


Hydrolysis of biomass: effect of different pretreatments and process strategies

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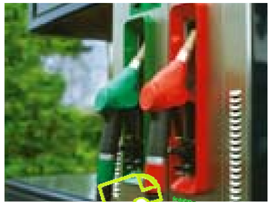
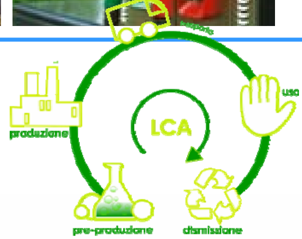
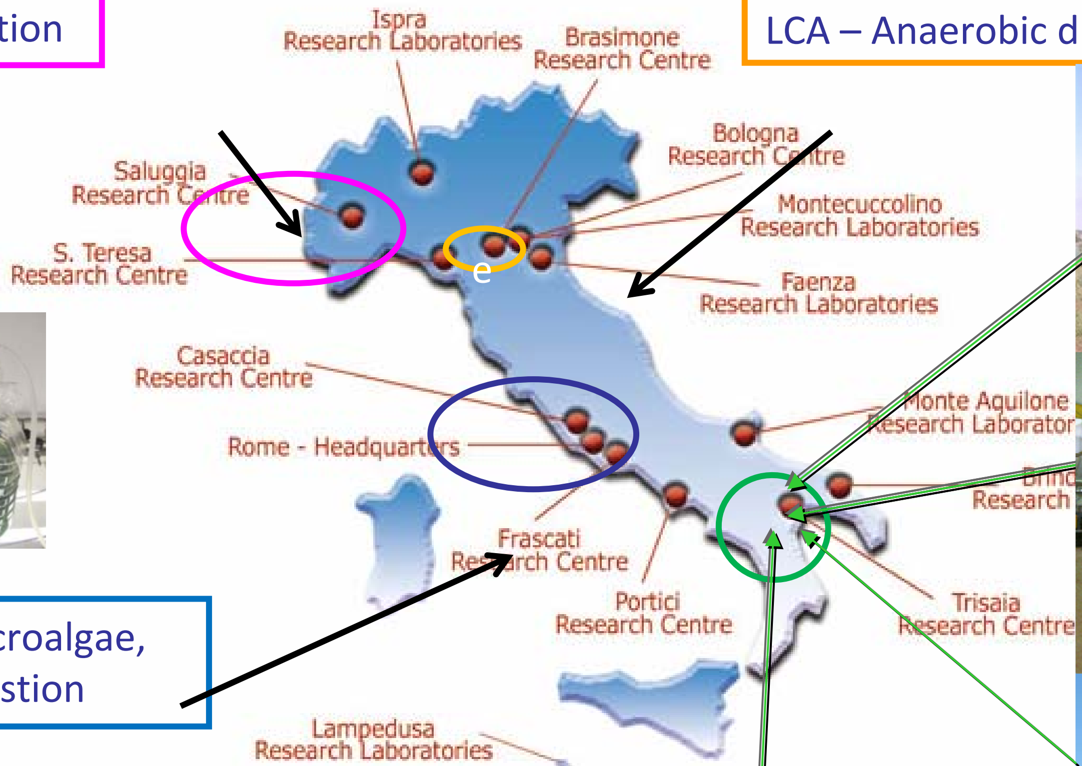


Biomass combustion

LCA – Anaerobic digestion





Biodiesel from microalgae, anaerobic digestion

ENEA TRISIAIA (involved in the BIOLYFE project)
Energy crops, thermochemical processes, biomass pretreatment and fractionation, biotechnological processes

ENEA’s role in the project

PARTNE	WP1	WP2	WP3	WP4	WP5	WP6	WP7	WP8	WP9	WP10	WP11	WP12	WP13	WP14
	SELECTION, SUPPLY AND PRETREATMENT OF FEEDSTOCK	SELECTION ENZYMES COCKTAILS	FERMENTATION TECHNOLOGY	BASIC DESIGN OF HIGH SOLID CONCENTRATION ENZYMATIC HYDROLYSIS REACTOR	BASIC DESIGN OF SSF REACTOR	PRELIMINARY DESIGN OF COMPLETE INDUSTRIAL DEMO PLANT	DETAIL DESIGN OF INDUSTRIAL DEMO PLANT	PURCHASE OF EQUIPMENT AND MATERIAL, PLANT CONSTRUCTION AND COMMISSIONING	PLANT TESTING, MONITORING, ENZYME COCKTAIL AND MICROORGANISM SUPPLY	PRODUCT UTILISATION	CREATION OF FUNCTIONING INFRASTRUCTURE	INTEGRATED ASSESSMENT OF SUSTAINABILITY	DISSEMINATION AND EXPLOITATION	COORDINATION AND IPR MANAGEMENT
ENEA	**S**	**S**	**S**	**S**	**S**	[illegible]	[illegible]		**S**			**S**	**S**	**S**




OUTLINE

- Effect of the pretreatment on the biomass hydrolizability
- High gravity hydrolysis of biomass
    - test of various enzymatic mixtures (*CTEC, CTEC2, NS22140, CTEC3*)
    - Effect of the process strategy
- Hybrid simultaneous saccharification and fermentation
- Conclusions




Raw Material

Biomass component	%
Glucan	34.75
Xylan	20.10
Galactan	0.27
Arabinan	2.12
Acetyl group	3.50
Lignin Klason	22.0
Ash	7.70
EtOH Extractives	10.22

Arundo donax contains significant percentages of C5 sugars. This implies the importance of selecting pretreatment conditions that minimizes the degradatior of pentoses




Biomass pretreatment

ENEA' CONTINUOS PLANT (300 kg/hr)

ENEA' BATCH PLANT

HIGH BIOMASS DESTRUCTURATION + HIGH C5 RECOVERY

1. mild thermal conditions along with small amounts of acid catalysts (i.e. SO_2, H_2SO_4)
2. Chemtex process , two steps process: hemicellulose is separated before steam explosion




Composition of the slurry after acid catalyzed steam pretreatment [200°C, 5 min]

Before

After

After the pretreatment , the solids were separated from the liquid and then recombined up to the desired DM level

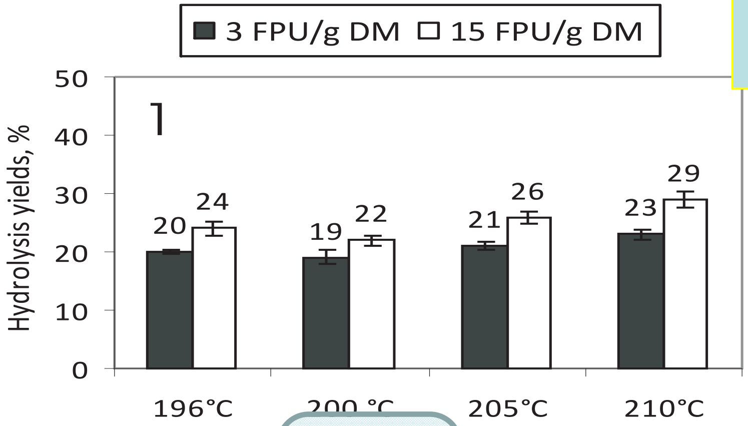
glucose monomers%	0.29±0.03
xylose monomers%	1.1 ± 0.1
glucose soluble olygomers%	0.07 ± 0.01
xylose soluble olygomers%	0.44 ± 0.03
insoluble glucose%	5.73 ± 0.38
insoluble xylose%	0.66 ± 0.05
acetic acid%	0.16 ± 0.01
5HMF%	0.05 ± 0.01
furfural%	0.09 ± 0.01
lignin%	3.85 ± 0.2

C5 RECOVERY: 71 %

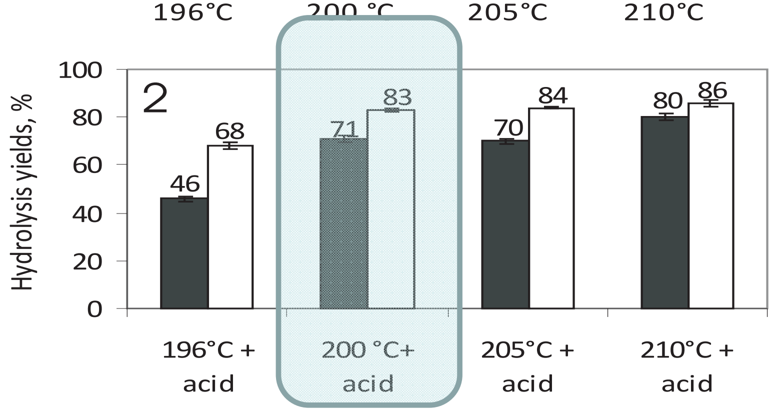



S/L 2%; pH 4.8; T 40°C,
Enzyme used: CTEC

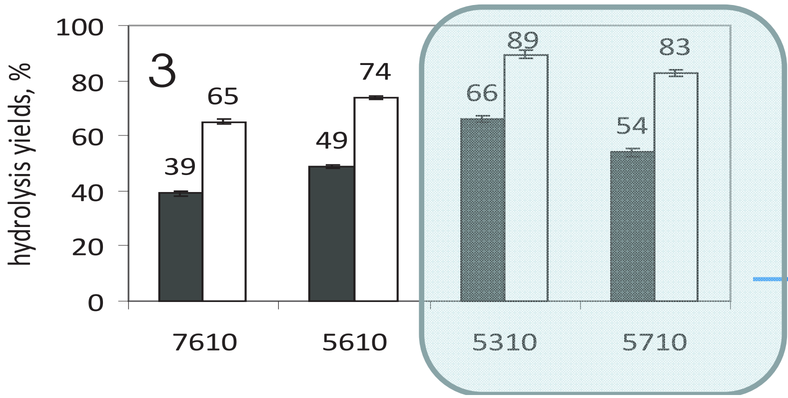
Improving the pretreatment



STEAM EXPLOSION IN BATCH DIGESTOR



ACID CATALIZED STEAM EXPLOSION IN BATCH DIGESTOR



HYDROLIZABILITY OF THE ARUNDO DONAX FIBER FROM THE TWO STEPS CHEMTEX PRETREATMENT

OUTLINE

- Effect of the pretreatment on the biomass hydrolizability
- High gravity hydrolysis of biomass
    - test of various enzymatic mixtures (*CTEC, CTEC2, NS22140, CTEC3*)
    - Effect of the process strategy
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High gravity hydrolysis = process in which the solids content is above 20%

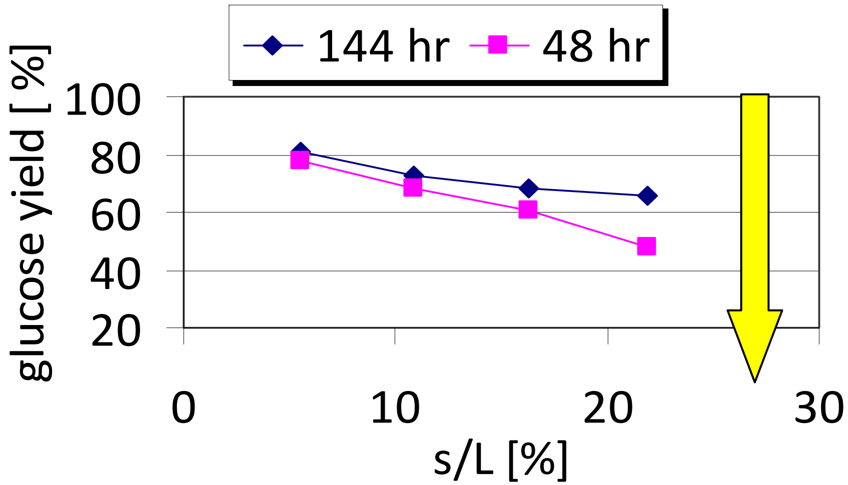
- Higher concentration of the final product
- Reduced distillation costs
- Reduced bioreactor capacity (→ lower installed costs)
- Lower amounts of waste waters

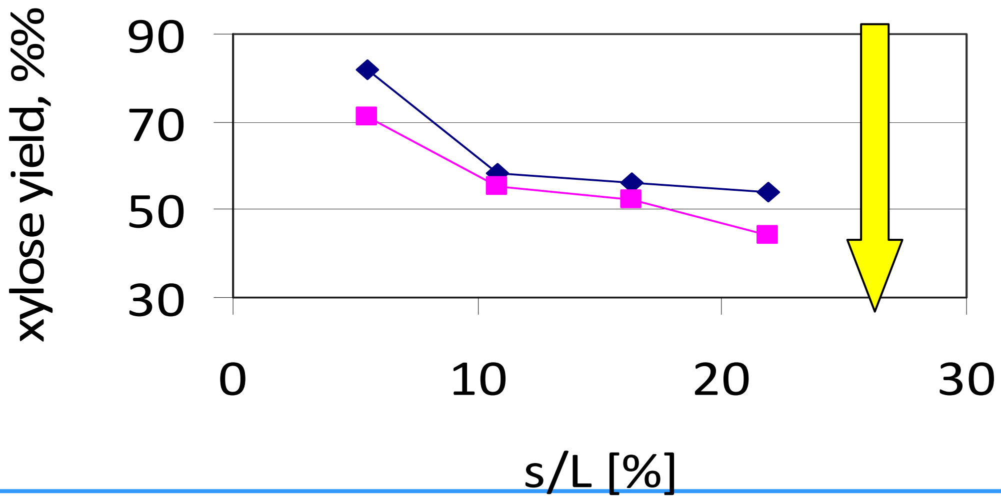
The Challenges of high gravity hydrolysis:

- High viscosities → mass transfer limitations→ poor mixing
- Inhibition by end-products

The Challenges of high gravity fermentation:

- High concentration of microbial inhibitors
- Osmotic stress due to high solutes concentration
- Toxic effect of ethanol (synergistic inhibition)





Process scale: 500 mL shaken flasks



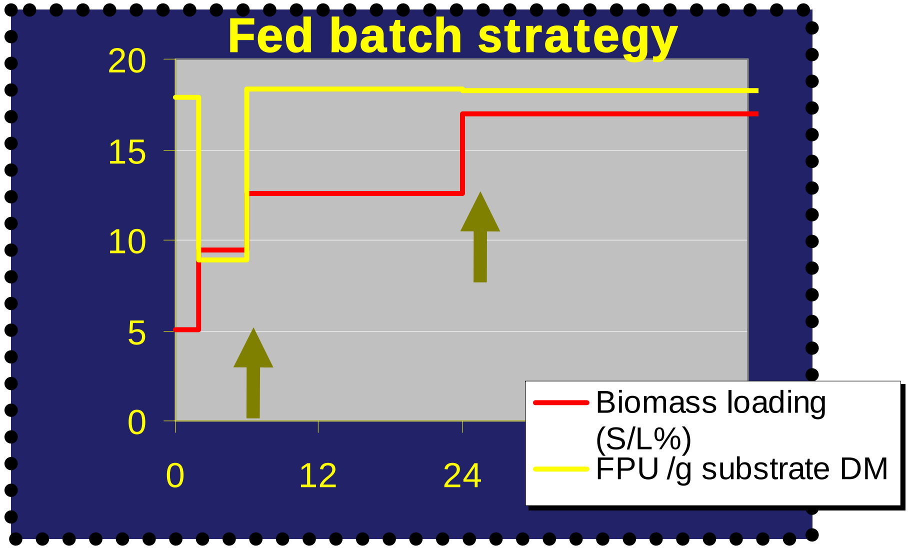

AIMS

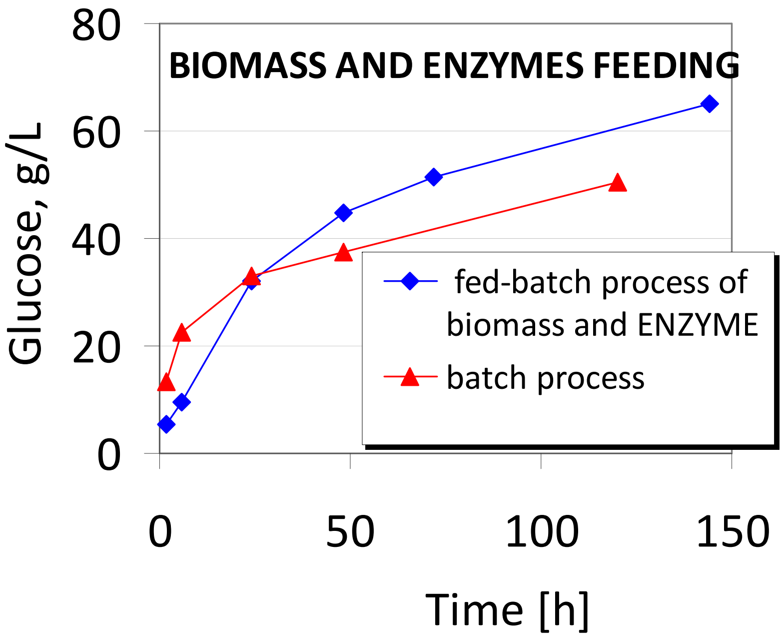
1. **Assess the effect of various feeding strategies on the cellulose hydrolysis**
2. **Assess the performances of the enzymatic blends provided by Novozymes**
3. **Assess the effect of the reactor geometry and mixing**
4. **Find the optimal process strategy (SSF, SHF, hybrid process)**
5. **Testing the resistance of some yeast strains at industrial relevant process conditions**

Fed-batch processes avoid high viscosities→ poor mixing
Fed batch of biomass and enzymes ensure that the same specific activity is maintained

- **Fed batch with respect to the biomass and extra β-glucosidase**
- **Fed batch with respect to the biomass and enzymes**
  - **Tests of various biomass feeding rates**
  - **Tests of various enzymes feeding rate**

biomass and enzymes were loaded to keep their internal ratio roughly constant



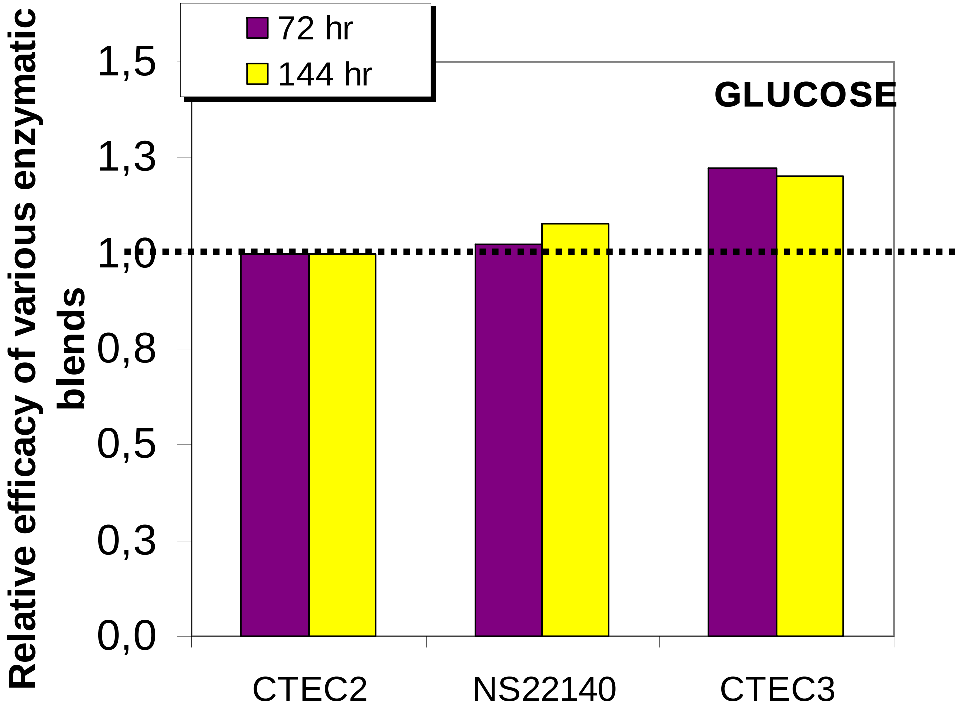


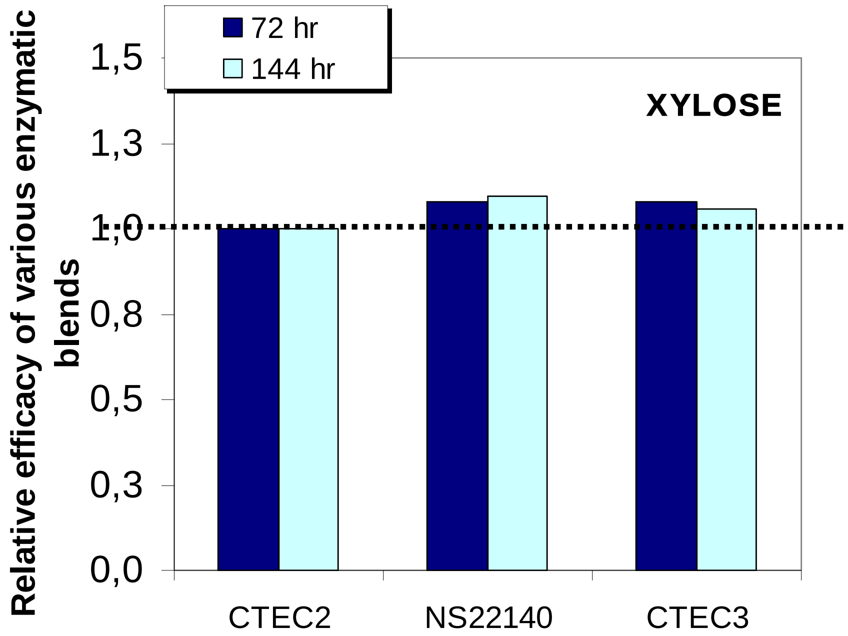
Glucose yields improved as effect of the feeding strategy.

Arundo fiber CTX 5710 pH5.5, 50°C.solids load 18%.

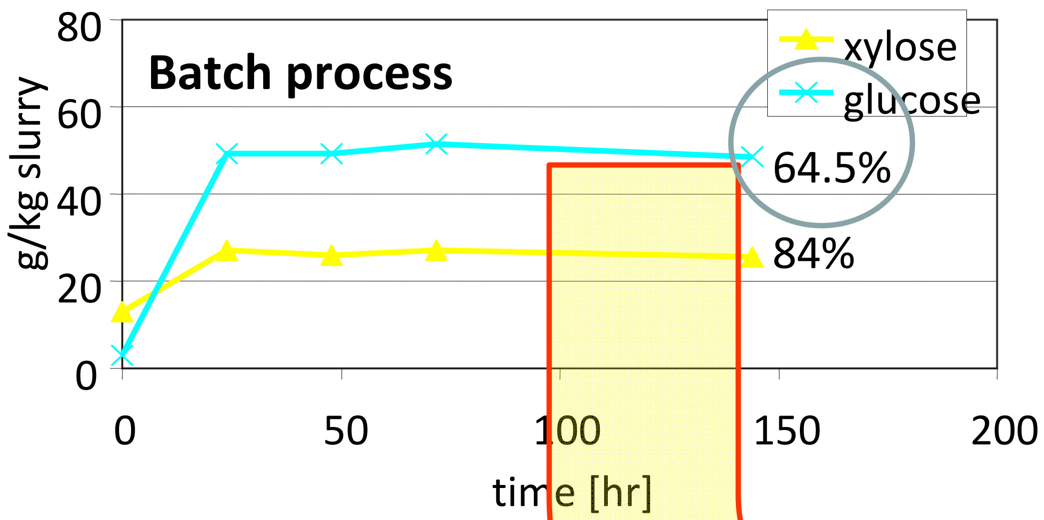
ENZYMATIC COCKTAILS

COMPARISON OF VARIOUS ENZYMATIC BLENDS DURING THE HYDROLYSIS OF CONCENTRATED SLURRY (27% S/L) OF ARUNDO STEAM PRETREATED (fiber).





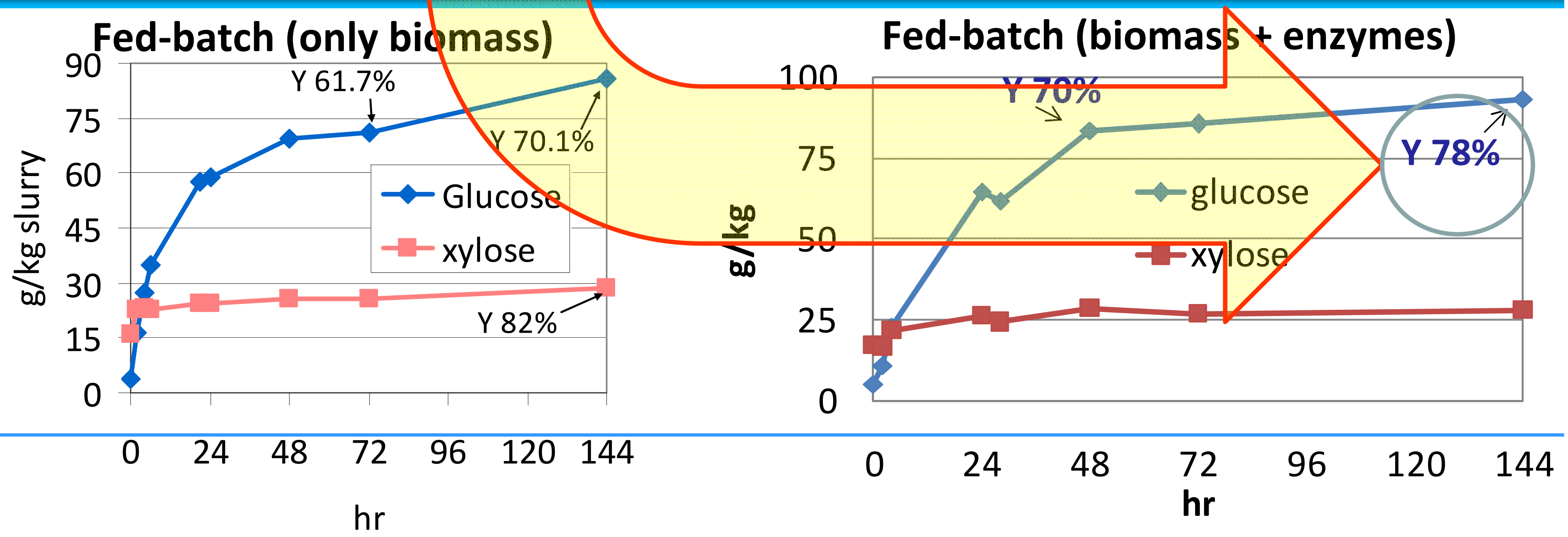
Inhibition by xylooligomers could have an important effect. Hemicellulases could improve the process yields



PROCESS STRATEGY+IMPROVED ENZYMATIC COCKTAIL

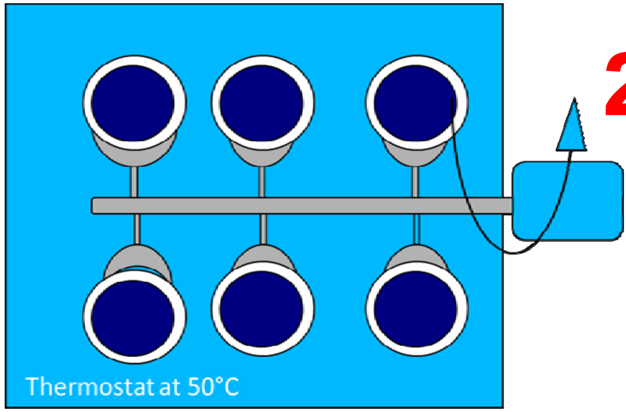
Pretreatment : acid catalyzed steam explosion at 200°C and 5 min

Process scale: stirred bioreactor;
pH 5; T 50°C, NS22140: 0.2 g_{enzyme} /g_{glucan}
s/L% 19



Effect of the mixing

1



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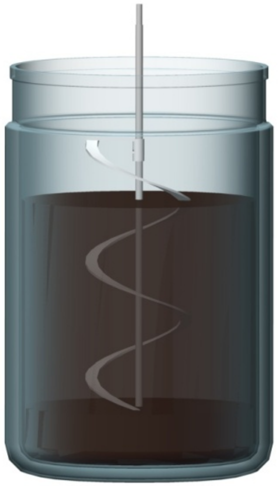

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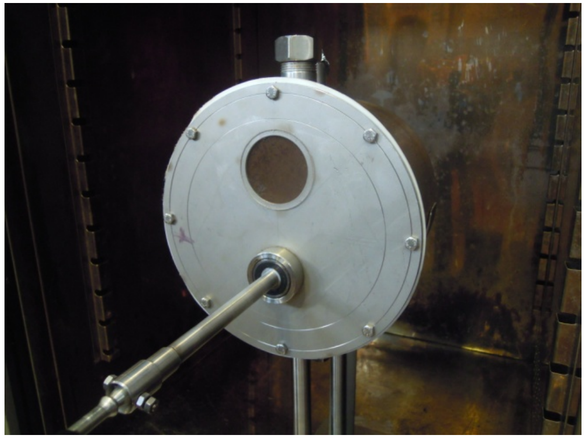

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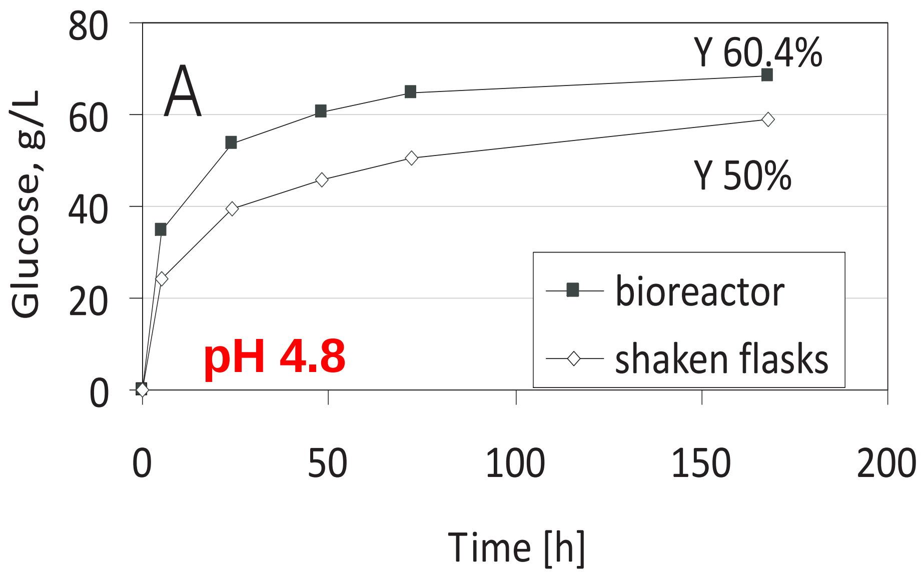
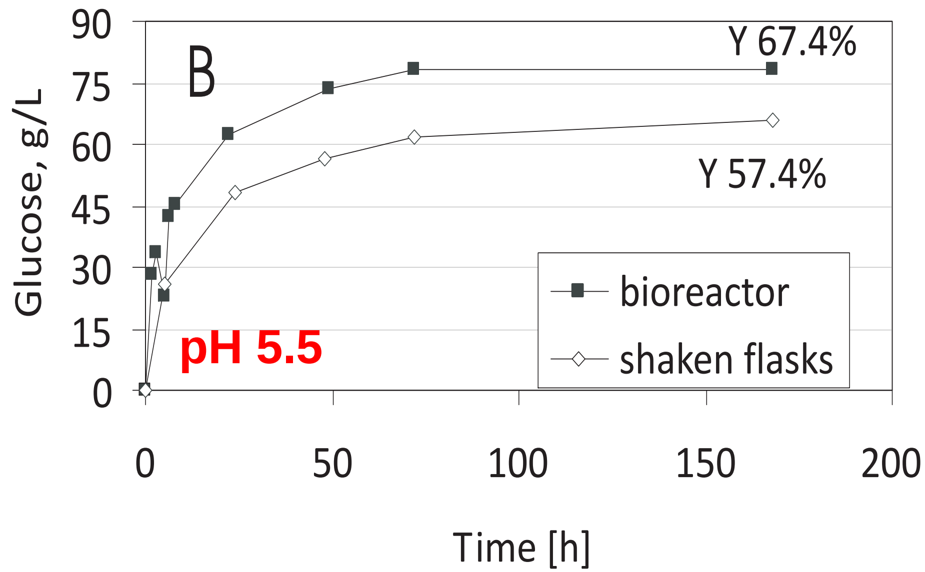
Mixing in shaken flasks and bioreactor (1 and 3)

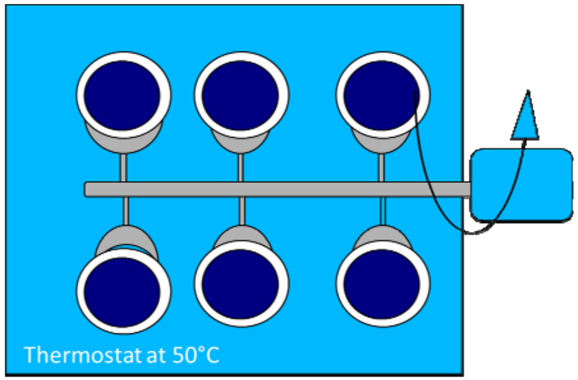


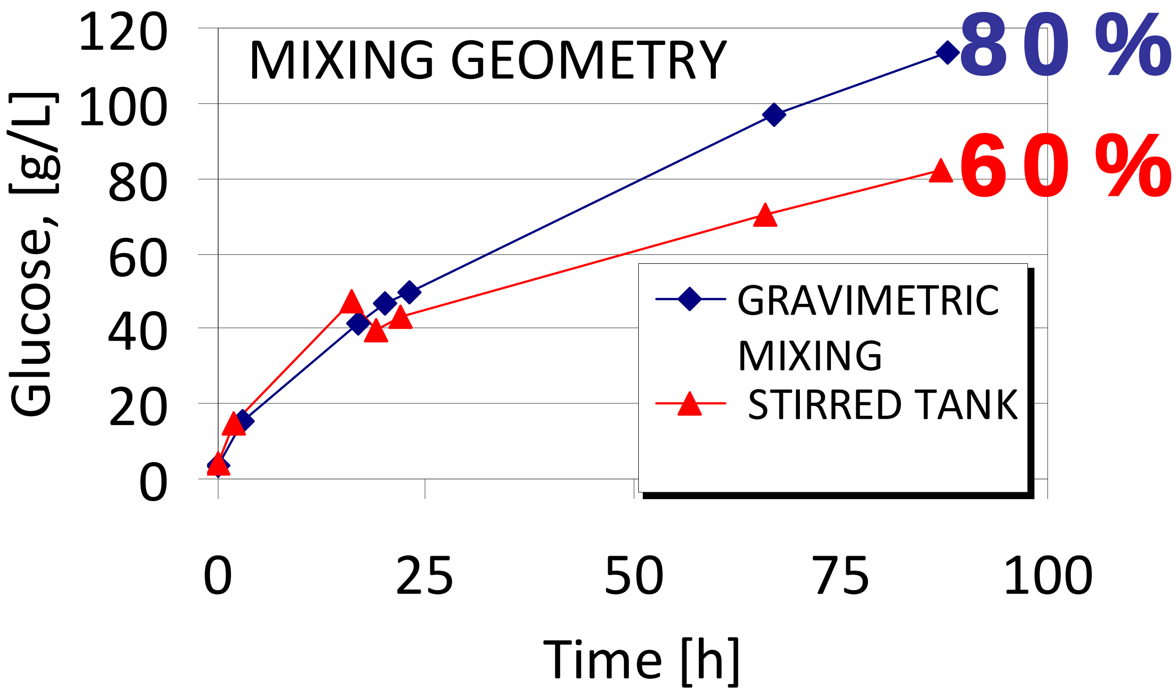


Arundo fiber 5310, 20% solids loading, 0.27 g Ctec2/g glucan, 50° C.

Mixing in stirred bioreactor and gravimetric shaker (3 and 2)

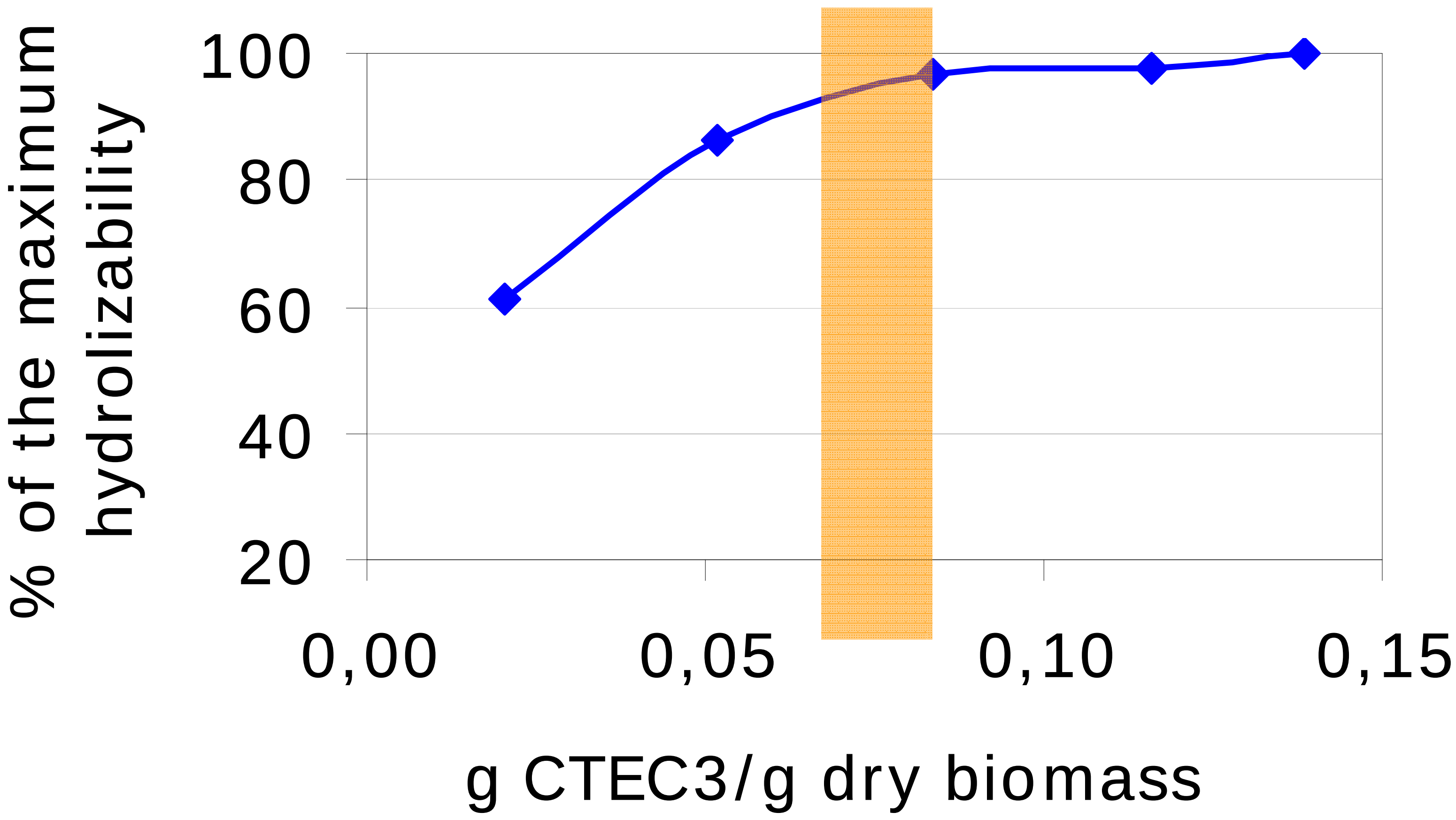
Gravimetric shaking in rotating drum system was much more effective. The final glucose yields are **80 %**





Composition of the substrate 30% S/L, CTEC3 0.08 g_{ENZYME} /g_{GLUCAN};

CTEC3



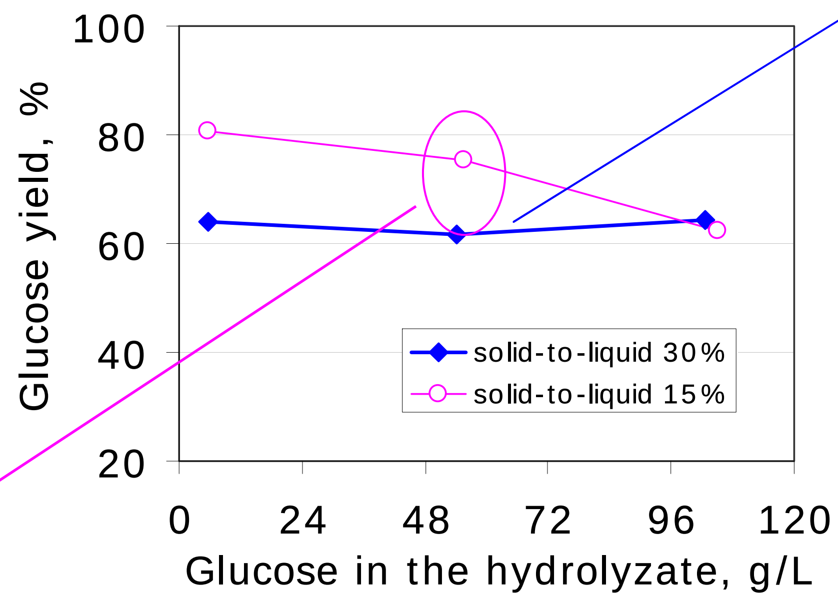
30% solids

Two main sources of inhibition: products and substrate inhibition

In a fed-batch process, hydrolysis of fresh biomass by enzymes can be affected by the amount of soluble glucose.

Substrate inhibition

Process conditions: T=50° pH5; CTEC3 0,10g/g glucan

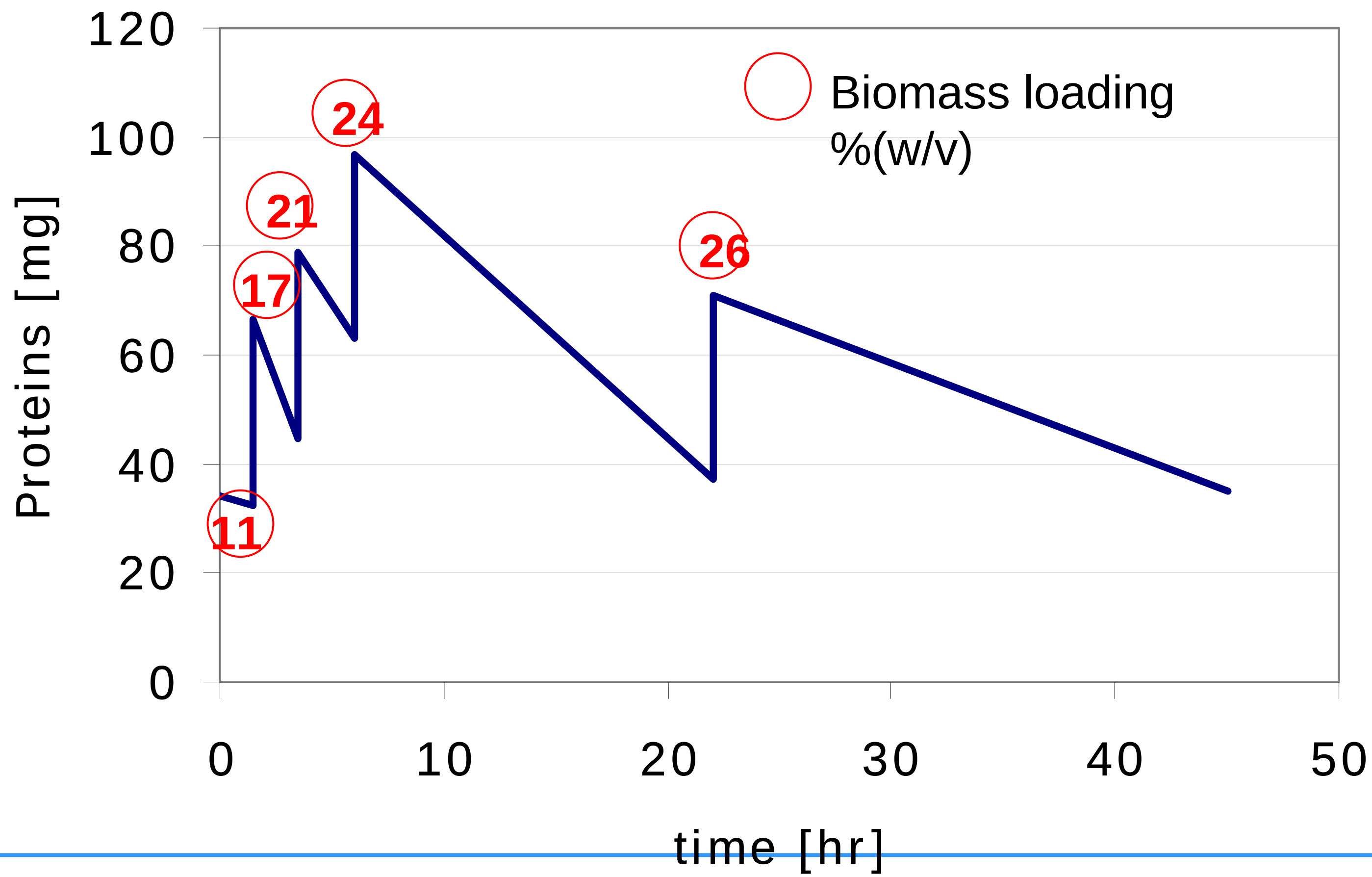


Glucose was slightly inhibiting toward the hydrolysis of fresh biomass at 15% s/L and enzyme-to-glucose ratio of 0.15 g/g.

Higher glucose concentrations corresponding to 0.080 g/g enzyme-to-glucose ratio reduced the hydrolysis yield by 18%→ product removal could reduce the enzymes inhibition

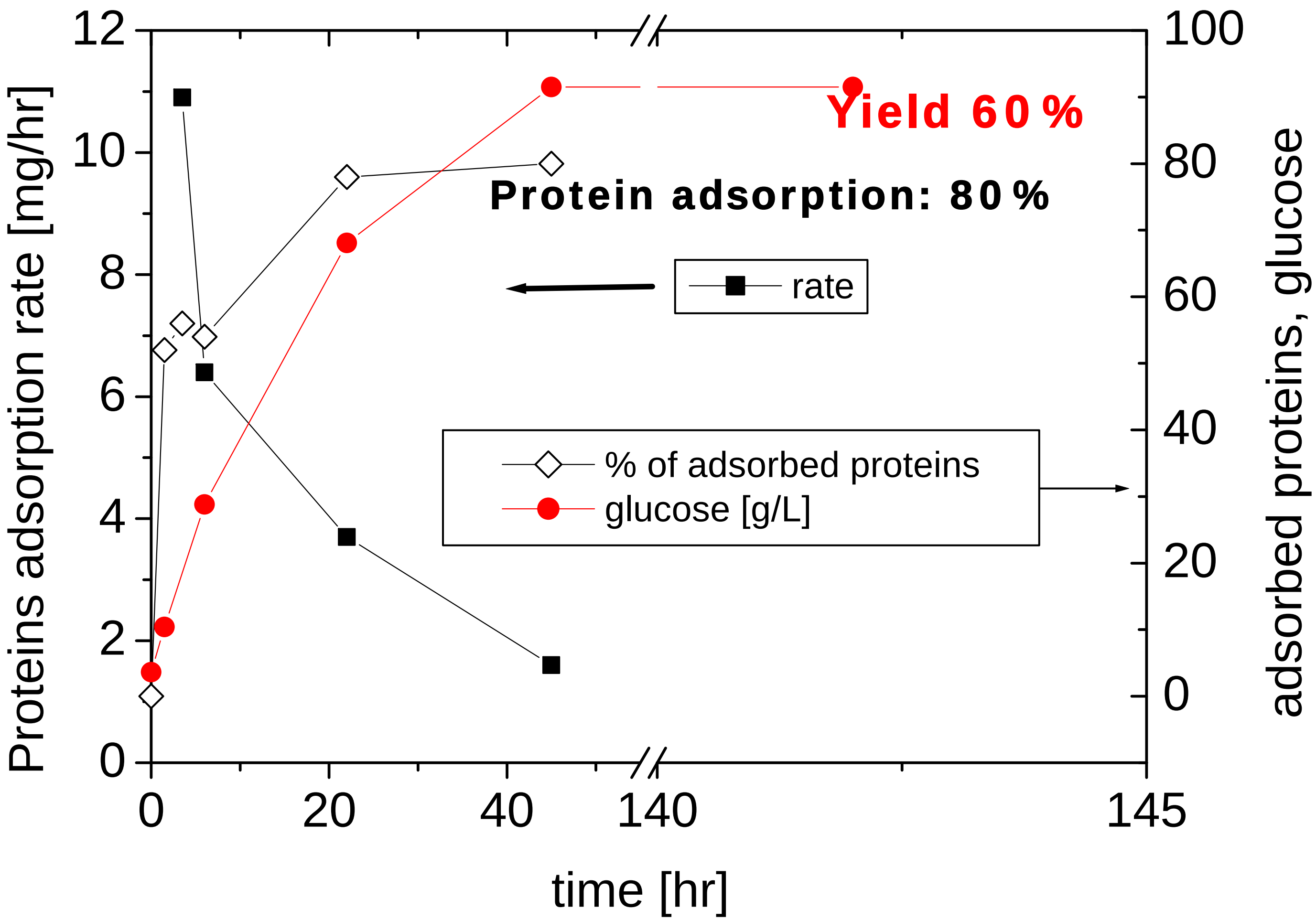
Enzymes adsorption

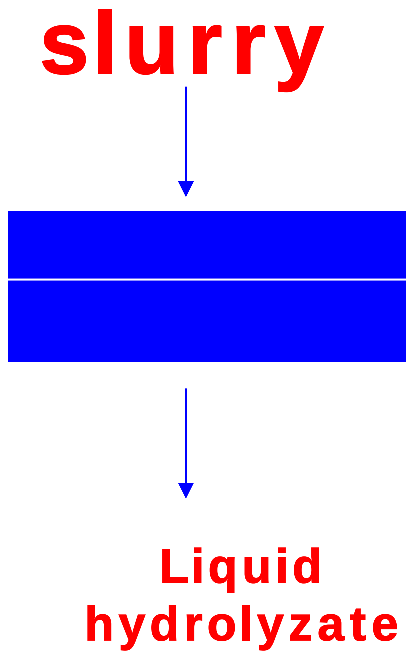
Proteins in solution during a typical fed batch process with respect to biomass and enzymes

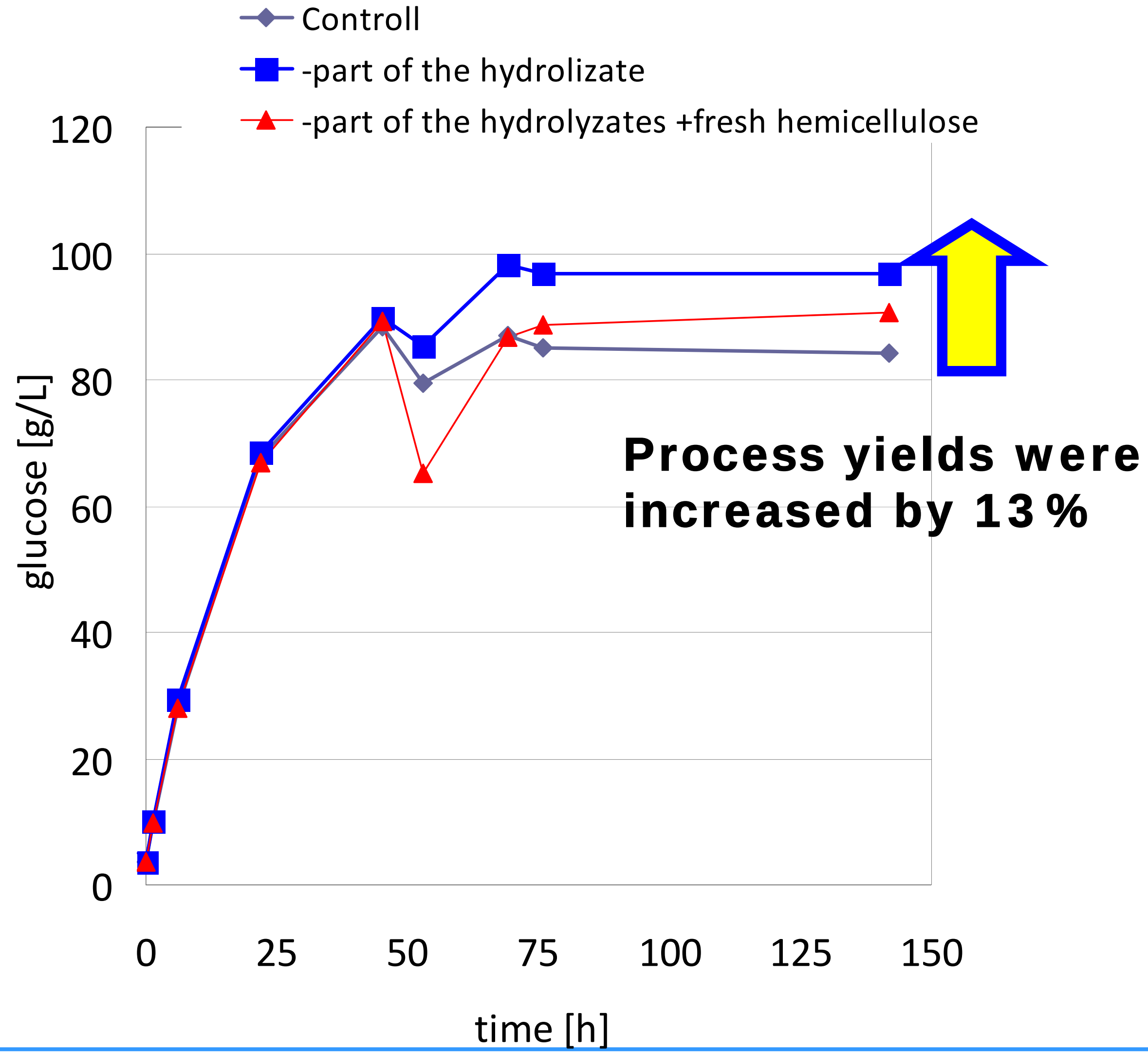





Substrate inhibition



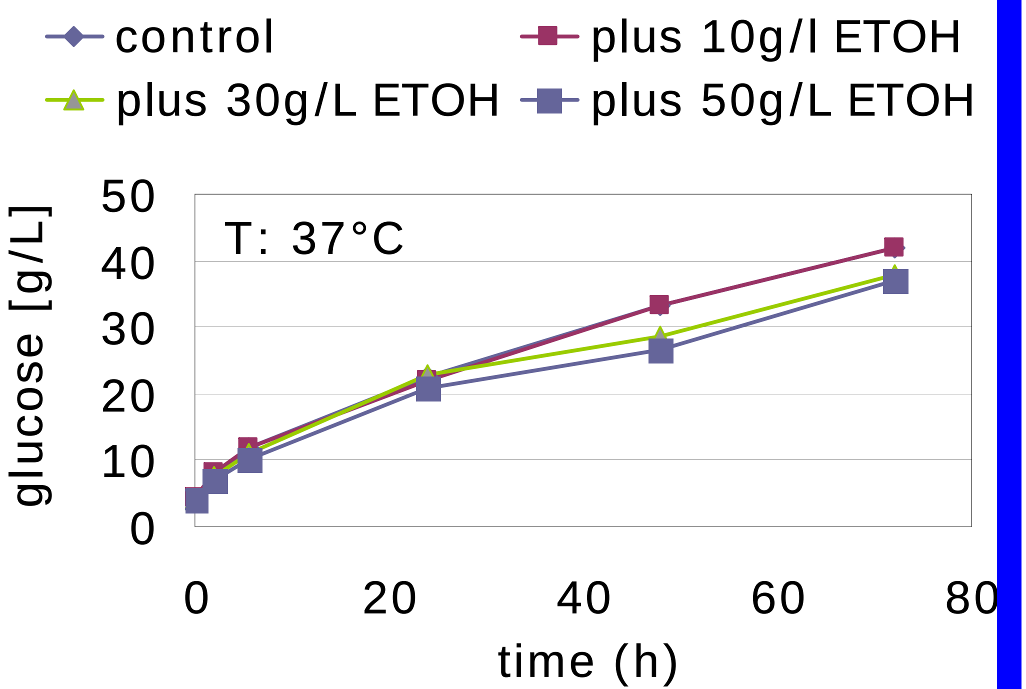




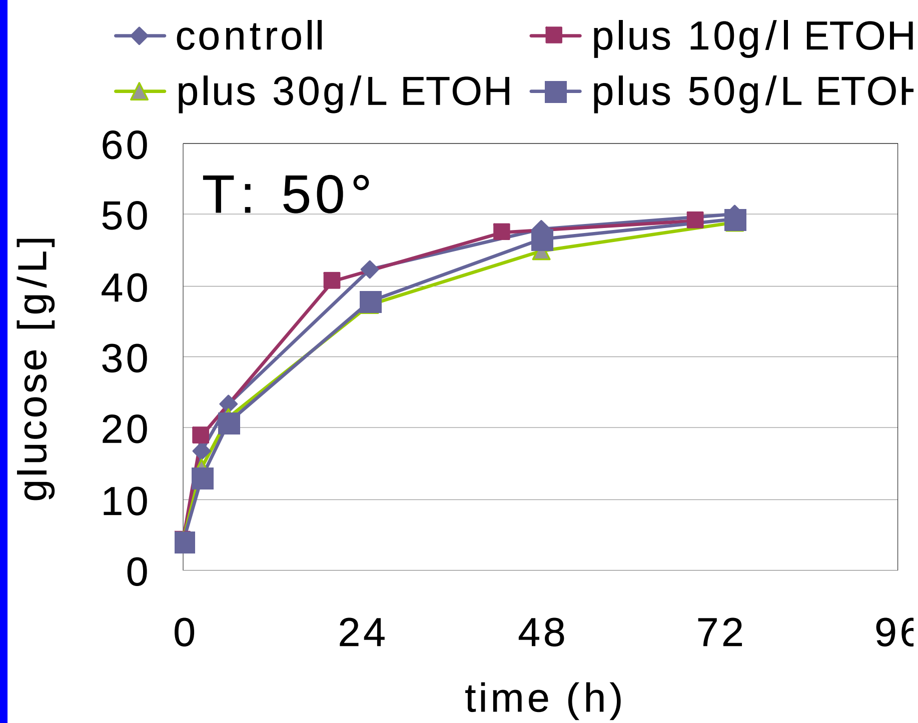
ENZYME loading 0.07 gCTEC3/g_{glucan})

Inhibition of CTEC3 by ethanol

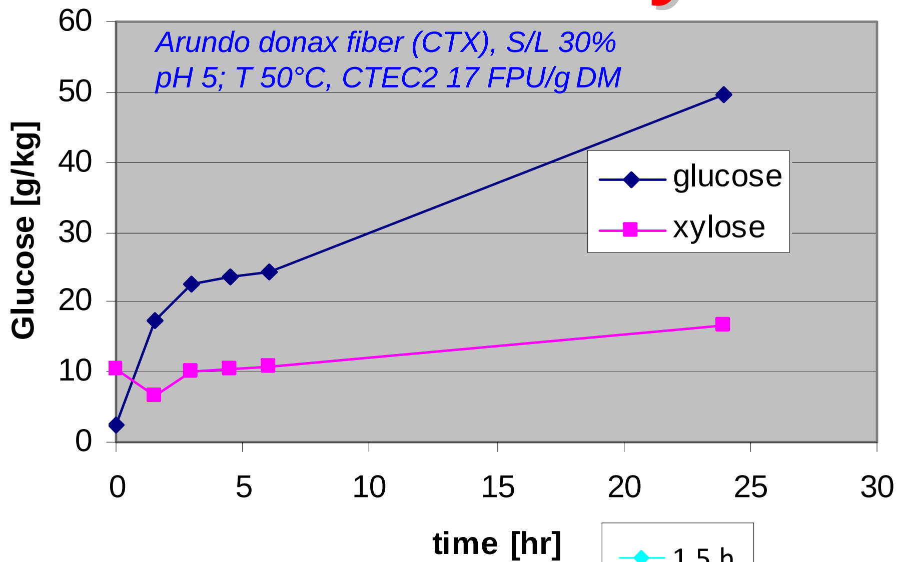
@37°C



@50°C

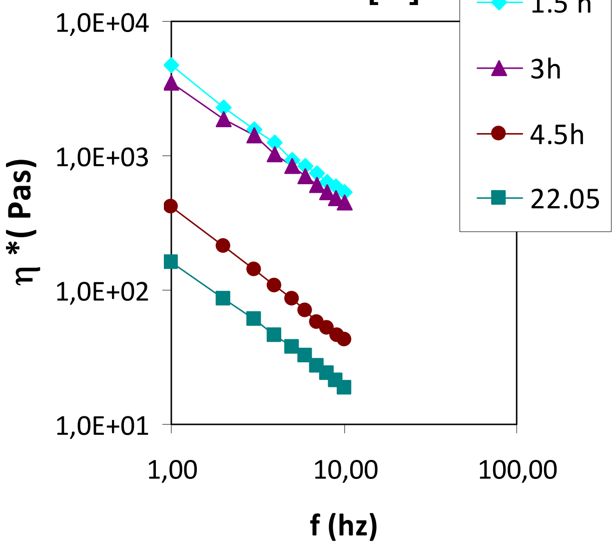


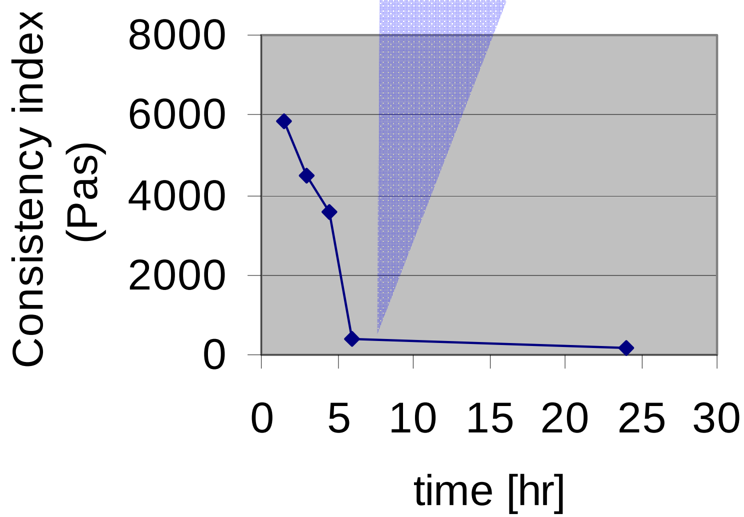
Pure and hybrid SSF (HSSF)



The Chemtex product was liquefied in a stirred bioreactor in five hours

The hydrolysis extent was 32% of the maximum achieved at prolonged process time.





Simultaneous saccharification and fermentation of pre-liquefied biomass

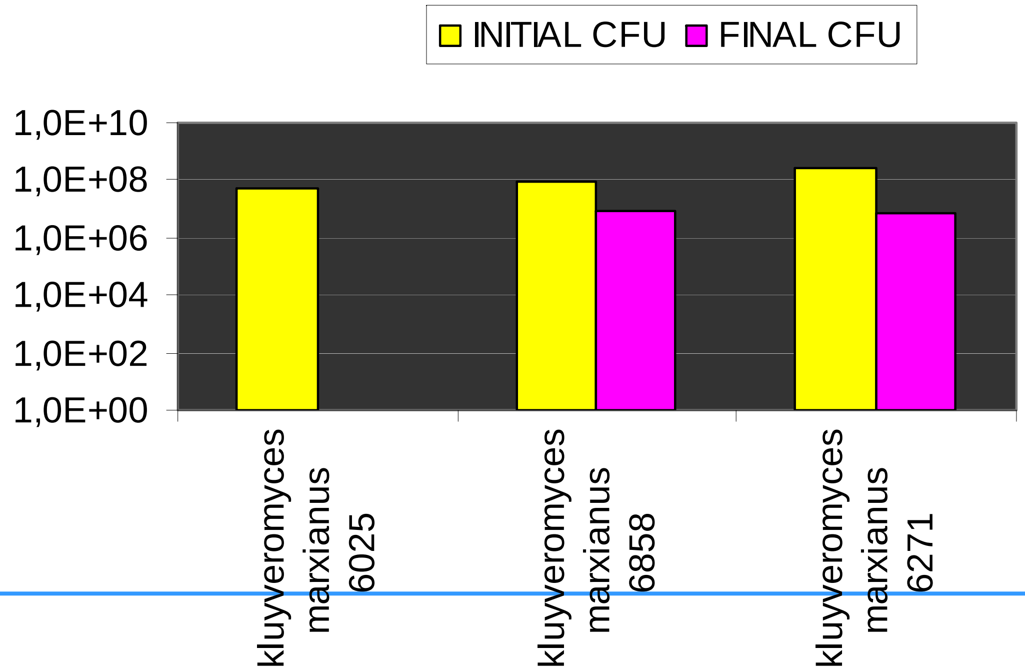
YEASTS FOR FERMENTATION TESTS

- ***Saccharomyces cerevisiae* (SIGMA II)**
- ***Saccharomyces cerevisiae M861 (isolated by ENEA and alcohol tolerant)***

- ***Kluyveromyces marxianus 6271 (DBVPG collection)***
- ***Kluyveromyces marxianus 6858 (DBVPG collection)***

Besides *S. cerevisiae*, some *kluyveromyces marxianus* species were tested with the aim of finding a temperature tolerant strain, able to coferment xylose

CELLS VIABILITY DURING GROWTH AND FERMENTATION ON CHEMTEX LIQUEFIED PRODUCT (VR)

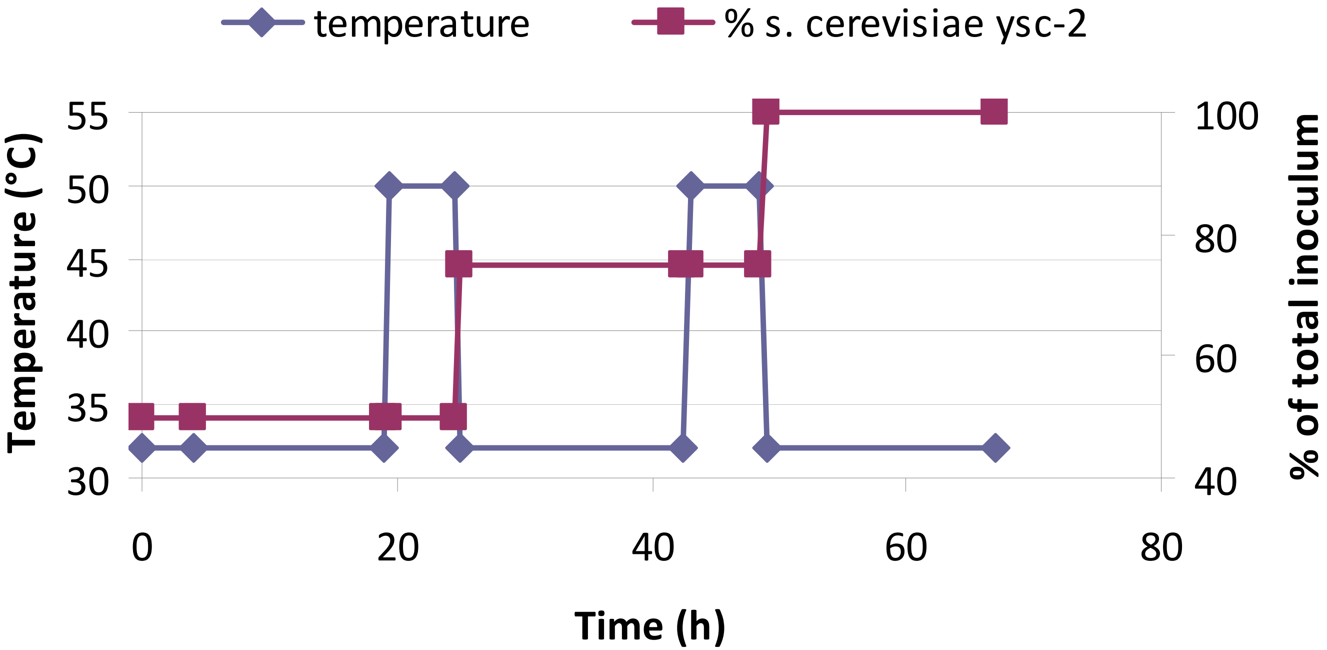


- Two of three *k.marxianus* strains showed a good tolerance to the VR composition
- One of the inhibitors tolerant *k.marxianus* consumed xylose in synthetic media



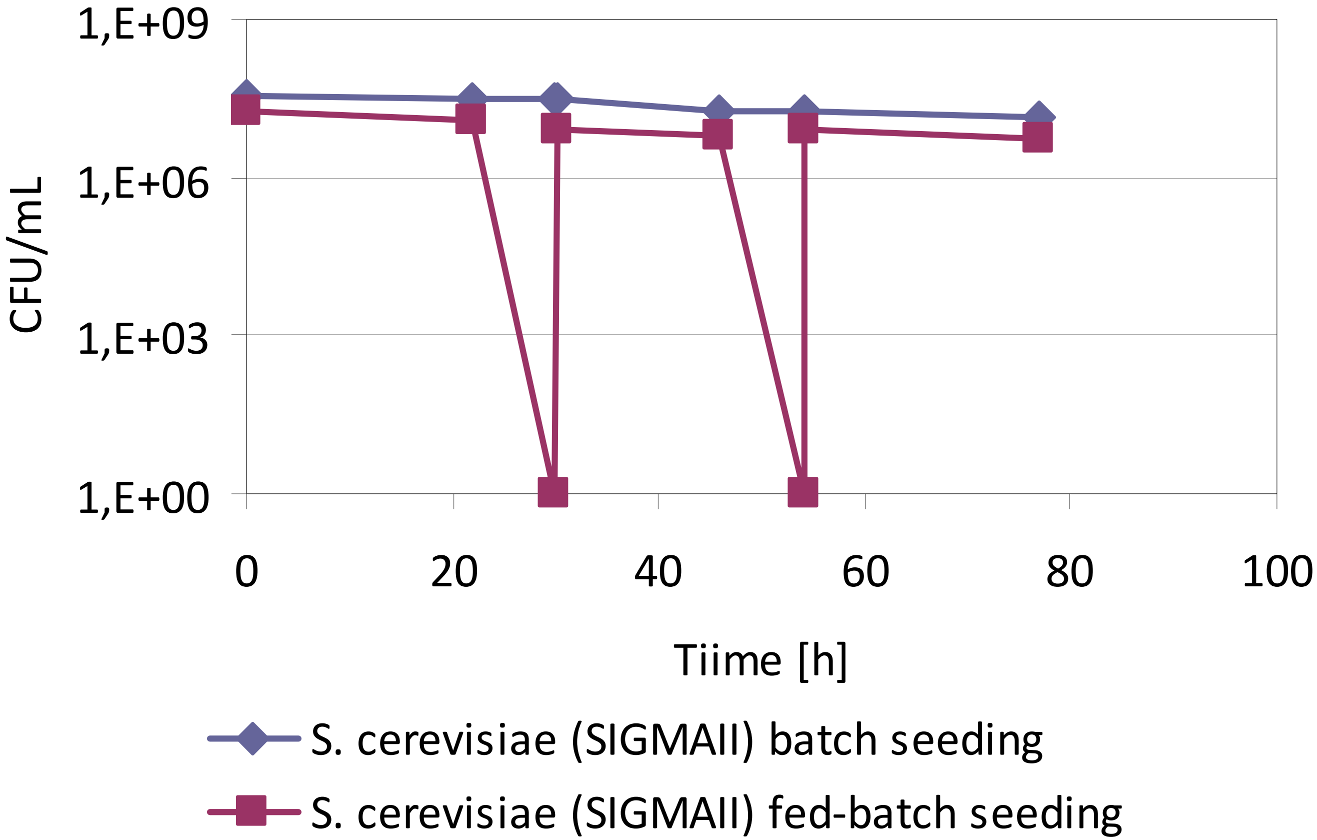

Two SSF set-ups were evaluated to *<u>increase the cellulose hydrolysis</u>*:

1. SSF at common process conditions
2. SSF with intermittent step-wise increase of temperature and fed-batch inoculation of the yeast

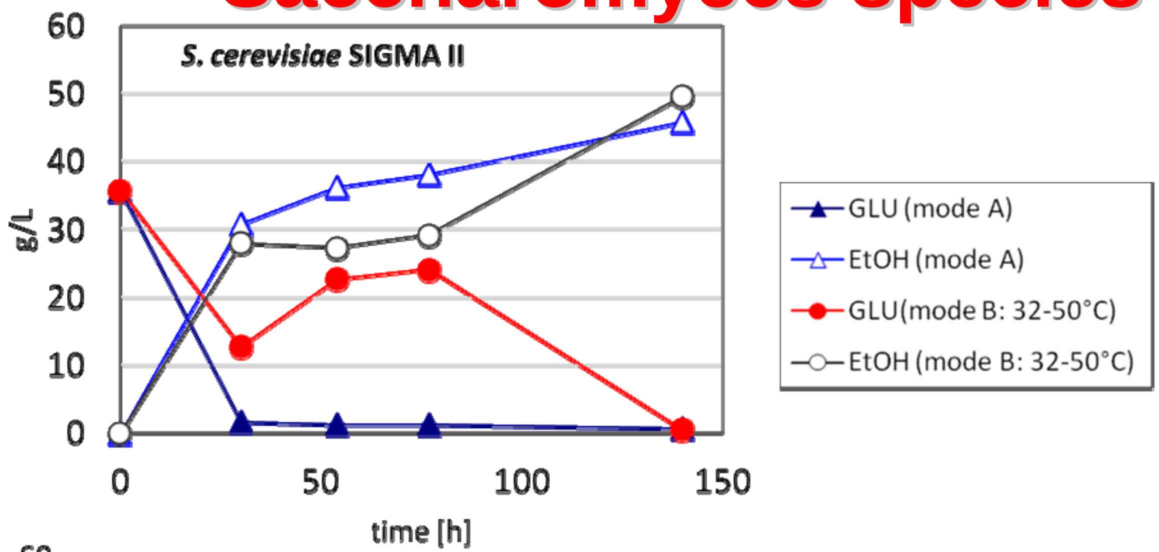


FB 1 **Fed Batch with 2 hydrolysis steps**
FB 2 **Fed Batch with 2 hydrolysis steps 1.6 times wider than 1**
FB 3 **Fed Batch with 1 hydrolysis step 1.8 times wider than 1**

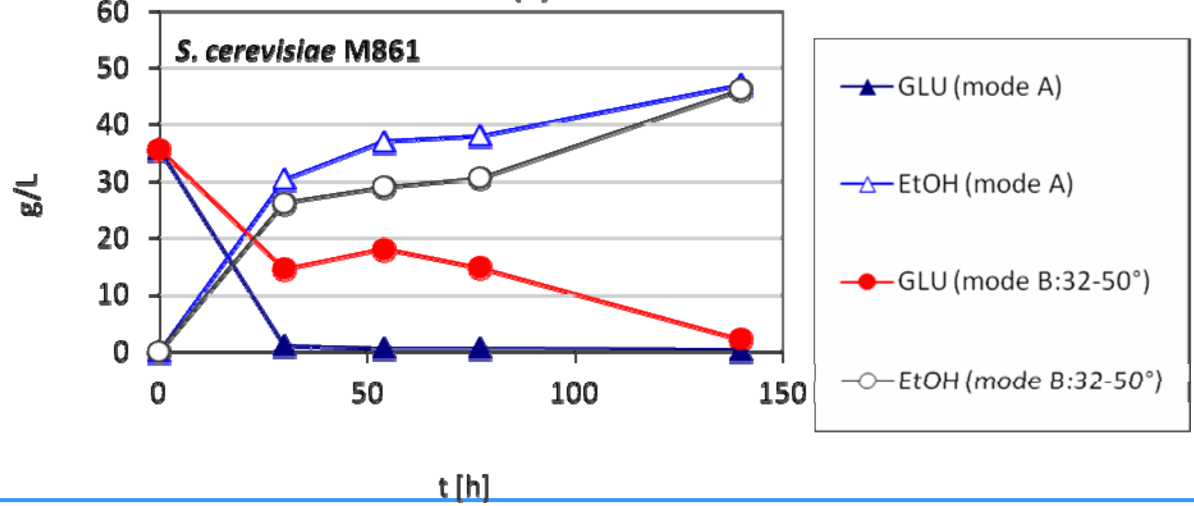
Typical cells viability during SSF with modulation of temperature



Saccharomyecs species

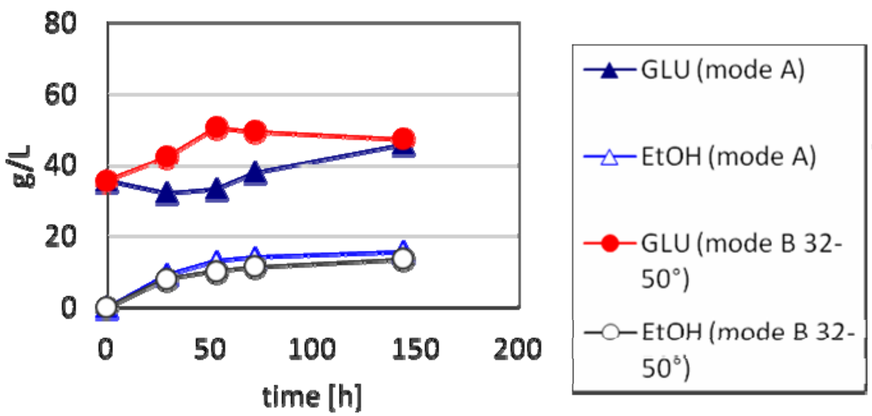


M861 more rapid in the glucose uptake

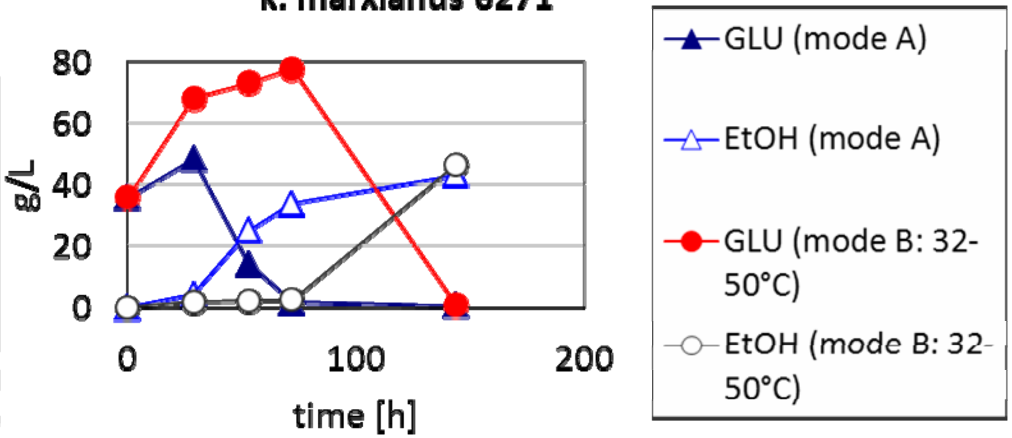


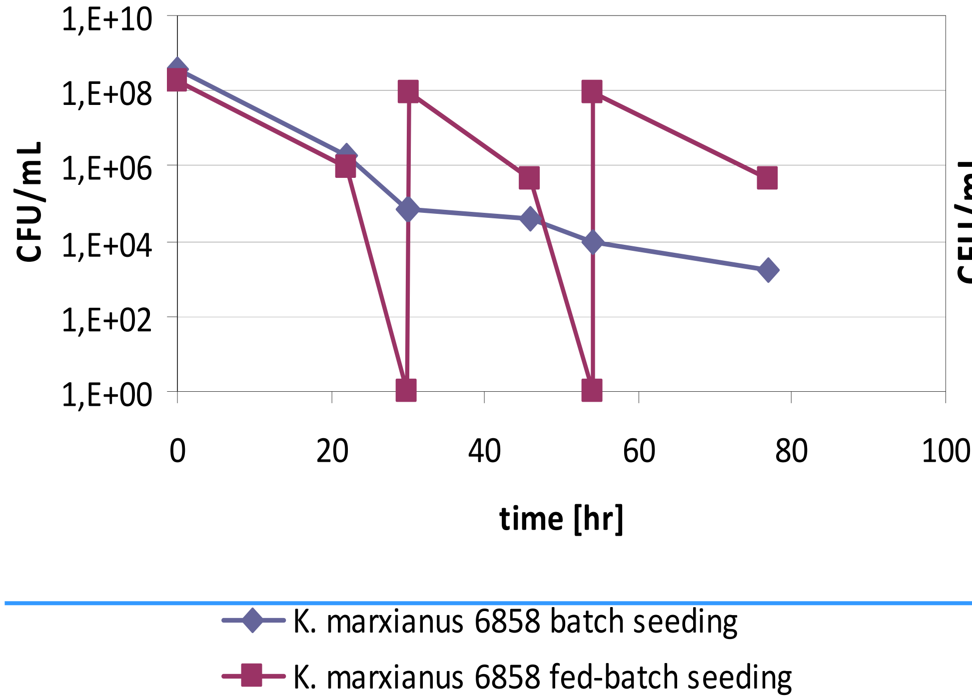


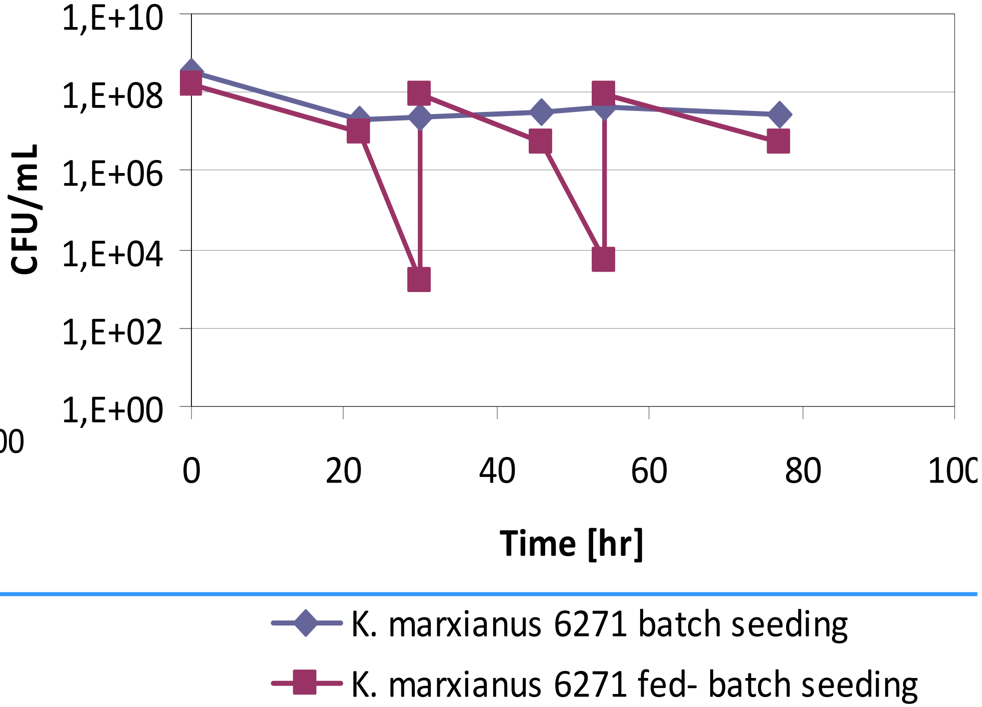

k marx. 6858



k. marxianus 6271

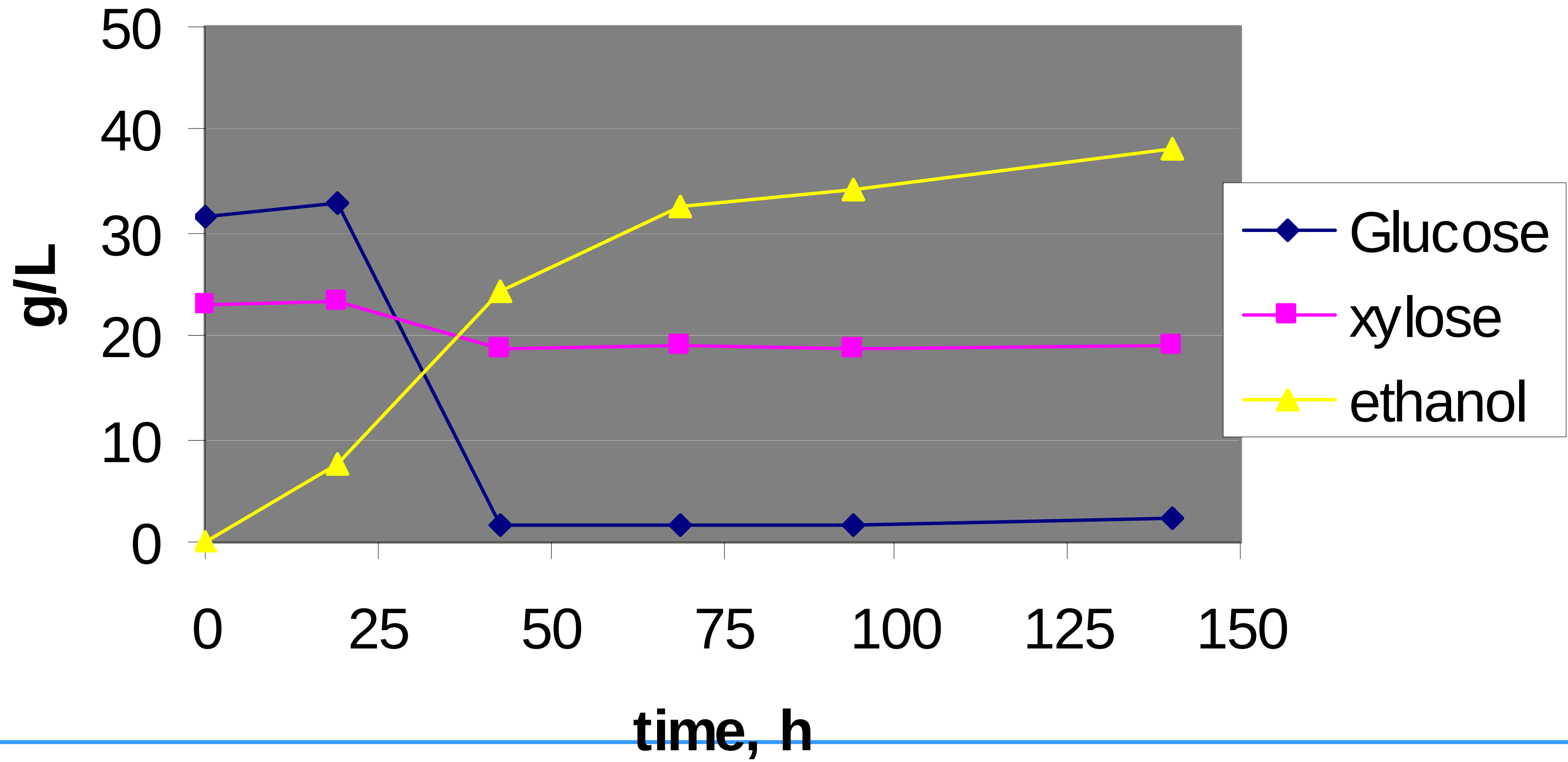






Xylose consumption by *k. marxianus* 6271 in biomass hydrolyzates

Xylose consumption was 10-17% but it mostly corresponded to xylitol production






Enzyme dosage [g/g glucan]	Microrganisms	Process Type	Yeast inoculation	T [°C]	Ethanol (%wt)	Overall glucose [g/L]
0,22	*S. cerevisiae (M861)*	SHF	B	32°C	3,5	68
0,085	*S. cerevisiae (SIGMA II)*	HSSF	B	32°C	3,3	66
0,22	*K marxianus k6858*	H SSF	FB 1	32-50°C	1,4	74
0,085	*S. cerevisiae (SIGMA II)*	HSSF	FB 1	37-50°C	3,8	75
0,085	*S. cerevisiae (SIGMA II)*	HSSF	FB 3	37-50°C	3,8	75
0,085	*S. cerevisiae (SIGMA II)*	HSSF	B	37°C	3,7	75
0,22	*K marxianus k6858*	H SSF	B	32°C	1,6	77
0,22	*S. cerevisiae (SIGMA II)*	HSSF	FB 3	37-50°C	4,2	84
0,22	*K. marxianus k6271*	H SSF	B	32°C	4,4	85
0,22	*S. cerevisiae (SIGMA II)*	HSSF	B	37°C	4,2	90
0,22	*S. cerevisiae (SIGMA II)*	H SSF	B	32°C	4,6	91
0,22	*K. marxianus k6271*	H SSF	FB 2	32-50°C	4,7	91
0,22	*S. cerevisiae (M861)*	H SSF	B	32°C	4,8	92
0,22	*S. cerevisiae (M861)*	H SSF	FB 2	32-50°C	4,7	93
0,22	*S. cerevisiae (SIGMA II)*	H SSF	FB 2	32-50°C	5,0	98

Reference

Glucose recovered

80%

Conclusions

1. Chemtex pretreatment in which the SE step was carried out at 206 and 4 min produced a biomass hydrolizability similar to ACSEP at 200°C

2. Hydrolysis at high DM content (~30%) by using 80 mgCTEC3/g GLUCAN produced 80% glucose yields (GLU+XYL=137 g/L)

3. Optimization of the process strategy includes *fed-batch* feeding of biomass and enzymes along with optimized mixing conditions

4. Inhibition of enzymes by glucose was observed at 100 g/L glucose and enzymes-to-glucose ratio of 0.08 g/g

5. Enzymes adsorption on fresh biomass and product removal enabled the enzymes recovery and reuse.

6. Hybrid SSF (HSSF) process produced yields 1.4 higher than SHF.

7. ~2.5 times more enzyme than in the hydrolysis tests at optimized conditions (T 50°C) is required to achieve SSF process yields of 80%.

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To all the project partners
for cooperation

Thanks for the attention

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