Fermentation Technology for Lignocellulose

Experiences from the BIOLYFE project

Benny Palmqvist and Gunnar Lidén
Chemical Engineering, Lund University

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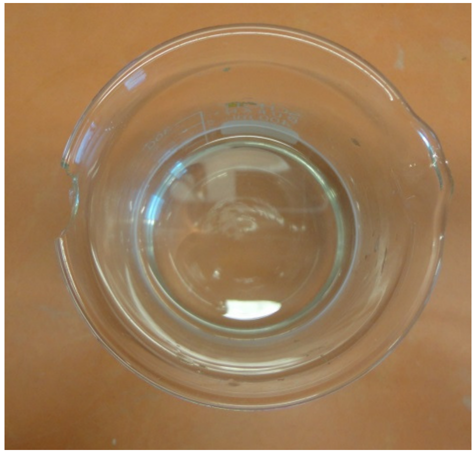
Biomass *is not* sugar – but it contains sugar



Sugar
200 g/L

This we can use in fermentation processes!

”Sugar”
≈ 200 g/L

This we can't…

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

The fermentation process

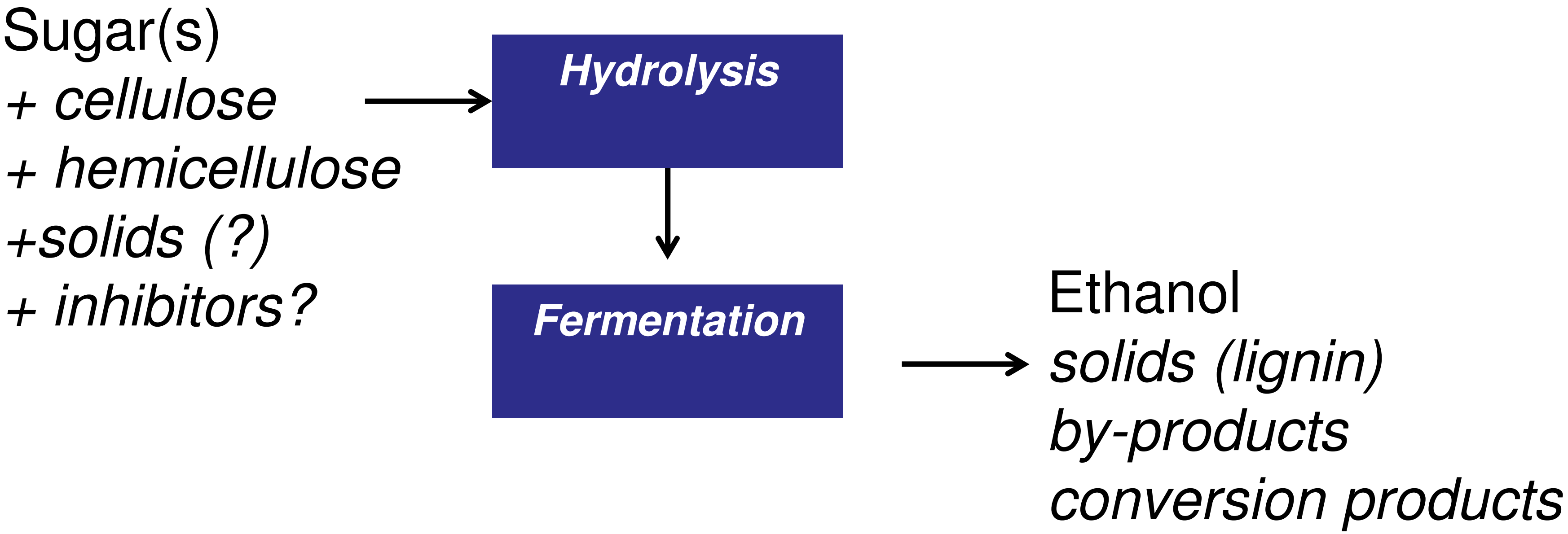
Sugar(s)
+solids
+ inhibitors

→ **Fermentation** →

Ethanol
solids (lignin)
by-products
conversion products from inhibitors

The fermentation process!

Maybe like this?



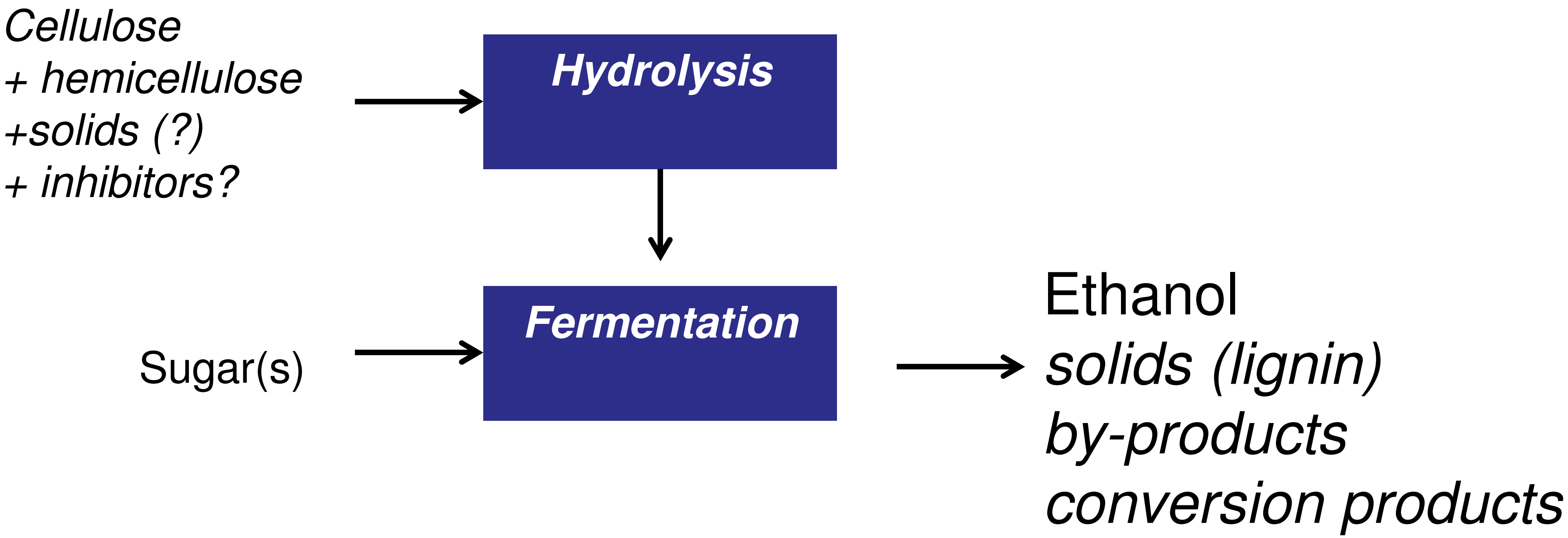
Sugar(s)
+ cellulose
+ hemicellulose
+solids (?)
+ inhibitors?

Hydrolysis

Fermentation

Ethanol
solids (lignin)
by-products
conversion products

Or this?



Cellulose
+ hemicellulose
+solids (?)
+ inhibitors?

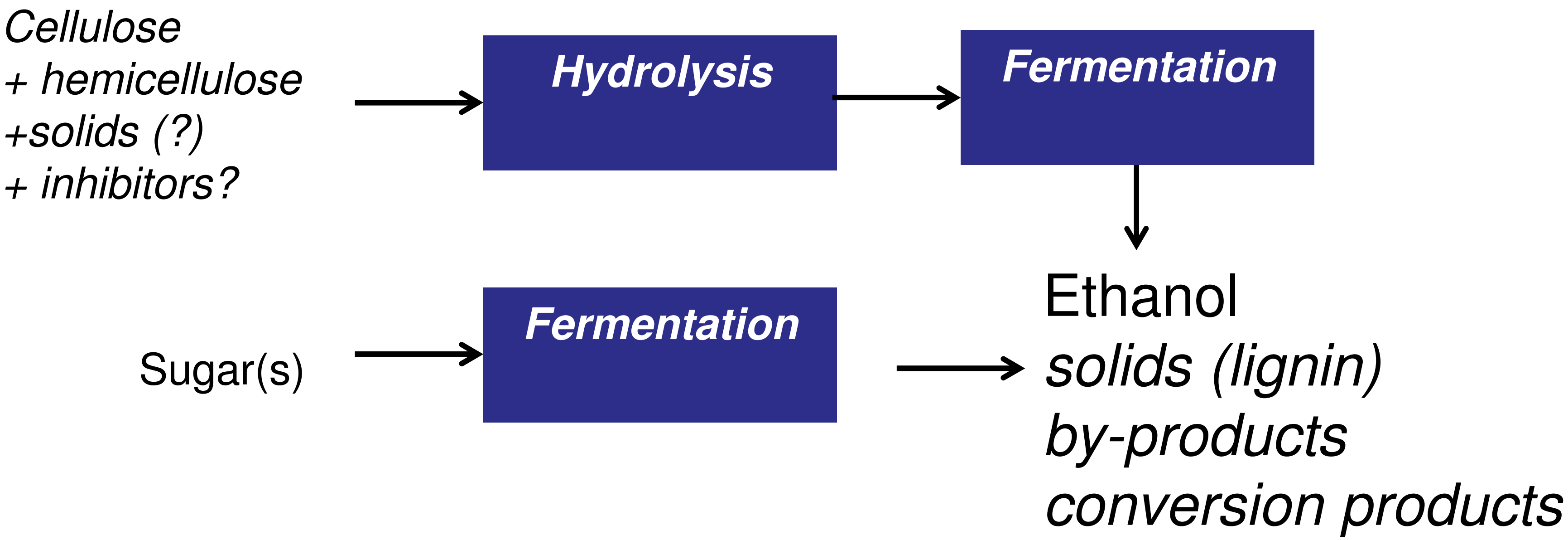
Hydrolysis

Sugar(s)

Fermentation

Ethanol
solids (lignin)
by-products
conversion products

Or this?



Cellulose
+ hemicellulose
+solids (?)
+ inhibitors?

Hydrolysis

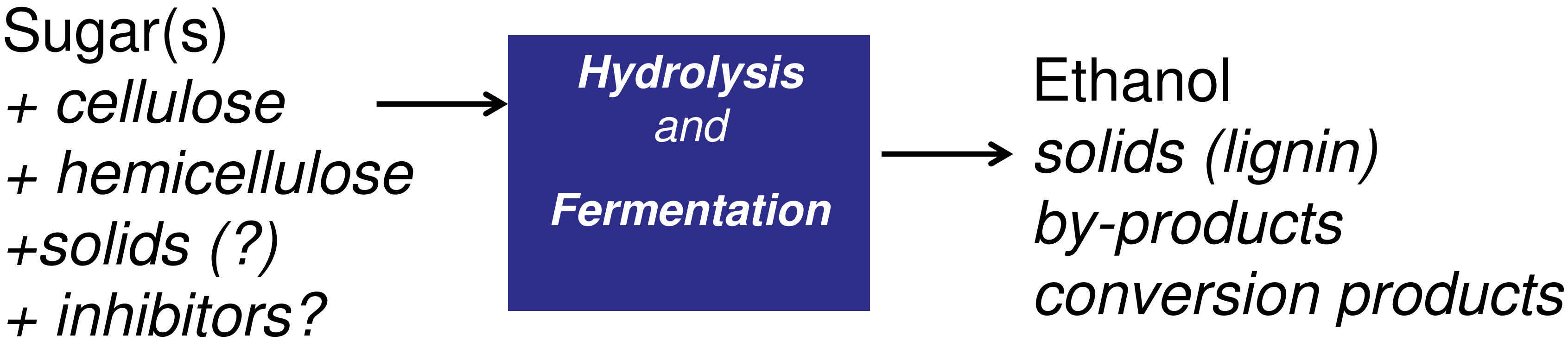
Fermentation

Sugar(s)

Fermentation

Ethanol
solids (lignin)
by-products
conversion products

Or this?



Sugar(s)
+ cellulose
+ hemicellulose
+solids (?)
+ inhibitors?

Hydrolysis
and
Fermentation

Ethanol
solids (lignin)
by-products
conversion products

THERE ARE OBVIOUSLY MANY OPTIONS IN THE PROCESS DESIGN!

Type	Plant	Glucan	Xylan	Arabinan	Mannan	Galactan	Acety	Lignin[a]	Extractives[b]	Ref.
Hardwood	Birch	38.2	18.5	NR	1.2	NR	NR	22.8	2.3	Hayn et al., 1993
	Willow	43	14.9	1.2	3.2	2.0	2.9	24.2	NR	Sassner et al., 2006
	Poplar	49.9	17.4	1.8	4.7	1.2	NR	18.1	NR	Wiselogel et al., 1996
	Red Maple	41.9	19.3	0.8	NR	NR	NR	24.9	NR	Jae et al., 2010
	Eucalyptus	42.9[c]	12.7[c]	2.3[c]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Vázquez et al., 2007
		46.1	17.1	0.8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Rencoret et al., 2010
	A[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Youngblood et al., 2010
Softwood	D[illegible]	[illegible]	[illegible]	[illegible]	13.0	2	NR	28	NR	Mabee et al., 2006
		[illegible]	[illegible]	[illegible]	12.7	4.3	NR	30.6	3.8	Johansson, 2010
	S[illegible]	[illegible]	[illegible]	[illegible]	12.0	1.8	NR	28.1	1.0	Tengborg et al., 1998
		[illegible]	[illegible]	[illegible]	14.3	NR	NR	27.1	3.8	Hayn et al., 1993
	Pine	46.4	8.8	2.4	11.7	NR	NR	29.4	NR	Wiselogel et al., 1996
		37.7	4.6	0	7.0	NR	NR	27.5	5.4	Hayn et al., 1993
		41.7	6.3	1.8	10.8	3.9	NR	26.9	NR	Youngblood et al., 2010
	Western Hemlock	41.4	3.3	1.0	12.0	1.8	NR	31.4	0.8	Johansson 2010
Crop residues	Wheat straw	38.2	21.2	2.5	[illegible]	0.7	NR	[illegible]	[illegible]	Wiselogel et al., 1996
		35.2	30.5	[illegible]	0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		36.5	18.4	2.2	0	NR	[illegible]	[illegible]	[illegible]	[illegible]
	Rice straw	34.2	24.5	NR	NR	NR	[illegible]	[illegible]	[illegible]	[illegible]
		38.9	[illegible]	3.4	[illegible]	0.5	[illegible]	[illegible]	[illegible]	[illegible]
	Corn stover	35.6	18.9	2.9	[illegible]	NR	NR	12.3	5.5	Hayn et al., 1993
		38.9	23.0	3.4	0.4	1.8	2.6	16.2	NR	Templeton et al., 2010
		36.4	18.0	3.0	0.6	1.0	NR	16.6	7.3	Wiselogel et al., 1996
	Corn cobs	37.0	[illegible]	2.2	NR	0.6	NR	13.9	NR	Wang et al., 2011
	Sugarcane Bagasse	39.0	22.1	2.1	0.4	0.5	NR	23.1	NR	DOE, USA
	Barley straw	38.1	[illegible]	3. 9	0	0	NR	20. 5	NR	Kim et al., 2011
	Cotton stalk	35.6	21.4	0	0	0	NR	27.8	NR	Akpinar et al., 2007
	Sweet Sorghum bagasse	41.3	18.0	1.94	0.85	1.26	NR	16.5	NR	Goshadrou et al., 2011
Dedicated crops	Switch grass	31.0	20.4	2.8	0.3	0.9	NR	17.6	17.0	Wiselogel et al., 1996
		34.2	22.8	3.1	0.3	1.4	NR	19.1	NR	DOE, USA
	Miscanthus	39.5[c]	19.0[c]	1.8[c]	NR	0.4	NR	24.1	4.2	Vrije et al., 2002
	Arundo donax L.	39.3	18.4	1.2	0.2	0.4	NR	26.2	NR	Bura et al., 2012
	Cassava pulp	19.1	4.2	1.4	0.7	0.5	NR	2.2	NR	Kosugi et al., 2009
	Bamboo	42.6	15.0	0	0	0	NR	26.2	NR	Sathitsuksanoh et al., 2010
		40.7	23.6	1.1	0.6	1.2	NR	27.1	NR	Tippayawong and Chanhom, 2012
	Hemp	37.4	21.1	2.9	NR	NR	2.9	18.0	NR	González-García et al., 2012
	Sorghum fiber	28.7	15.8	2.03	0.4	0.4	NR	NR	NR	Godin et al., 2011
2° & 3°	Newspaper	35.1[c]	5.0[c]	3.9[c]	10.7[c]	2.3[c]	NR	39.1[c]	NR	Foyle et al., 2007
	White office paper	65.4[c]	14.4[c]	0	0	0	NR	9.5[c]	NR	Foyle et al., 2007

Different distribution of carbohydrates

Here is some acid..

And here are lots of stuff..

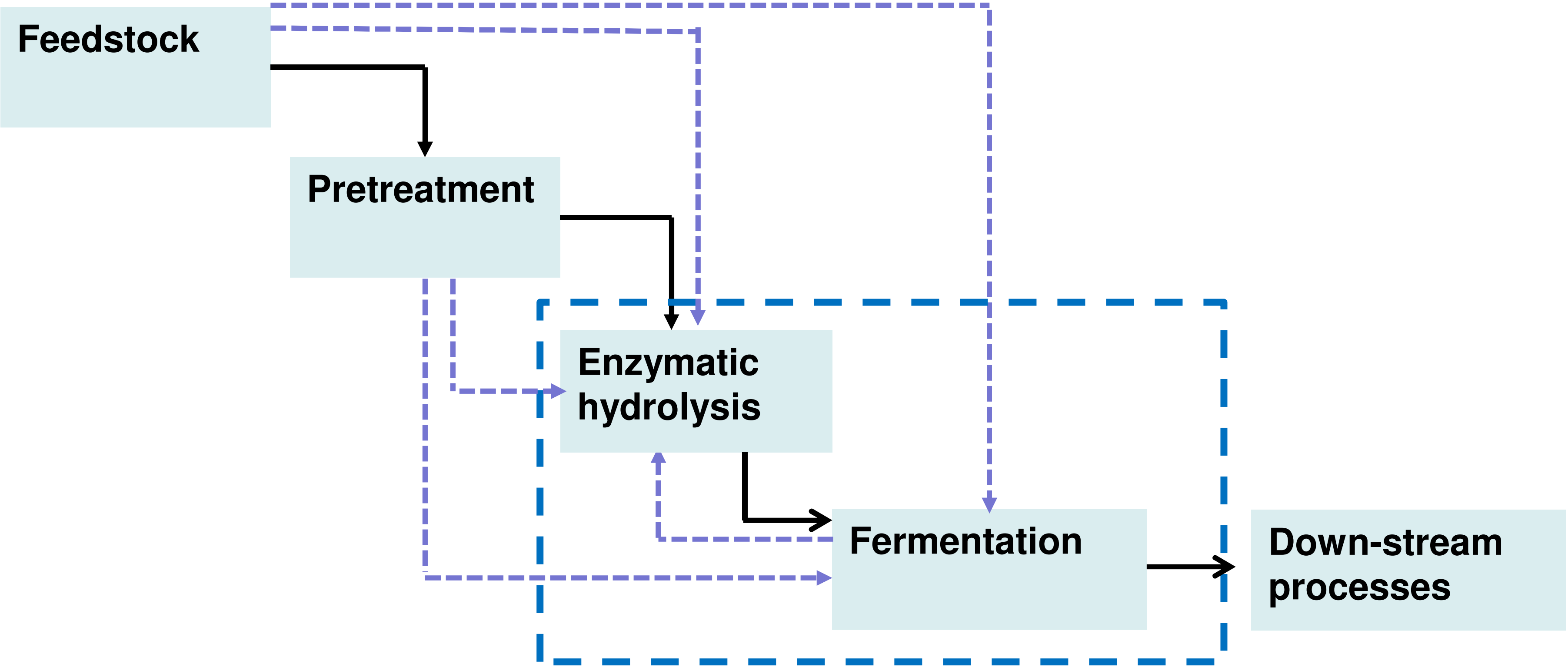
BIOMASS –

What biomass?

tion

ocks

Fermentation – a part in the integrated process



Feedstock

Pretreatment

Enzymatic hydrolysis

Fermentation

Down-stream processes

Fermentation – a part in the integrated process

The challenges in the fermentation are connected to all upstream steps:

- The *feedstock defines the sugars* to be converted, and also contains some components which may be inhibitory
- The *pretreatment (may) produce (or liberate) inhibitory* compounds to the fermentation and enzymatic hydrolysis.
- The hydrolysis - if simultaneous to the fermentation – affects desired process temperature.
- The *process design (feeding strategies) defines the relative ratios between sugars and also the level of inhibitors* in case these are converted in the process (in-situ detoxification)

Feedstock

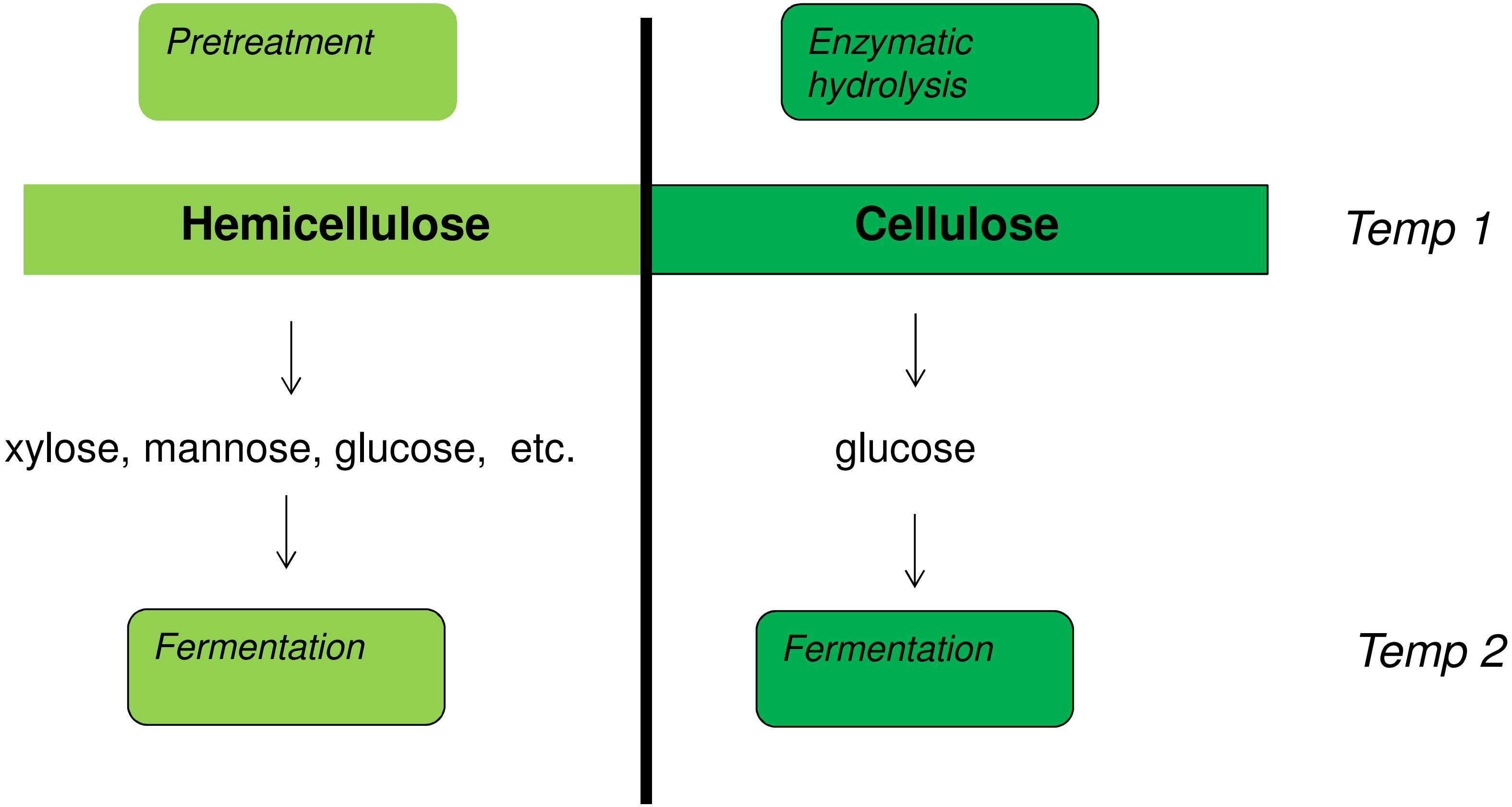
Arundo Donax

Component	Content (% on oven-dry matter)
Cellulose	33.8%
Hemicellulose	25.6%
Lignin	24.0%
Extractives	12.2%
Ash	5.0%

Shatalov AA and Pereira H, (2010). Xylose production from giant reed (Arundo donax L.): Modeling and optimization of dilute acid hydrolysis, Carbohydrate Polymers, doi:10.1016/j.carbpol.2011.07.041

Pretreatment

- The pretreatment defines the structure and chemical composition going into enzymatic hydrolysis.
- It also defines the relative ratios of oligosaccharides to monosaccharides in the liquid fraction
- and – to a large extent – the amount of inhibitors to be handled



Pretreatment

Enzymatic hydrolysis

Hemicellulose

Cellulose

Temp 1

↓

xylose, mannose, glucose, etc.

↓

Fermentation

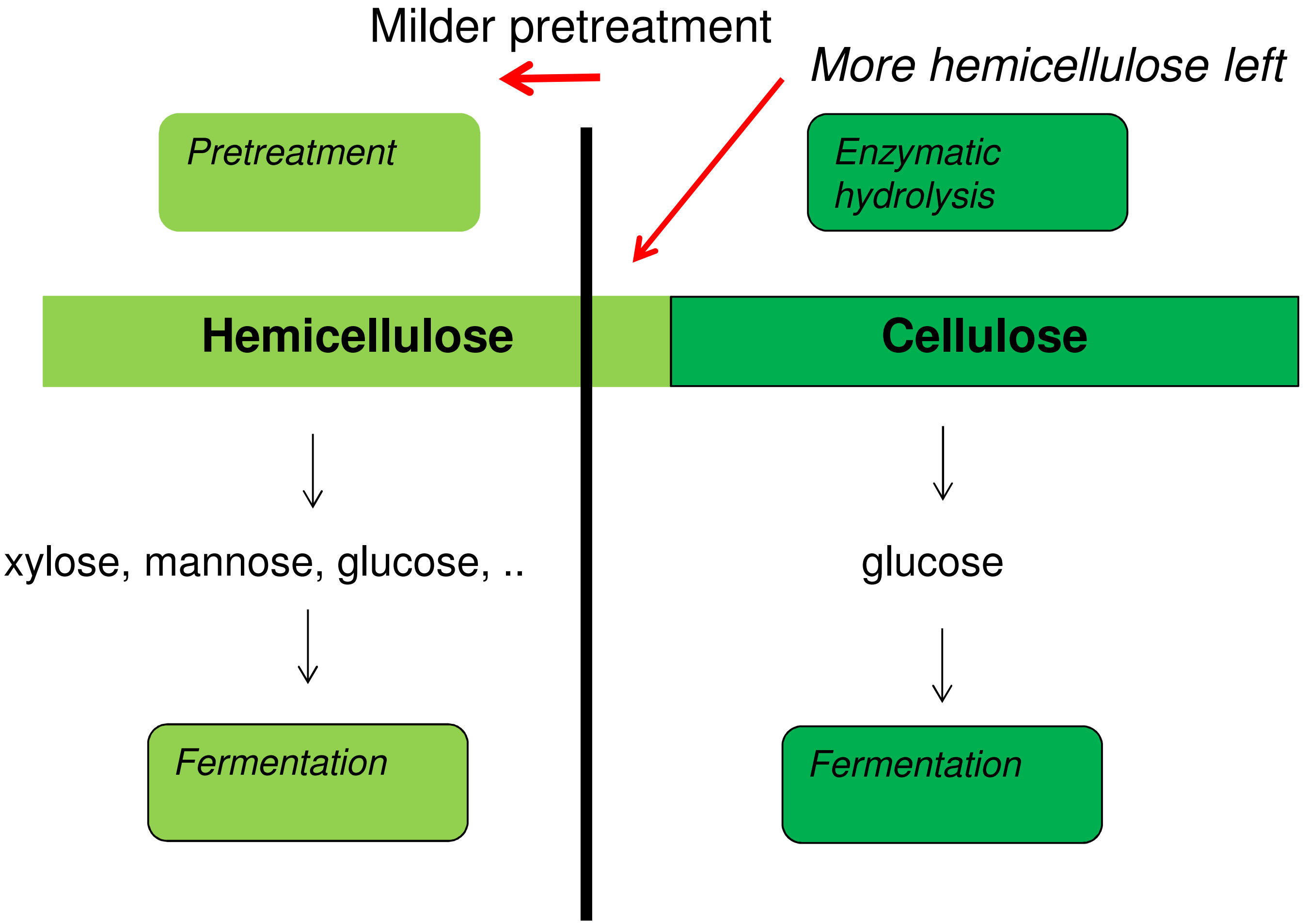
↓

glucose

↓

Fermentation

Temp 2



Milder pretreatment

More hemicellulose left

Pretreatment

Enzymatic hydrolysis

Hemicellulose

Cellulose

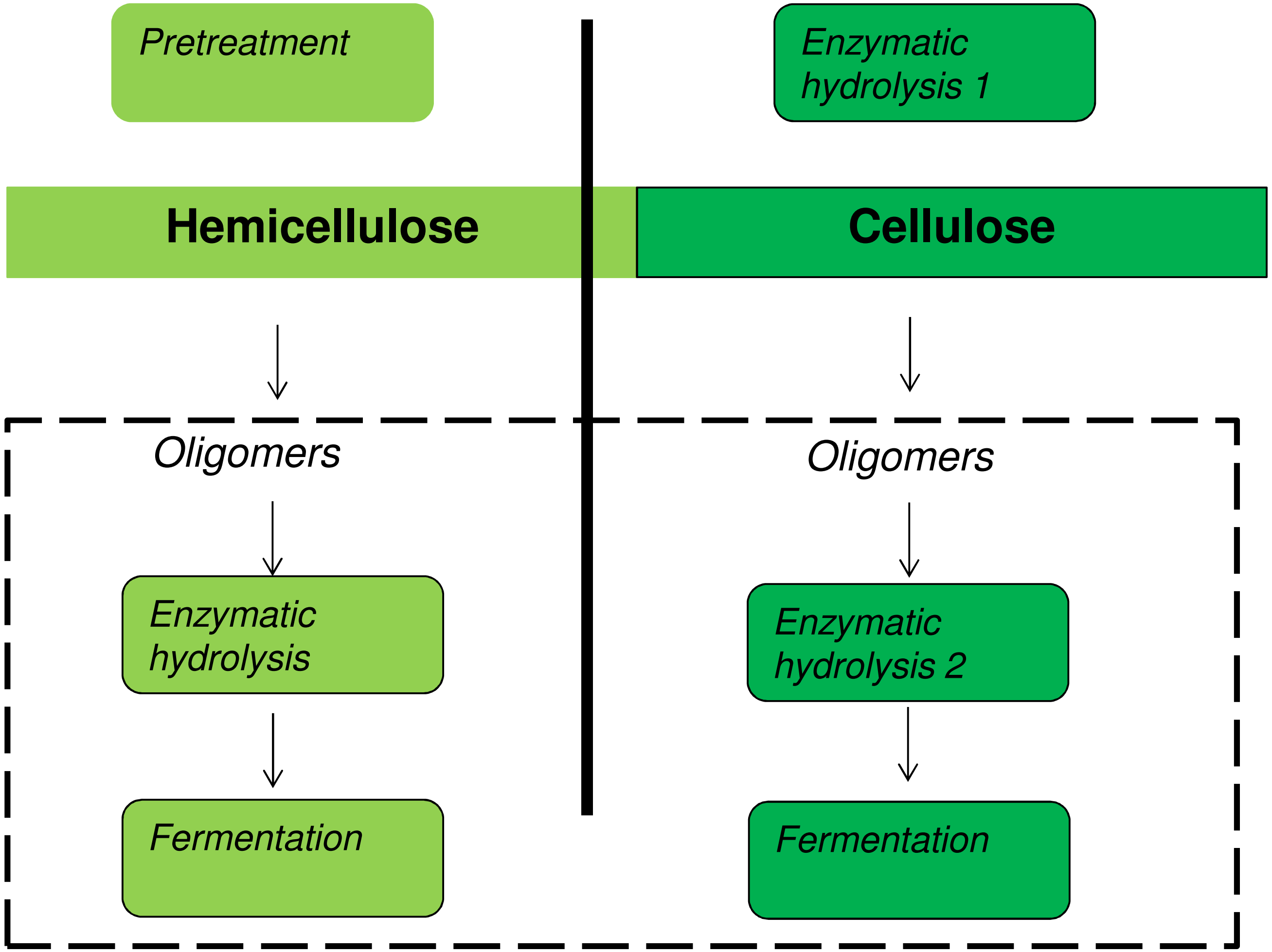
xylose, mannose, glucose, ..

glucose

Fermentation

Fermentation

Less acid conditions also leaves more oligosaccharides in the liquid phase.



Pretreatment

Enzymatic hydrolysis 1

Hemicellulose

Cellulose

Oligomers

Oligomers

Enzymatic hydrolysis

Enzymatic hydrolysis 2

Fermentation

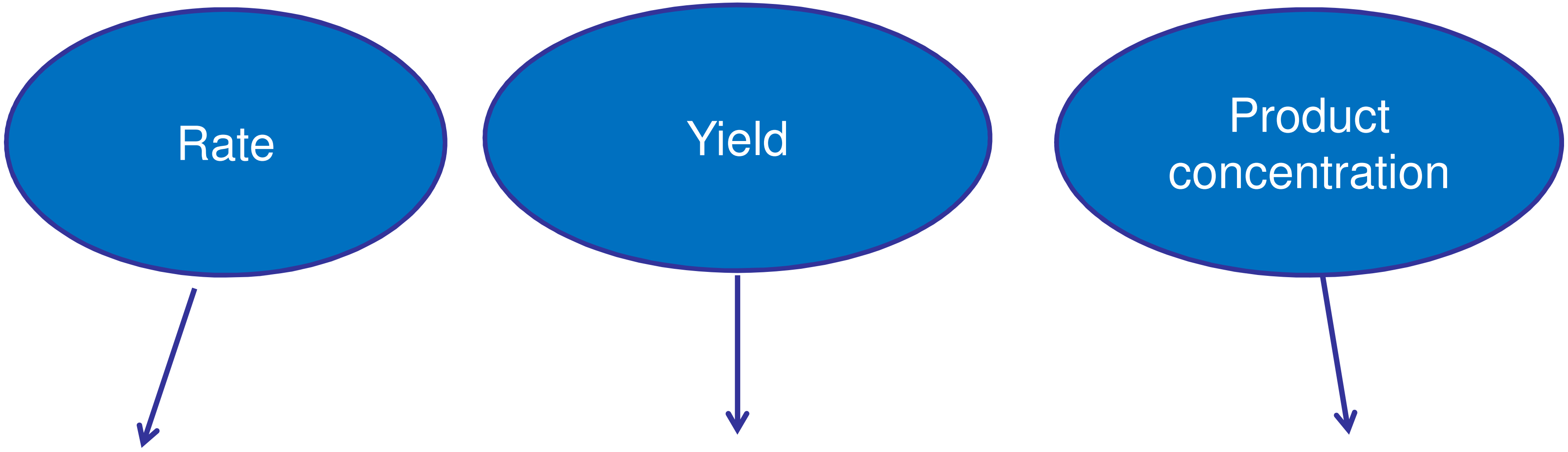
Fermentation

Fibre composition	
Glucan	48.2%
Xylan	3.8%
Lignin	41.7%
Furfural	0.2 g/L

Soluble components		
Sugars	Monomers	Total sugars
Glucose	2.5 g/L	14.2 g/L
Xylose	4.0 g/L	18.4 g/L
Acetic Acid	5.6 g/L	↑
HMF	n.d	
Furfural	0.2 g/L	

A lot of oligos

The eternal challenges..



Rate

Yield

Product concentration

Investment costs
"CAPEX"

Raw material costs
"OPEX"

Operating costs
(downstream)
"OPEX"

Rate

Yield

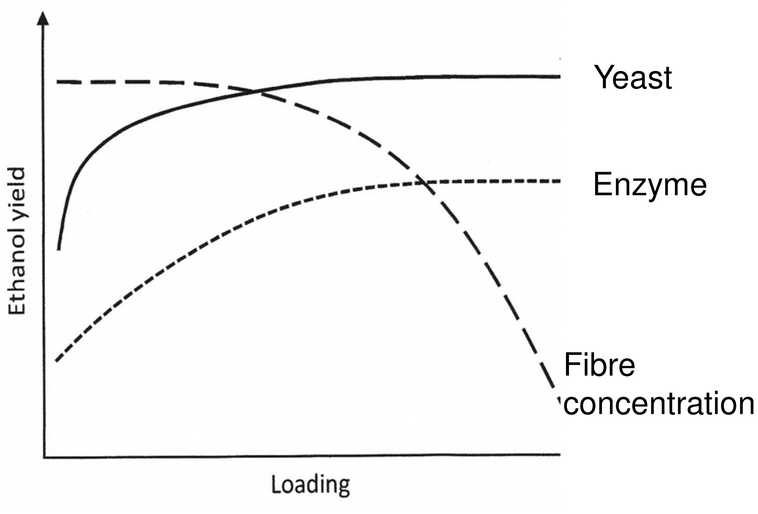
Product concentration

High solids handling in enzymatic hydrolysis

Inhibition in fermentation

Increased final ethanol titer → Higher fiber contents to be handled

Lower yield



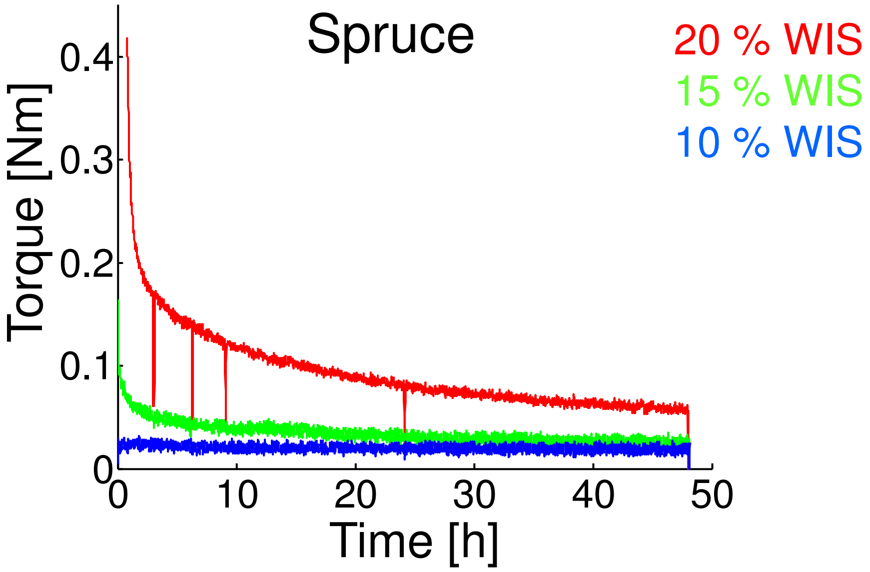
Inhibitor problems

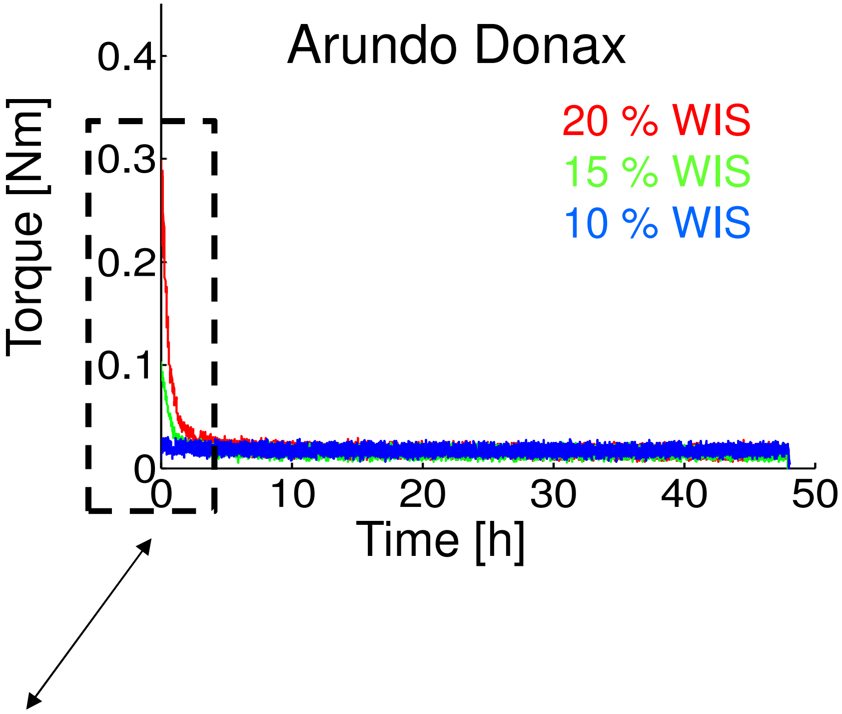
- *Effects yeast metabolism*
- *Effects on enzymatic hydrolysis*

Mixing issues

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

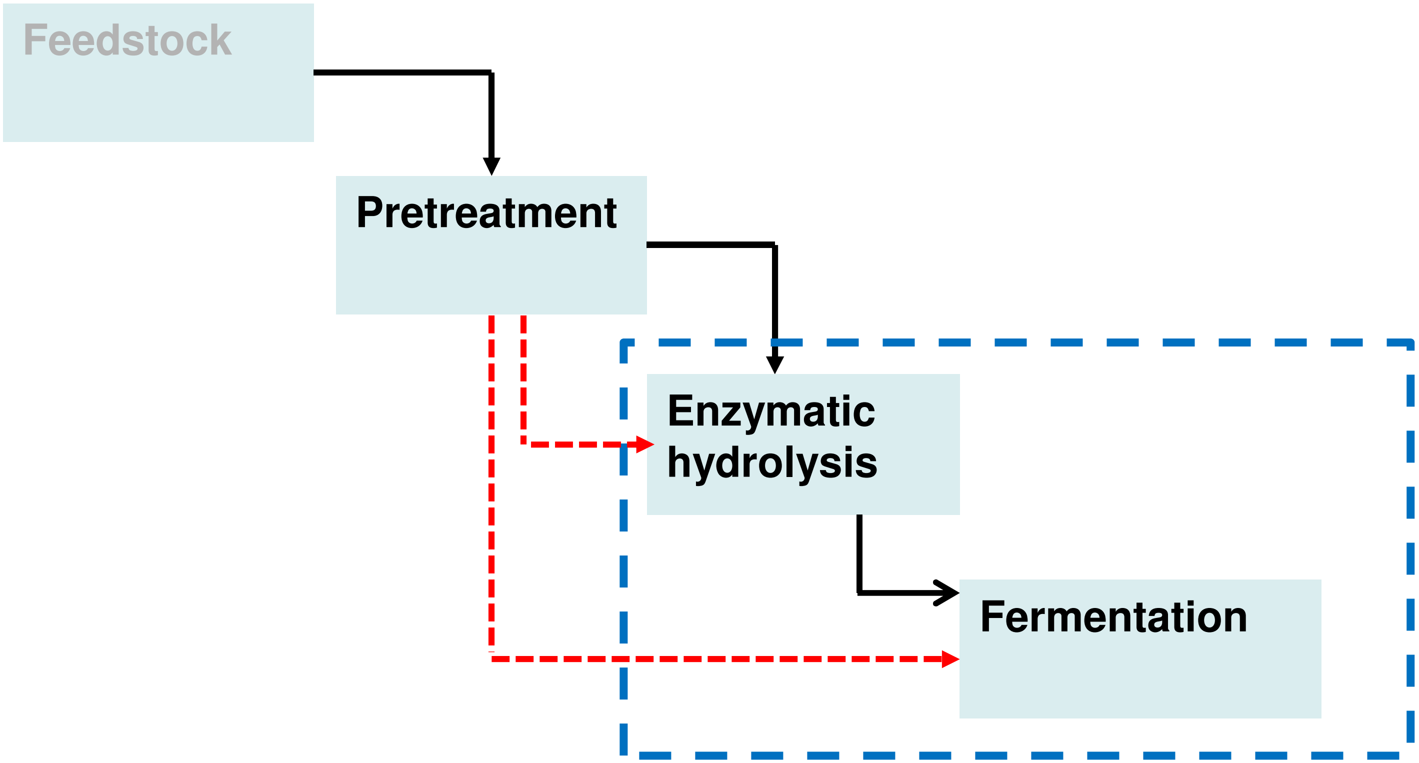
Viscosity is a significant factor in the processing





Very rapid loss of viscosity in the Arundo case!
→Low mixing energy requirements in hydrolysis

What is the impact of pretreatment on SSF?

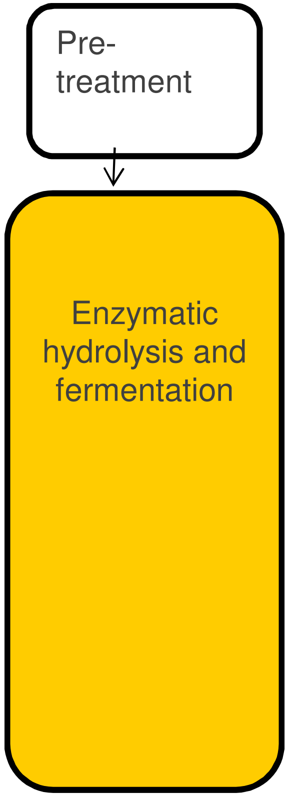


Feedstock

Pretreatment

Enzymatic hydrolysis

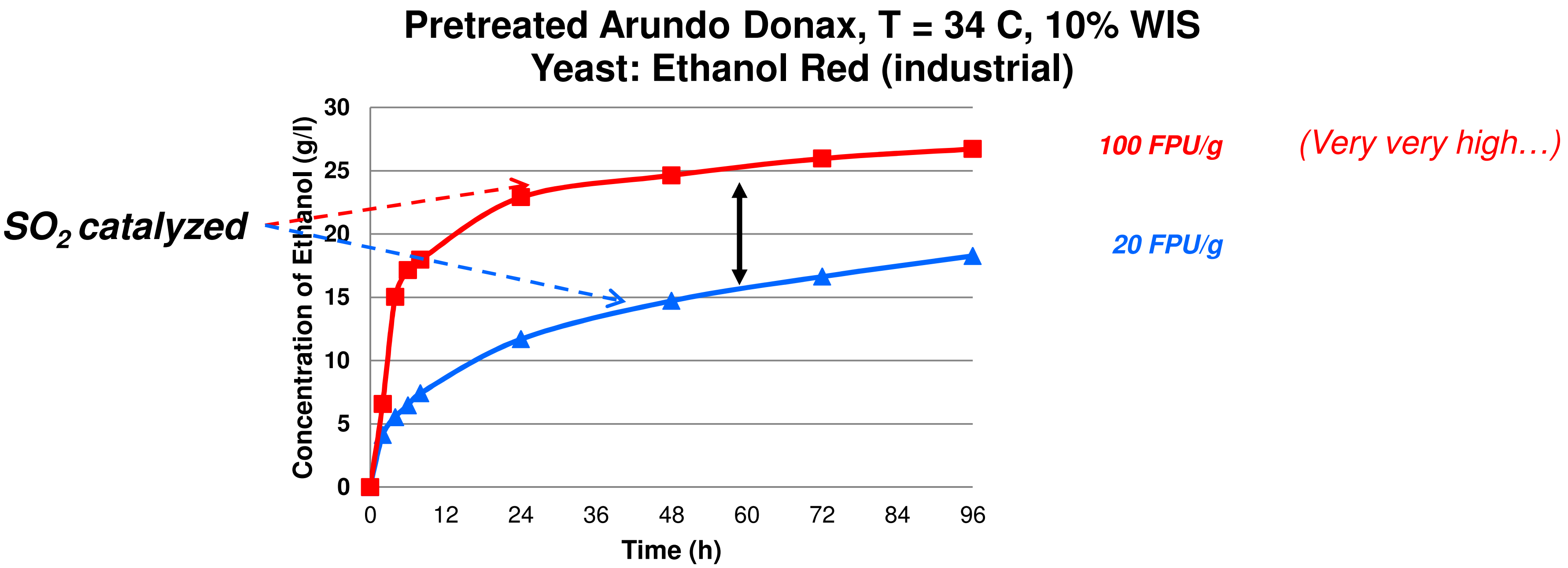
Fermentation



Pre-treatment

Enzymatic hydrolysis and fermentation

SSF



Pretreated Arundo Donax, T = 34 C, 10% WIS
Yeast: Ethanol Red (industrial)

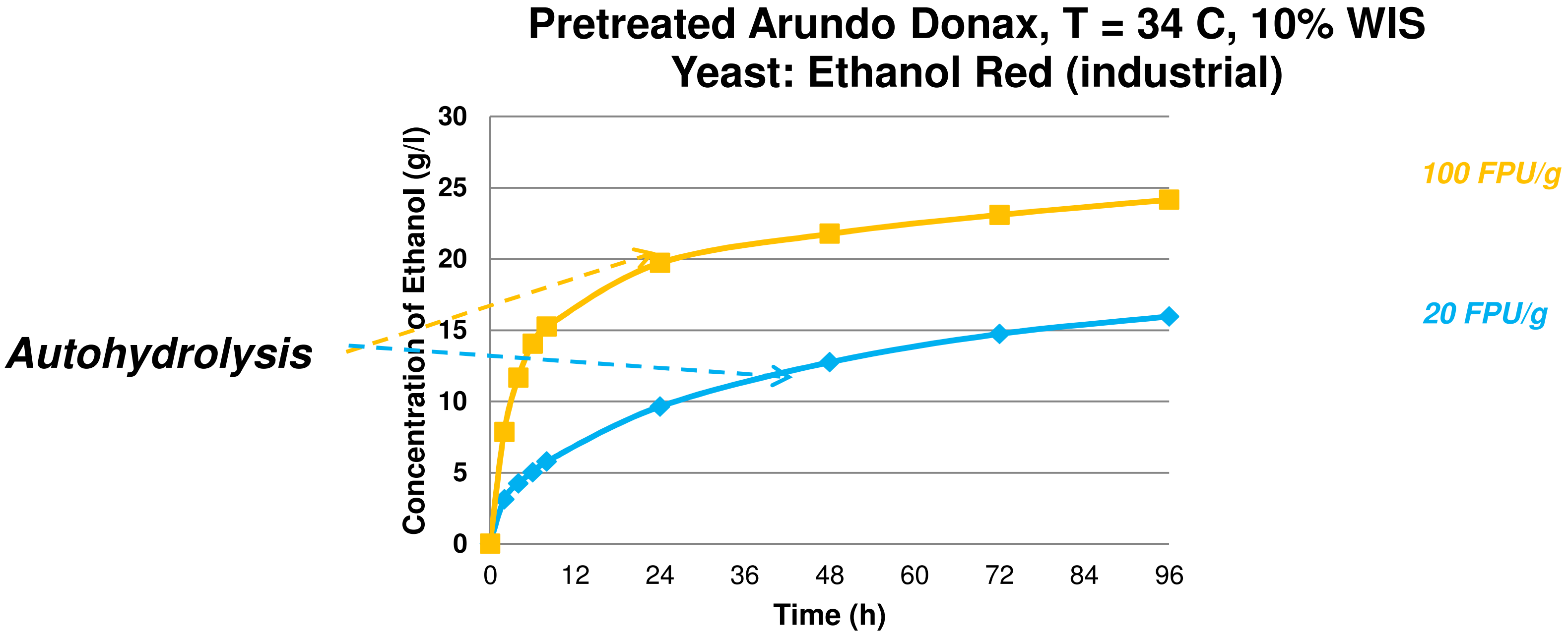
SO_2 catalyzed

100 FPU/g *(Very very high…)*

20 FPU/g

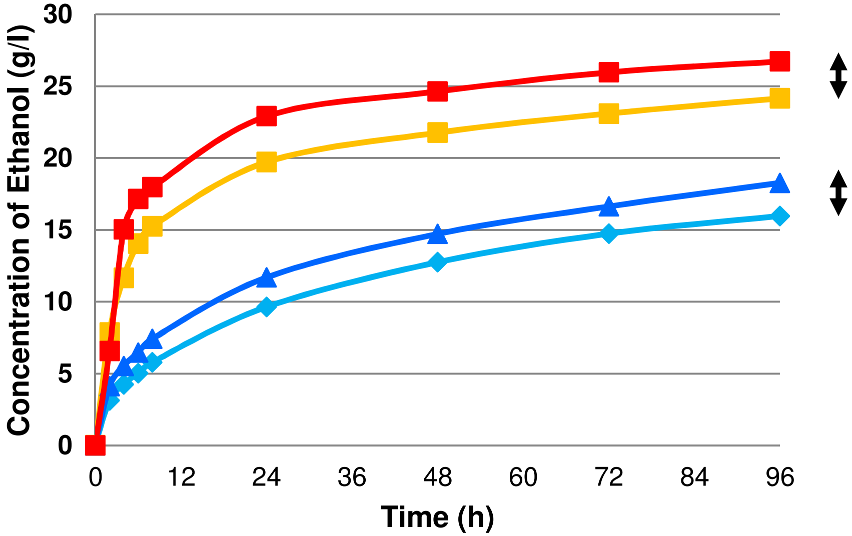
Bhargav Prasad Kodaganti, M. Sc. Thesis, Lund Univ. 2011

SSF



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

SSF



Difference due to pretreatment

Pretreatment without acid catalysts leaves a material which is more difficult to enzymatically hydrolyse

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

Rate

Yield

Product concentration

Recalcitrance in enzymatic hydrolysis

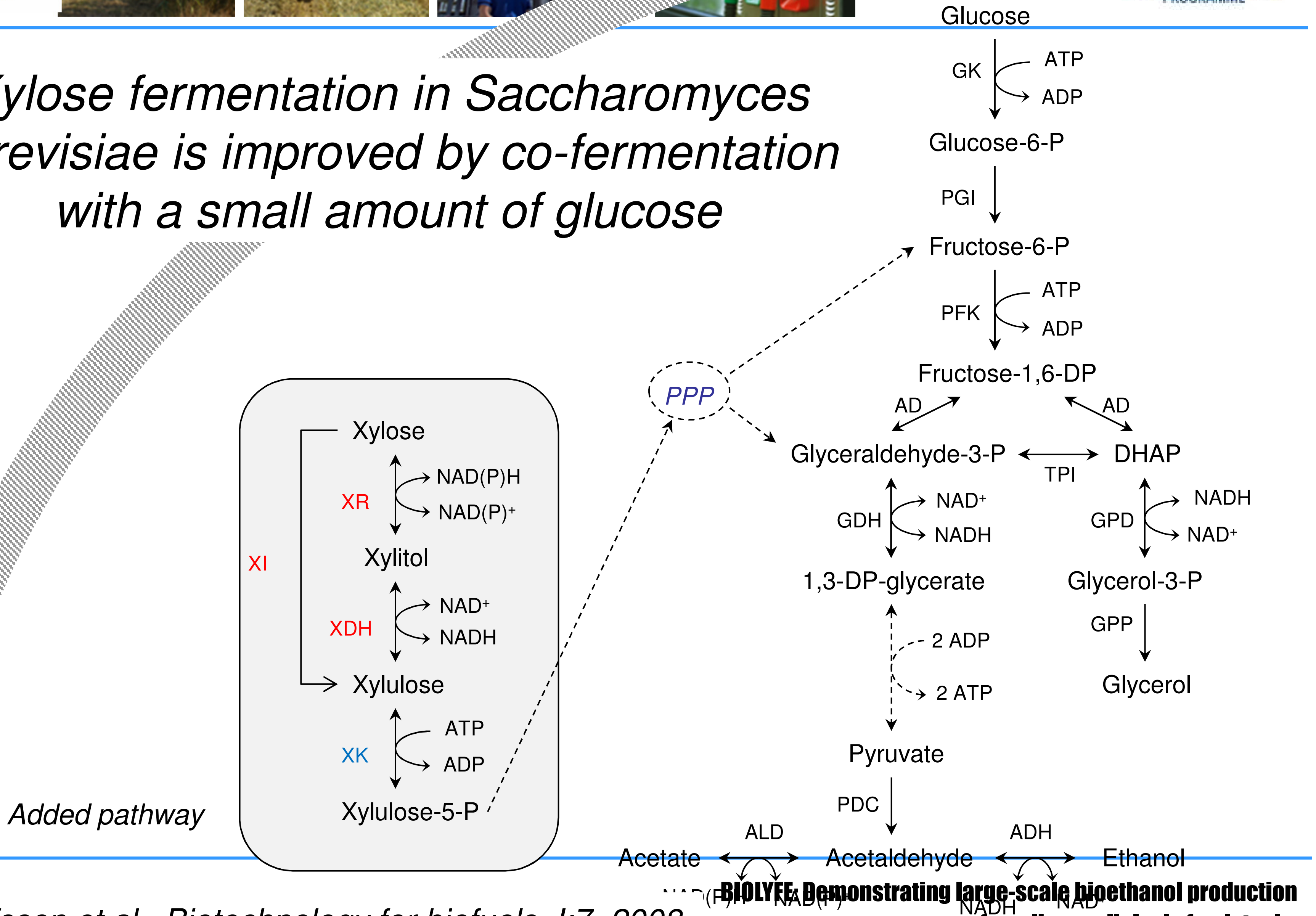
Conversion of xylose/by-products in fermentation

Recalcitrance in enzymatic hydrolysis – Improved enzymes

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.

Xylose fermentation in Saccharomyces cerevisiae is improved by co-fermentation with a small amount of glucose



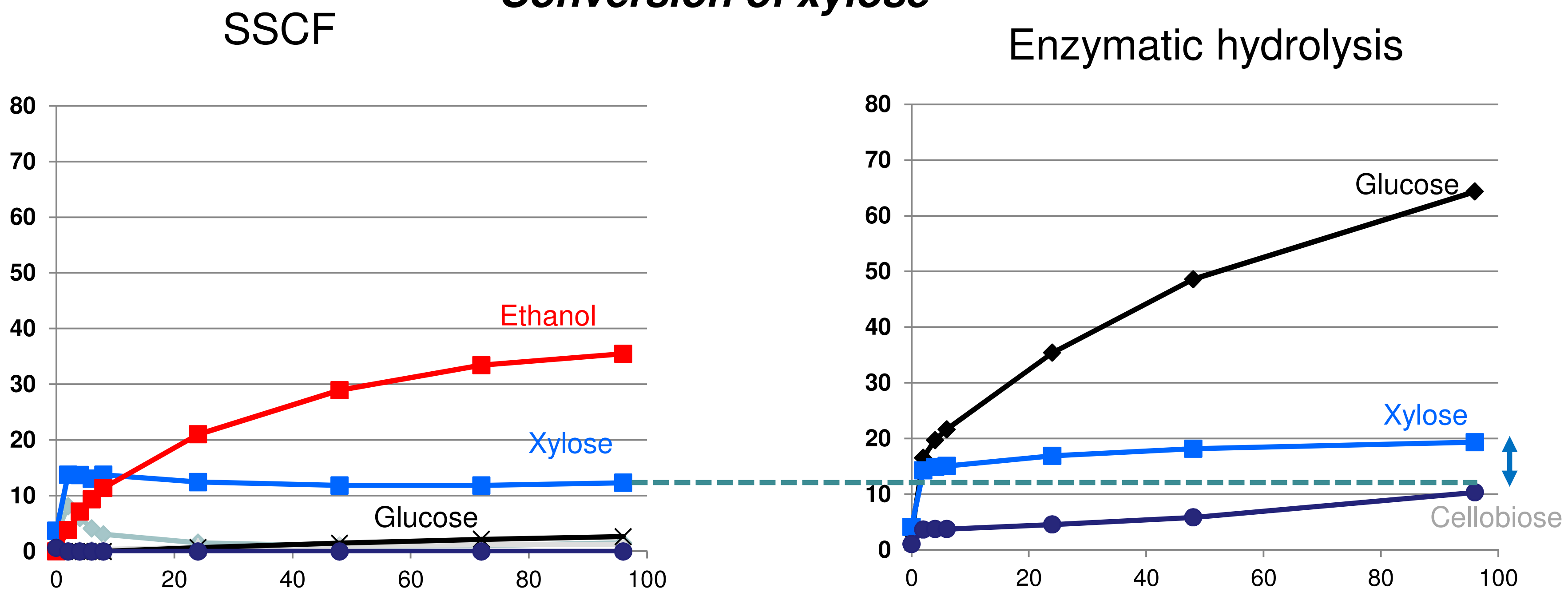
Glucose
GK
ATP
ADP
Glucose-6-P
PGI
Fructose-6-P
PFK
ATP
ADP
Fructose-1,6-DP
AD
AD
PPP
Glyceraldehyde-3-P
TPI
DHAP
GDH
NAD+
NADH
GPD
NADH
NAD+
1,3-DP-glycerate
Glycerol-3-P
2 ADP
2 ATP
GPP
Glycerol
Pyruvate
PDC
ALD
ADH
Acetate
Acetaldehyde
Ethanol
NAD(P)H
NAD(P)+
NADH
NAD+

Xylose
XR
NAD(P)H
NAD(P)+
XI
Xylitol
XDH
NAD+
NADH
Xylulose
XK
ATP
ADP
Xylulose-5-P

Added pathway

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

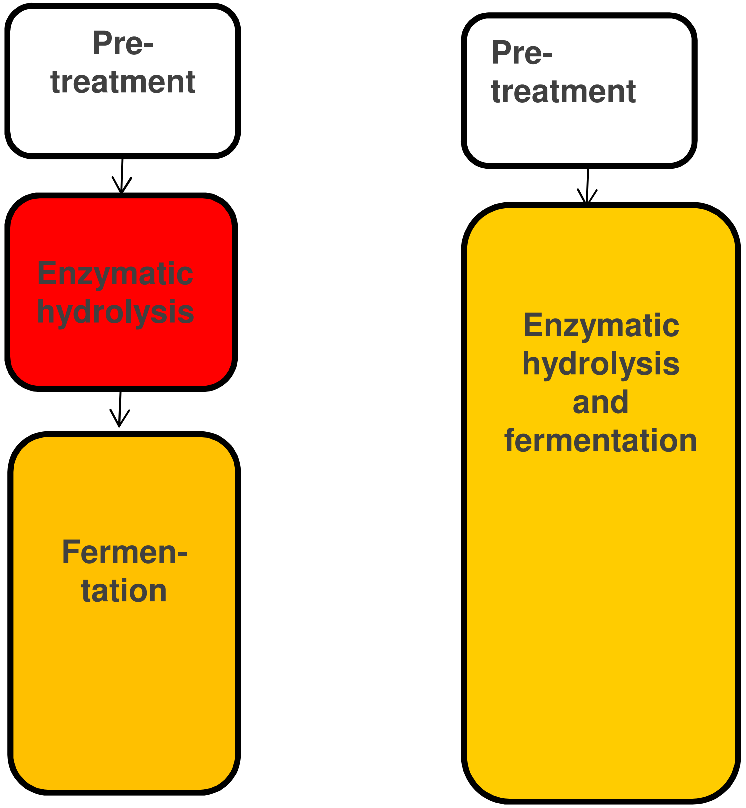
Conversion of xylose



34˚C, pH 5.0, WIS content 22 %
Xylose fermenting yeast TMB3400
CTec3 (0.075 g enzyme solution/g glucan)

34˚C, pH 5.0, WIS content 22 %
CTec3 (0.075 g enzyme solution/g glucan)

The basic process layouts



Temperature 180-210ºC

Pre-treatment

Temperature 45 -50ºC

Enzymatic hydrolysis

Temperature 30 -32ºC

Fermen-tation

SHF

Pre-treatment

Temperature 180-210ºC

Enzymatic hydrolysis and fermentation

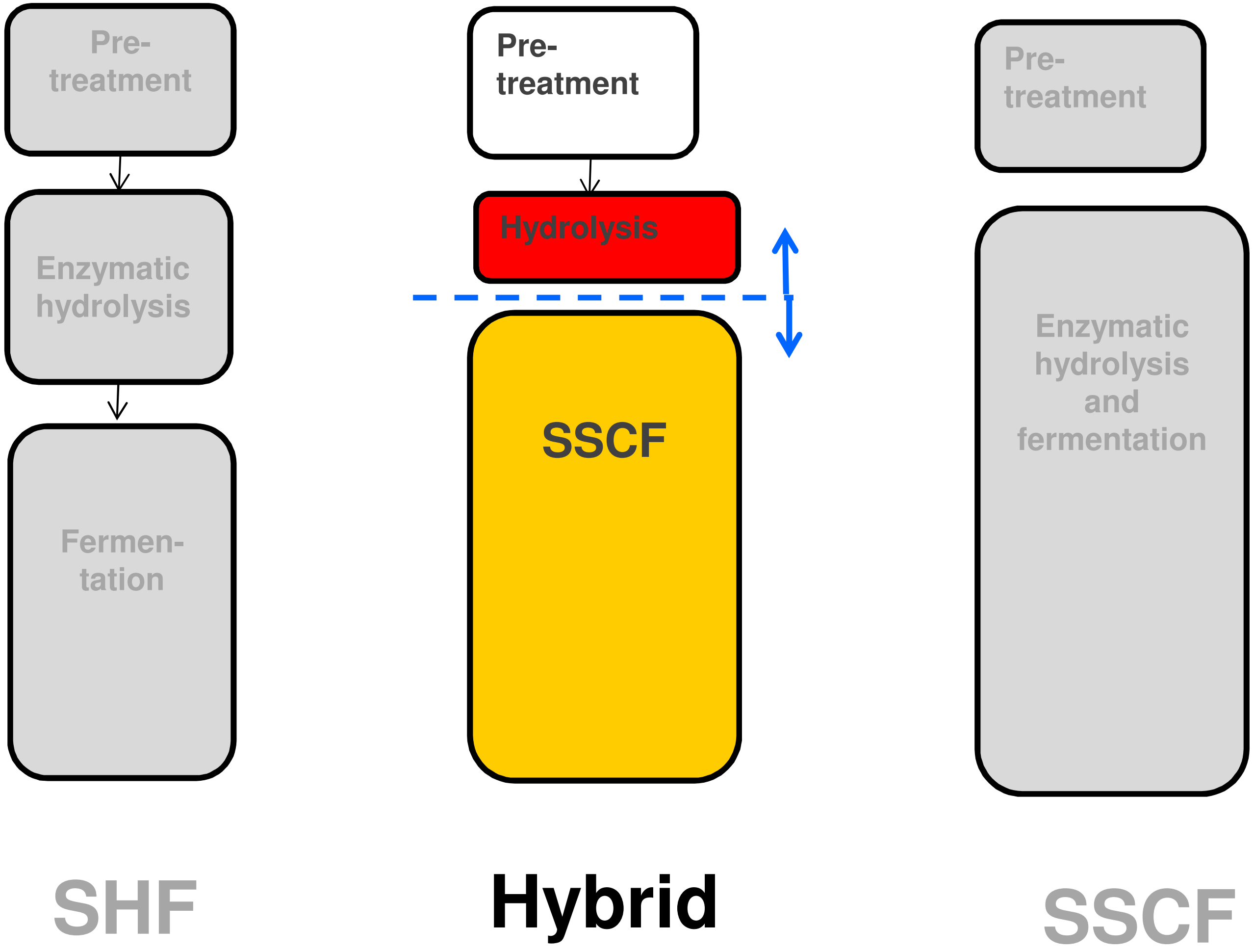
Temperature 32 -37ºC

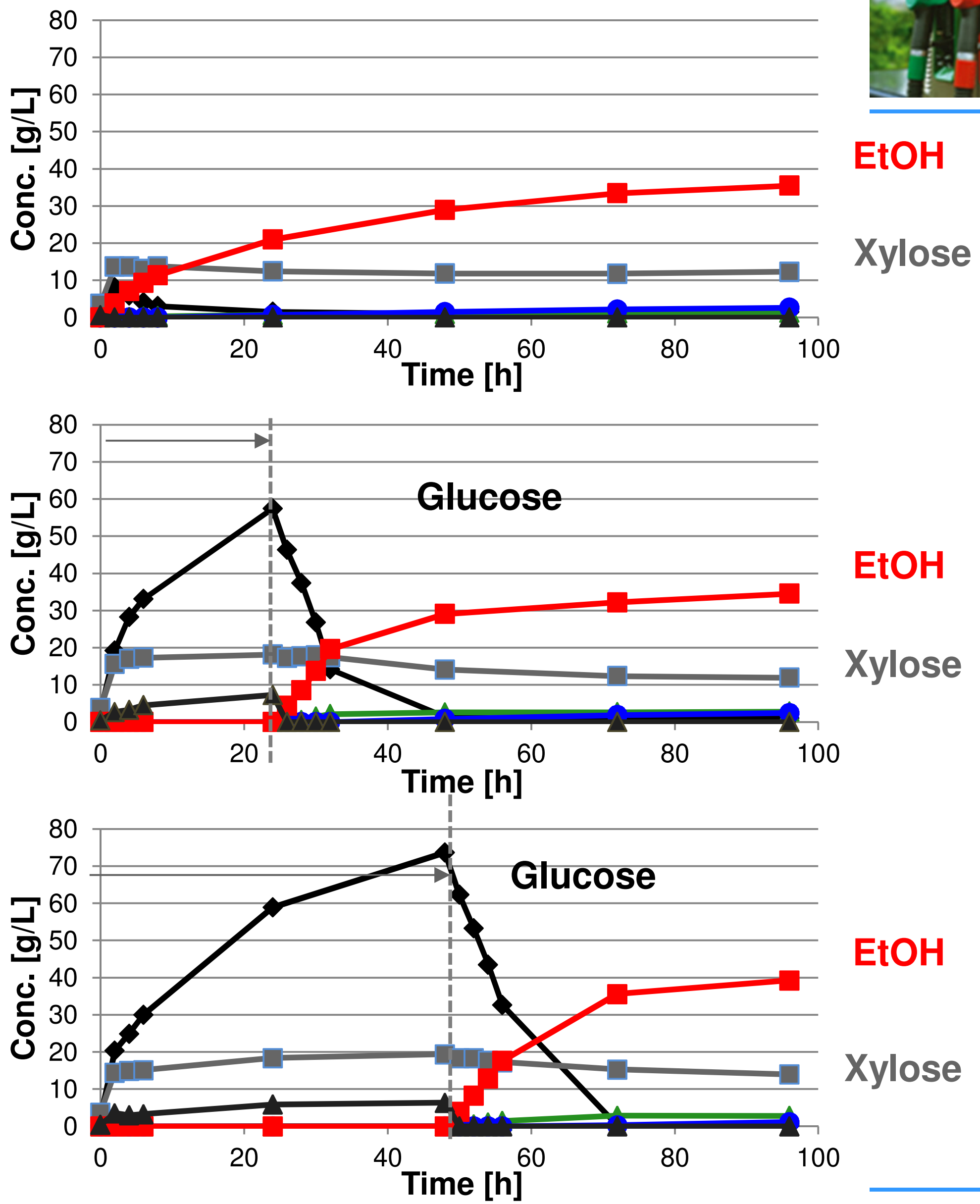
SSCF

But the enzymatic hydrolysis is favored in SHF (high T)

Xylose fermentation is favored in SSF (co-utilization)

So maybe a hybrid in between these is a good idea





pH 5, 22% WIS

Increased time for EH increases overall ethanol yielc

Xylose conversion relatively low (about 40%)

Palmqvist and Liden, submitte

Rate Yield Product concentration

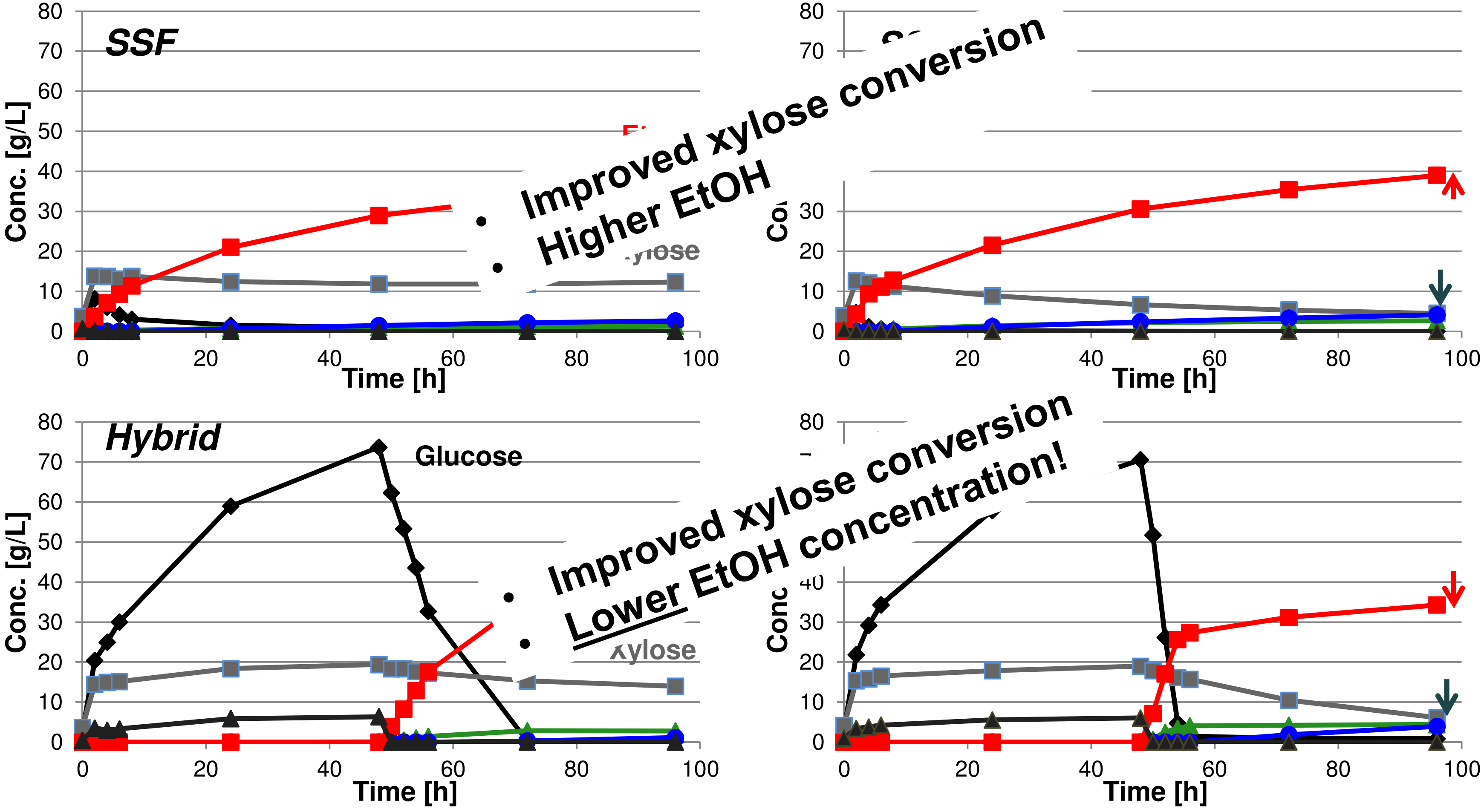
Recalcitrance in enzymatic hydrolysis

Inhibition in fermentation

Soluble components		
Sugars	Monomers	Total sugars
Glucose	2.5 g/L	14.2 g/L
Xylose	4.0 g/L	18.4 g/L
Acetic Acid	**5.6 g/L**	
HMF	n.d	
Furfural	0.2 g/L	

Acetic acid inhibition is pH-dependent.
Xylose fermentation is particularly inhibited by acetic acid

$HAc \leftrightarrow H^+ + Ac^-$



- Improved xylose conversion
- Higher EtOH

- Improved xylose conversion
- Lower EtOH concentration!

pH 5

pH 5.5

- The reason behind these, at first surprising results, lies in the fact that a higher pH favors growth and glycerol production.
- However, the effect is different under the carbon starved conditions in the SSF in comparison to the hybrid process.

Conclusion

- In the fermentation everything matters! – feedstock, pretreatment, process design and process conditions
- With a judicious choice of process design and conditions, significant improvements – in both hydrolysis yields and fermentation yields - can be made on top of the improvements in enzyme performance or strain performance

Partners WP3

LUND UNIVERSITY

biochemtex

ENEA

novozymes
Rethink Tomorrow

TAURUS ENERGY
Provider of yeast

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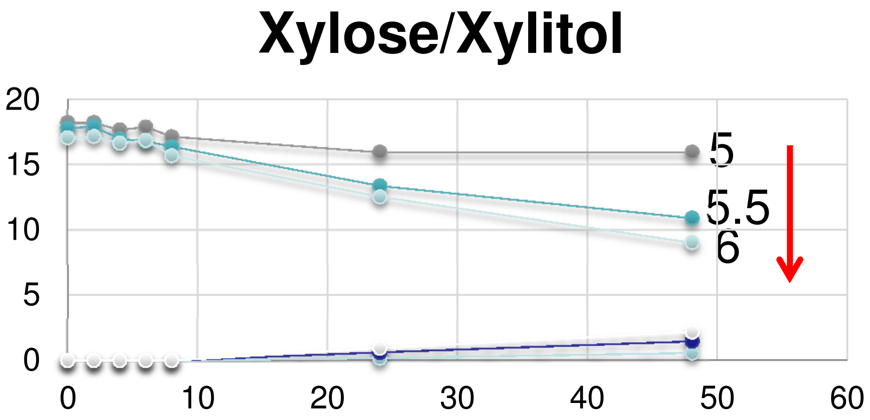
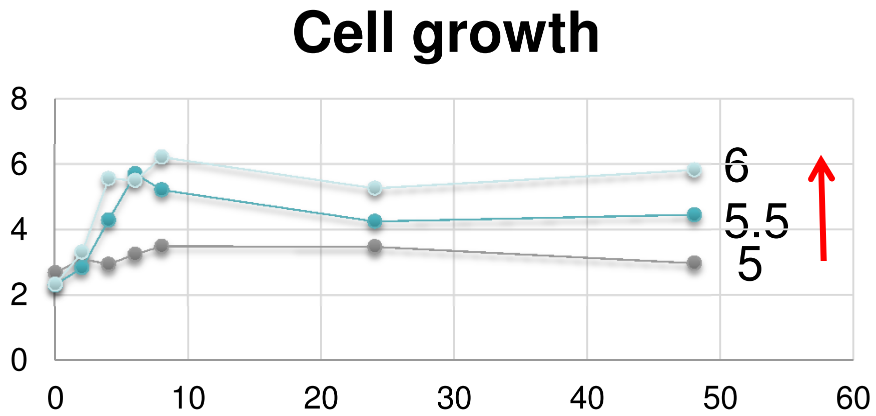


Xylose/Xylitol

Improved xylose conversion



Cell growth

Improved growth

Synthetic model medium with 8 g/L HAc