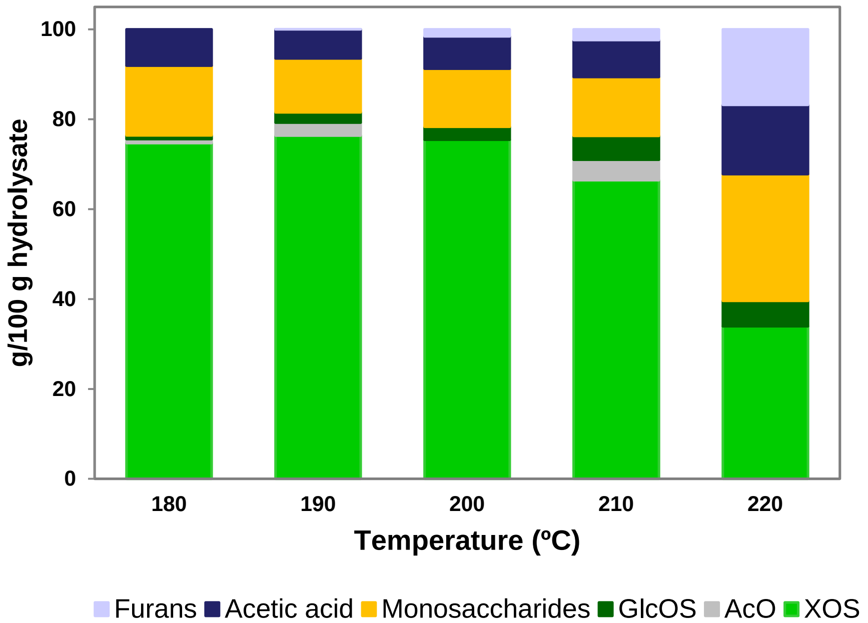
Pilot-scale

(Lanxess Deutschland GmbH,
Group Function Innovation and Technology)

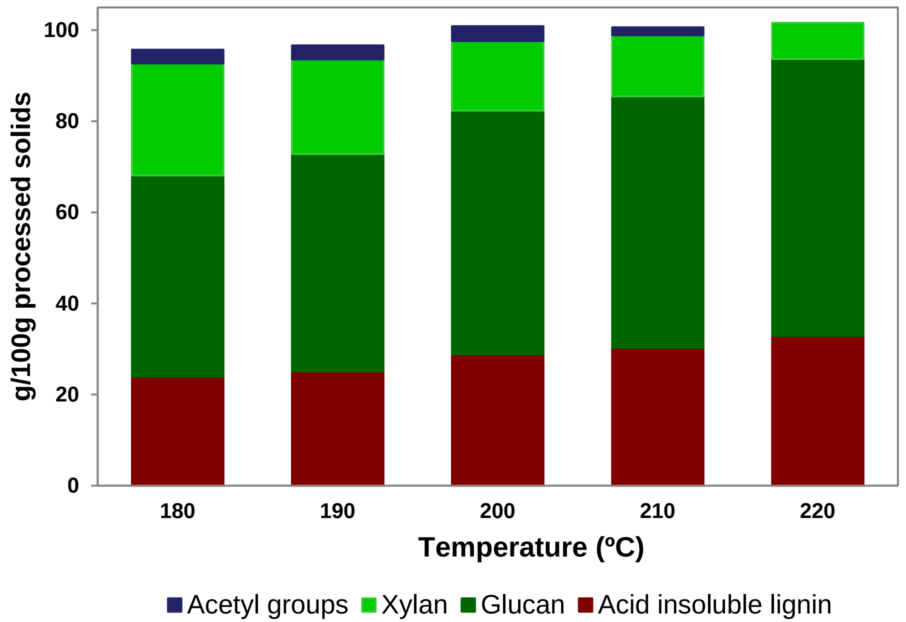
Hydrothermal pre-treatment (autohydrolysis)

Optimization 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)

from EIB.10.013: Florbela Carvalheiro, LNEG, Lisbon

Influence of pre-treatment on properties of pulp and lignin

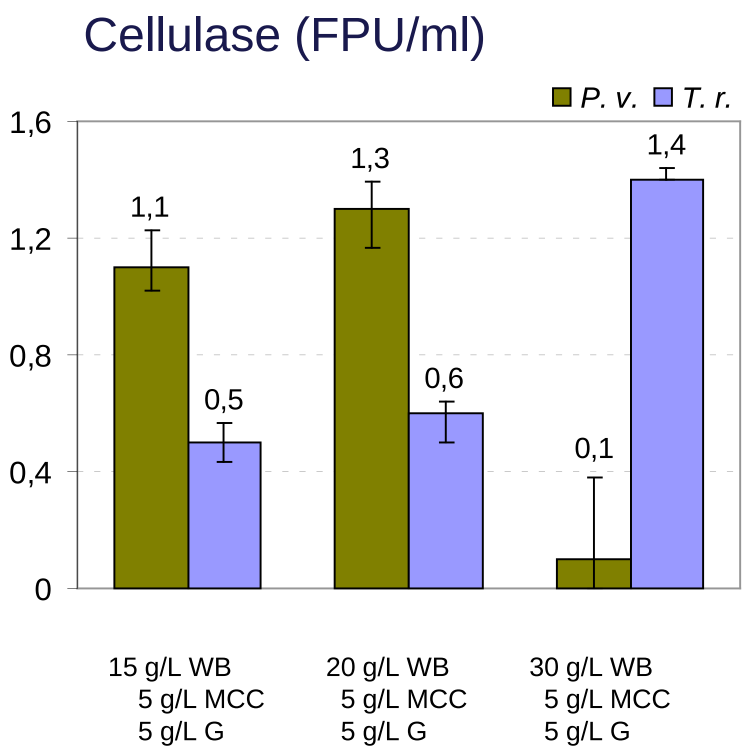
Analytic of pulp:	➢ **Intrinsic viscosity** ➢ Determination of crystallinity by X-ray diffractometry ➢ Scanning electron microscopy ➢ **Composition of pulp; lignin, cellulose and holocellulose content** ➢ Xylo-oligosaccharides at autohydrolysis
Analytic of lignin:	➢ Influence of pulping duration / liquid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

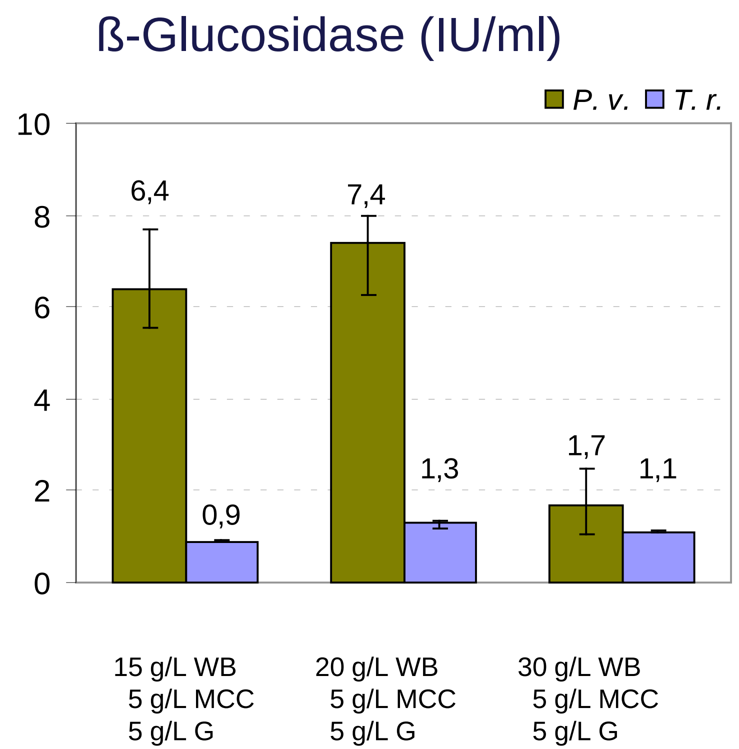
Comparison of all pre-treatments

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

***Penicillium verruculosum* enzyme complex**

Production of the *P. verruculosum* cellulase





Production of cellulase based on wheat bran, glucose and MCC as substrate

(lab-scale in shake flask)

from the Bachelor-Thesis
Denise Lachmann, 2013

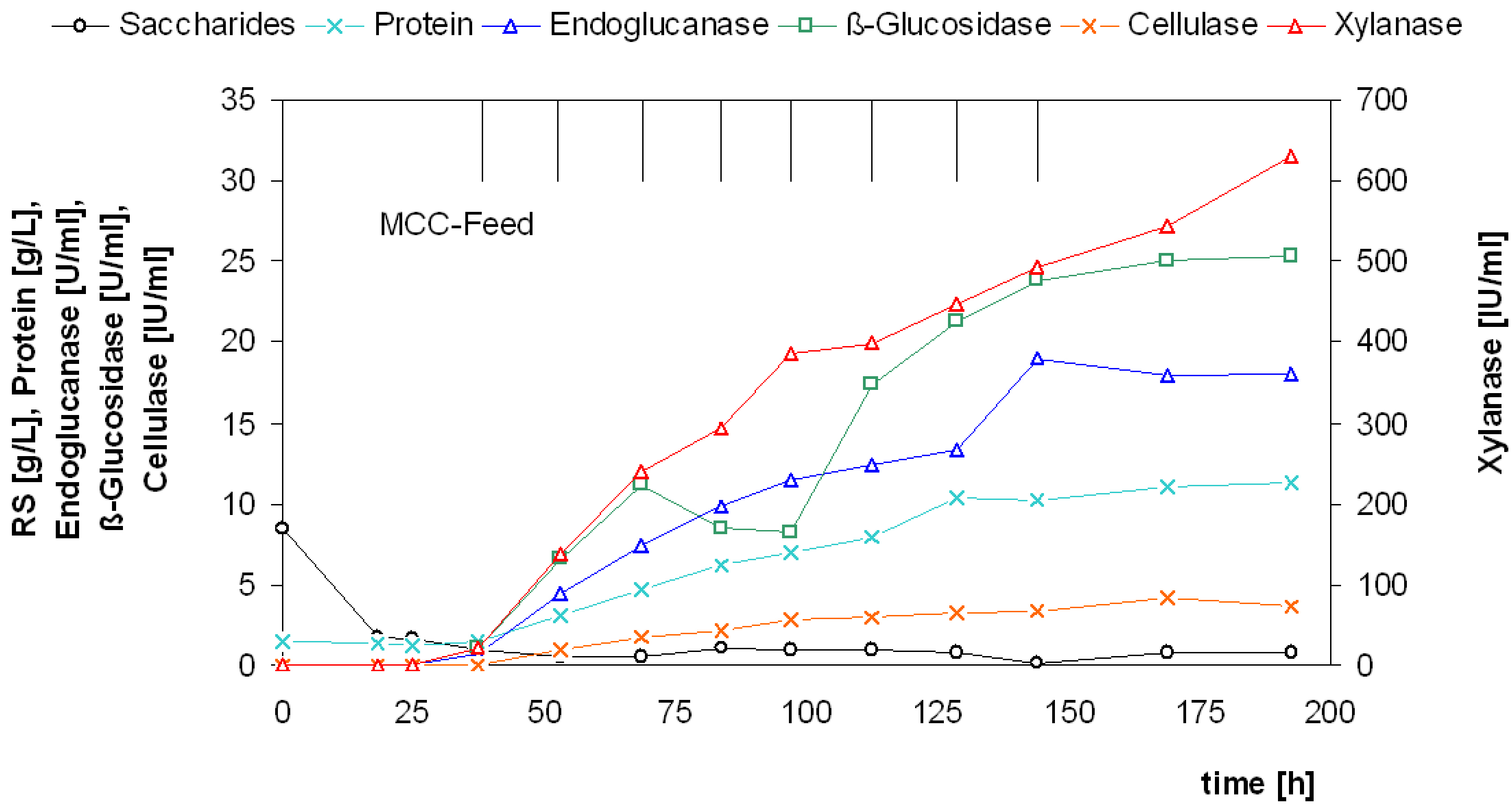
Production of cellulase from *P. verruculosum* M28-10

- 600L Bioreactor
- V_F = 400L
- Agitation: Rushton (x3)
- Aeration 0,2-0,8 vvm (air)

- Medium composition: glucose, wheat bran, MCC

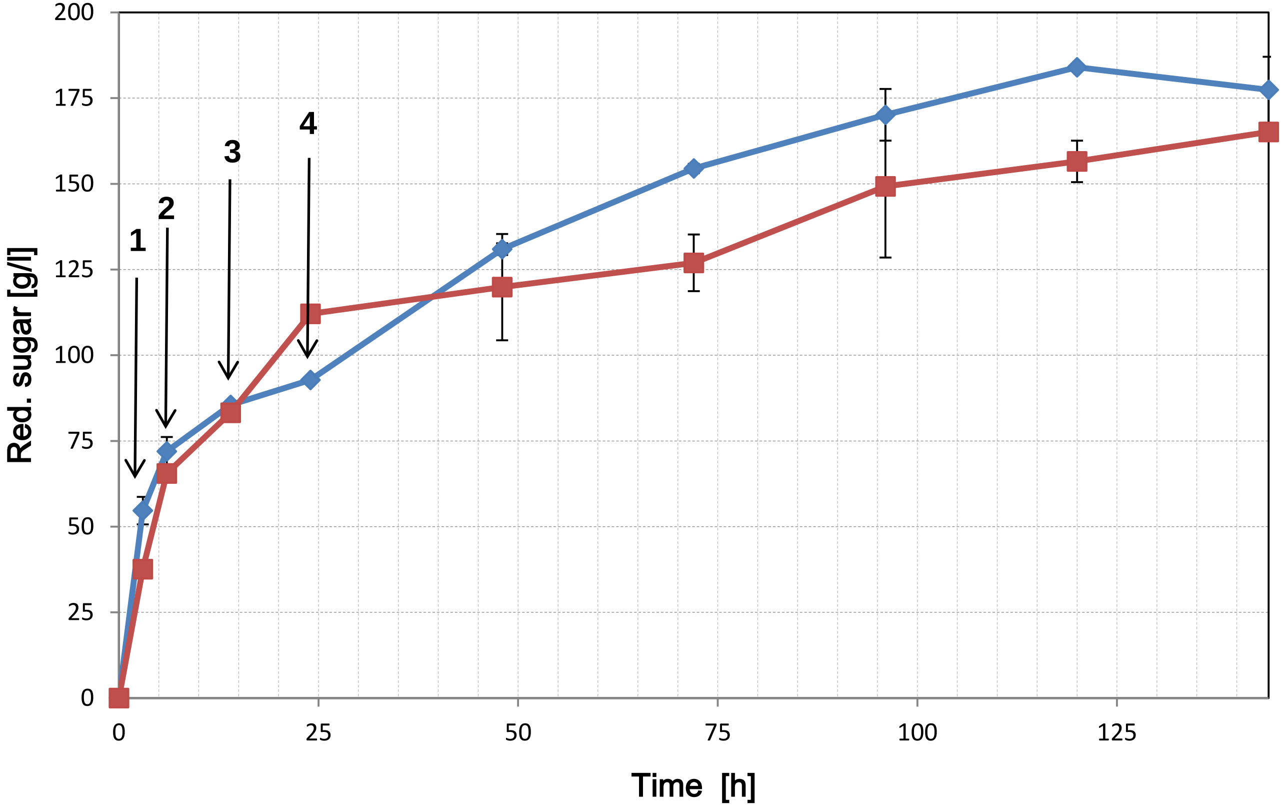
- fed-batch technique: feeding of MCC

Fermentation of *P. verruculosum* - course of enzyme activity



Saccharification of pulp by *P. verruculosum* cellulase

Enzymatic saccharification of NP- and alkaline pulp: Feeding of pulp in fed-batch technique



125 FPU/g pulp

(dry weight)

alkaline pulp

NP-pulp

1 feeding of pulp (in both experiments)
2 feeding of pulp (in both experiments)
3 feeding of NP pulp
4 feeding of alkaline P.

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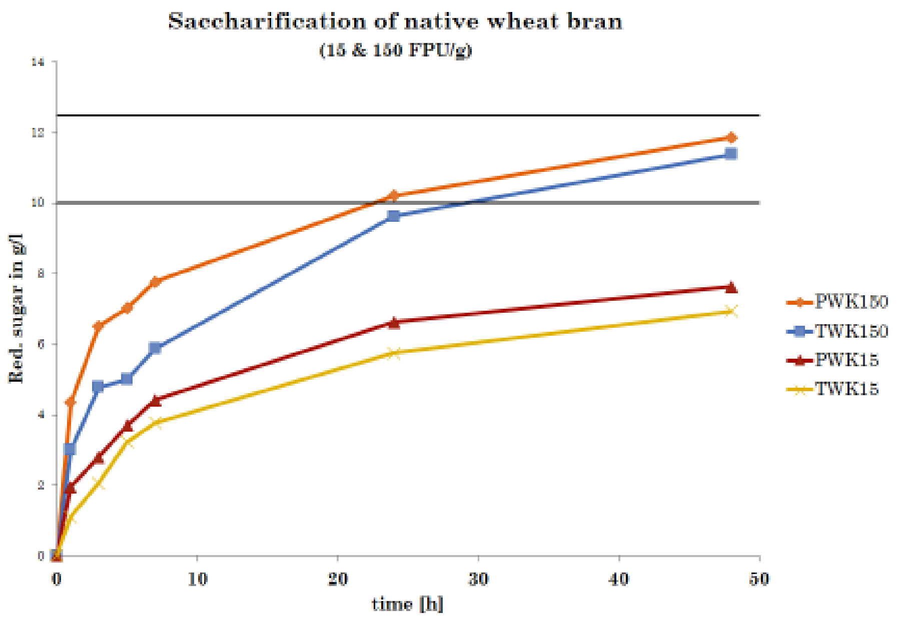
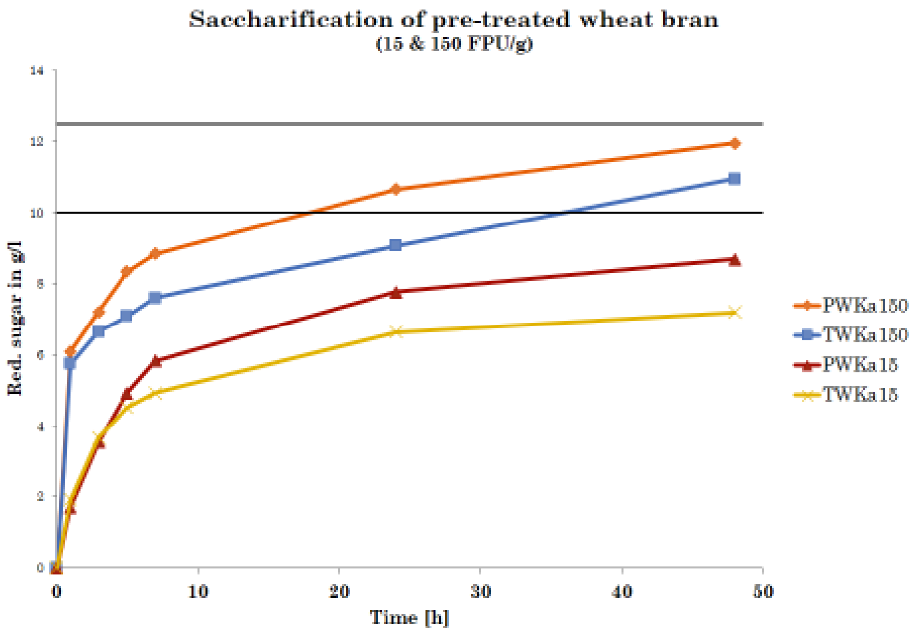
Comparison between *T. reesei*- and *P. verruculosum*-cellulase in hydrolysis of untreated and pre-treated wheat bran

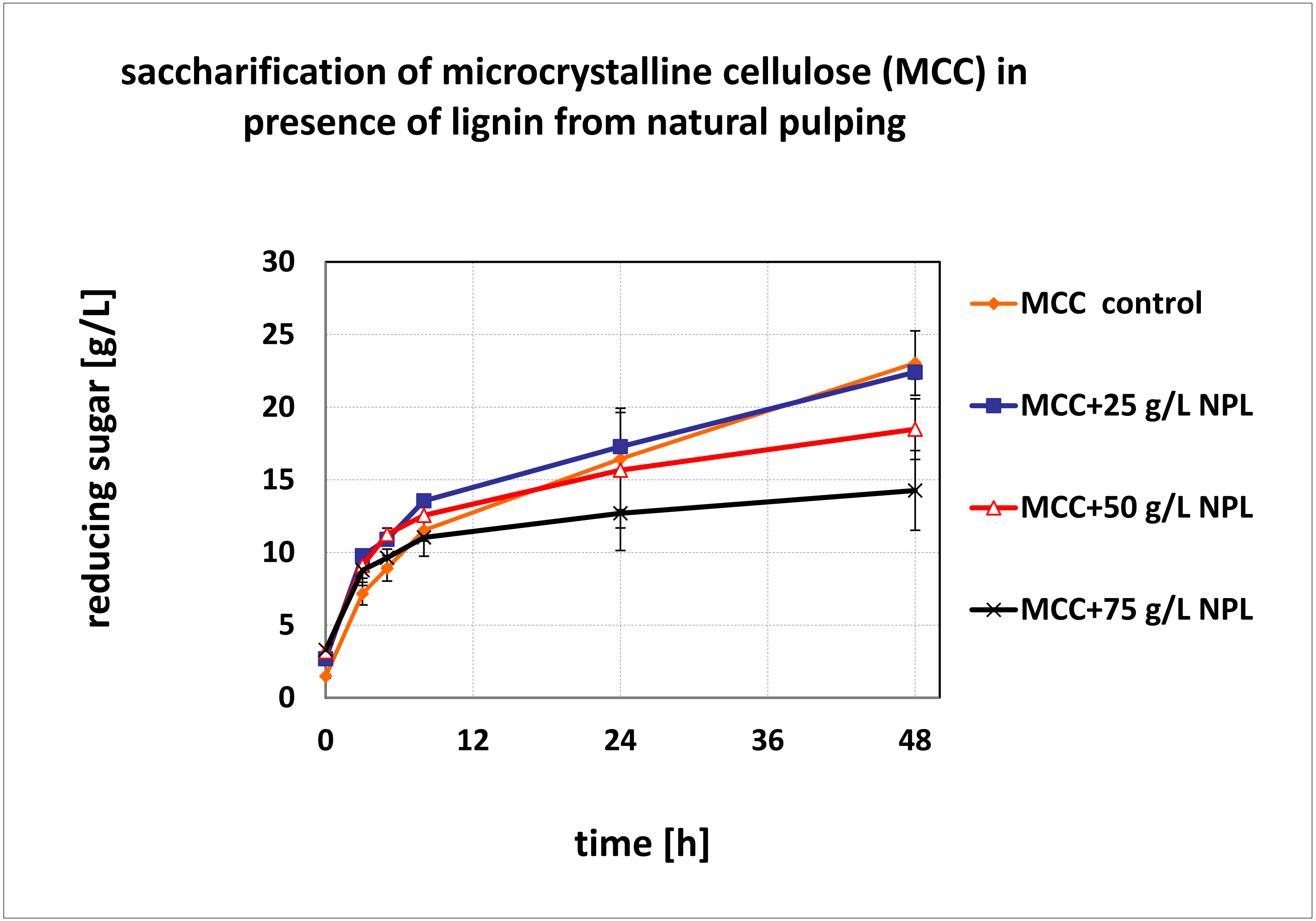




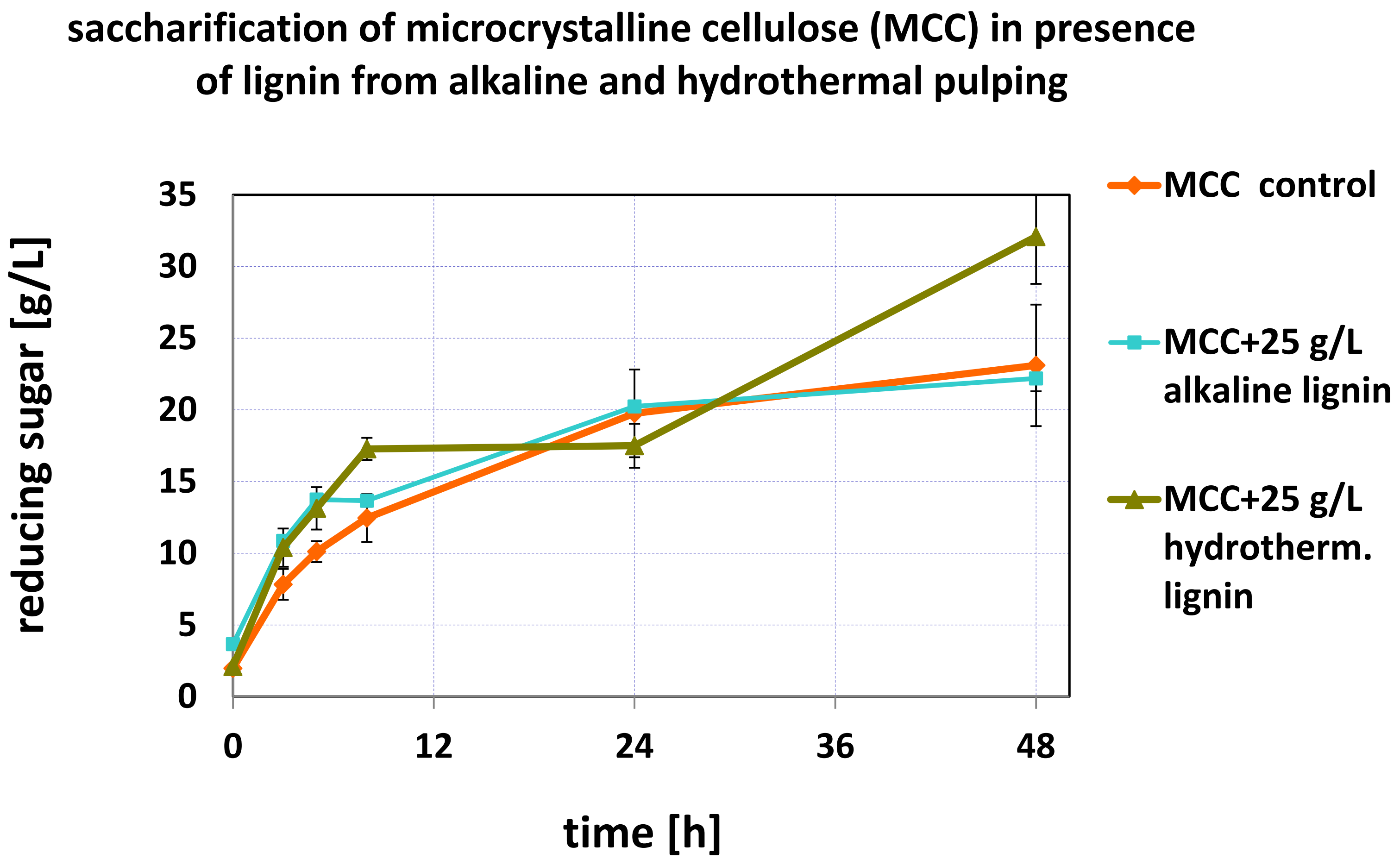
from the Bachelor-Thesis
Robert Koksch, 2012

Inhibition of *P. verruculosum* cellulase by lignin and ethanol

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



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



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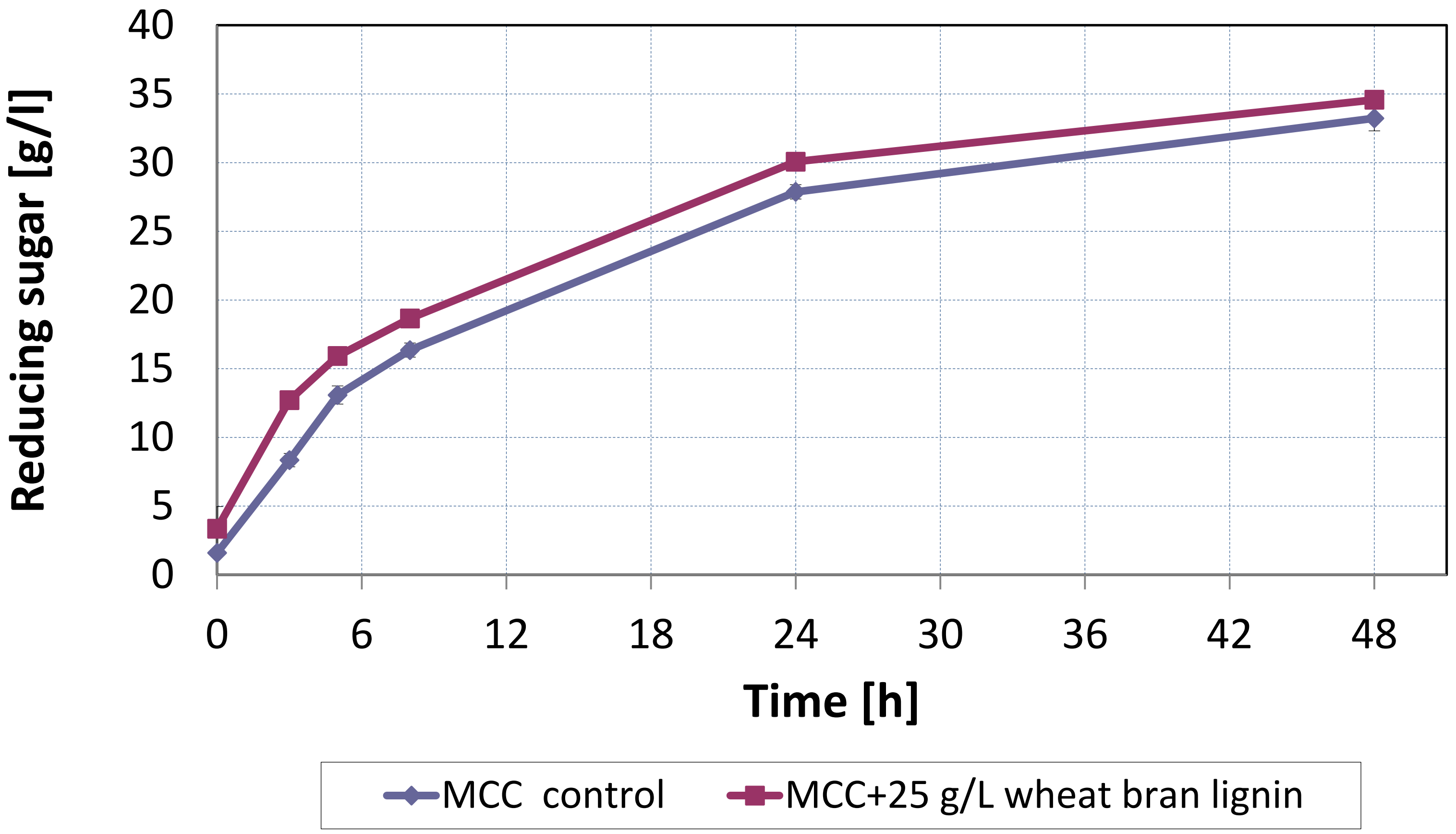
Saccharification of MCC by *P. verruculosum* cellulase in presence of lignin from wheat bran by Natural Pulping

(FPU= 50 U/g_{DM}; substrate 50 g/L_{DM} MCC)



Investigations on the SSF-process

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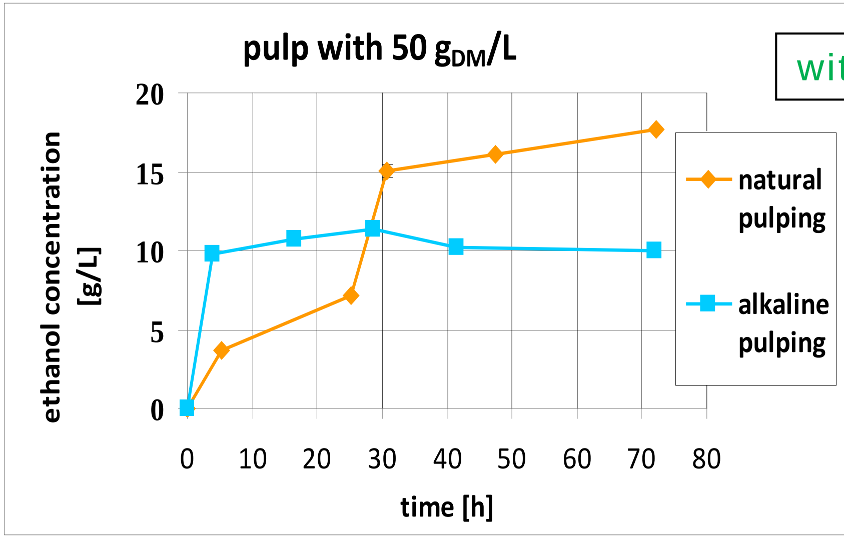
Investigations in 3-L-bioreactor

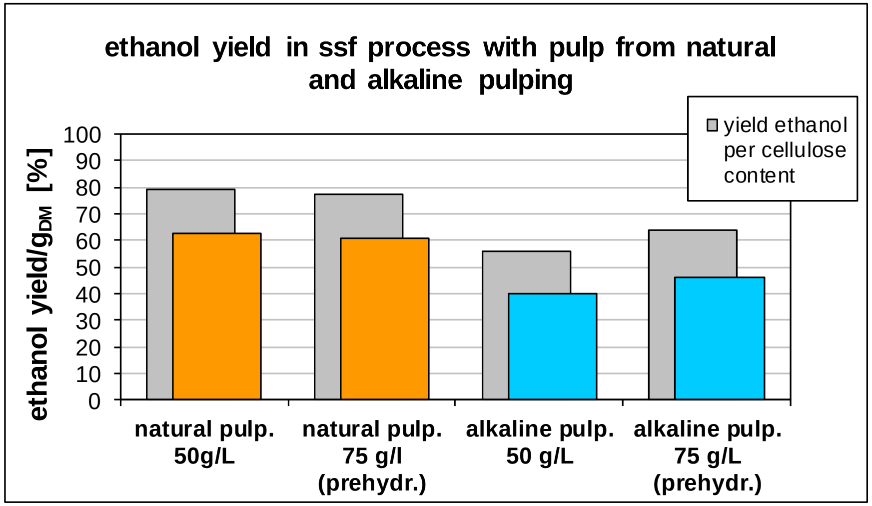
Experimental setup - process parameters

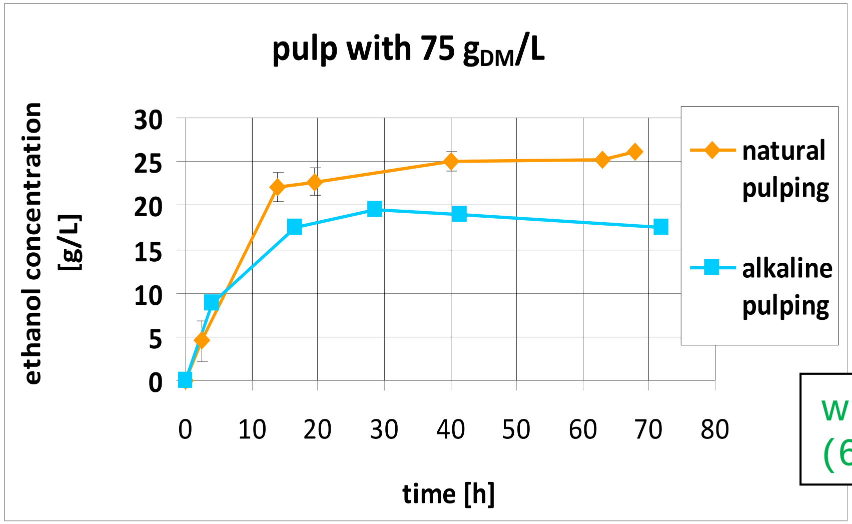
Working volume	1,700 ml
FPU/g $_{DM}$	50
Yeast inoculum (dry yeast, ZFT)	5 g/L
pulp content (g/L) -natural pulping -alkaline pulping	 50 - 75 75 - 100
NH_4Cl	2 g
KH_2PO_4	1 g
pH	5
temperature	35°C
trial time	~ 72 h
pre-hydrolysis (45°C)	6 h / 8 h

Comparison of SSF between pulp from natural and alkaline pulping



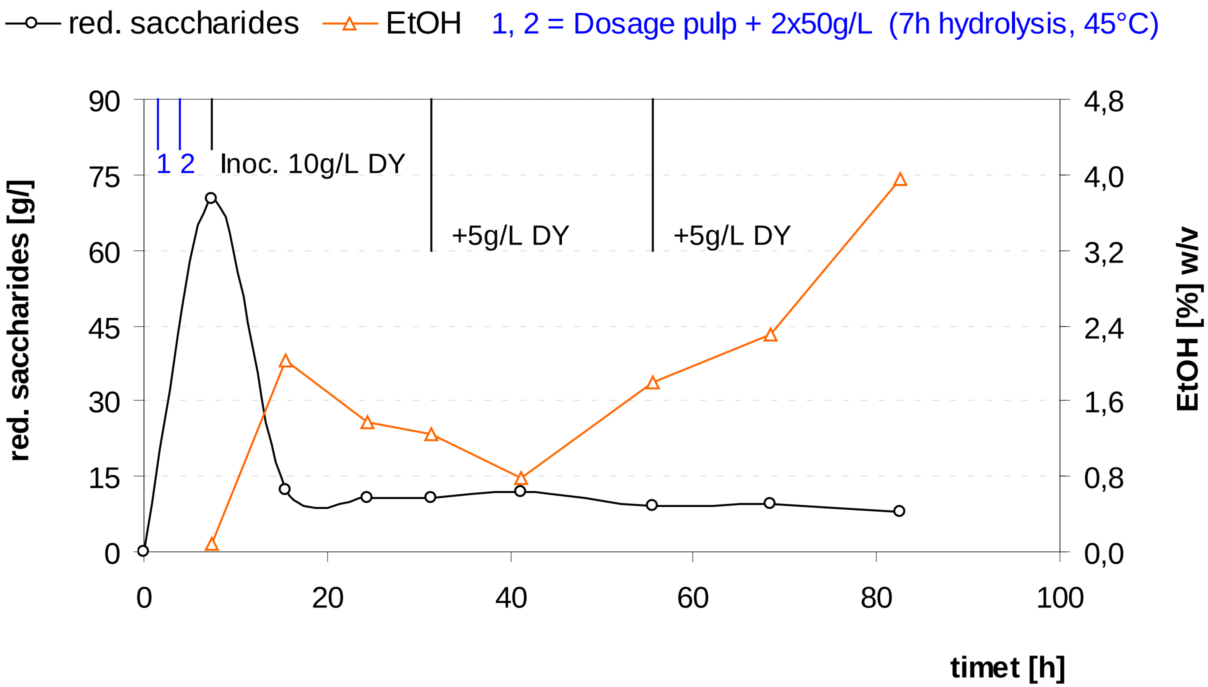
without pre-hydrolyses





with pre-hydrolyses (6h, 45°C)

SSF process with feeding of yeast in fed batch



- 5L stirred bioreactor, V_F = 2L, 200g/L pulp (natural pulping), 30FPU/gDM
- EtOH-conc. = 4% (w/v) after 72h
- EtOH-yield = 35% (Pulp), 44% (Cellulose)
- Pulp-reduction = 57% (pulp-residue = 43%)

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: 73,7%
  (related to cellulose-content in pulp)

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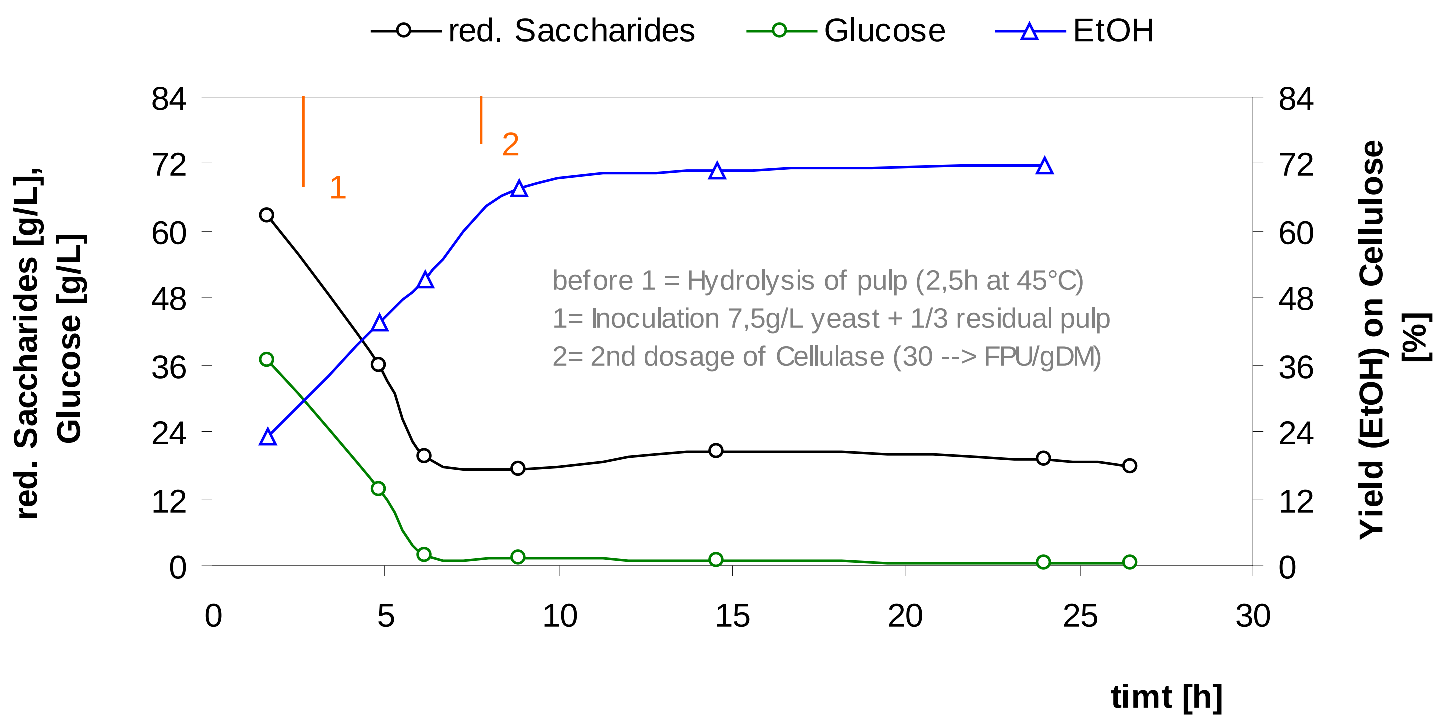
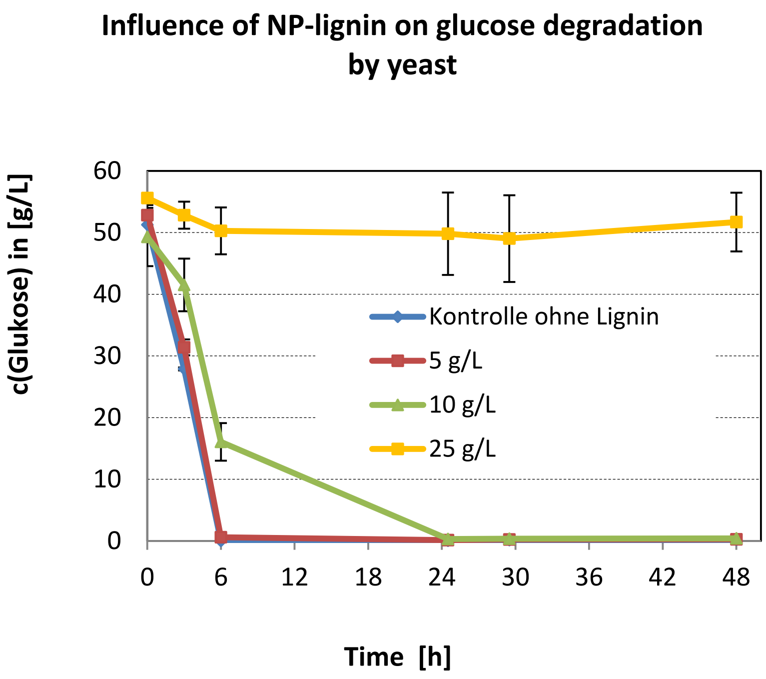
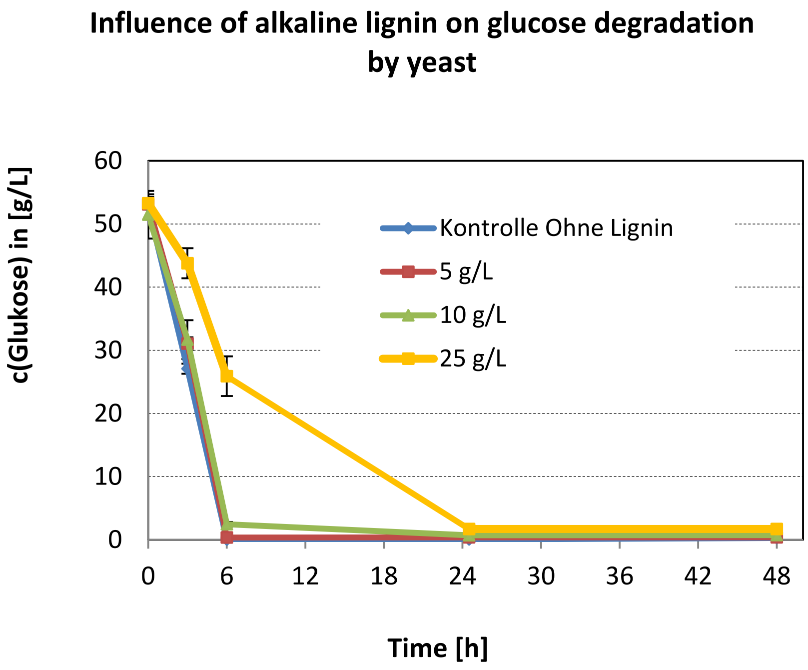
Course of the anaerobic fermentation at CBP



- final EtOH-conc. ~ 3.8% (w/v) after 15h
- EtOH-yield = 55% (based on Pulp), =73% (based on Cellulose)
- successful fermentation on technical scale (further optimization necessary)





Influence of lignin on glucose utilization by yeast

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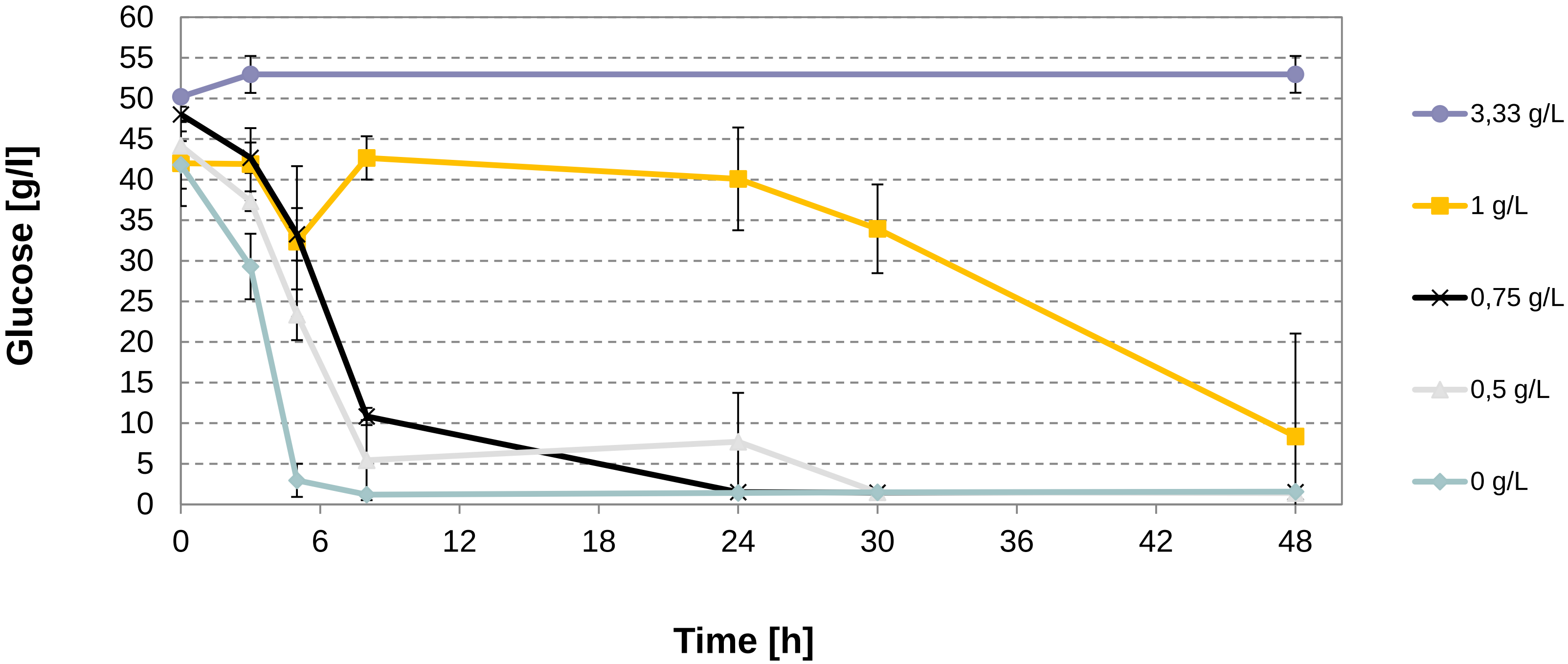
Influence of formic acid on yeast growth

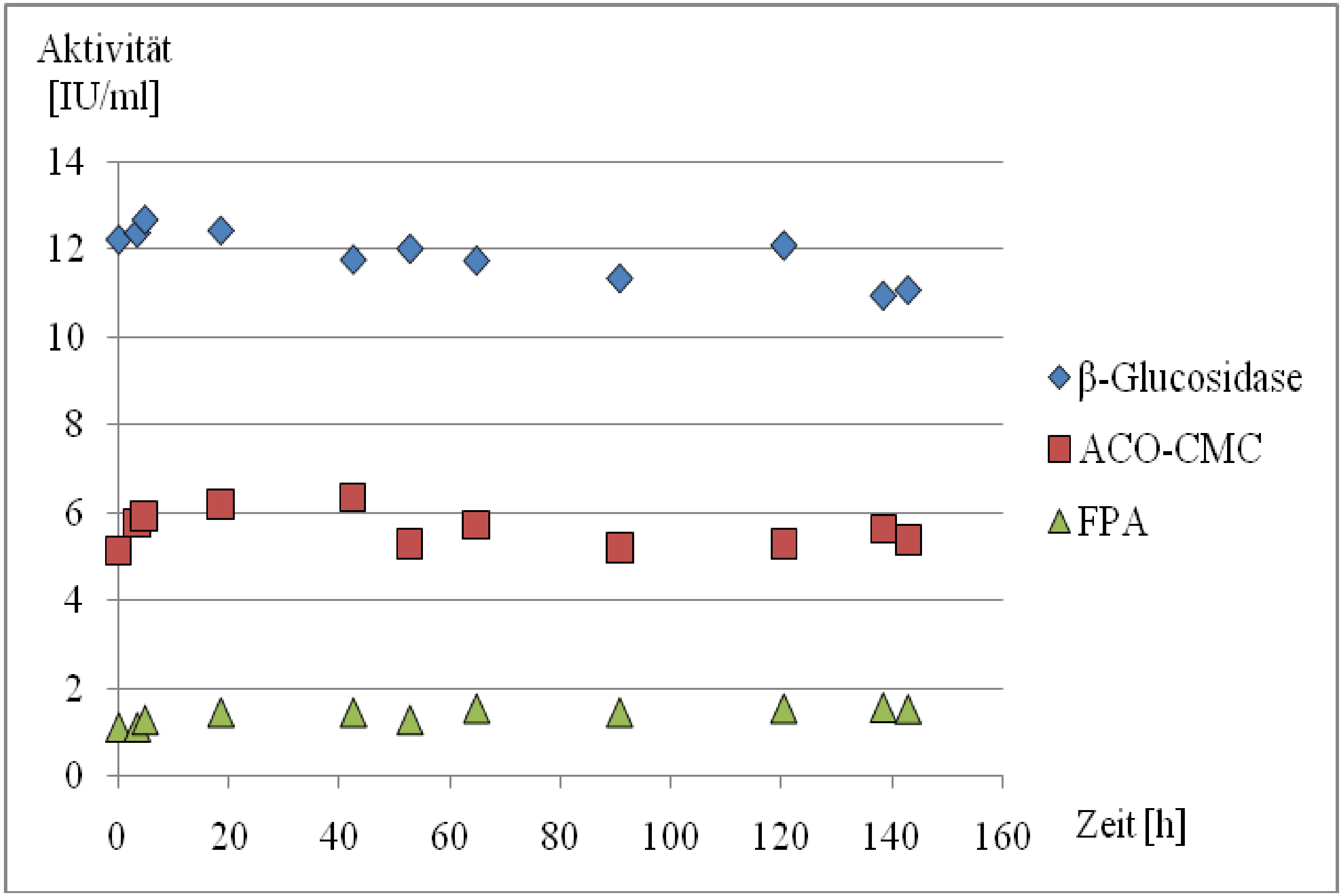


Inhibition of aerobe glucose utilization by formic acid

Concentration: 0.5 g/L - 3.33 g/L formic acid

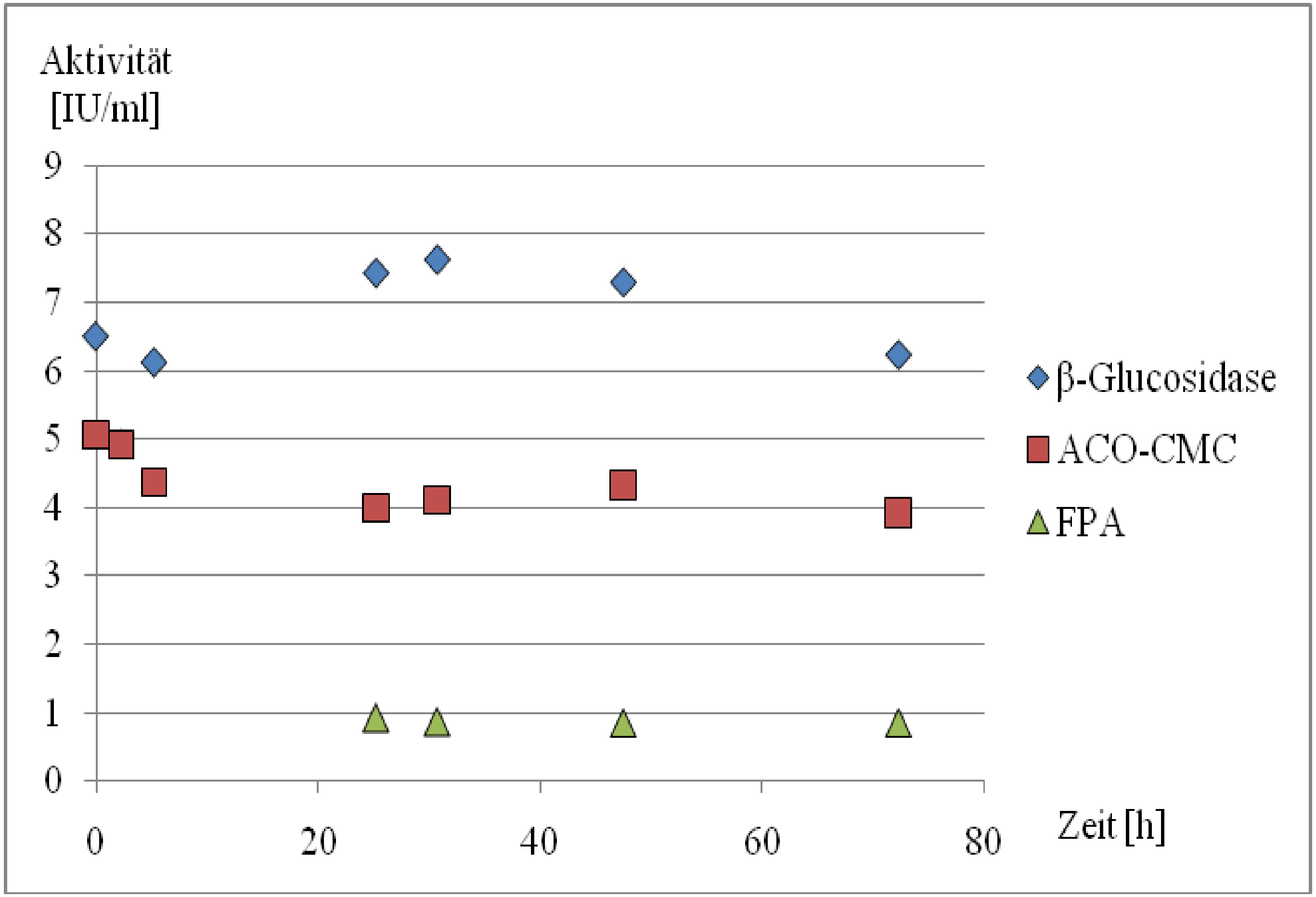
Stability of *P. verruculosum* cellulase in the SSF-process

Cellulase activity in the course of the SSF-process with a-cellulose as substrate



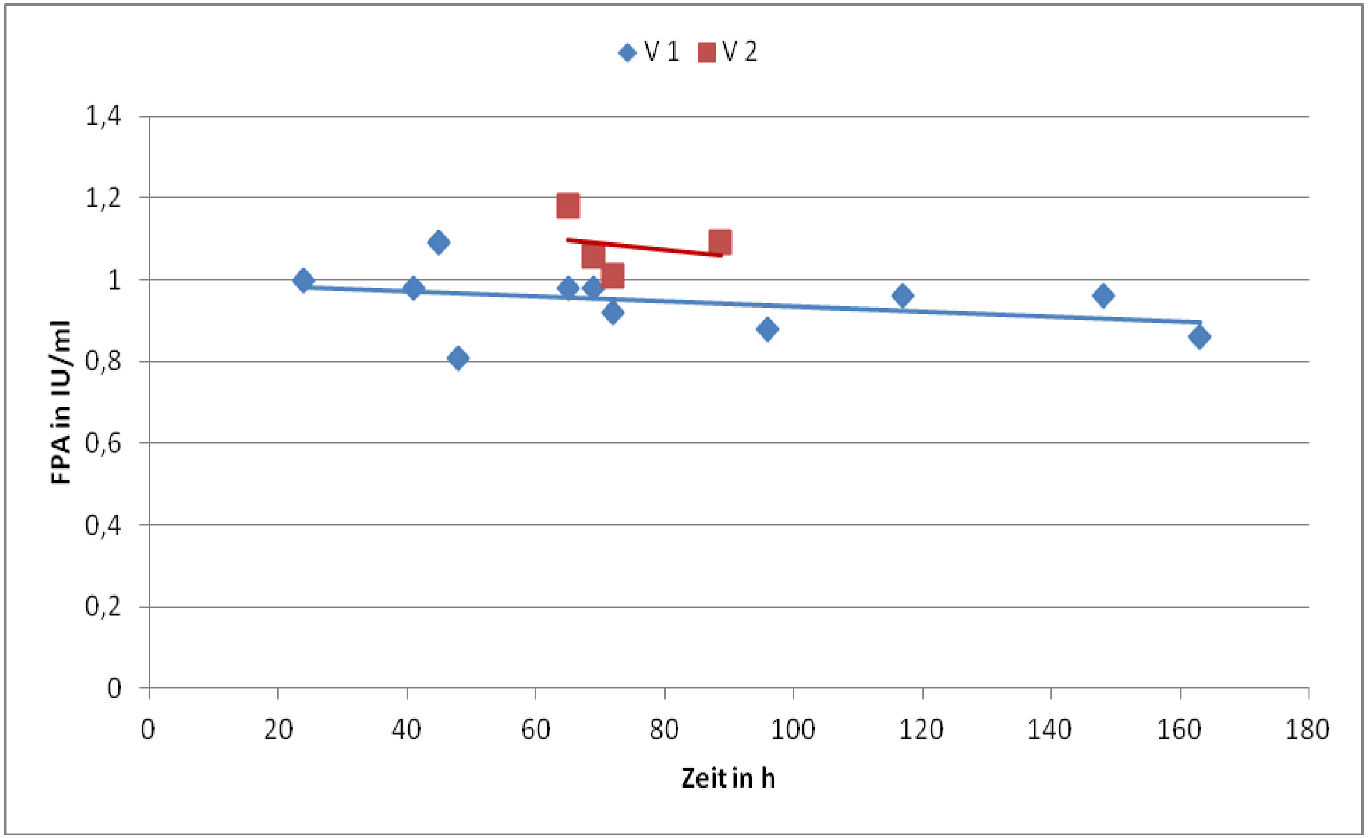
42-L-reactor; *P. verruculosum*-cellulase, 50 FPU/g DM

Cellulase activity in the course of the SSF-process with NP pulp as substrate



3-L-reactor; *P. verruculosum*-cellulase, 50 FPU/g DM

Cellulase activity in the course of the SSF-process with wheat bran as substrate



42-L-reactor; *P. verruculosum*-cellulase, 15 FPU/g DM

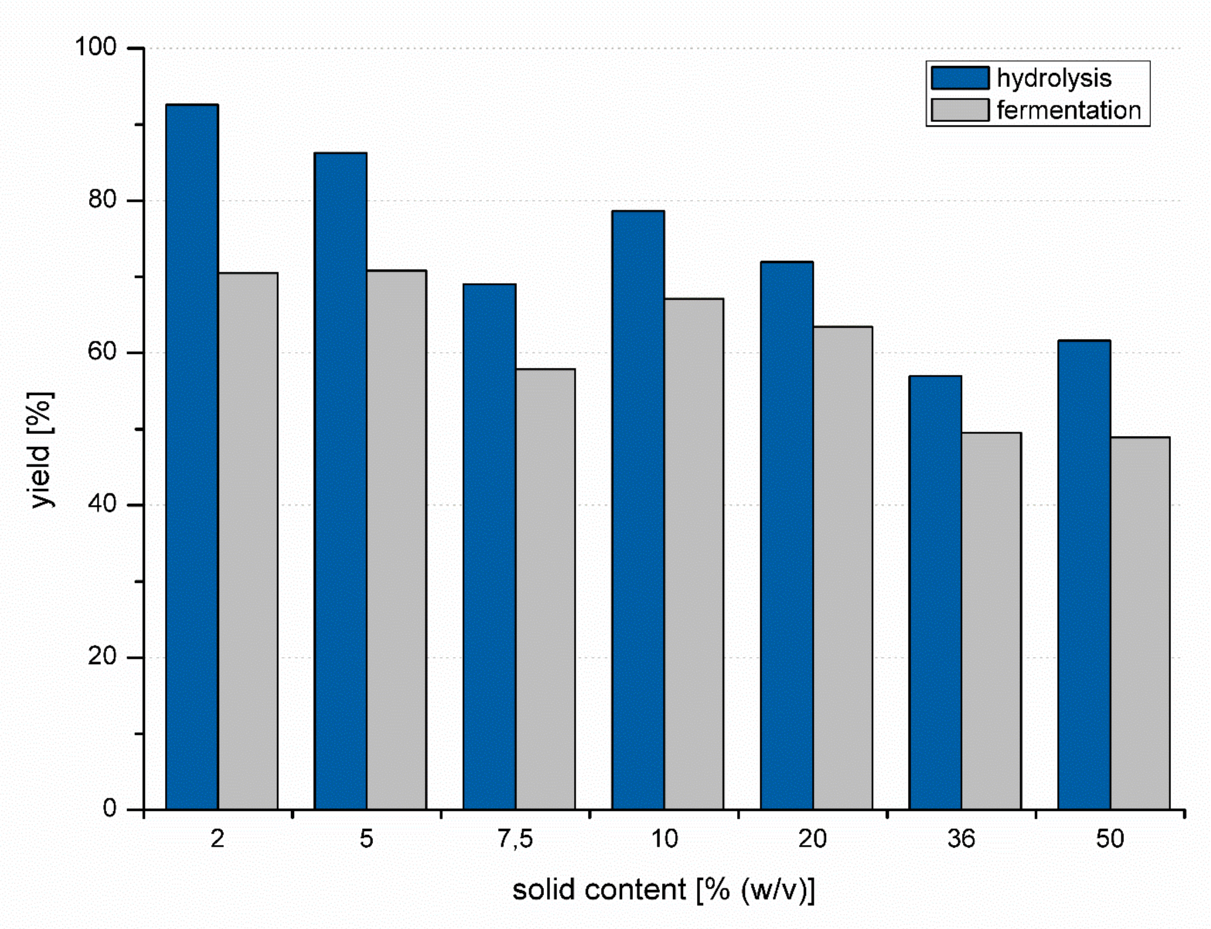
from Master thesis Manuel Meißner, 2013

Problems to supply the required amount of pulp for > 10% ethanol in the SSF process

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



from EIB.10.013: Doreen Steffien, FUMT, Freiberg

Solid state reactor for high pulp concentration

- economical ethanol production:

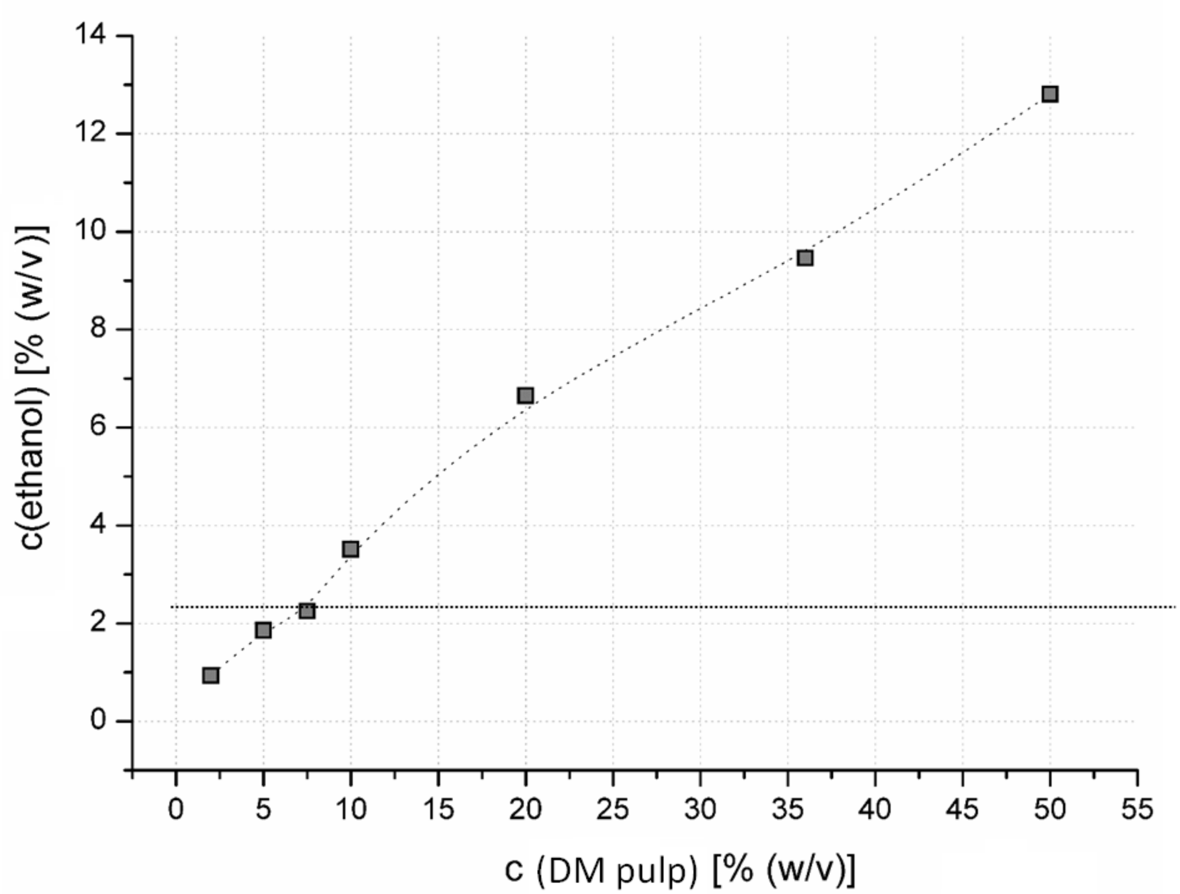
  ethanol conc. **>4 % (v/v)** required

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

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

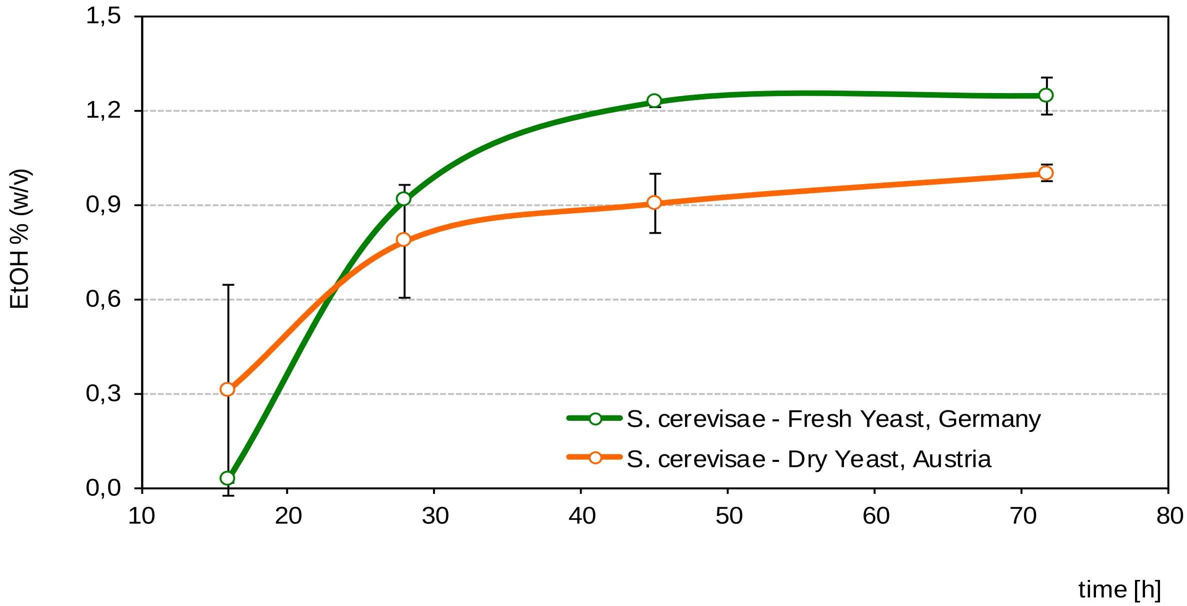
SSF-process in solid state reactor

High solid content



from EIB.10.013: Doreen Steffien, FUMT, Freiberg

Comparison of different yeast strains



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

initial OD_{600}=12, Temp.=35°C

Interaction between yeast and enzyme

		t [h]	FPA [IU/mL]
aerob			
	Yeast + enzyme + yeast extract /peptone	144	**1,06**
	yeast + enzyme	144	**0,00**
anaerob			
	yeast + enzyme + yeast extract/peptone	144	**0,95**
	yeast + enzyme	144	**0,00**
	yeast + enzyme + stillage	**93**	**0,85**

Kluyveromyces marxianus; 37 °C; 90 rpm, pH=5.0, control: 1.0 FPU/mL

from the Diploma thesis: Anne-Catrin Letzel, 2010, FUMT, Freiberg

Results to the SSF-process

Cellulase complex

- The *P. verruculosum*-cellulase complex 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 → max. 12.8 %(w/v) ethanol
- The yeast needs to be optimized for the SSF process

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 and 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 fermentation of high pulp concentration in a solid-state-fermenter.

P. verruculosum production strain:

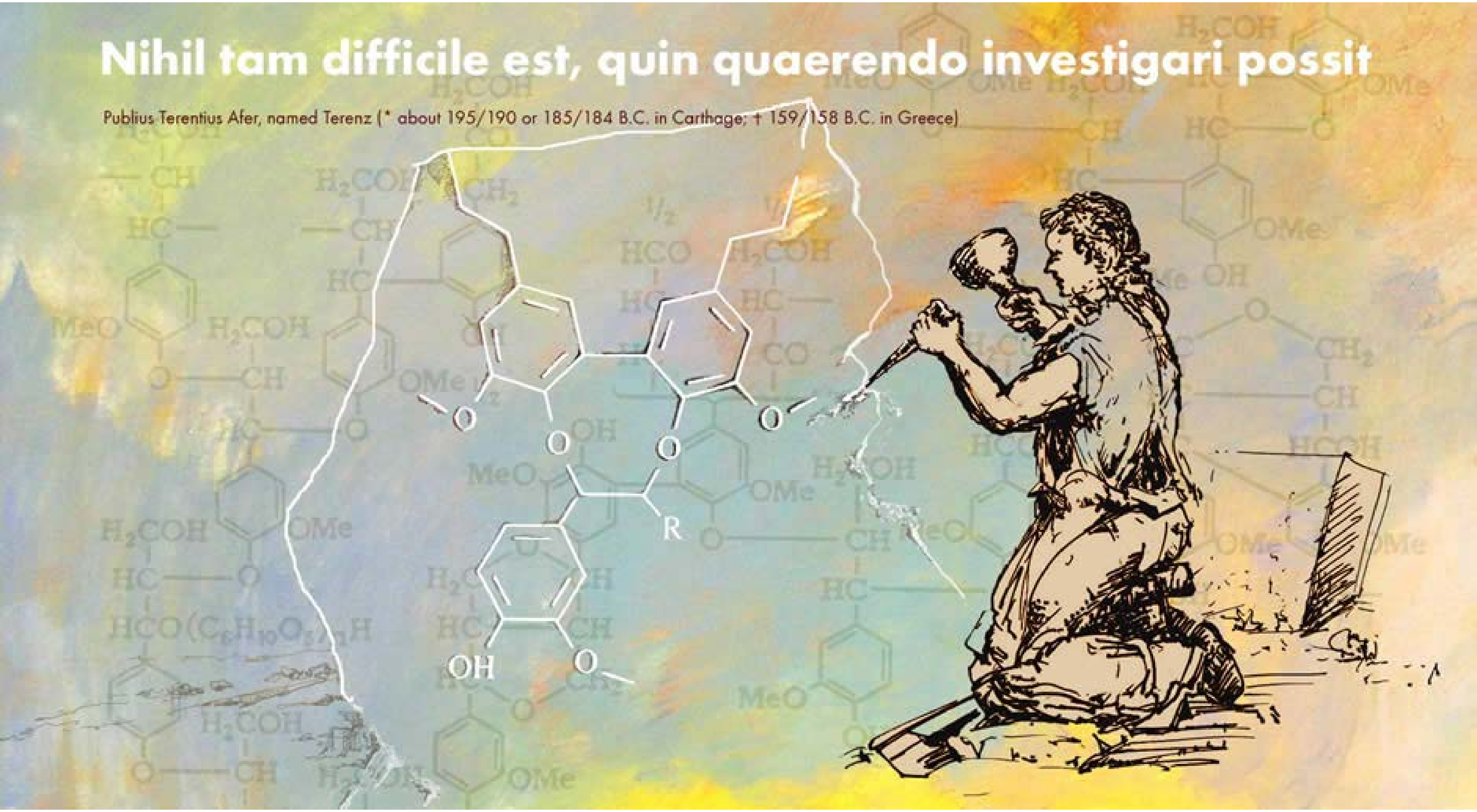
The *P. verruculosum*-enzyme complex is favoured for the SSF-process in “second generation”. For industrial scale, the used *P. verruculosum* production strain must be improved, in particular to eliminate the carbon catabolite repression by classical genetic methods. This has the advantage that the strain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

Partner of the EIB.10.013-Consortium:

(www.era-ib-lignocellulose.eu)

SIAB, UL	SIAB and Leipzig University, Leipzig
IPWC	Technical University of Dresden
IWPT	
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

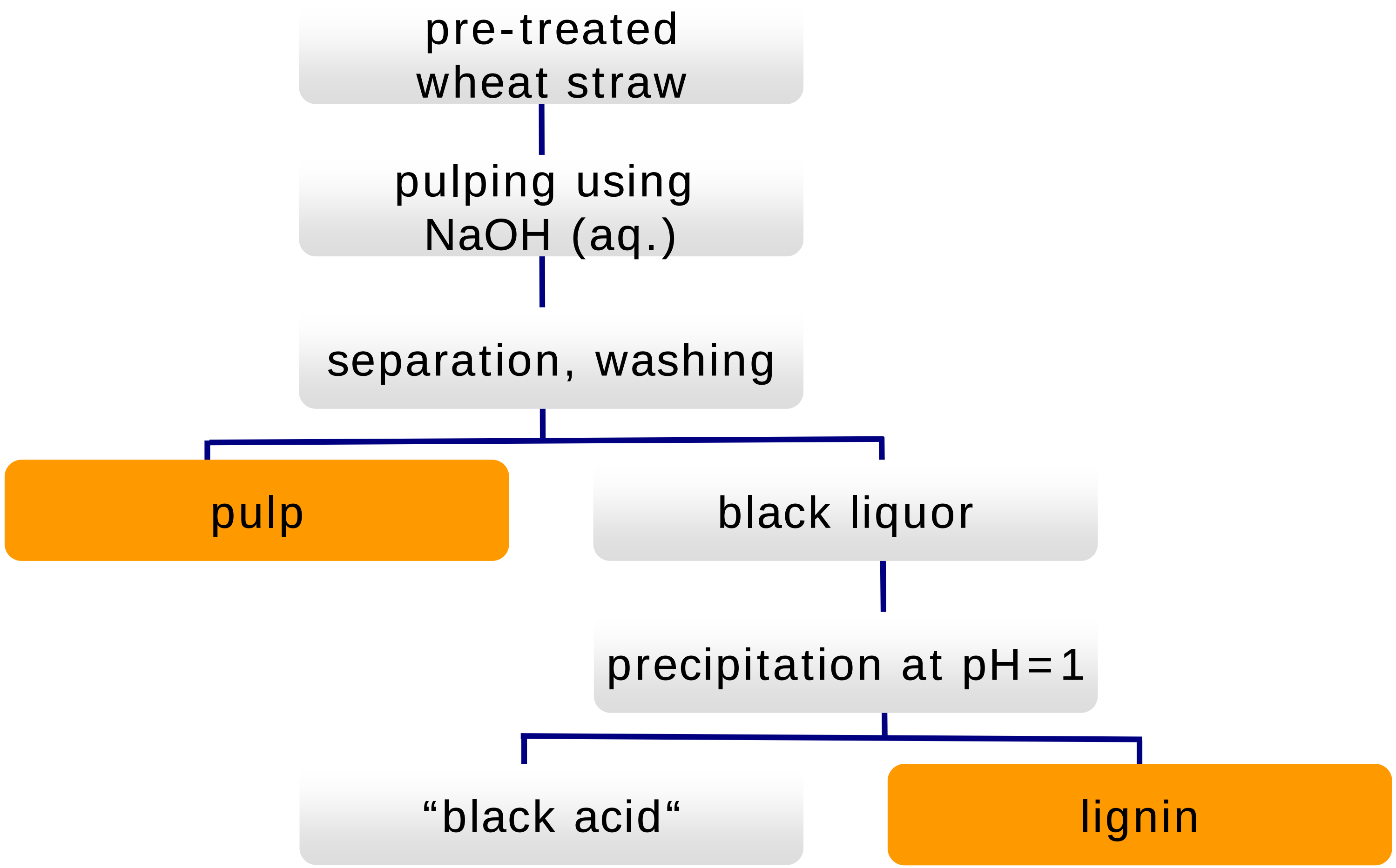
Consortium at project meeting in Bucharest, 2013



Thank you for your attention!

Notes
to the presentation

alkaline pulping of wheat straw



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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

Comparison of the pre-treatment:

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

* in relation to wheat straw

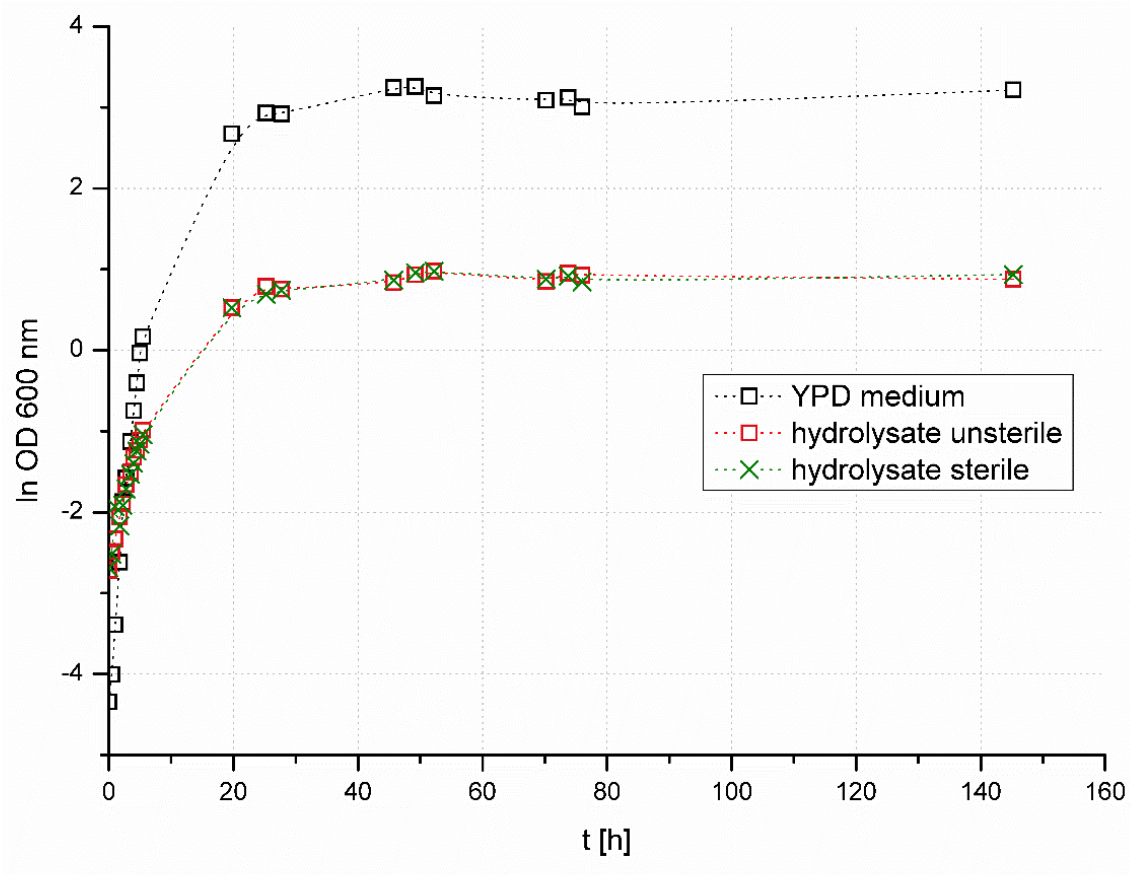
To the SSF-process

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)

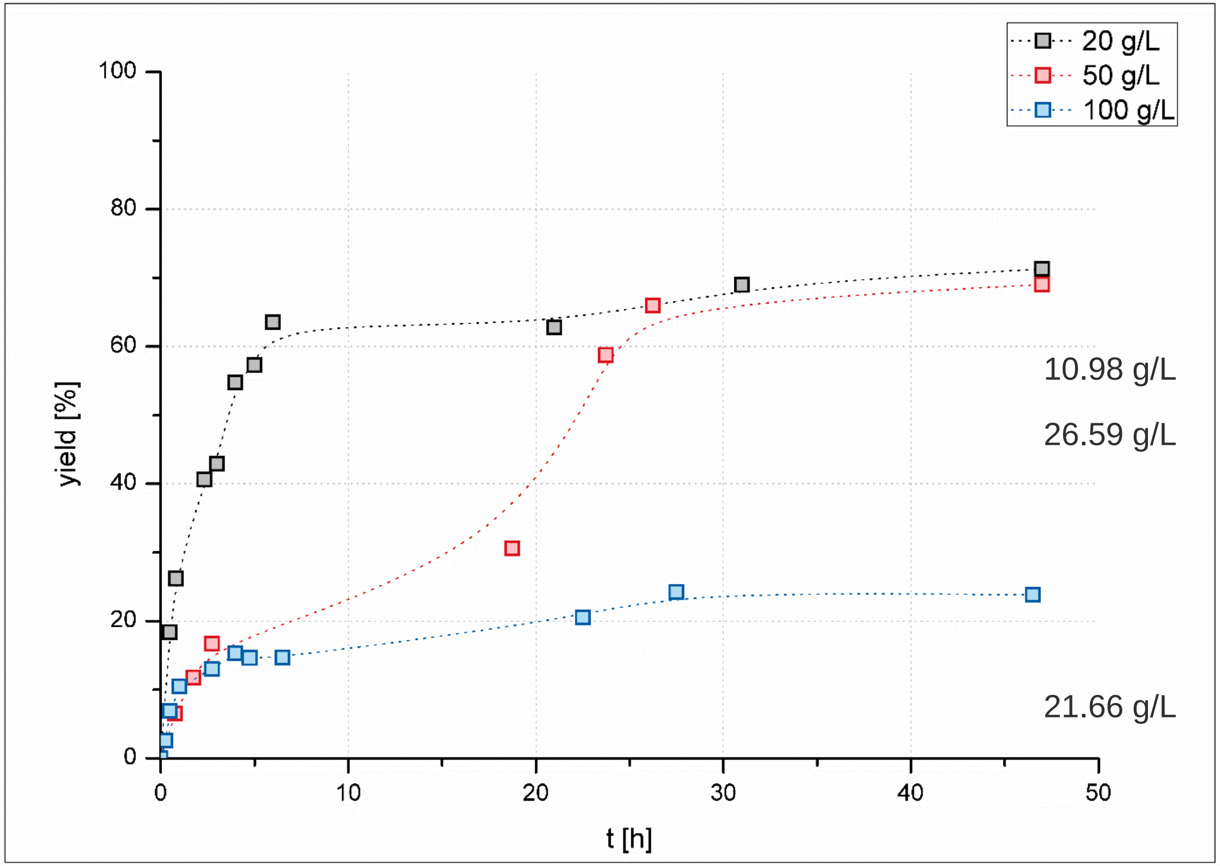


Conclusion:

- Yeast grows on hydrolysate,
- no sterilization necessary

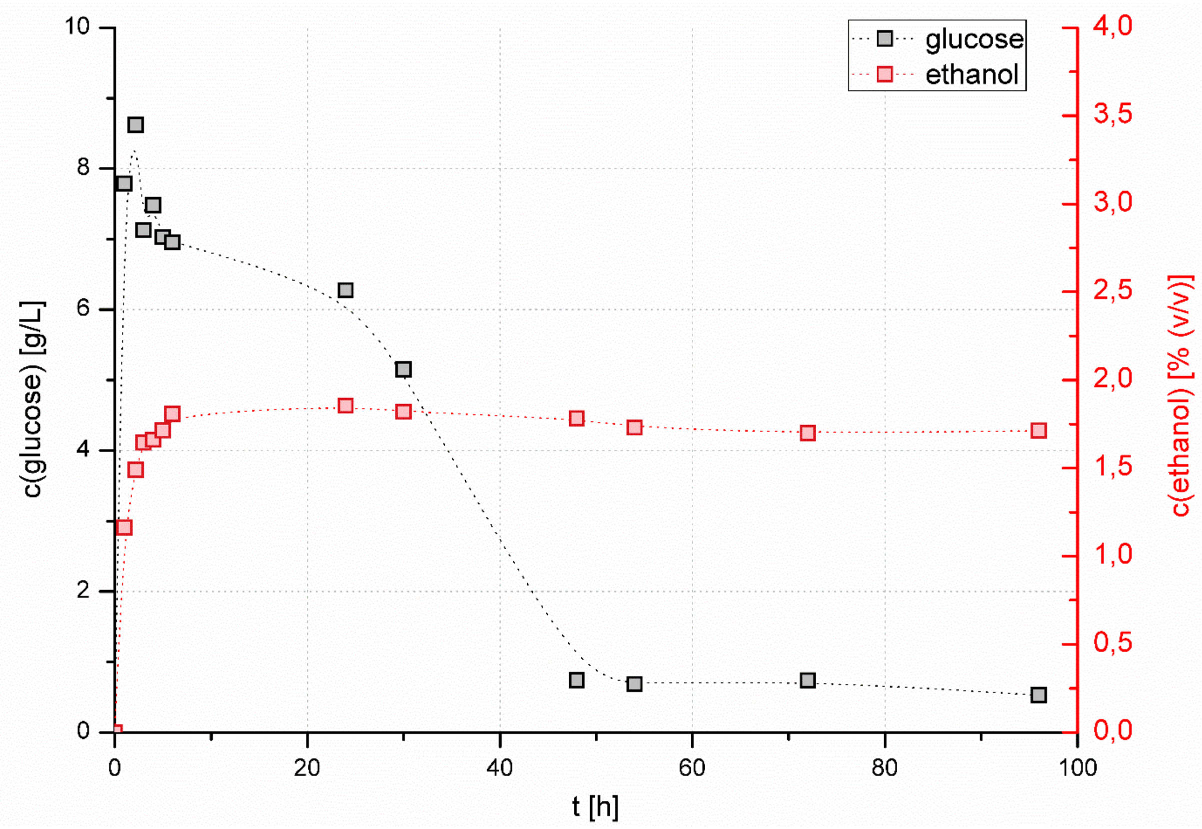
Enzymatic hydrolysis with *P. verruculosum*

- Influence of solid concentration



SSF-process

- 5 %(w/v) solid concentration



➔ max. 1.85 %(v/v) ethanol

➔ Hydrolysis: yield = 86 %

➔ Fermentation: yield = 71 %

TMP–wheat straw

from TU Dresden

Components	percent
Dry matter	97.8
Ash«	2.7
Extract	2.5
Cellulose	49.0
Polyosen	76.4
Hemicellulose	27.4
Lignin	22.8