Fermentation Technology for Lignocellulose

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Background

Lund University one of the partners in Workpage 3 - dealing with Fermentation technology - in the project BIOLYFE

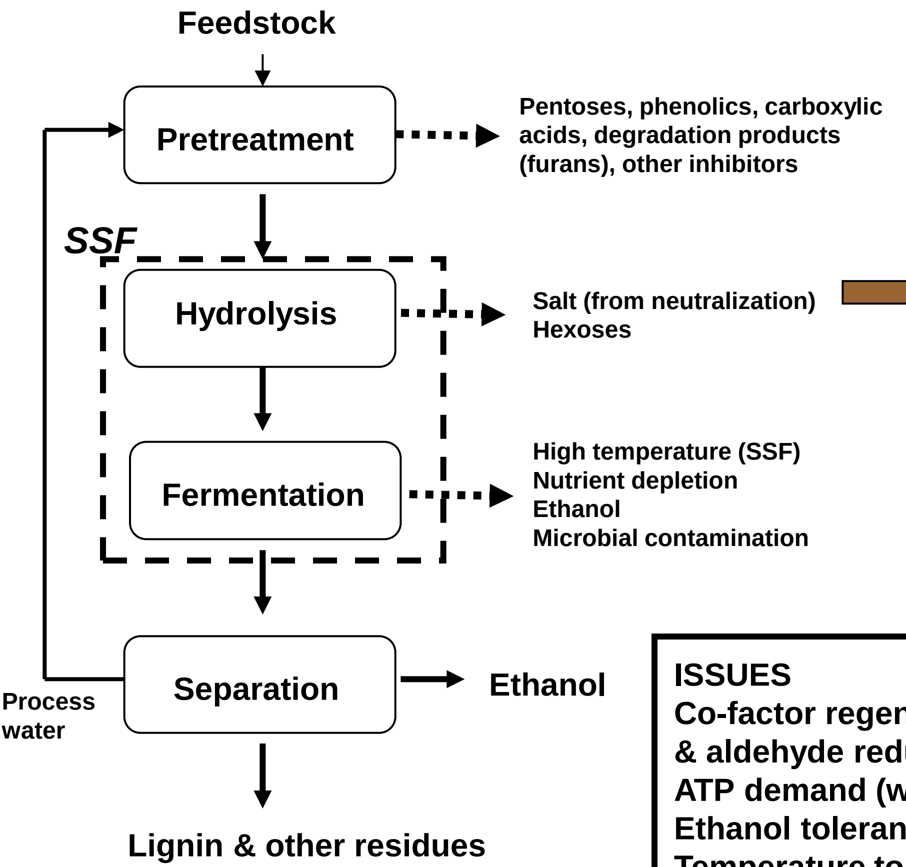
Subcontractor

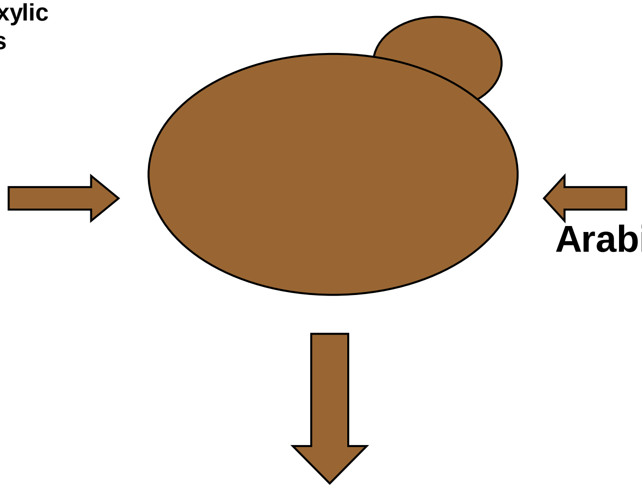
Outline

- Challenges in lignocellulose fermentation
- Mixing
- Fermentation process design
- Xylose fermentation
- Conclusions

Lignocellulose conversion – Fermentation challenges

Environmental factors *Genetic factors*





Xylose pathway
- oxidation/reduction
  XR/XDH/XK
- isomerization
  XI/XK

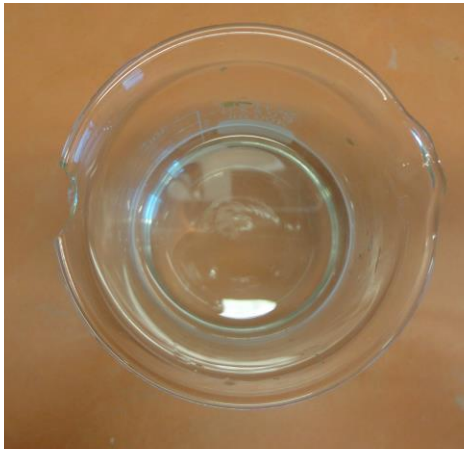
Arabinose pathway
- Oxidation/reduction
  XR/LAD/LXR/XDH
- Isomerization
  AI/RK/RE

Activation of PPP

ISSUES
Co-factor regeneration (xylose & arabinose pathways & aldehyde reduction)
ATP demand (weak acid & transport)
Ethanol tolerance
Temperature tolerance
Osmotic stress & compatible solute formation
Other uncharacterized toxic effects
Competition/contamination

Almeida et al. Biotechnol. J, 3, 286-299, 2011

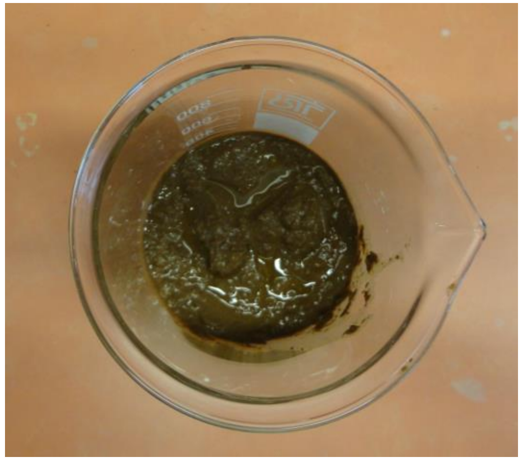
Sugars – different forms..



Sugar
200 g/L

Sugar
≈ 200 g/L

Milled pine wood, moisture content 50%, Glucan 35%, Mannan 12%

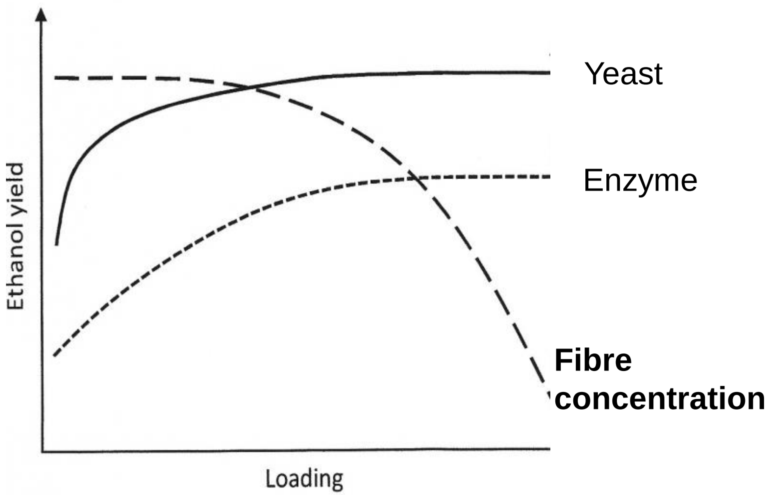


Sugar
≈ 130 g/L

Pretreated pine, 17%WIS, glucan 45%, dissolved glucose 30 g/L, mannose 25 g/L

Increased final ethanol titer → Higher fiber contents to be handled

Lower yield



Mixing issues

- Temperature control
- Distribution & blending
- Effects on process performance

Inhibitor problems

- Effects yeast metabolism
- Effects on enzymatic hydrolysis

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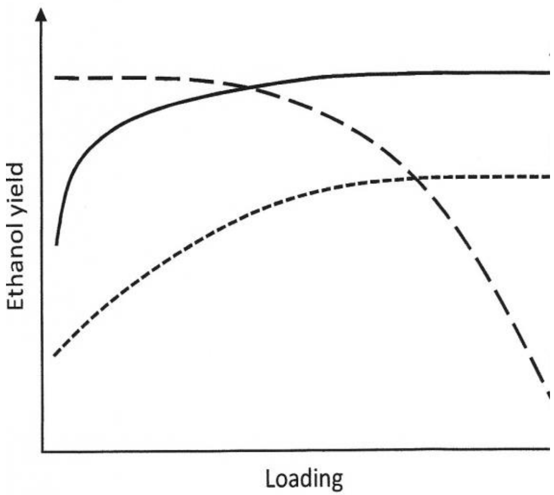
Increased final ethanol titer → Higher fiber contents to be handled

Lower yield



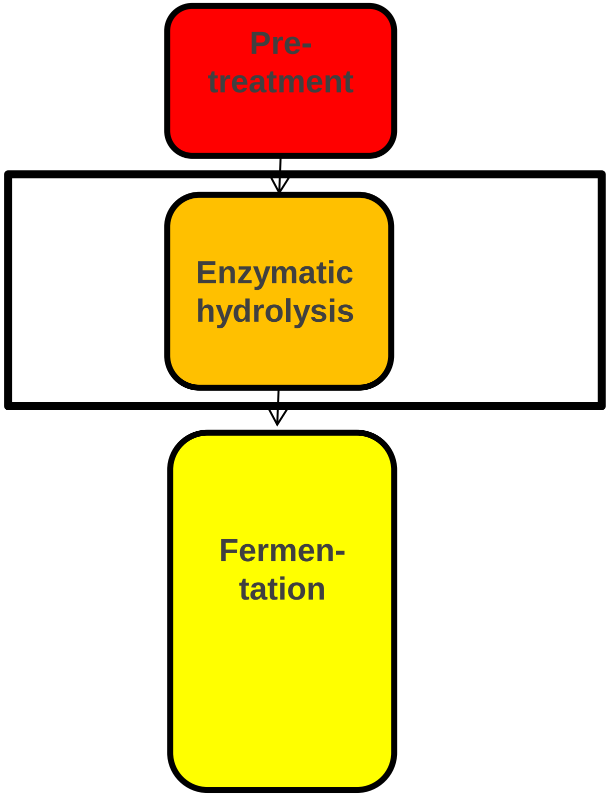
Mixing issues

- Temperature control
- Distribution & blending
- **Effects on process performance**

Inhibitor problems

- Effects yeast metabolism
- Effects on enzymatic hydrolysis

The basic process layouts



Temperature 180-210ºC

Temperature 45 -50ºC

Temperature 30 -35ºC

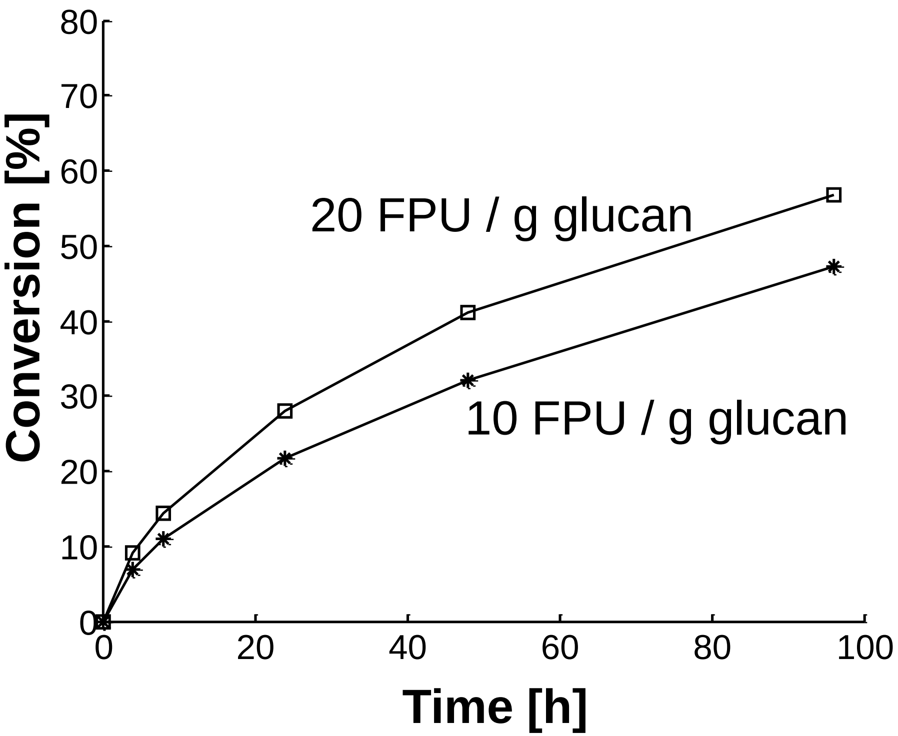
SHF

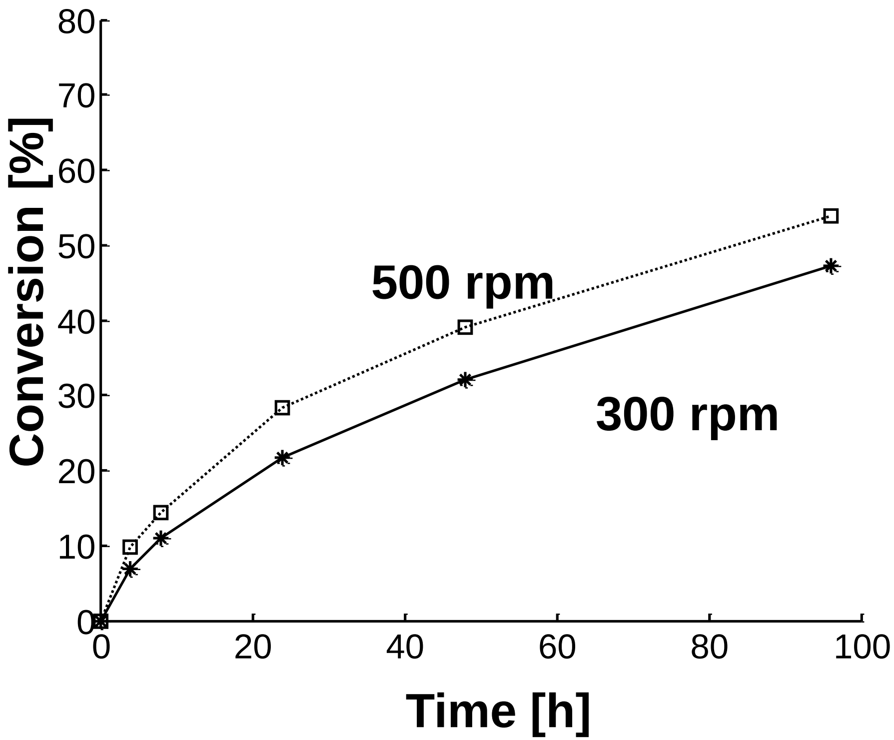
Separate hydrolysis and fermentation

Mixing – example 1:
Spruce – a softwood material

Glucan	43%
Lignin	46%

Mixing





10 FPU / g glucan

Hydrolysis of pretreated spruce
Enzyme used Cellic CTec

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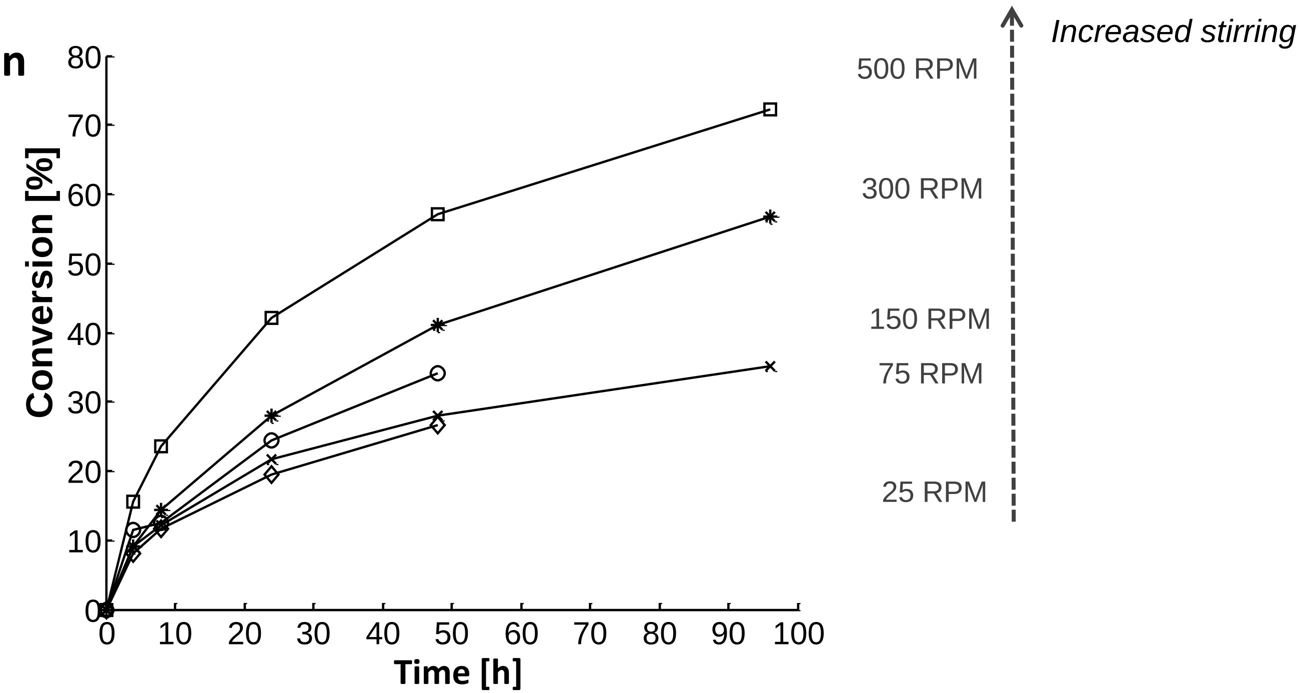
Mixing

Hydrolysis of pretreated spruce

20 FPU/g glucan



→ **MIXING MATTERS!!**

Palmqvist et al. Biotechnol Biofuels. 4: 10, 2011

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Mixing at high solids contents

Powerful, geared servo motor

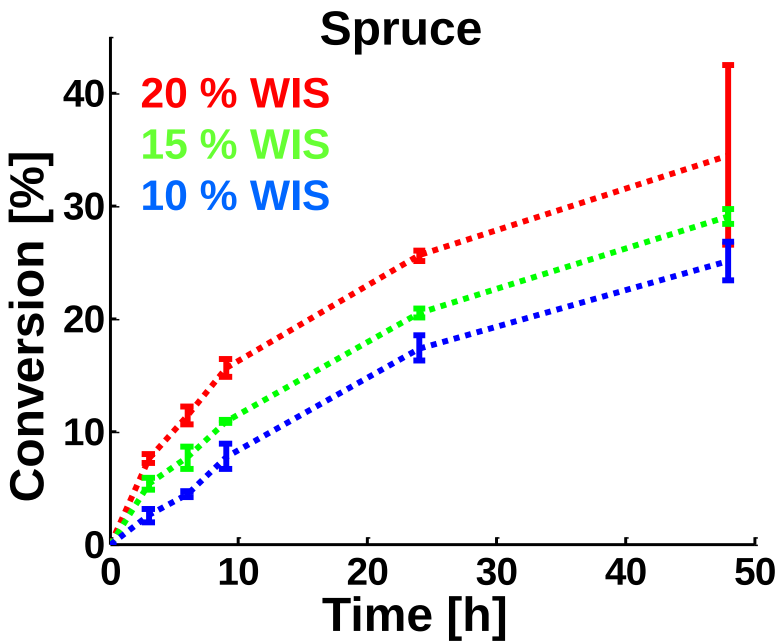
Heating/cooling with water jacket (Control on jacket or vessel temperature)

"Anchor type" stirrer

Torque measurement (and hence measured power consumption)

$$P = 2 * \pi * N_i * M$$

What happens when we increase the WIS content?



Yield increases with increased WIS content!!

Enzymatic hydrolysis at 10, 15 and 20 % WIS

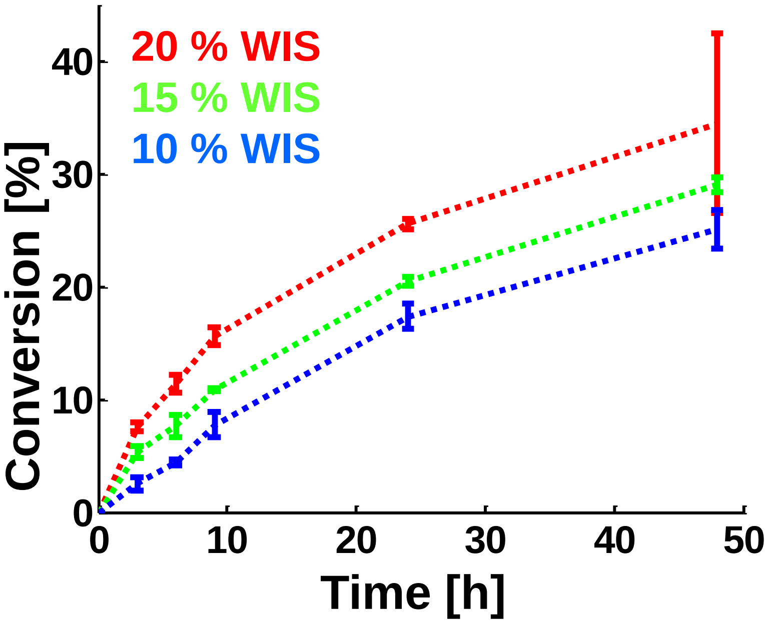
Enzyme used: Cellic CTec2

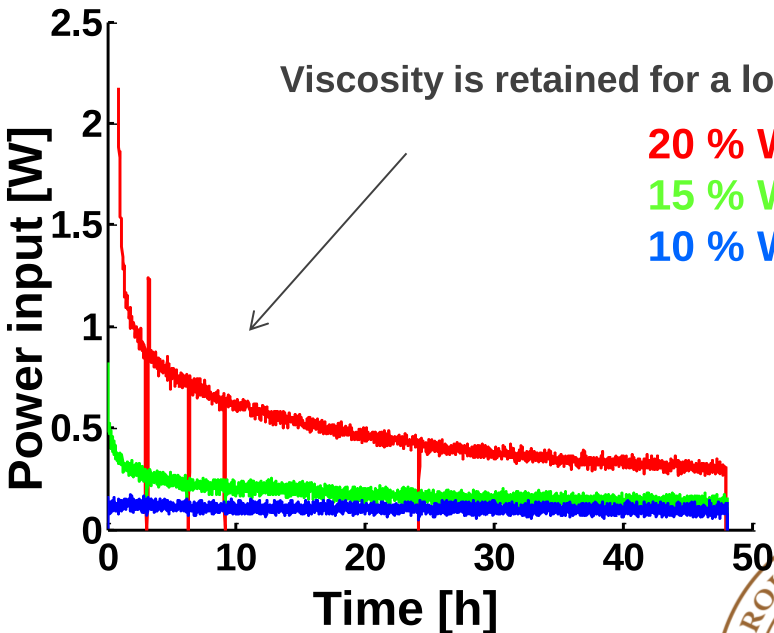
Palmqvist et al. Biotechnology for Biofuels, 2012, 5:57

Why?

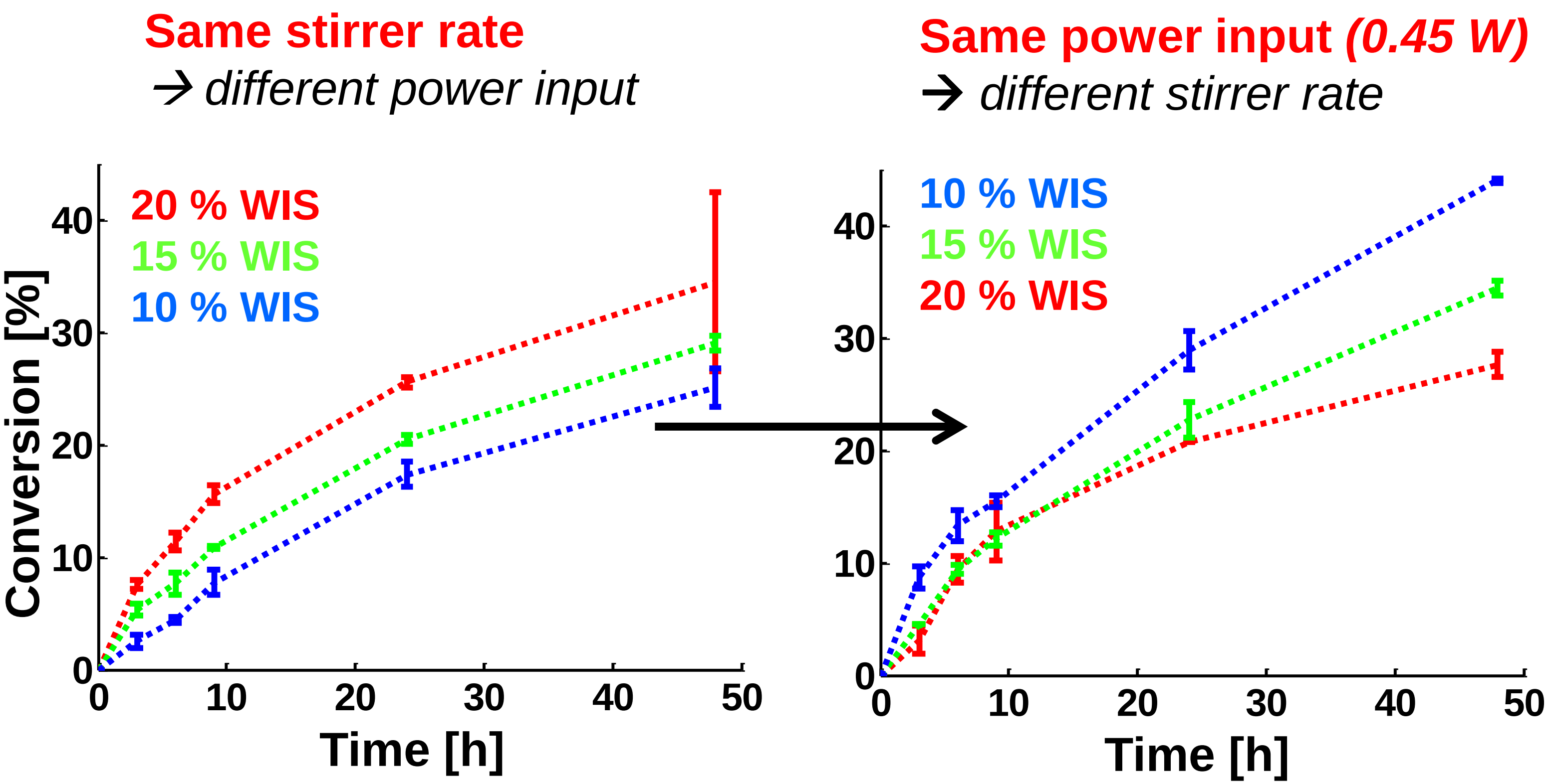
The mixing power is very different!

~ five fold higher total energy input at 20 % WIS





If the same mixing power (rather than stirring rate) is used, the behaviour is as expected



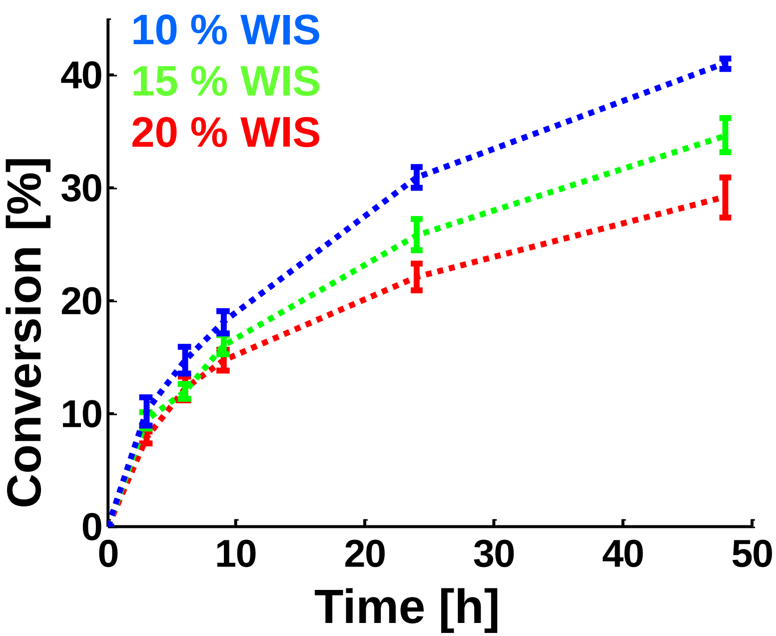
Palmqvist et al. Biotechnology for Biofuels, 2012, 5:57

Arundo Donax

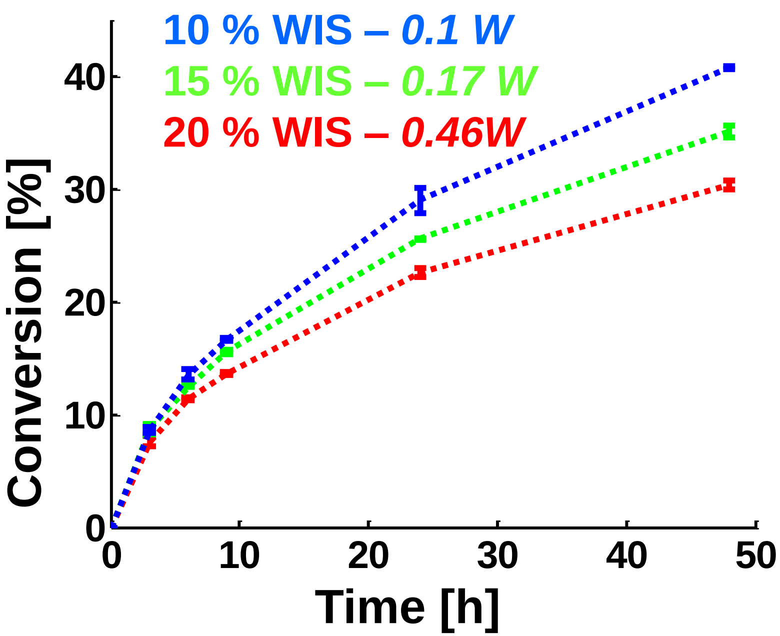
Pretreated Arundo Donax

So let's try Arundo!

Same power input *(0.1 W)*



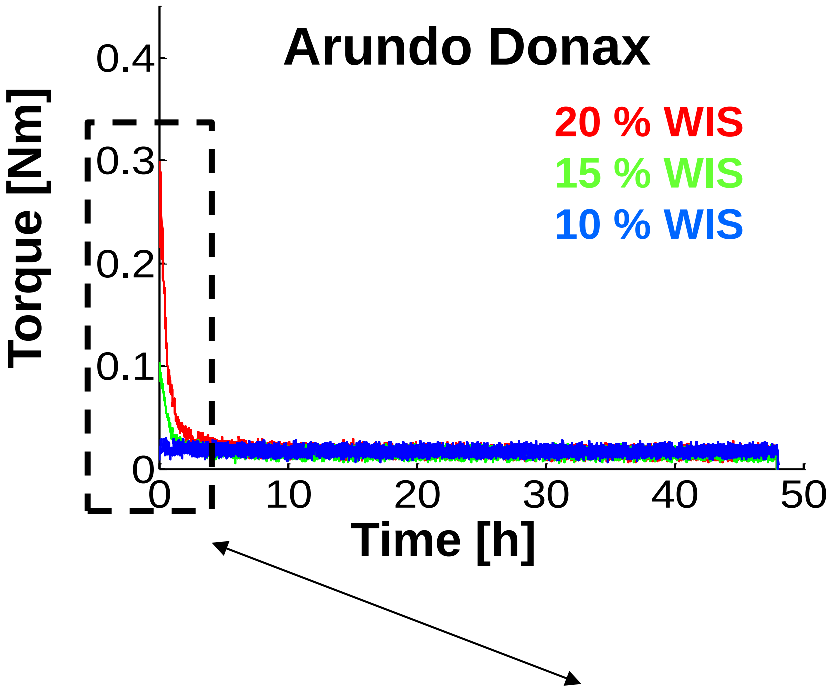
Different power input

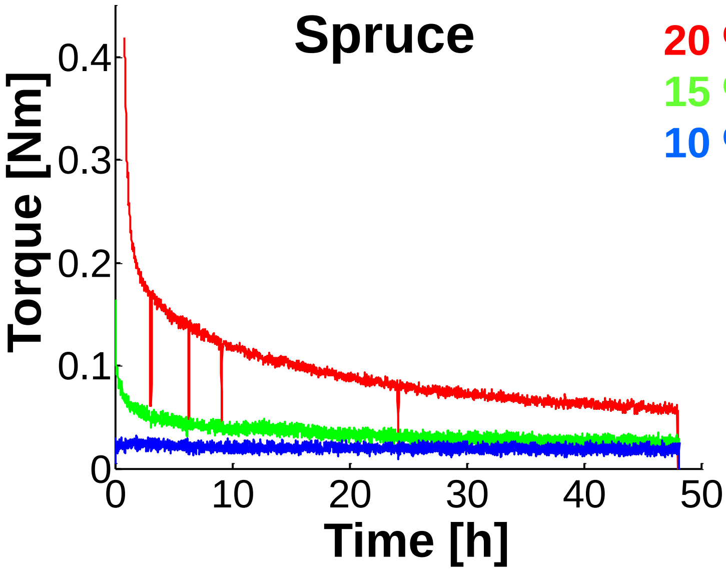


→ **MIXING DOES NOT MATTER!**

→ **Arundo is different!**

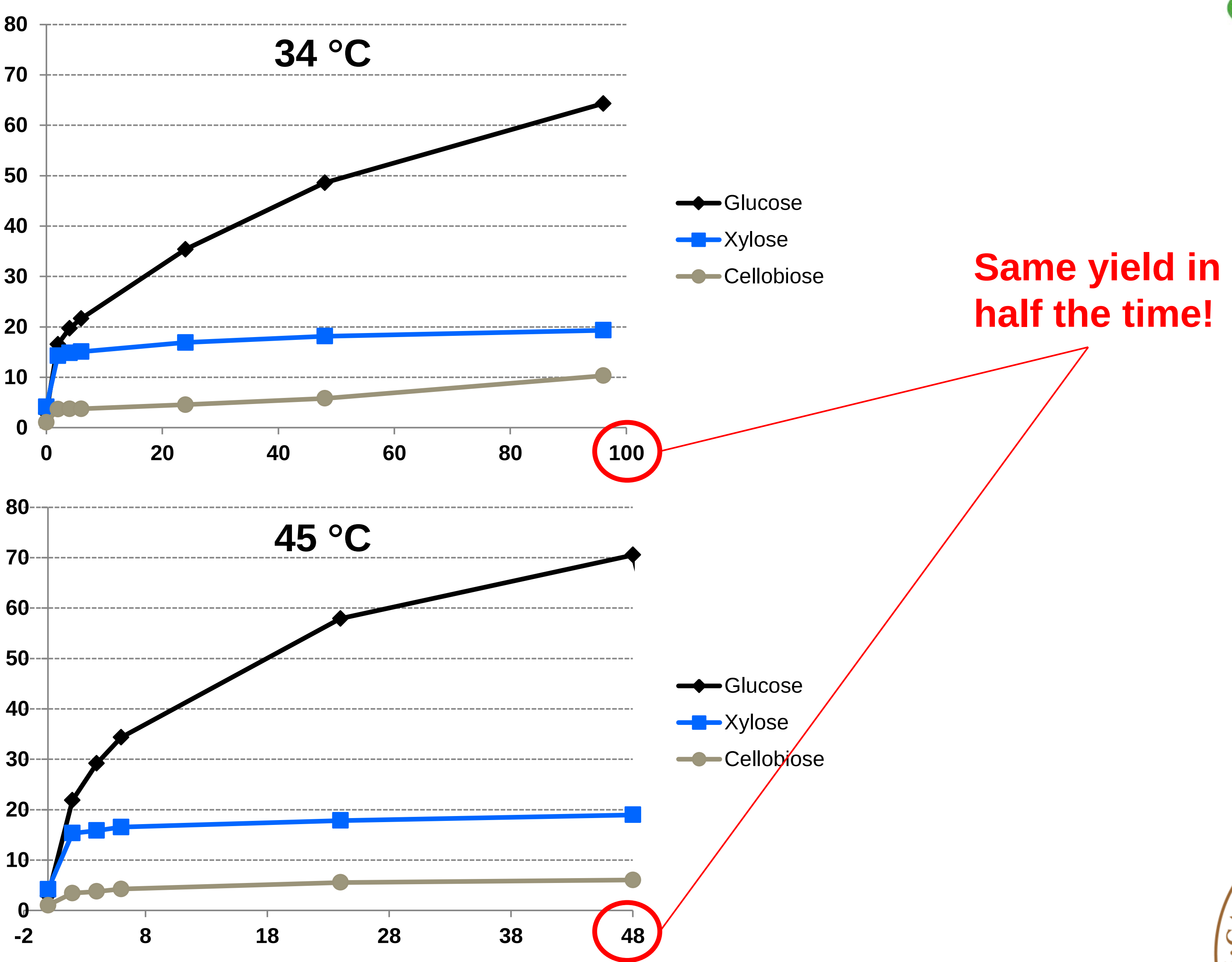
Torque-profiles during hydrolysis



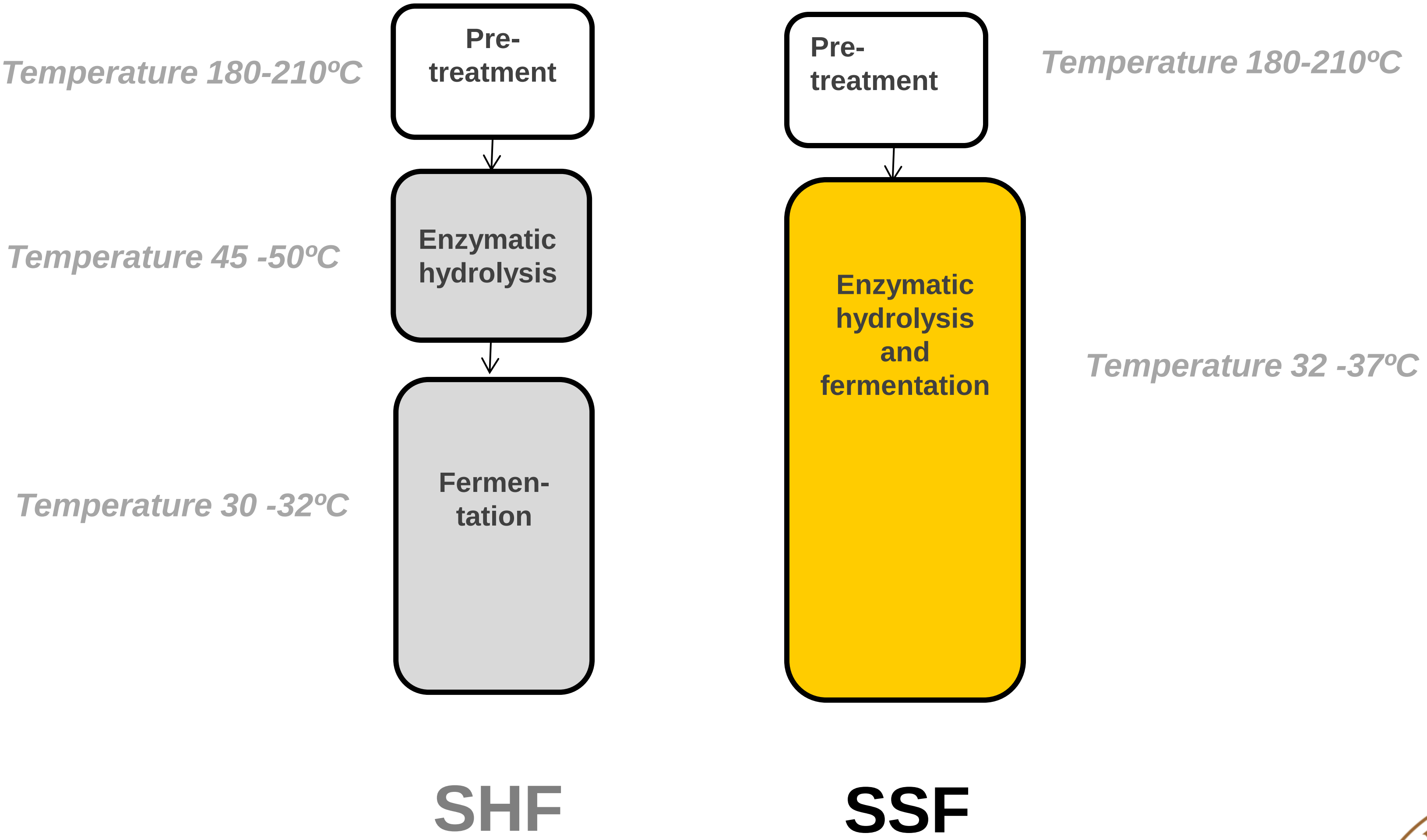


Very rapid loss of viscosity in the Arundo case!

Temperature effects on hydrolysis

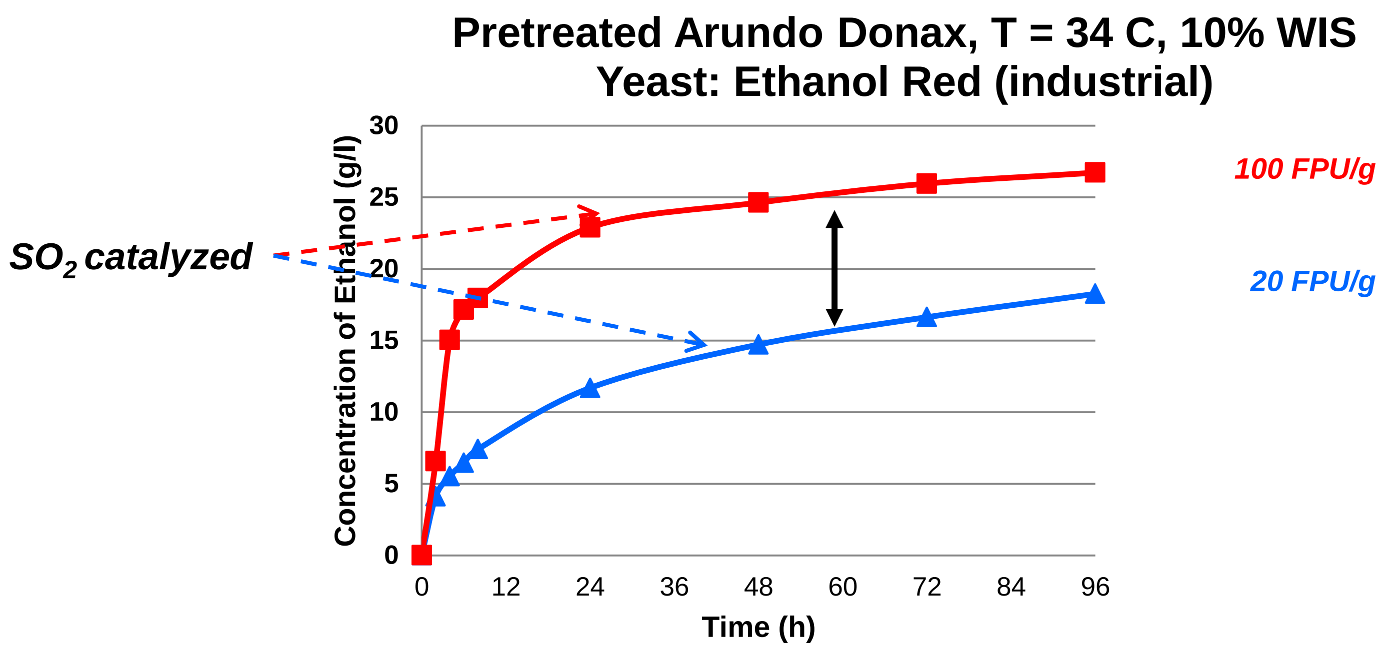


The basic process layouts



Simultaneous saccharification and fermentation

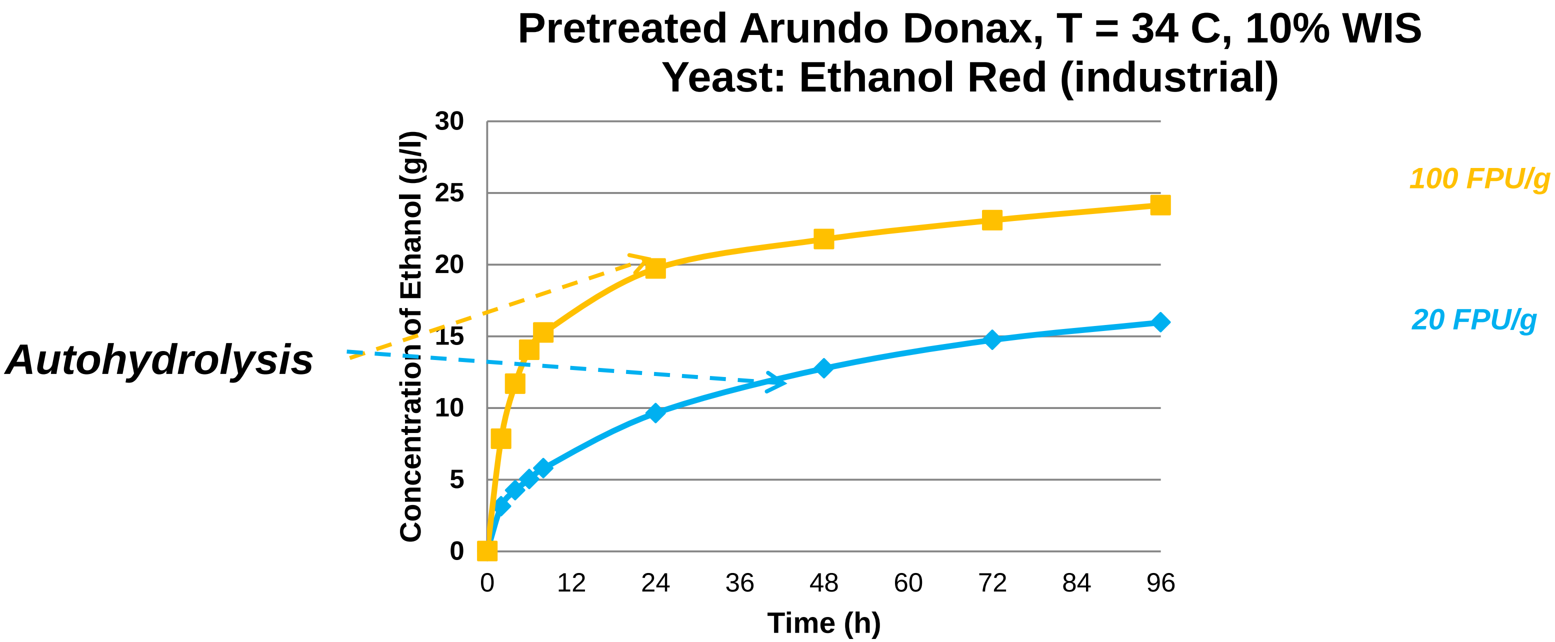
SSF – enzyme dose effect



(Very very high…)

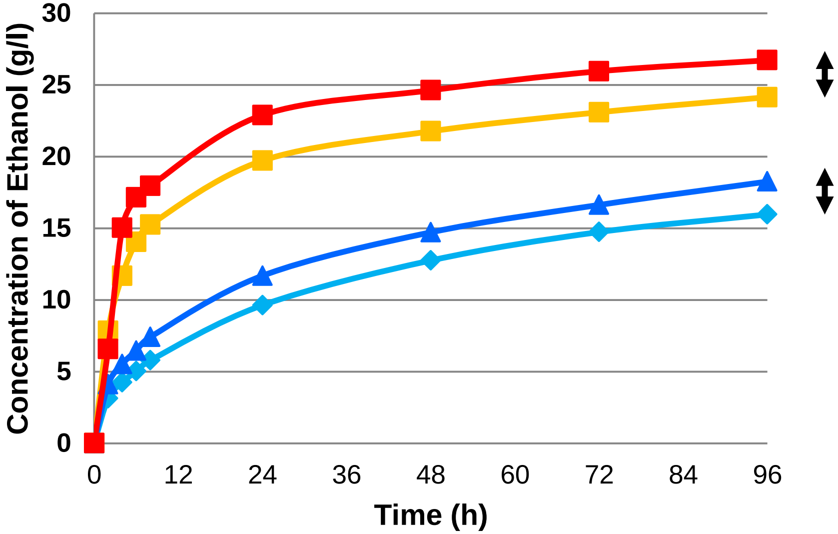
Bhargav Prasad Kodaganti, M. Sc. Thesis, Lund Univ. 2011

SSF – enzyme dose effect



Bhargav Prasad Kodaganti, M. Sc. Thesis, Lund Univ. 2011

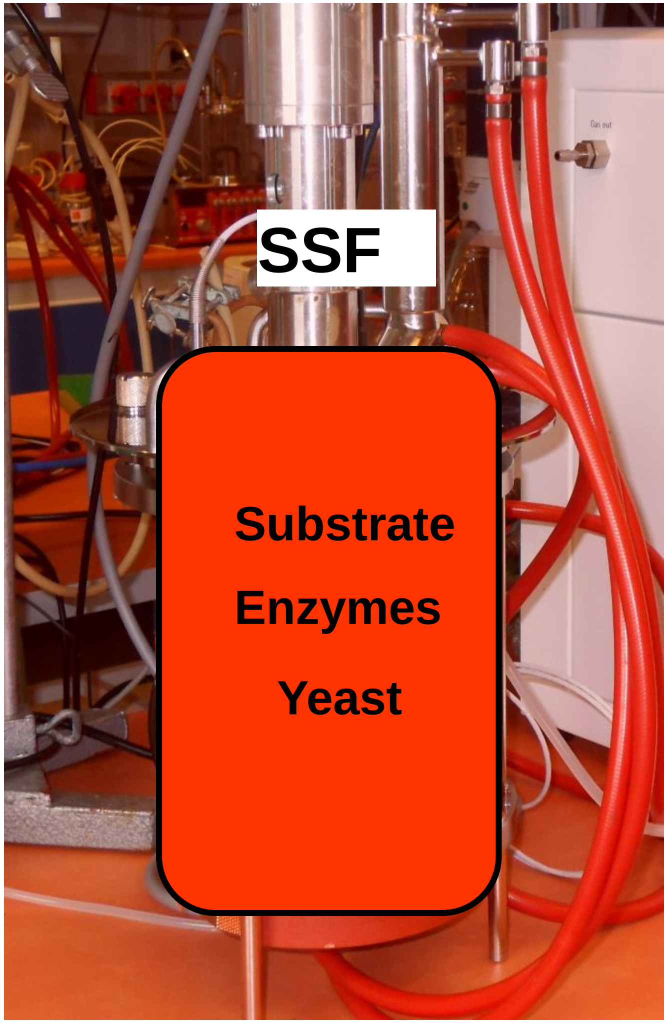
SSF – pretreatment effect



Difference due to pretreatment

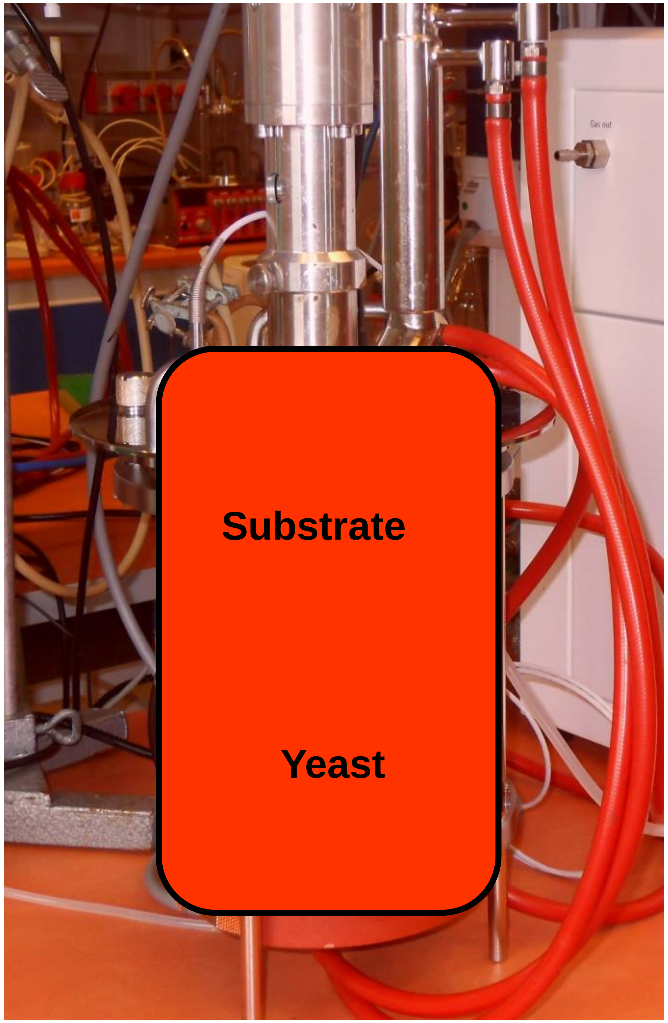
Bhargav Prasad Kodaganti, M. Sc. Thesis, Lund Univ. 2011

The batch SSF

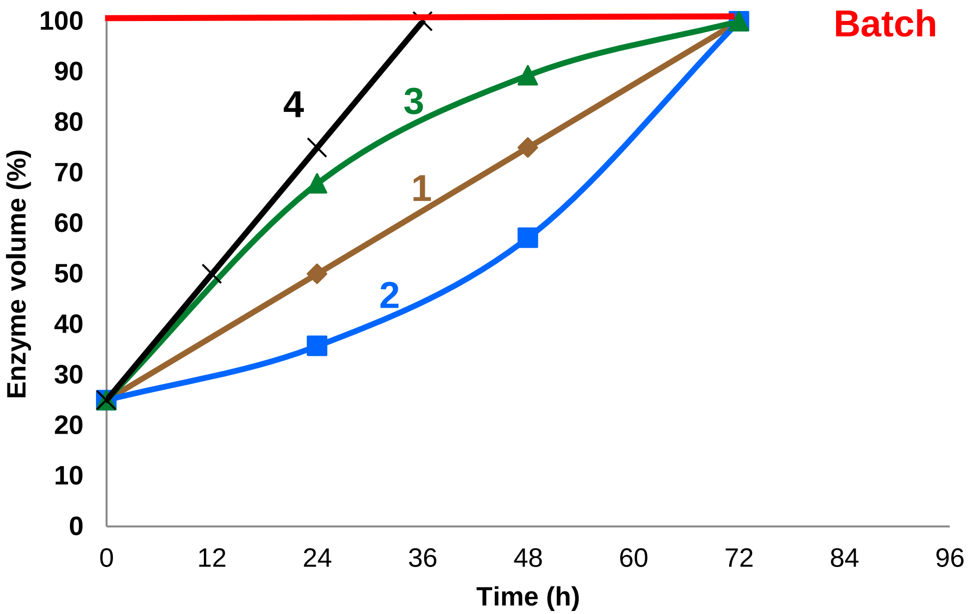


Fed-batch SSF

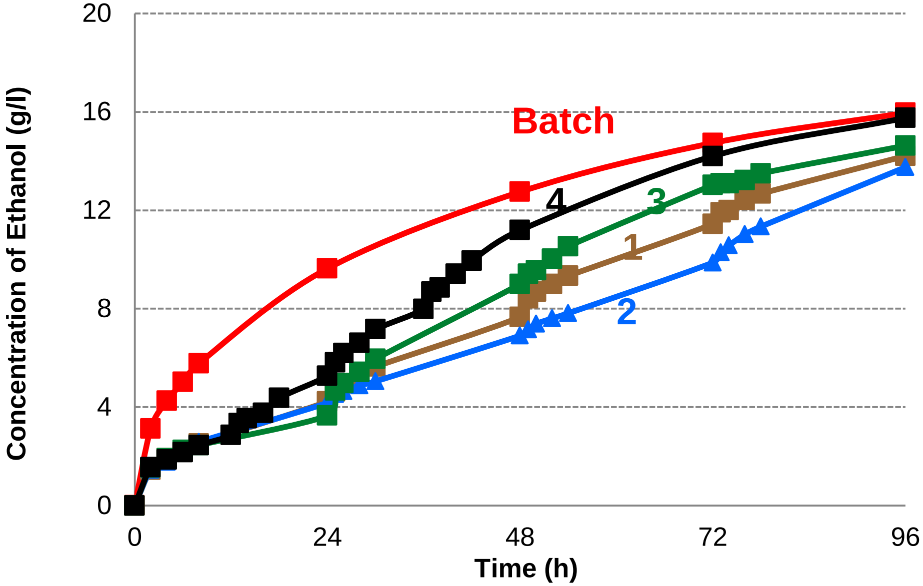
Enzymes



Enzyme feeding strategies



Enzyme fed-batch SSF feeding strategies

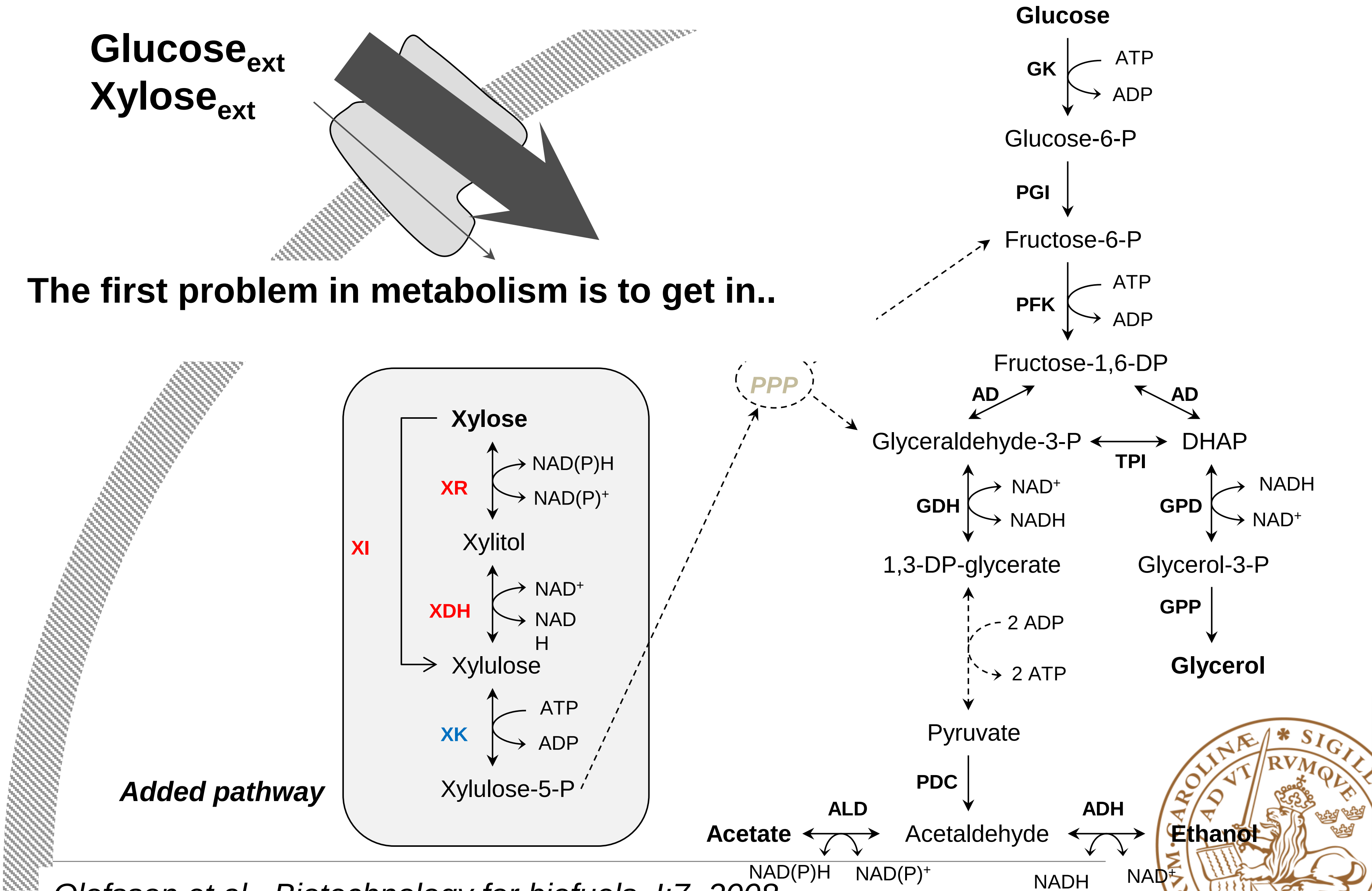


Results obtained with four different enzyme feeding strategies
20 FPU/g glucan Cellic CTEC 2, Ethanol Red, T 34 C

No improvement from enzyme feeding
”Batch is best”

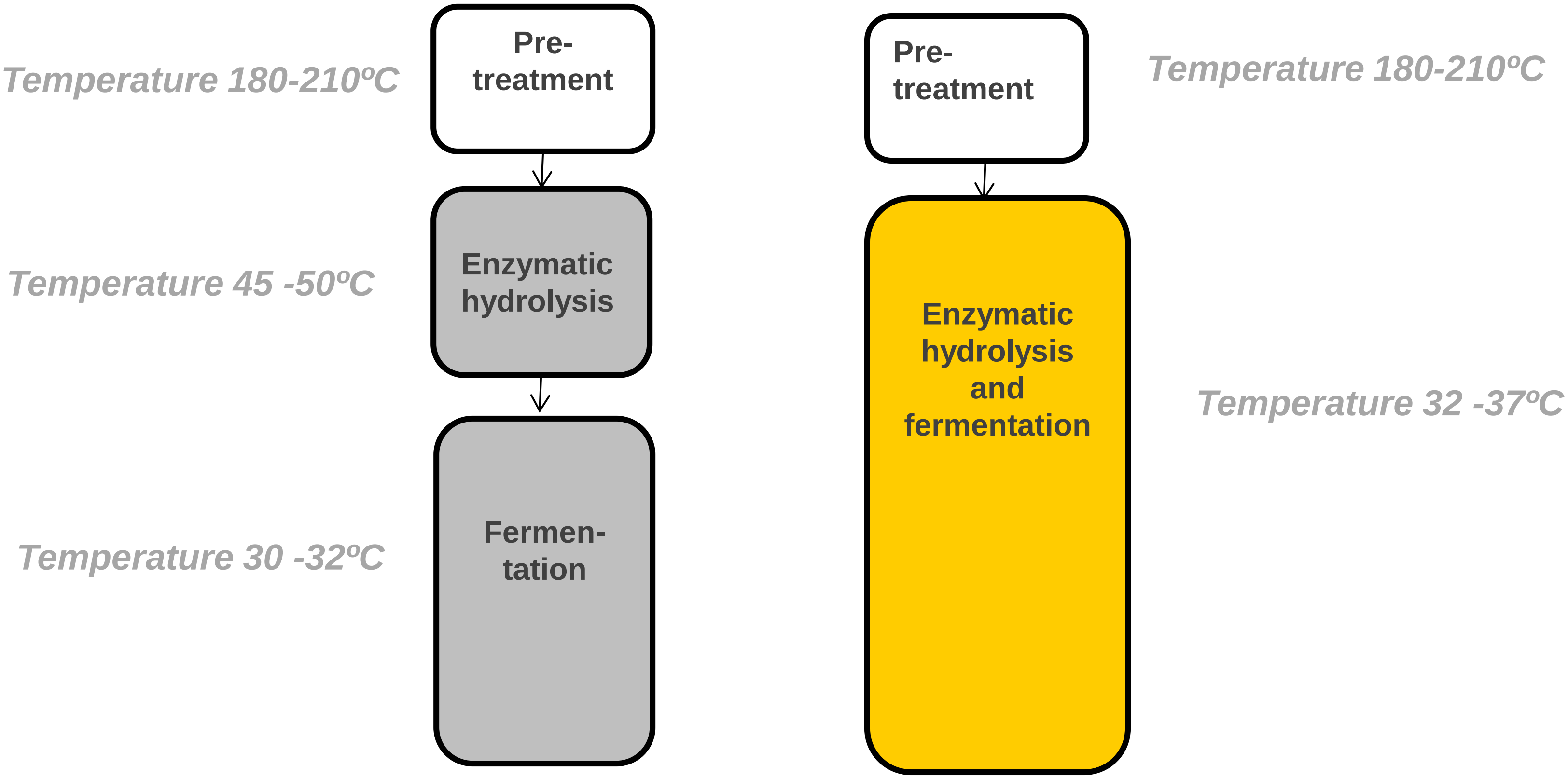
Bhargav Prasad Kodaganti, M. Sc. Thesis, Lund Univ. 2011

Glucose and xylose co-fermentation in Saccharomyces cerevisiae



Olofsson et al., Biotechnology for biofuels, I:7, 2008

The basic process layouts

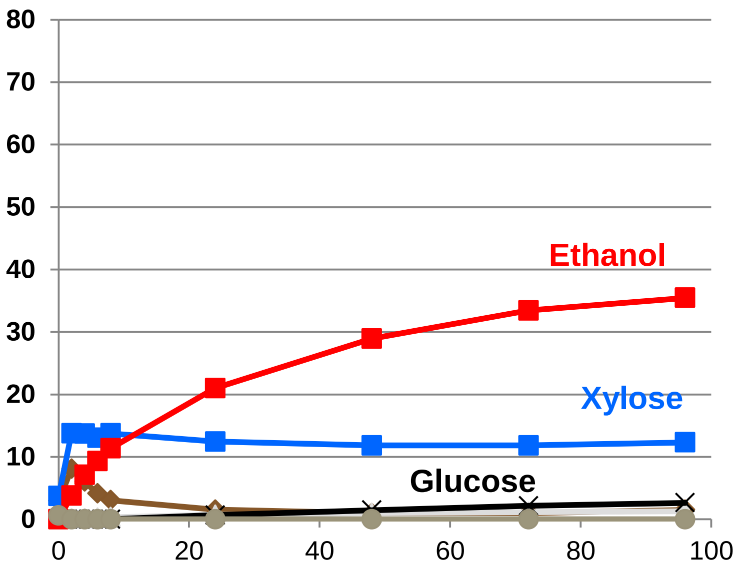


SHF

SSCF

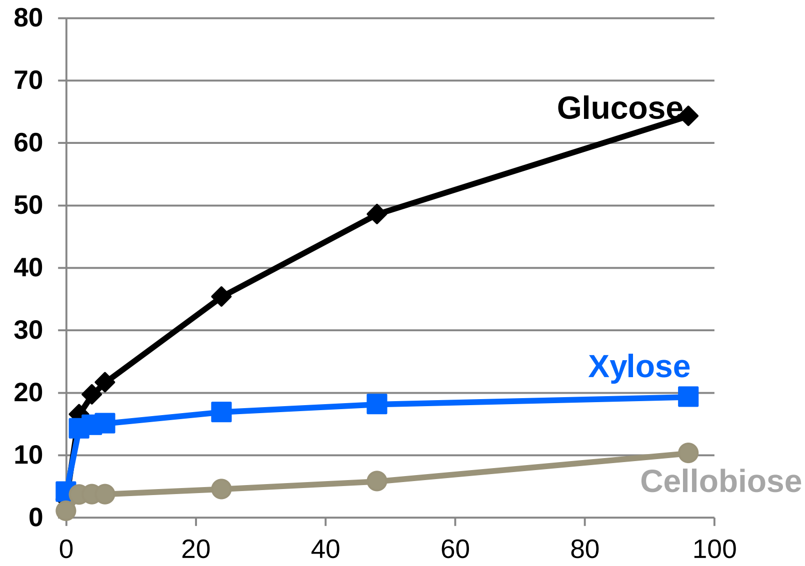
Simultaneous saccharification and co-fermentation

SSCF



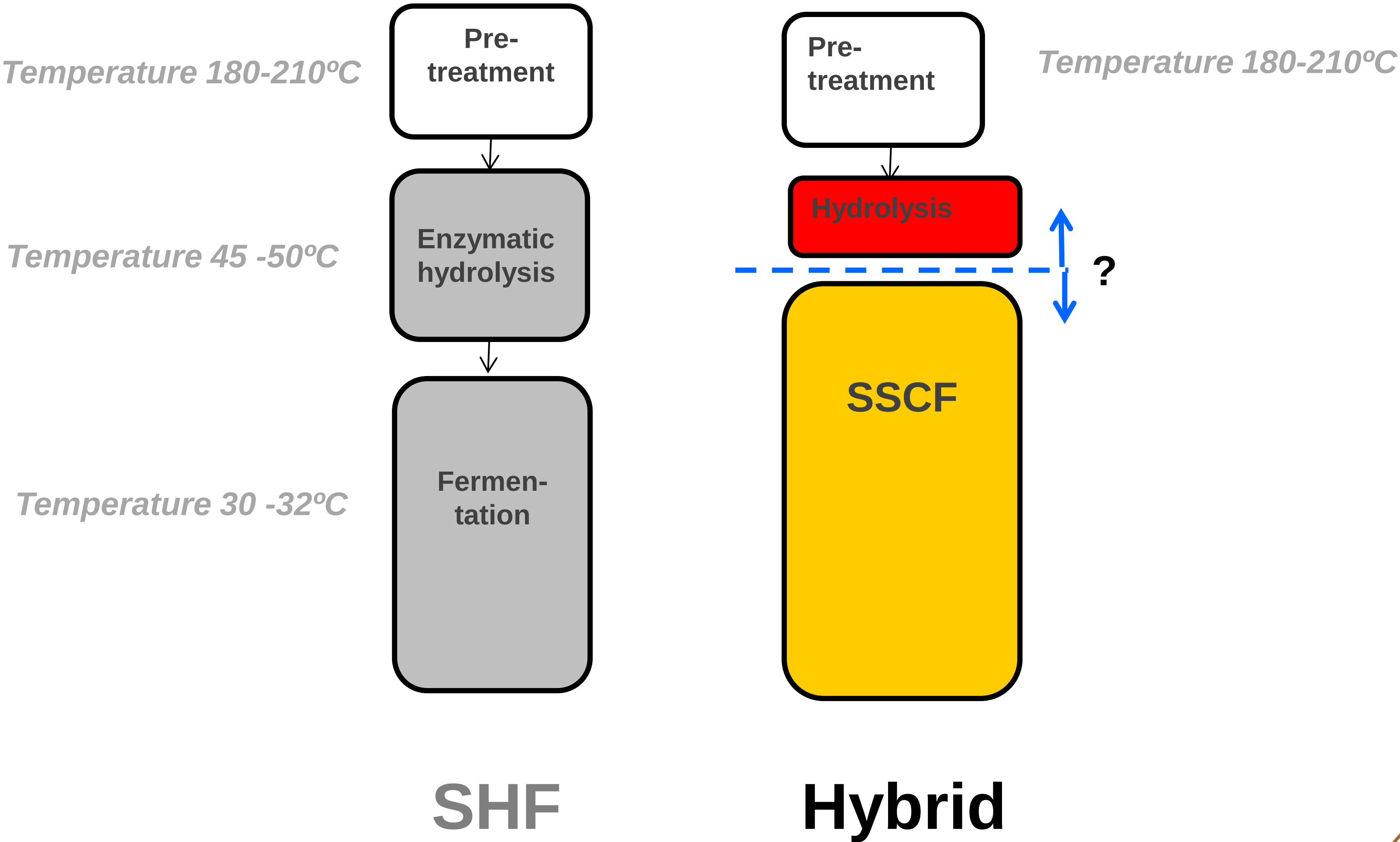
34˚C, pH 5.0,
Xylose fermenting yeast TMB3400
Enzyme CTec3

Enzymatic hydrolysis

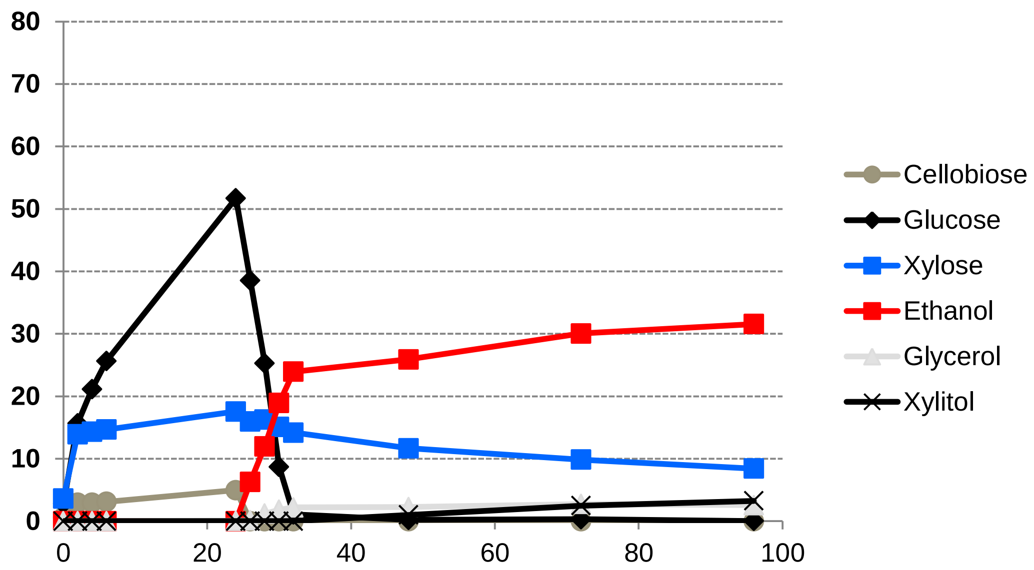


34˚C, pH 5.0,
Enzyme CTec3

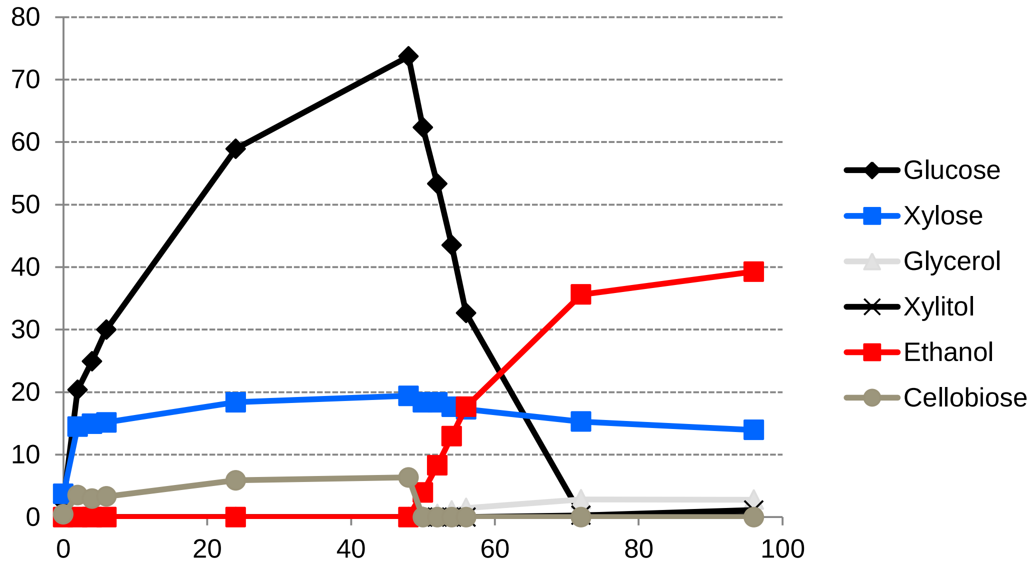
The basic process layouts



24 h hydrolysis - 45˚C, pH 5.0
72 h SSCF - 34˚C, pH 5.0



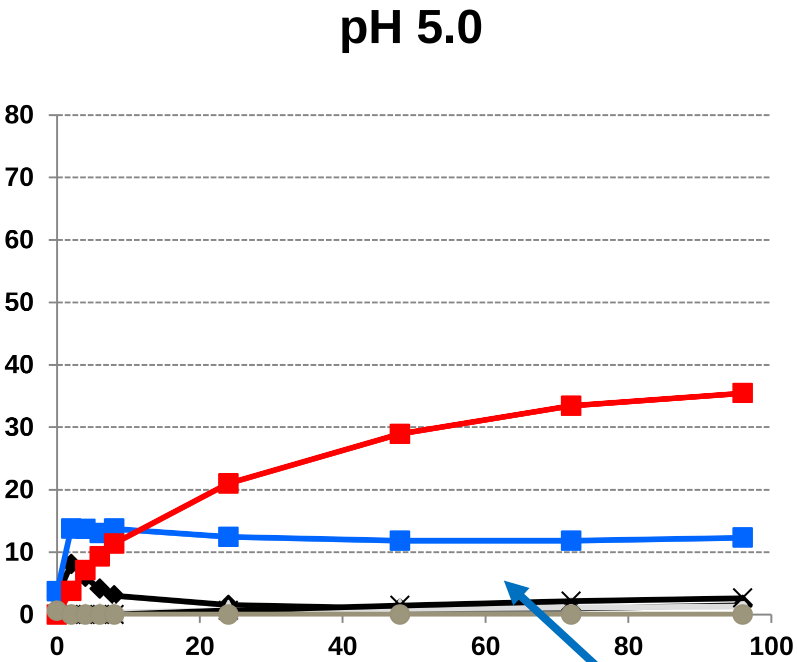
48 h hydrolysis - 45˚C, pH 5.0
48 h SSCF - 34˚C, pH 5.0

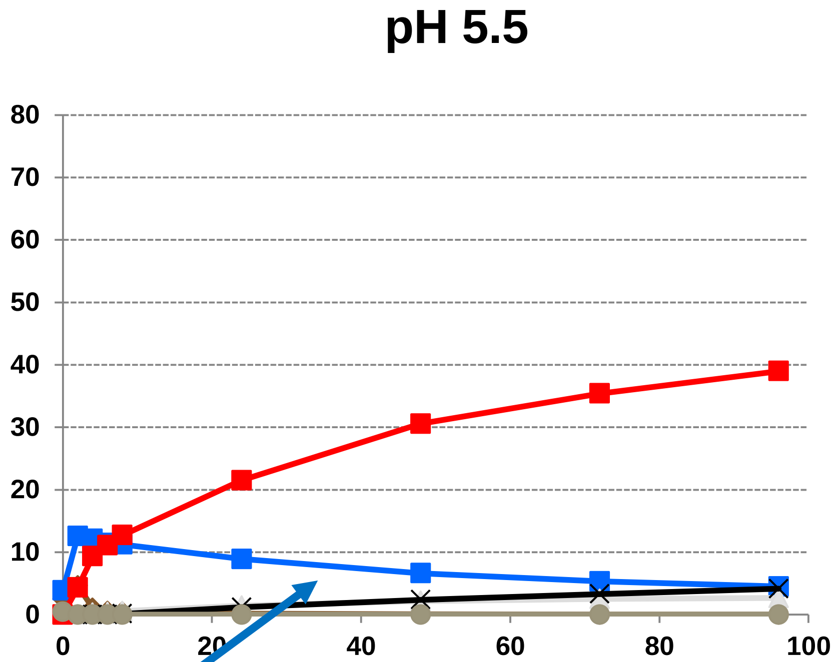


25% higher ethanol yield!

Small things that matter..

Don’t forget Biochemistry 101!!





34˚C,
Xylose fermenting yeast TMB3400
Enzyme used CTec3

Significant effect on xylose consumption!

Development trends

Decrease enzyme use → Harsher pretreatment / lignin removal

Decrease use of chemicals → Milder pretreatment

Inhibitor problems

Problems with enzymatic hydrolysis

Higher enzyme use
<u>or novel enzymes</u>

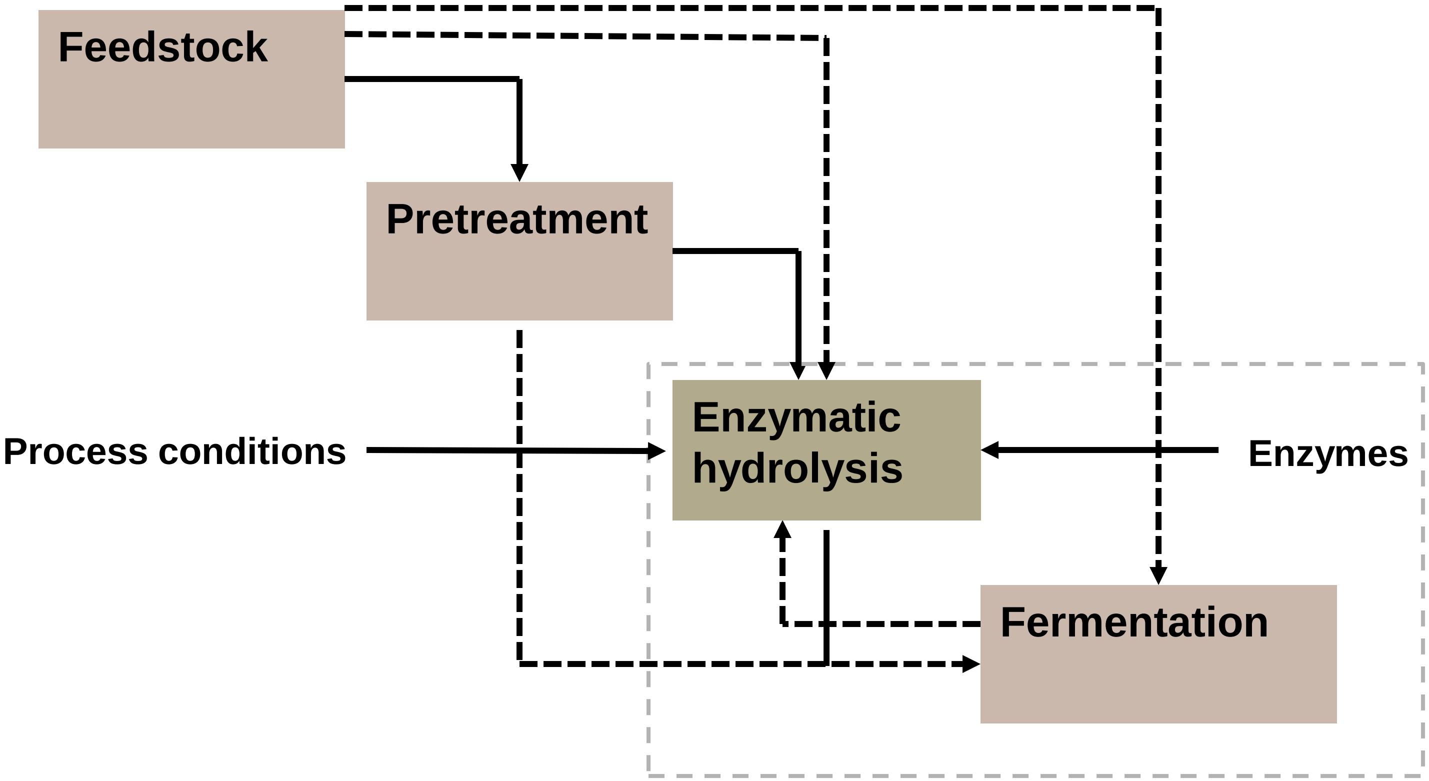
Tolerant yeasts
Detoxification
Modified pretreatment

Increase in ethanol yields due to improved enzyme cocktails

Reference enzyme mixture		Improved enzyme mixture	Change in ethanol yield
Cellic CTec (+ HTec)	→	Cellic CTec2	No significant increase
Cellic CTec2	→	Intermediate enzyme blend	~ 15 % increase
Intermediate enzyme blend	→	Cellic CTec 3	~ 8 % increase
Overall increase			**~ 24 %**

Batch SSF experiments at a WIS loading of 10 %.
Yeast used: TMB3400 (Taurus Energy). T = 34 C.

Conclusions



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Chemtex, Italy
Arianna Giovannini

SEKAB, Sweden
Sune Wännström

See also poster:
Process development of a hybrid saccharification and co-fermentation process for Arundo donax
Palmqvist et al