Hydrolysis of biomass at high dry matter content: effect of different pretreatments and process strategies

Isabella De Bari, **Federico Liuzzi**, Alfredo Ambrico, Mario Trupo, Giacobbe Braccio
ENEA CR TRISAIA, ITALY

Workshop BioLYFE

Copenhagen - June 4th, 2013

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	S	S		S			S	S	S

Outline on processes

- Biomass hydrolysis after two different pretreatments
- Biomass hydrolysis with several enzymatic mixtures at high biomass loading (*CTEC, CTEC2, NS22140, CTEC3*)
- Various process configurations, including biomass, and enzymes fed-batch
- Biomass hydrolysis during SSF
- 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 degradation of pentoses

Biomass pretreatments

ENEA
AGENZIA NAZIONALE
PER LE NUOVE TECNOLOGIE, L'ENERGIA
E LO SVILUPPO ECONOMICO SOSTENIBILE

ENEA's pretreatment facilities

TARGET: 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 the acid catalyzed steam explosion pretreatment

CONDITIONS: 200° 5’ 1.4% sulphuric acid

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
H20	**~ 85**

Chemtex products

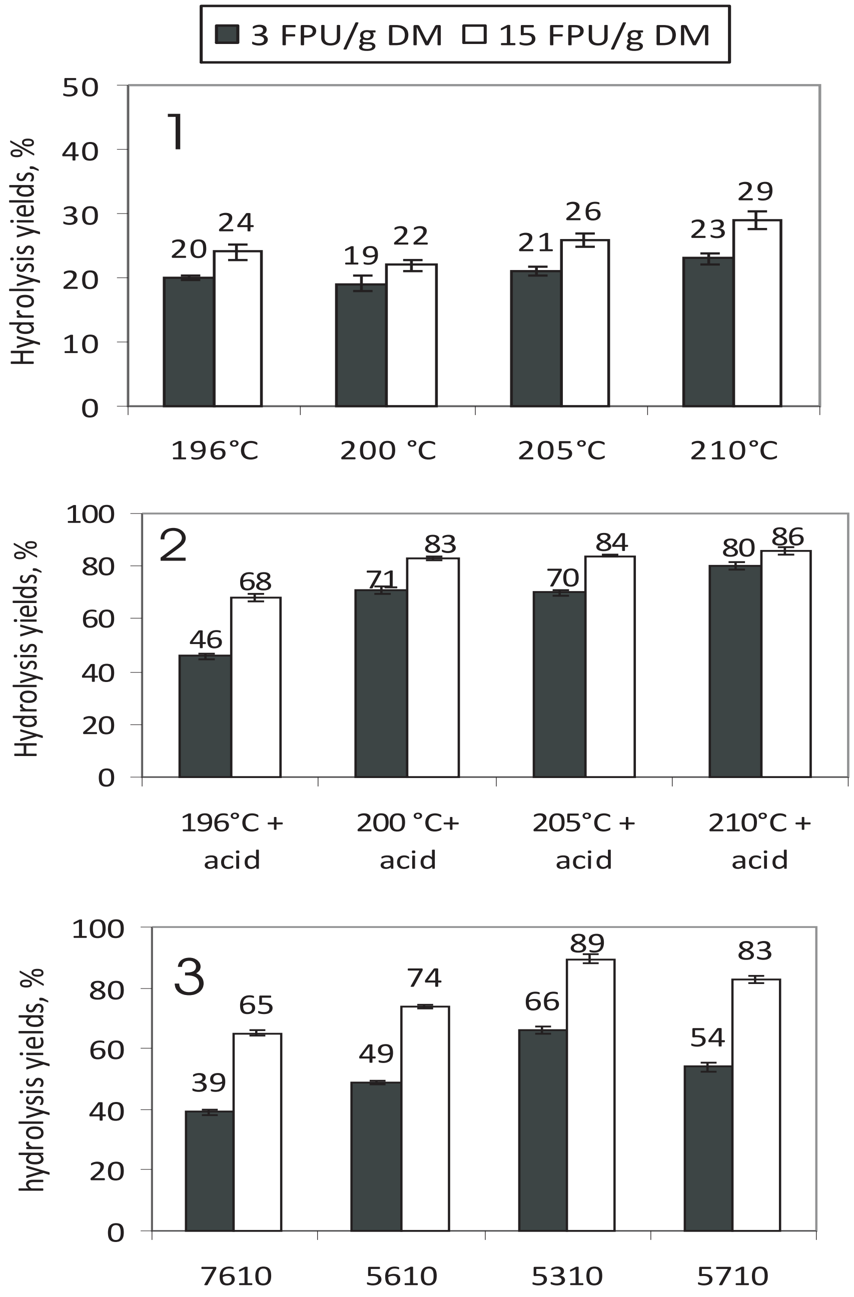
DOUBLE-STEPS CHEMTEX PRE-TREATMENTS CONDITIONS

Code of product	Conditions during the hemicellulose separation	Steam explosion temperature [°C]	Steaming time [min]	logR0
7610	-	198°C	4	3.49
5310	-	206°C	4	3.72
5610	+ water	198°C	4	3.49
5710	+water	206°C	4	3.72

Hydrolizability of Arundo donax after different pretreatments

ENEA
AGENZIA NAZIONALE PER LE NUOVE TECNOLOGIE, L'ENERGIA E LO SVILUPPO ECONOMICO SOSTENIBILE

S/L 2%;
pH 4.8;
T 40°C,
CTEC 0.24g/$g_{biomass}$



STEAM EXPLOSION IN BATCH DIGESTOR

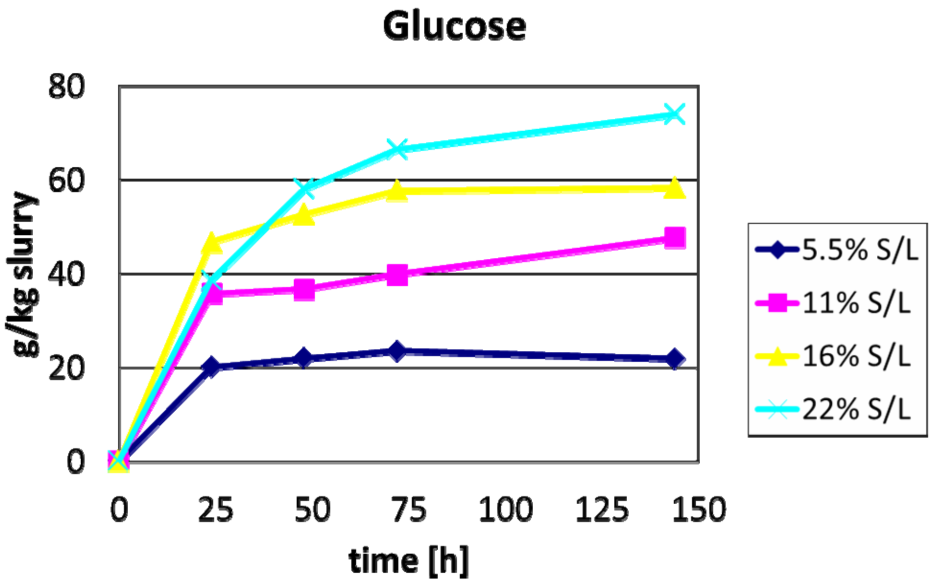
ACID CATALIZED STEAM EXPLOSION IN BATCH DIGESTOR

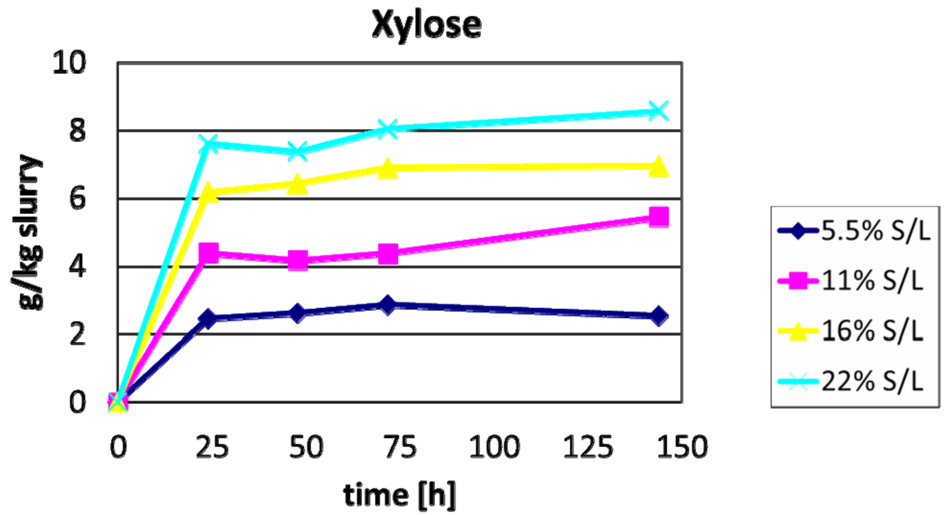
HYDROLIZABILITY OF THE ARUNDO DONAX FIBER FROM THE TWO STEPS CHEMTEX PRETREATMENT

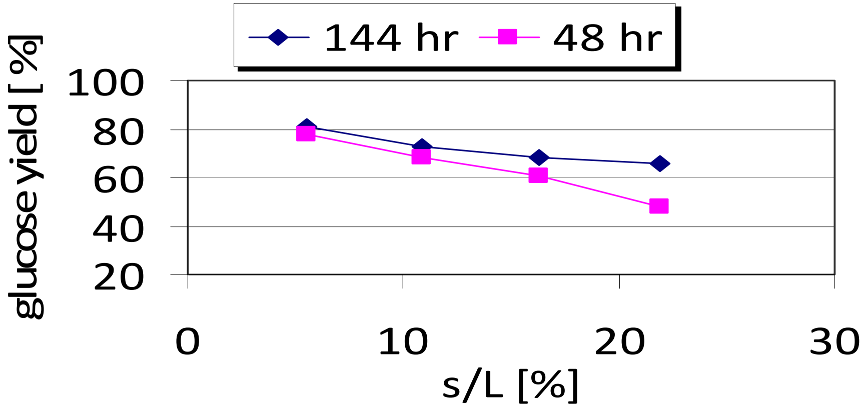
Summary on process assessments

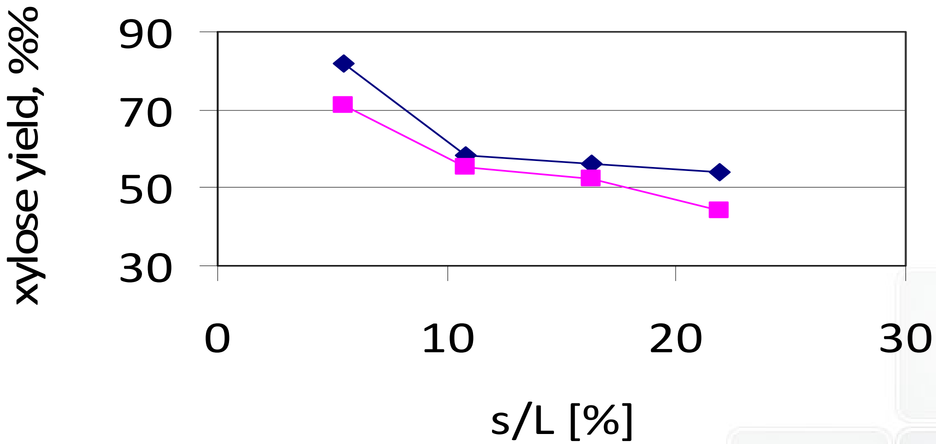
- Biomass hydrolysis after two different pretreatments
- Biomass hydrolysis with several enzymatic mixtures at high biomass loading (*CTEC, CTEC2, NS22140, CTEC3*)
- Various process configurations, including biomass, and enzymes fed-batch
- Biomass hydrolysis during SSF
- Conclusions

Hydrolizability of *arundo donax* (washed fibers) from acid catalyzed steam pretreatment (enzymes mixture NS22140)







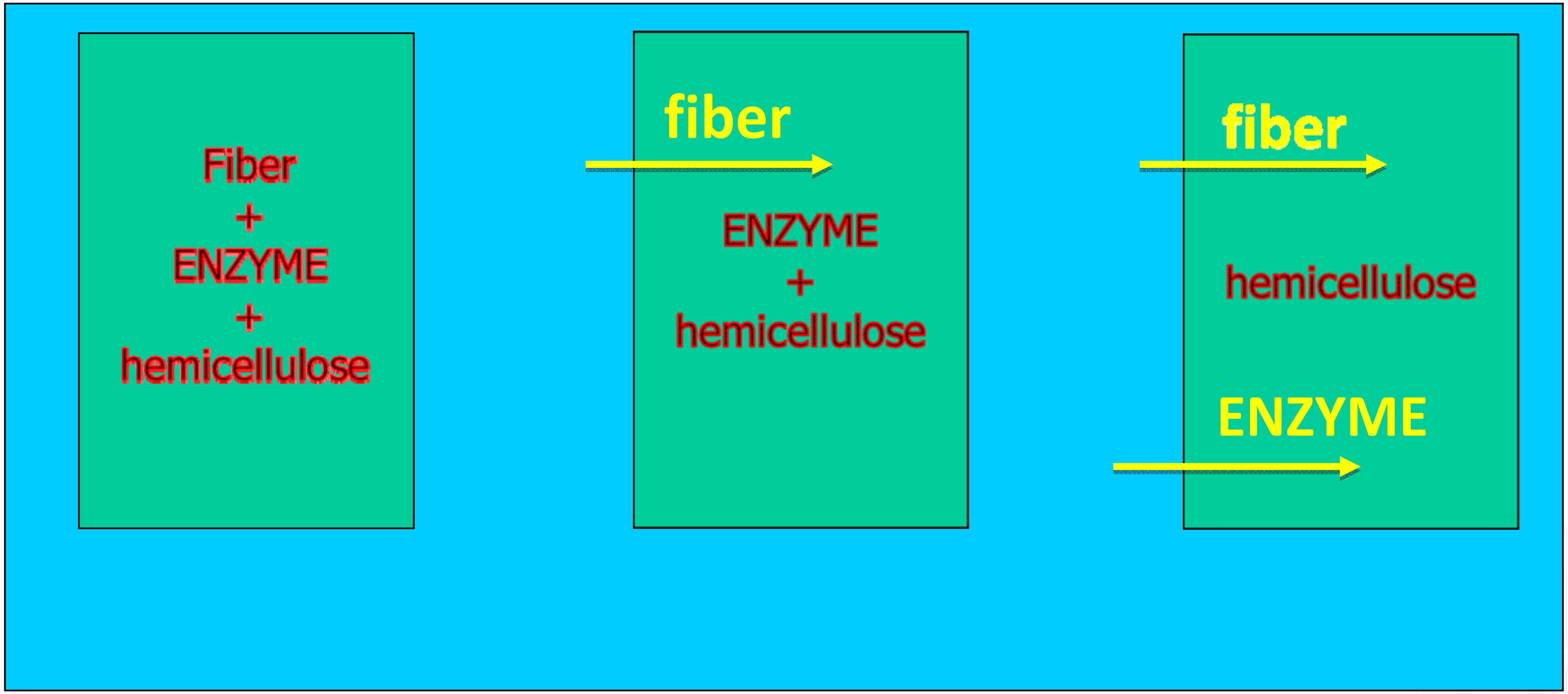


Composition of the substrate: Glucan: 53.91%, Xylan: 6.02%
Process conditions: T 50°C; pH 5; 0.17 g_{ENZYME} /g_{GLUCAN}
Process scale: 500 mL shaken flasks

Effect of the feeding strategy during hydrolysis at high DM content

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

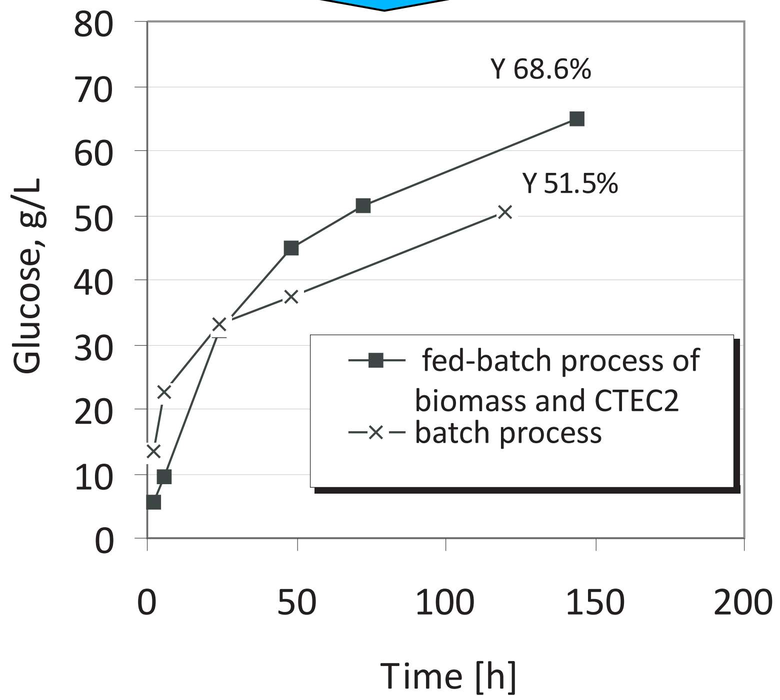
Batch | **Fed-batch fiber** | **Fed-batch fiber and enzyme**



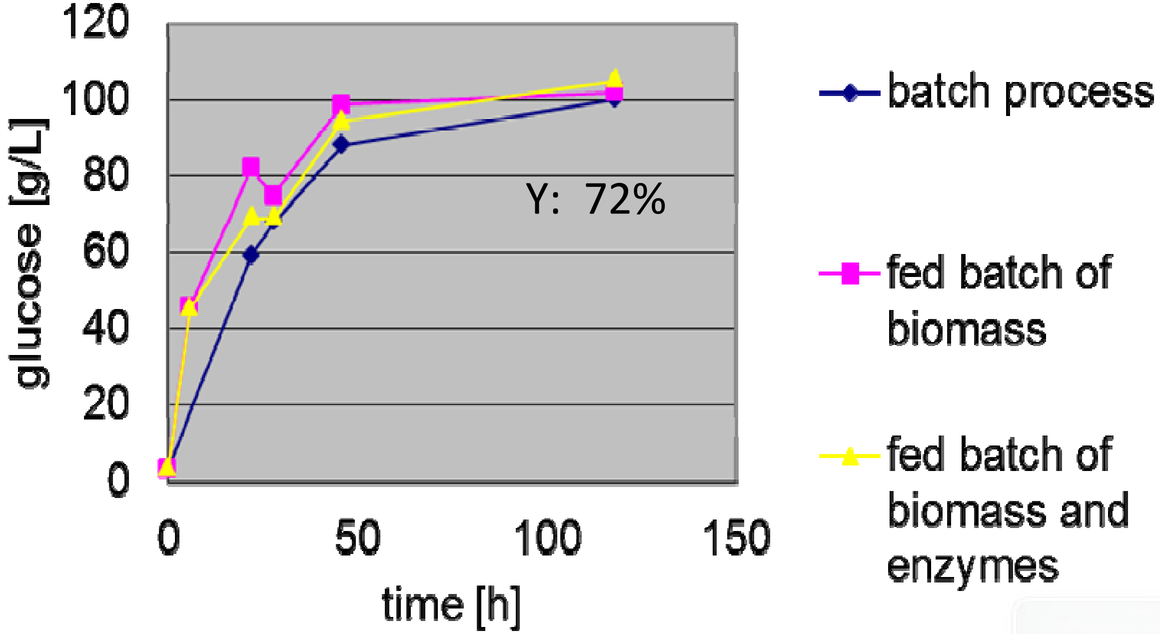
Effect of feeding strategies - Results

Different feeding strategies produced different results depending on the substrate, and hydrolysis set-up (namely, enzymatic cocktail, pH, bioreactor geometry, etc)

Meaningful differences



No meaningful differences

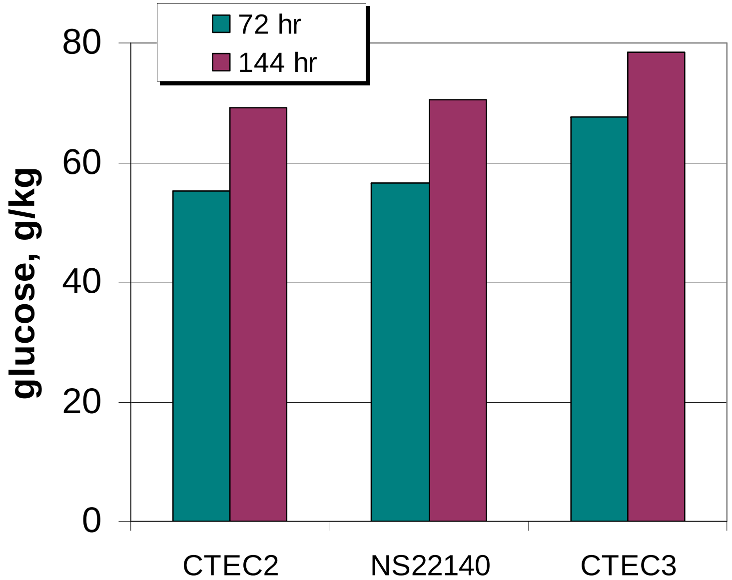


Substrate: *Arundo* fiber CTX 5710 (206°C + water);
Process conditions: T 50°C; pH 5.5; 18% s/l; 0.27 g_{CTEC2} /g_{GLUCAN}

Substrate: Arundo donax slurry, from ENEA acid catalyzed SE pretreatment
Process conditions: T 50°C; pH 5; 30% s/l; 0.27 $g_{ns22140}$ /g_{GLUCAN}

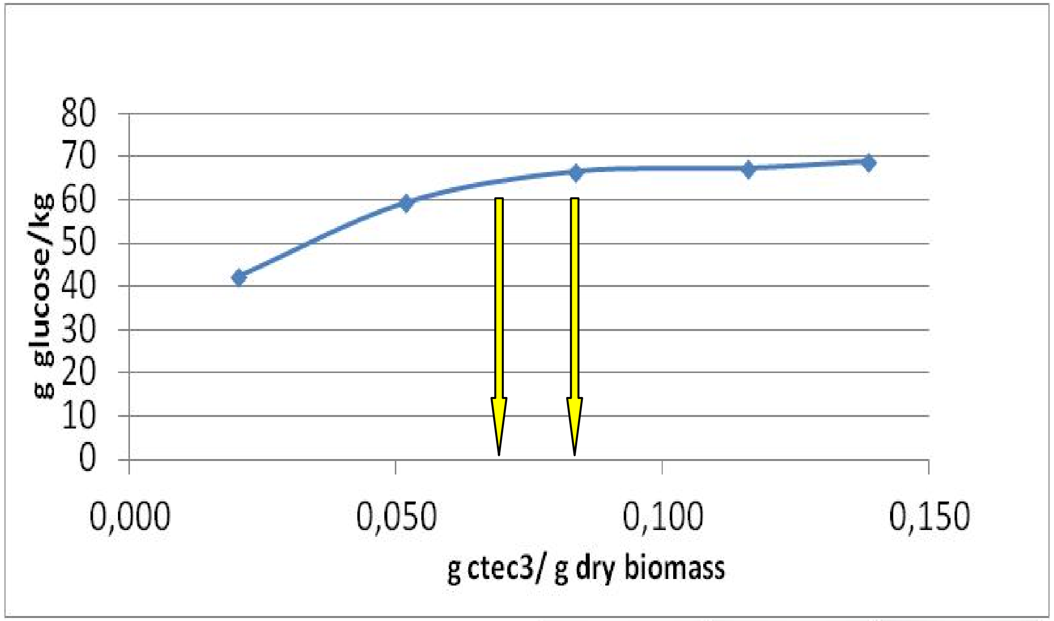
CTEC3 Performance

Hydrolyisis of the cellulose rich fraction of *Arundo donax* pretreated by acid-catalyzed steam explosion and water-washed (ENEA batch plant).



Experimental setup
T: 50°; 20%s/L; pH5
0.17g of enzyme/gram of biomass
Shaken flasks 500mL

ASSESSMENT OF THE MINIMUM CTEC3 DOSAGE

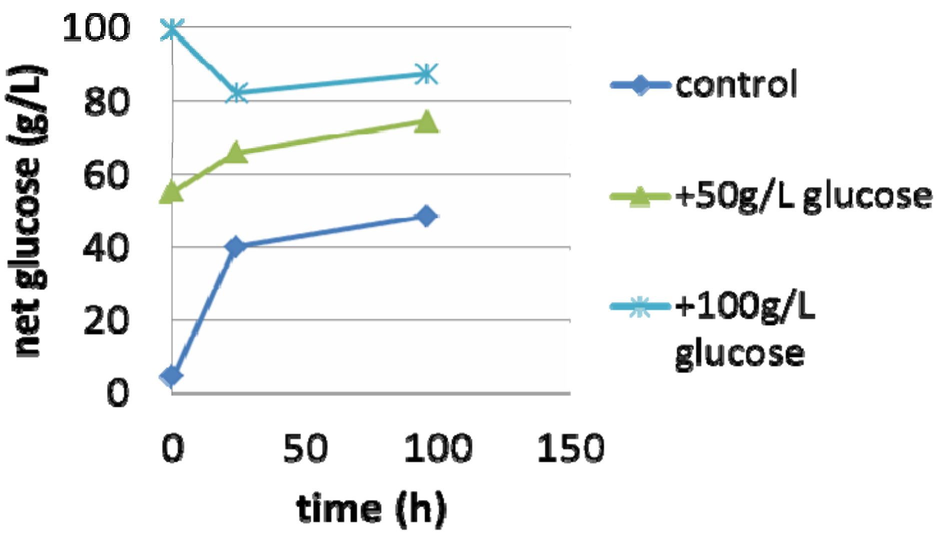


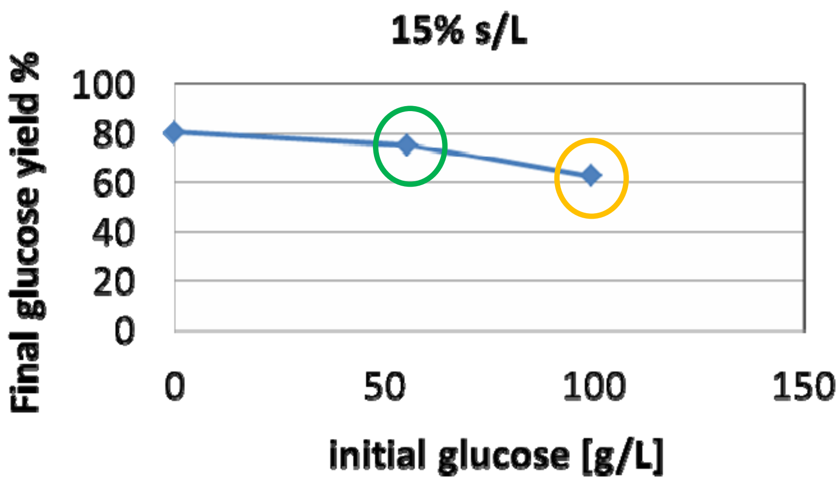
Experimental setup
T: 50°; 27%s/L; pH5; t: 48h
Shaken flask 500mL

CTEC3 inhibition by soluble glucose

In a fed-batch process, hydrolysis of fresh biomass by enzymes can be affected by the amount of soluble products.
The inhibition effect of soluble glucose was investigated during the hydrolysis

Process conditions: T=50° pH5; CTEC3 0,11g/g glucan





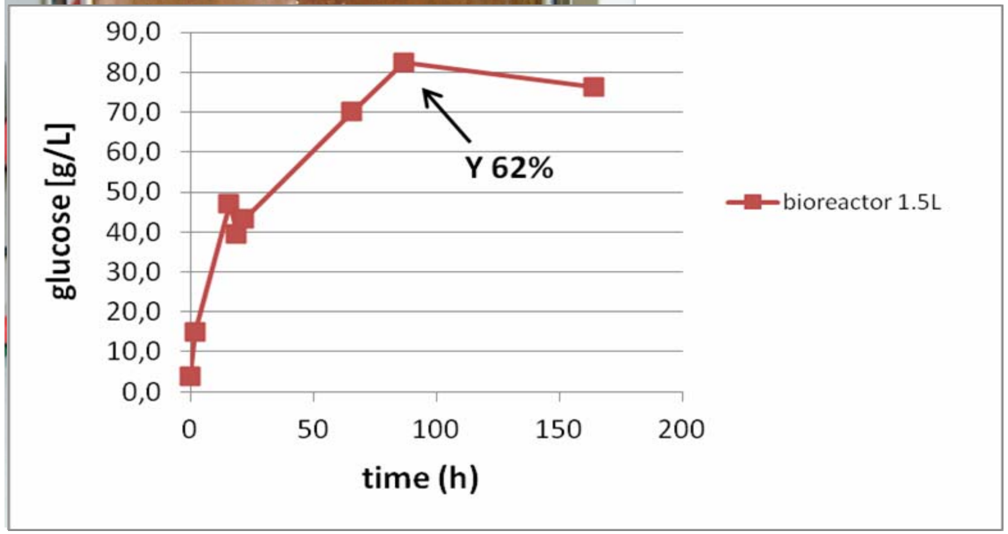
Glucose was slightly inhibiting toward the hydrolysis of fresh biomass at 15% s/L and enzyme-to-glucose ratio of 0.15 g/g.

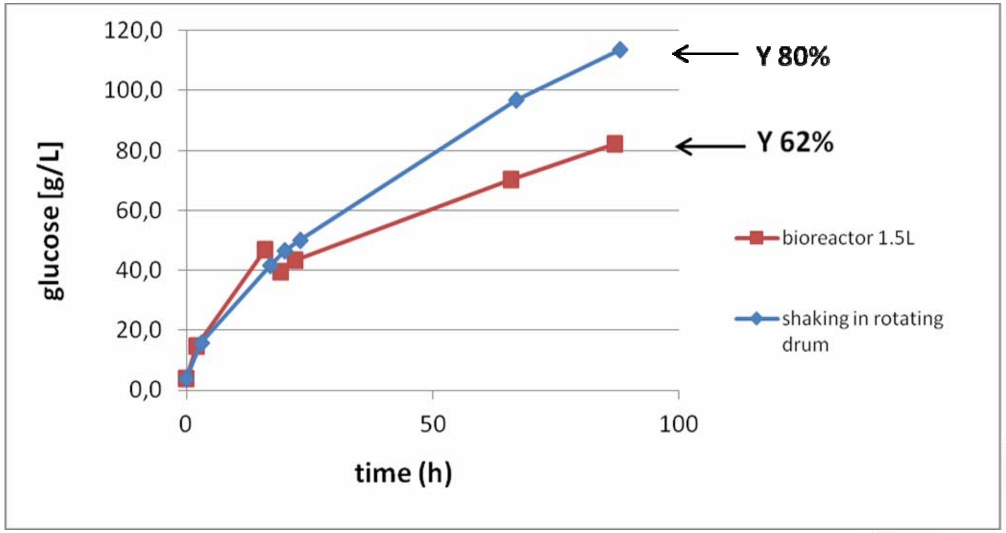
Higher glucose concentrations corresponding to 0.080 g/g enzyme-to-glucose ratio reduced the hydrolysis yield by 18%→ product removal could reduce the enzymes inhibition

Effect of the mixing geometry

At high DM content an effective mixing of the slurry could produce different process yields

Composition of the substrate: overall slurry from acid catalyzed steam explosion at 30% S/L
Process conditions: T 50°C; pH 5; CTEC3 0.08 g_{ENZYME} /$g_{GLUCAN;}$ fed-batch strategy





In vertical bioreactor, the addition of fresh biomass at 72 hours did not increase the glucose yield

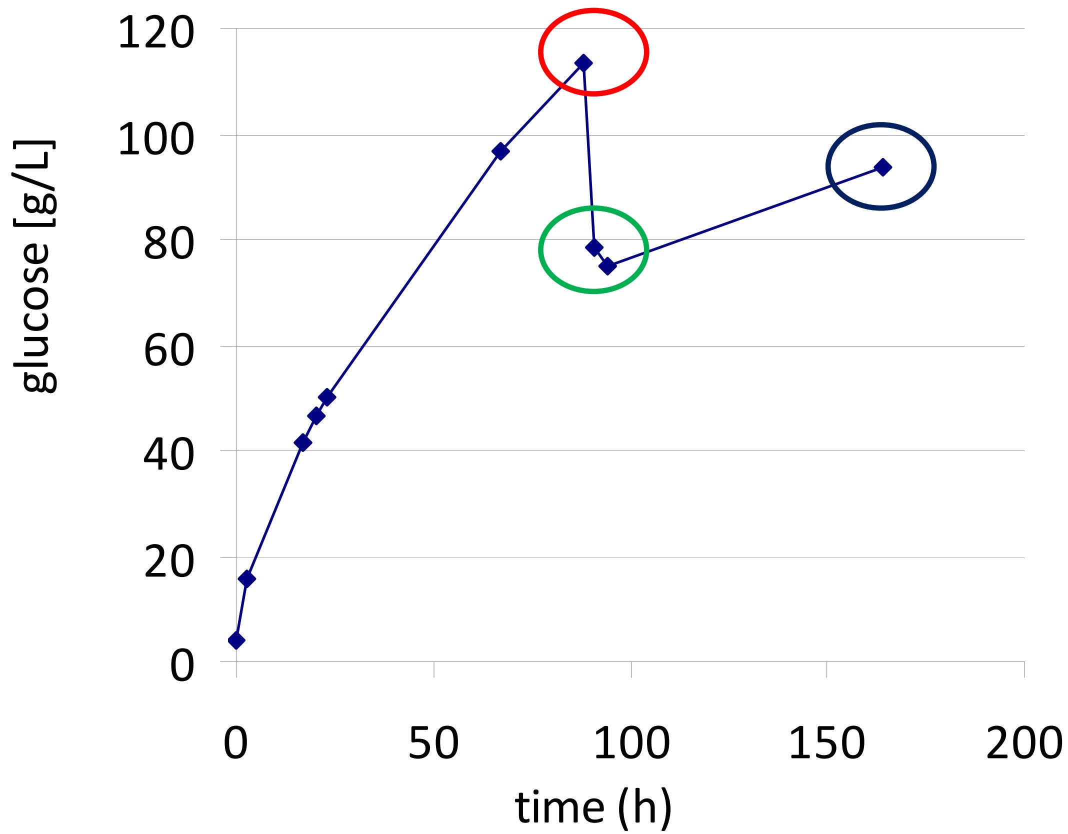
Gravimetric shaking in rotating drum system (containing flasks) was much more effective. The final glucose yield was **80%**

Bioreactor and impeller geometry are very important to improve the process yields

Hydrolysis by CTEC3 with product removal

TEST BY USING PRODUCT REMOVAL + GRAVIMETRIC SHAKING SYSTEM

Glucose yield at 88h: 80%



- Removal of part of the liquid hydrolyzate;
- addition of hemicellulose fraction
- Addition of fresh biomass;
- no addition of further enzyme

glucose concentration increased again: new **Y** 75%

Ctec3 maintained its activity in prolonged operations → recovery and reuse for several process batches.

Biomass liquefaction

BIOLYFE PROJECT

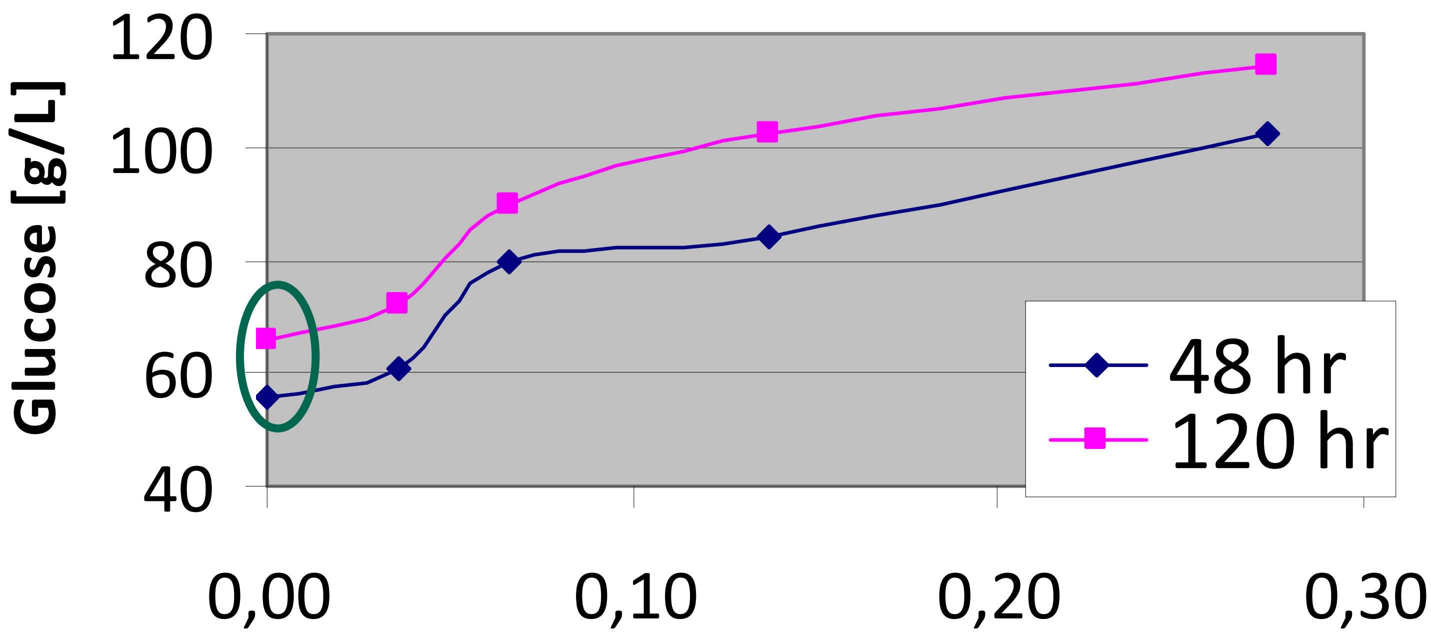
Arundo donax

Double-steps steam explosion pretreatment

Enzymatic liquefaction at high dry matter level

Simultaneous saccharification and cofermentation (SSF) with different microrganisms

Hydrolizability of the product from CTXI viscosity reduction step



g_{CTEC3}/g_{GLUCAN}

Conditions: 50° pH5
500mL shaken flasks
Enzyme: CTEC3

- The activity of the original enzymes can be reduced by various handling steps
- Need to adding fresh CTEC3 to enhance the cellulose conversion

Simultaneous saccharification and fermentation of pre-liquefied biomass

YEASTS FOR FERMENTATION TESTS

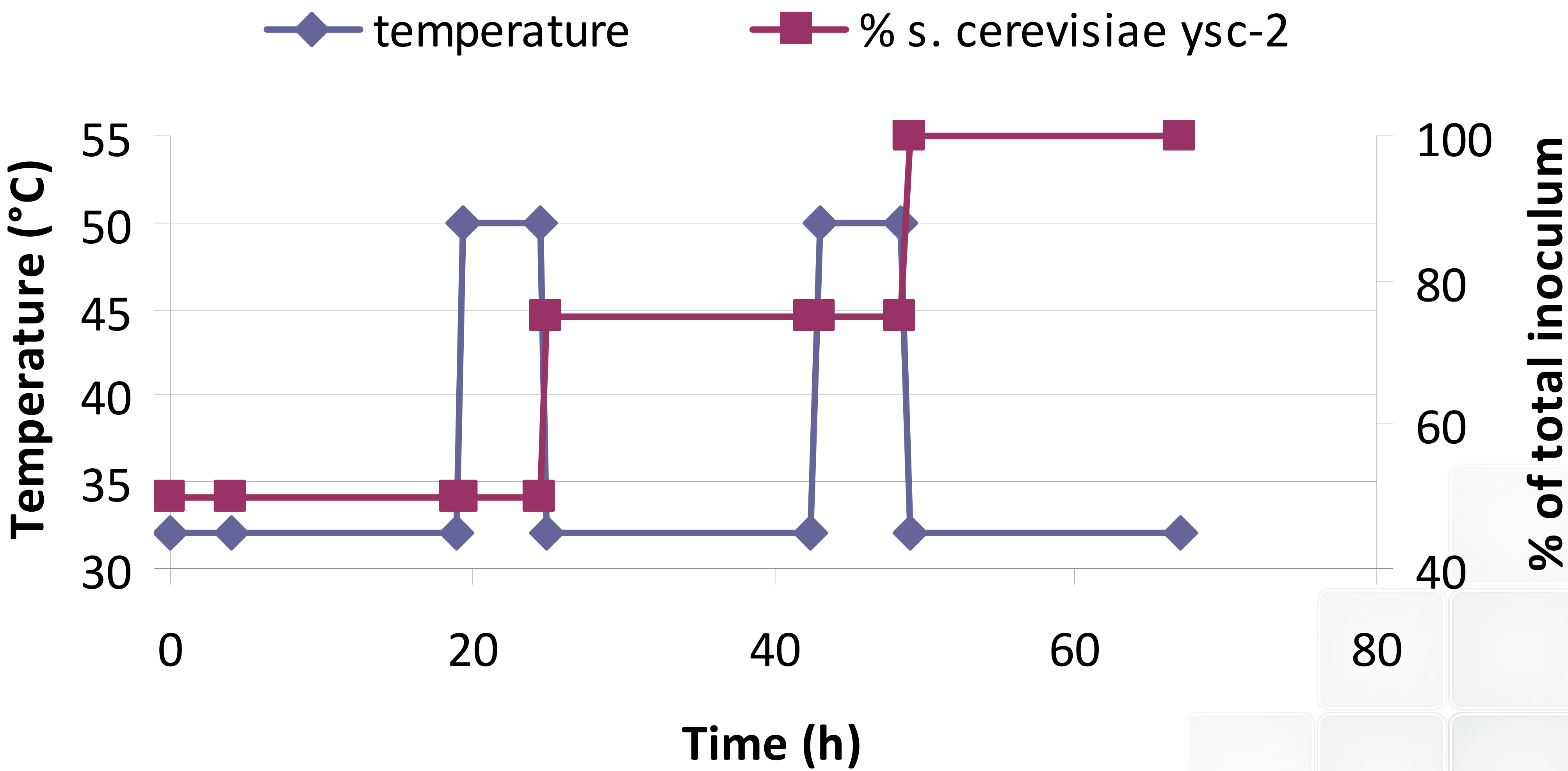
- ***Saccharomyces cerevisiae* (SIGMA II)**
- ***Saccharomyces cerevisiae M861 (isolated by ENEA and alcohol tolerant)***

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

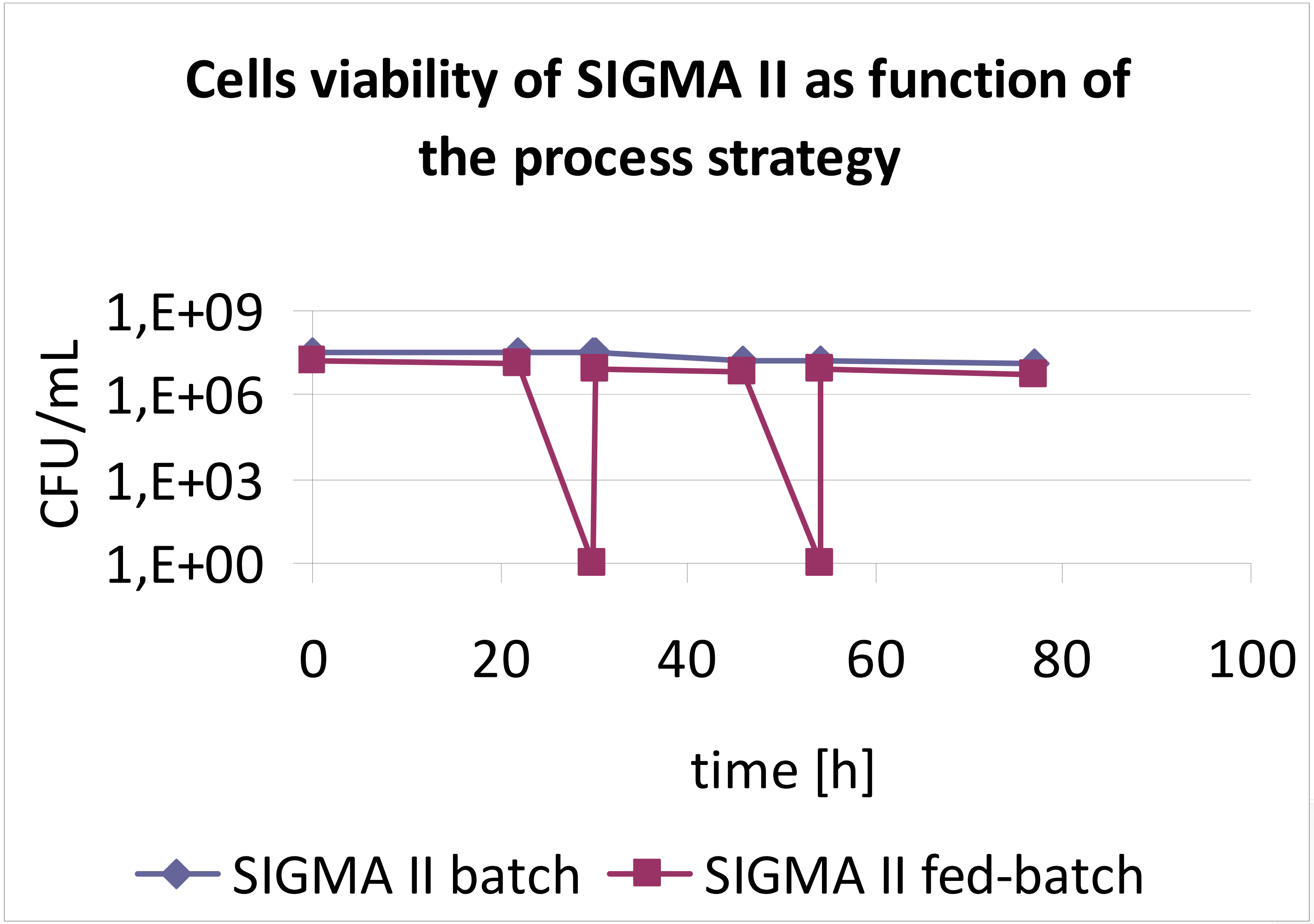
SSF set-up

Two SSF set-ups were evaluated to *increase the cellulose hydrolysis*:

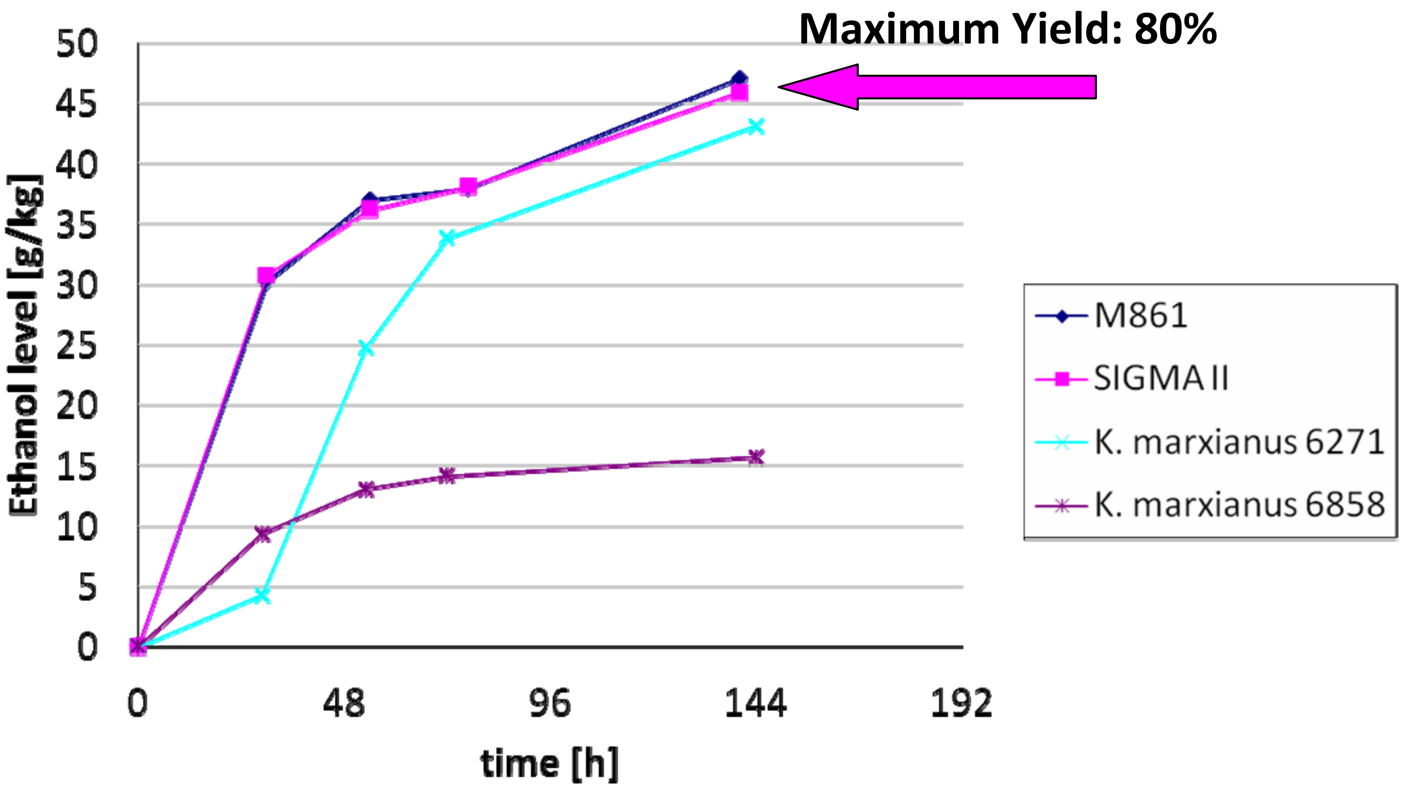
1. SSF at common process conditions
2. SSF with intermittent step-wise increase of temperature



Typical cells viability during SSF with modulation of temperature



Traditional SSF

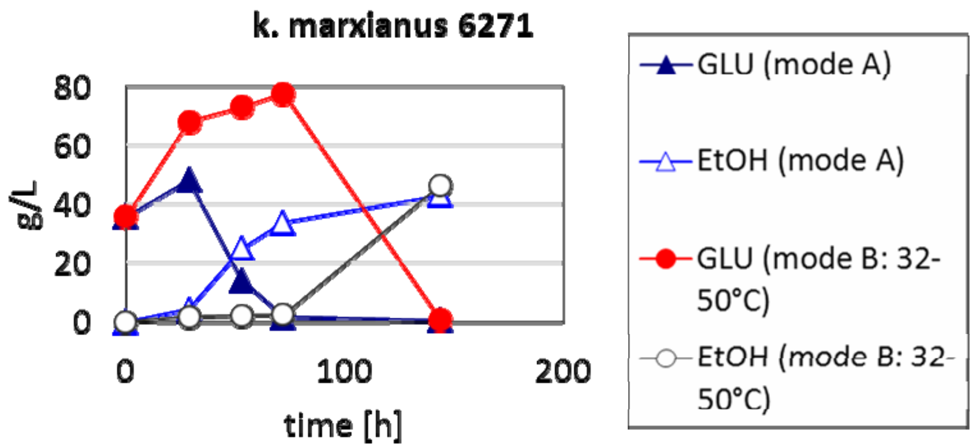


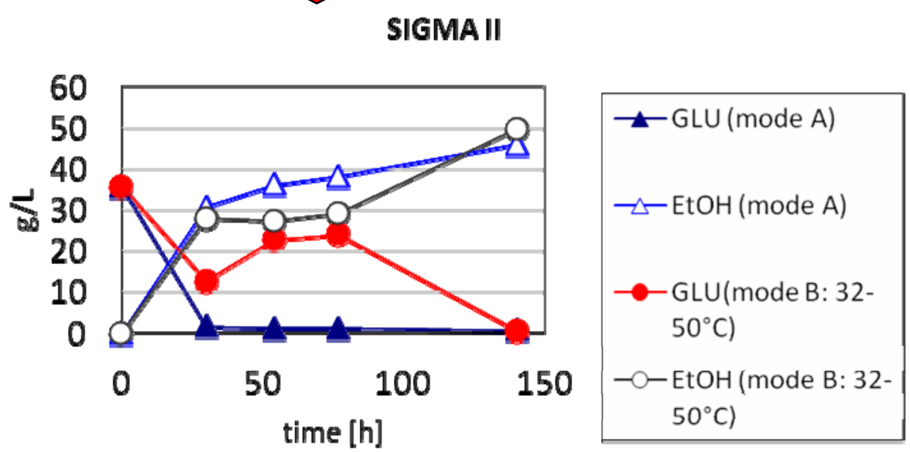
Substrate: product from the CHEMTEX viscosity reduction step
Conditions: 32° pH5
500mL shaken flasks
Enzyme: CTEC3 0.23g/g of residual glucan
Yeast seeding: 4g/L

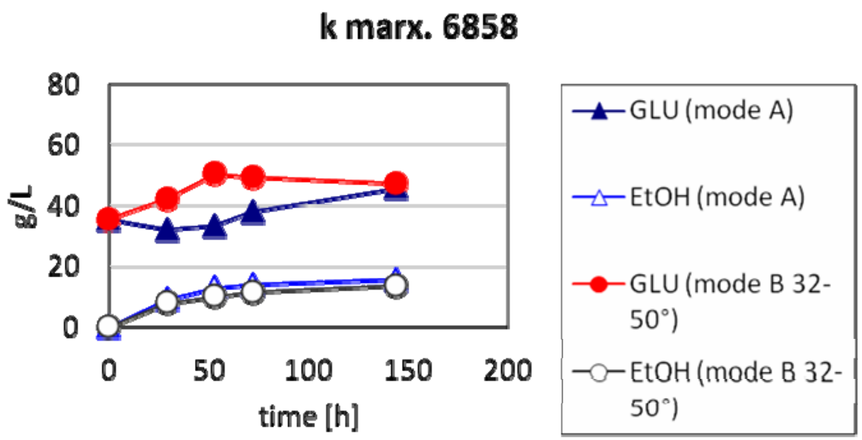
Traditional SSF compared to SSF with the temperature modulation

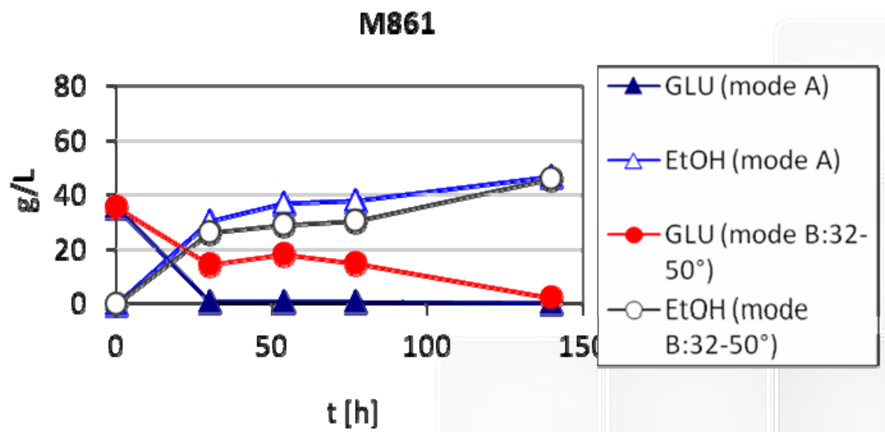
K. marxianus strains

S. cerevisiae strains









SSF at different process conditions

yeast	temperature	pH	g CTEC3/g residual glucan	ethanol yield%
SIGMA II	32°	5	0,22	80
SIGMA II	32°	5	0,09	55
SIGMA II	37°	5	0,09	61

The maximum ethanol yields from glucose was 80 % by using 0.22 g ENZYME/g glucan (46 g/L)
The process yields at higher temperature and lower enzymes loading were lower (36 g/L)

Hydrolysis of residual cellulose in the hybrid SSF process is the bottle neck of the process.
~2.5 times more enzyme than in the hydrolysis tests at optimized conditions (T 50°C) is required to achieve comparable process yields around 80%.

Conclusions

1. ACSEP at 200°C and the Chemtex process in which the SE step was carried out at 206 and 4 min had similar hydrolizability
2. CTEC3 is 1.5 more effective than CTEC2 and NS22140
3. Hydrolysis at high DM content (~30%) by using 80 mgCTEC3/g GLUCAN produced 80% glucose yields (GLU+XYL=137 g/L)
4. Optimization of the process strategy includes *fed-batch* feeding of biomass and enzymes along with optimized mixing conditions
5. Inhibition of enzymes by glucose was observed at 100 g/L glucose and enzymes-to-glucose ratio of 0.08 g/g
6. Enzymes adsorption on fresh biomass and product removal enables the enzymes recovery and reuse.
7. ~2.5 times more enzyme than in the hydrolysis tests at optimized conditions (T 50°C) is required to achieve comparable SSF process yields of 80%.
8. Hydrolysis of residual cellulose in the hybrid SSF process is the bottle neck of the process.

Thanks for the attention

For further information: isabella.debari@enea.it