2nd International Conference on Sustainable Solid Waste Management

12- 14 June, 2014 - Athens, Greece

The effect of acid pretreatment on bio-ethanol and bio-hydrogen production from sunflower straw

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FORTH/ICE-HT

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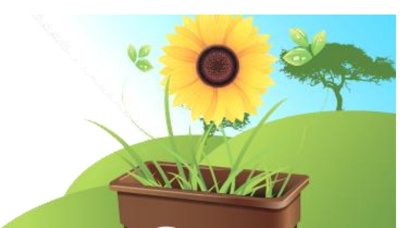
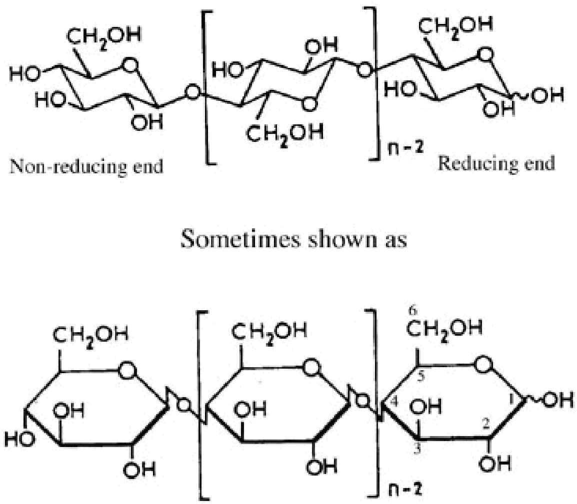
Sunflower straw (SS)

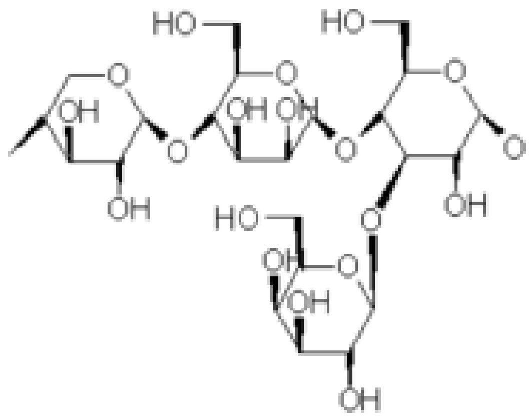
- Sunflower is the fourth oil-seed source worldwide with more than 25 million hectares cultivated land surface
- Sunflower has a high-lipid content seed and represents an important resource for biodiesel production.
- While it is mainly harvested for its oil, the remainder of the plant, such as the straw, remains to a large extent unutilized.
- Each hectare of sunflower culture could produce 3-7tons of dry biomass, including heads and stalks *(Marechal and Rigal, 1999)*
- In order to maintain a competitive advantage in the world market and ensure a sustained economic return in the production of this oil crop, the potential of conversion of this **lignocellulosic residue** in bioenergy should be explored and exploited.



Lignocellulosic biomass

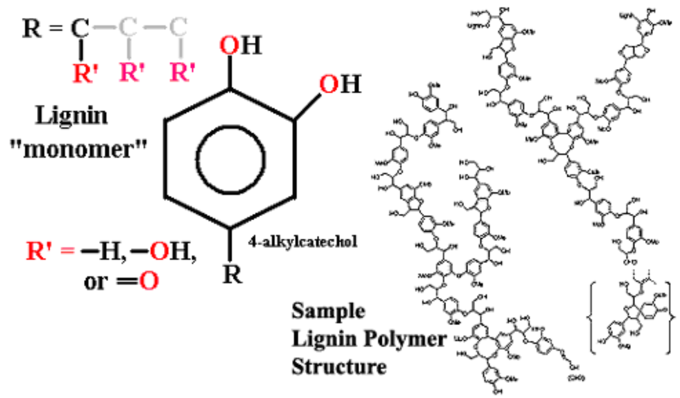


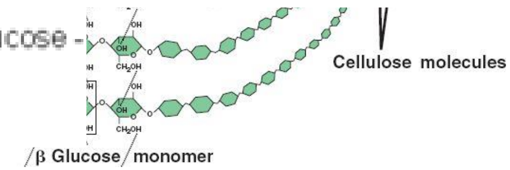
Cellulose

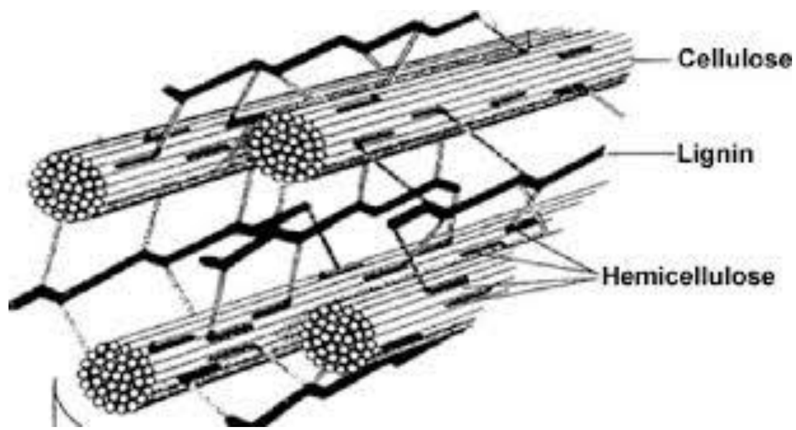


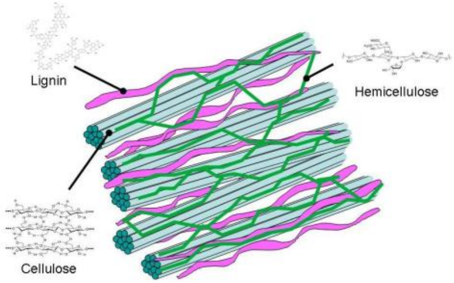
- Xylose - ß(1,4) - Mannose - ß(1,4) - Glucose -
- alpha(1,3) - Galactose

Hemicellulose



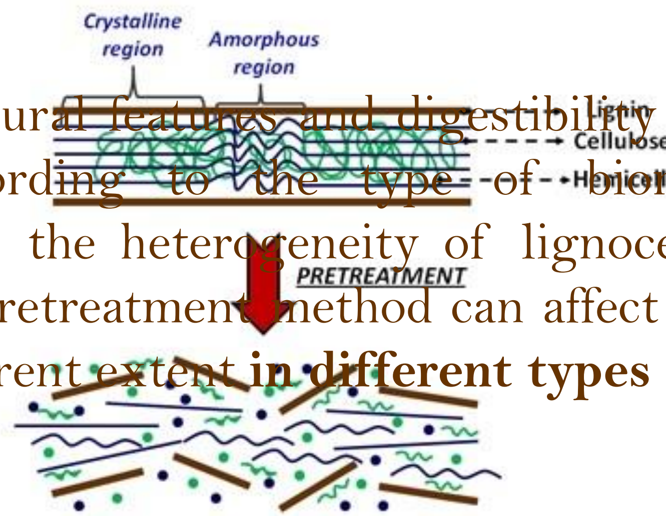






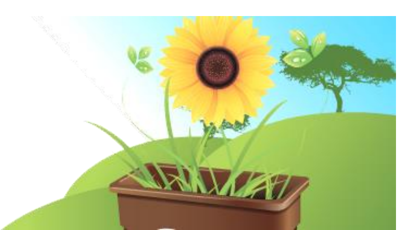
Pretreatment

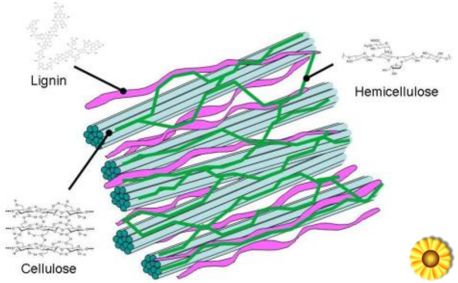
- Pretreatment is commonly accepted to be an essential prerequisite to make lignocellulosic biomass accessible to enzymatic attack, by breaking the lignin seal, removing hemicellulose, or disrupting the crystalline structure of cellulose (*Fan et al., 1981*)



- The correlation of structural features and digestibility is reported to be of variant importance according to the type of biomass to be treated Consequently, because of the heterogeneity of lignocellulosic biomass the application of the same pretreatment method can affect in a different degree and have an effect of different extent **in different types of biomass**.

Best pretreatment method for **intended bioconversion**





Pretreatment

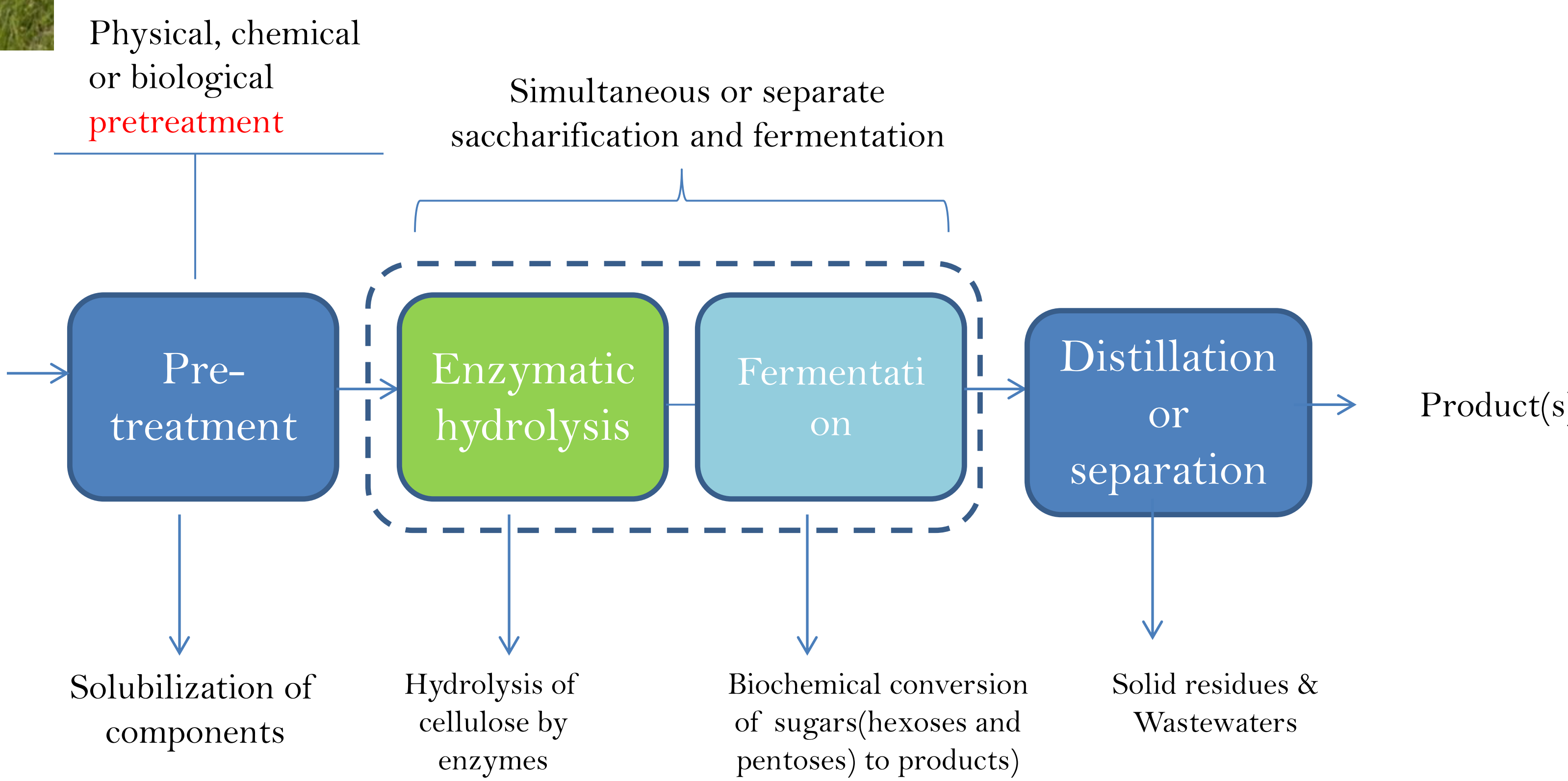
- A variety of pretreatment including mechanical, thermochemical or thermal processes, have been developed for changing the chemical and structural composition of biomass and improving the enzymatic conversion efficiency *(Hendriks and Zeeman, 2009).*

- Acid pretreatment, has been proposed as an efficient pretreatment method for ethanol or fermentative hydrogen production *(Schell et al., 2003; Sanchez et al., 2004; Garcia et al., 2014)*

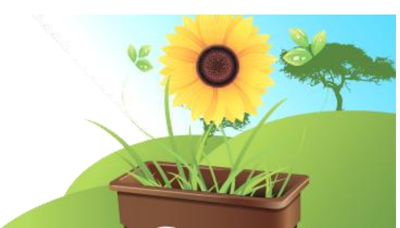
- During acid pretreatment, hemicellulosic fraction of biomass is hydrolyzed to soluble sugars and compounds such as furfural and/or hydroxylmethylfurfural (HMF), formic and acetic acids can also be observed *(Ramos, 2003).*

- These compounds might have an inhibitory or toxic effect on bacteria or yeasts that are used in subsequent bioconversions.

Biochemical processing



✓Cost efficient production of sugars from biomass is a key pre-requisite for any biorefinary based on sugar route.



The process followed….

Acid Pre-treatment

Simultaneous saccharification and fermentation (SSF)

Chemical agent	Concentration (g/100gTS)	Conditions
H_2SO_4	2, 10, 20	1h, 120°C
H_3PO_4	2, 10, 20	1h, 120°C
HCl	2, 10, 20	1h, 120°C
no chemical	thermal	1h, 120°C

Ethanol

Hydrogen

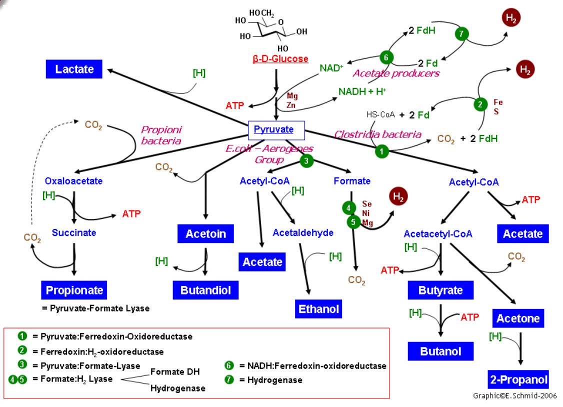
Solubilization of hemicellulose

Hydrolysis of cellulose by enzymes

Biochemical conversion of sugars(hexoses and pentoses) to products)

- **For all pretreatment methods**: mass ratio of solid(g) to liquid(mL) was 5:100 (organic load 5% w/v)

Why hydrogen?



- A clean and environmentally friendly fuel which produces water instead of greenhouse gases, when combusted
- Possesses a high-energy yield (122 kJ/g)
- Could be directly used to produce electricity through fuel cells
- Can be produced by renewable raw materials, such as wastes / wastewaters and energy crops through biological processes

Fermentative hydrogen production

Acetic acid production
- $C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$

Butyric acid production
- $C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$

Propionic acid production
- $C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$

Lactate production
- $C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH$

Ethanol production
- $C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$

Mixed acid fermentations

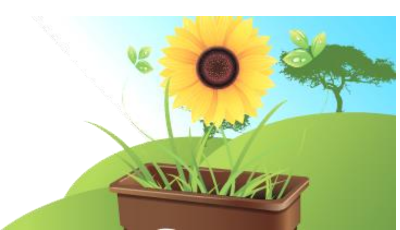
↓

lower yields of H_2

✓The absence or presence of hydrogen-consuming microorganisms in the microbial consortium also affects the microbial metabolic balance

✓Mixed cultures need to be pretreated in order to suppress hydrogen-consuming bacterial activity while still preserving the activity of the hydrogen-producing bacteria

→ Heat treatment (100°C, 15min)



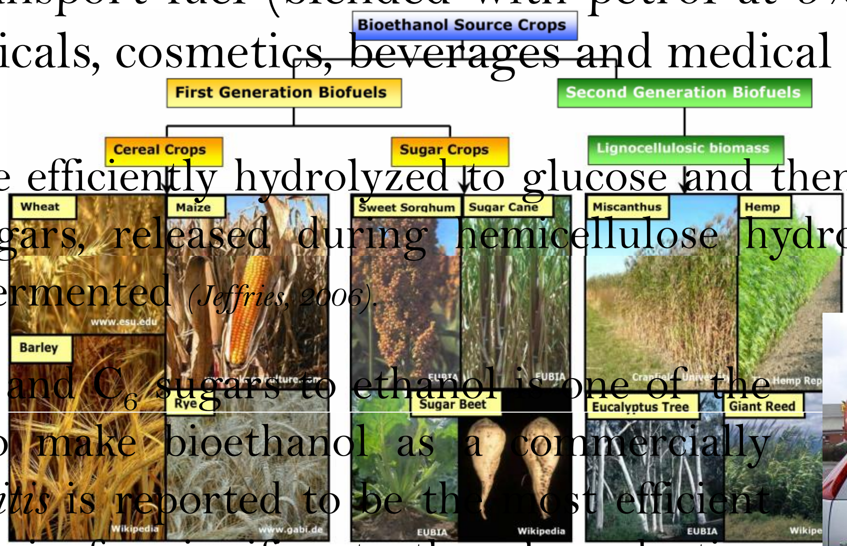
Bioethanol production

- The production of bioethanol from traditional means, or ***1st generation bioethanol*** is based upon ***starch crops*** (corn and wheat) and from ***sugar crops*** (sugar cane and sugar beet)

- It could be used as transport fuel (blended with petrol at 5%). A wide range of uses in the pharmaceuticals, cosmetics, beverages and medical sectors

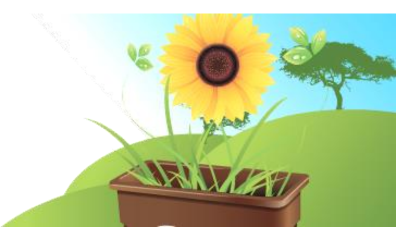
- Although cellulose can be efficiently hydrolyzed to glucose and then fermented to ethanol, xylose and other C_5 sugars, released during hemicellulose hydrolysis, are much more difficult to be efficiently fermented *(Jeffries, 2006)*.

- Bioconversion of both C_5 and C_6 sugars to ethanol is one of the most important issues to make bioethanol as a commercially viable product. *Pichia stipitis* is reported to be the most efficient and highly productive strain for significant ethanol production from xylose *(Cheng et al., 2008)*.



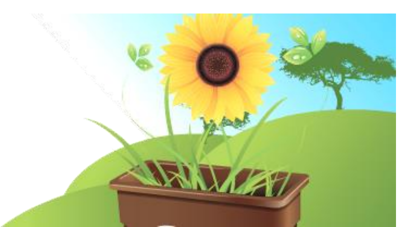
Chemical composition

Characteristic	Value
TS (%)	90.90 ± 0.04
VS (%TS	79.54 ± 0.09
Cellulose (%TS)	27.79 ± 2.57
Hemicellulose (%TS)	18.73 ± 2.37
Lignin (%TS)	22.34±0.26
Ash (%TS)	1.09±0.56



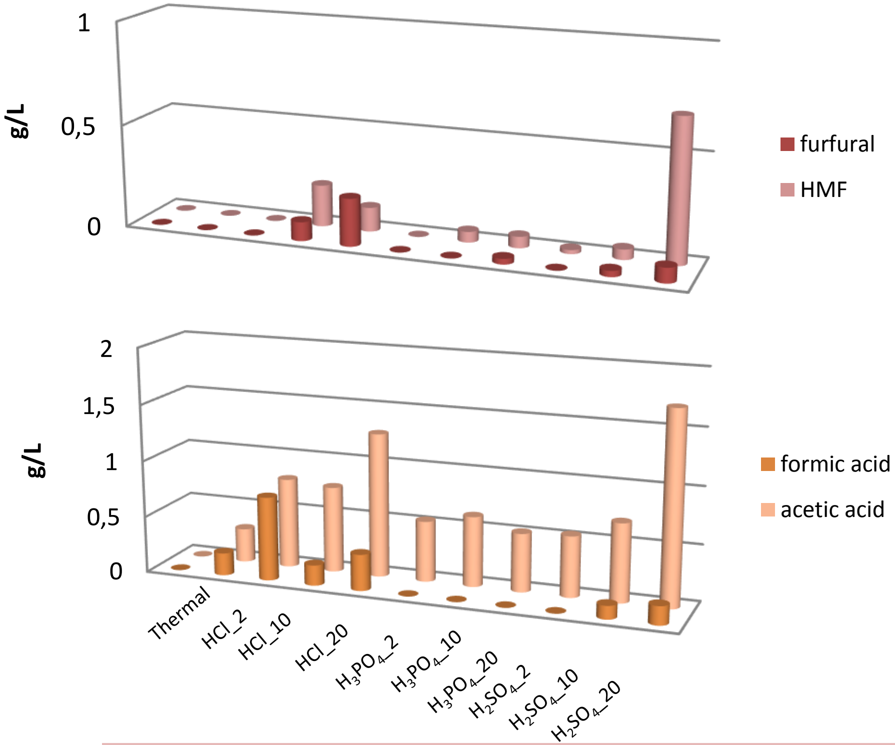
Chemical composition after pretreatment

Pretreatment		Cellulose (g/100gTS)	Hemicellulose (g/100gTS)	Lignin (g/100gTS)
no	thermal	28.49 ± 0.21	18.26 ± 0.37	22.11 ± 1.31
H_2SO_4	2 g/100gTS	27.74 ± 1.22	13.41 ± 1.00	20.80± 0.35
	10 g/100gTS	29.87 ± 0.27	10.51 ± 0.36	21.36 ± 1.28
	20 g/100gTS	29.09 ± 0.14	7.60 ± 2.80	20.78± 0.14
H_3PO_4	2 g/100gTS	26.46 ± 2.11	13.81 ± 2.14	20.55 ± 0.77
	10 g/100gTS	27.22 ± 0.42	11.89 ± 0.62	20.06 ± 0.07
	20 g/100gTS	27.65 ± 0.60	10.65 ± 0.29	19.41 ± 0.35
HCl	2 g/100gTS	27.24 ± 1.97	13.53 ± 0.41	19.11 ± 0.76
	10 g/100gTS	27.59 ± 0.58	6.66 ± 2.36	19.21 ± 1.56
	20 g/100gTS	26.46 ± 0.26	4.32 ± 0.35	18.56 ± 0.56



Chemical composition after pretreatment

Pretreatment		Sugars (g/100gTS)
no	raw	2.71 ± 0.16
	thermal	2.80 ± 0.13
H_2SO_4	2 g/100gTS	3.74 ± 1.22
	10 g/100gTS	8.69 ± 0.12
	20 g/100gTS	13.79 ± 0.12
H_3PO_4	2 g/100gTS	2.78 ± 0.19
	10 g/100gTS	3.48 ± 0.01
	20 g/100gTS	5.30± 0.60
HCl	2 g/100gTS	2.87± 0.01
	10 g/100gTS	11.36 ± 0.92
	20 g/100gTS	12.68 ± 2.01

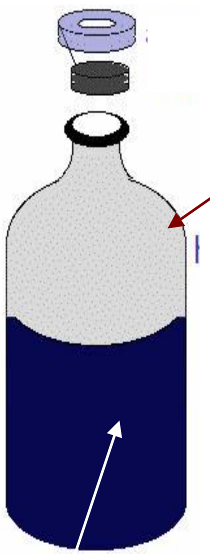


✓Treatment with HCl of 10 and 20g/100gTS and H_2SO_4 at 20g /100gTS, led to higher hemicellulose solubilisation and higher furfural and HMF concentration values

✓The higher the acid concentration, the higher the HMF and furfural which was produced (*Hendriks, and Zeeman, 2009*).

Fermentative hydrogen production

160 mL serum vials



Headspace

Inoculum (20%v/v)

Biogas measurement

Hydrogen content

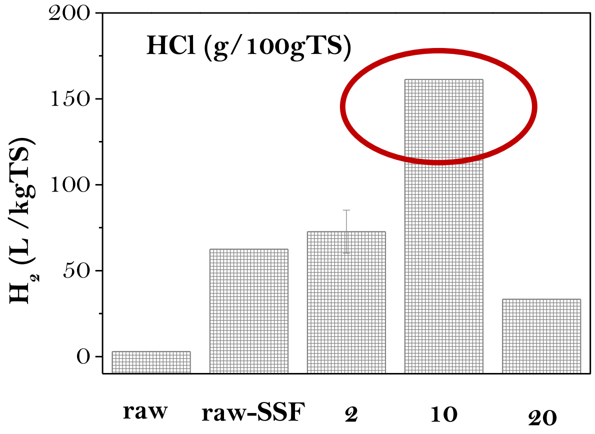
- **Inoculum:** Anaerobic sludge from the anaerobic digester of Patras wastewater treatment plant: **boiled for 15min at 100°C**
- **Assays**: In 160mL serum bottles, at 35°C: **10mL inoculum** + **30mL synthetic medium** + 10mL whole pretreated biomass (0. 5gTS SS + 10 mL of each chemical agent)→5% organic load
- **Mixture of Celluclast 1.5L + Novozyme at a ratio of 3:1, (30FPU Celluclast/gTS)**

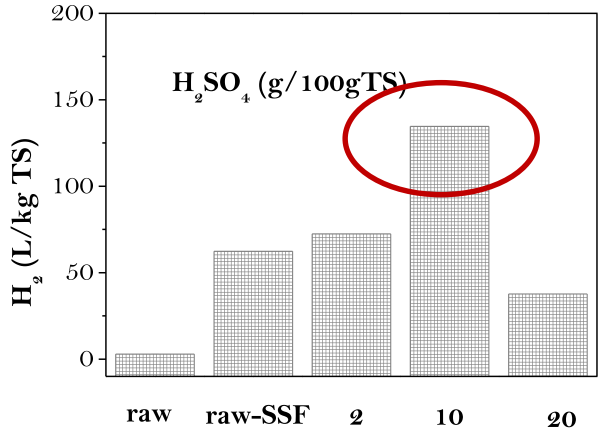
Celluclast 1.5L: Cellulase from *Trichoderma reesei*, ATCC 26921

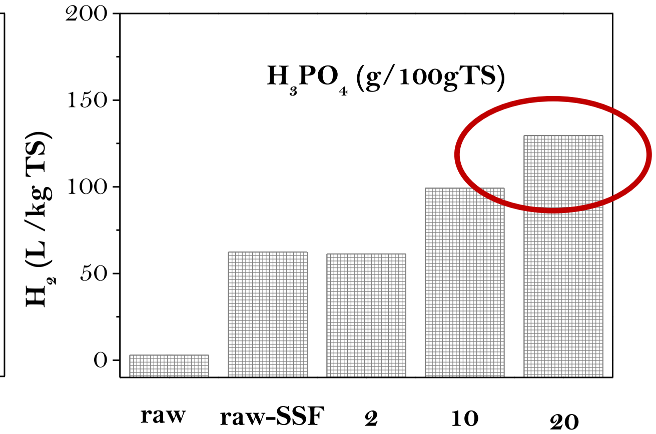
Novozyme: Cellobiase from *Aspergillus niger*

- **Agitation at 100rpm at 35°C**

Fermentative hydrogen production

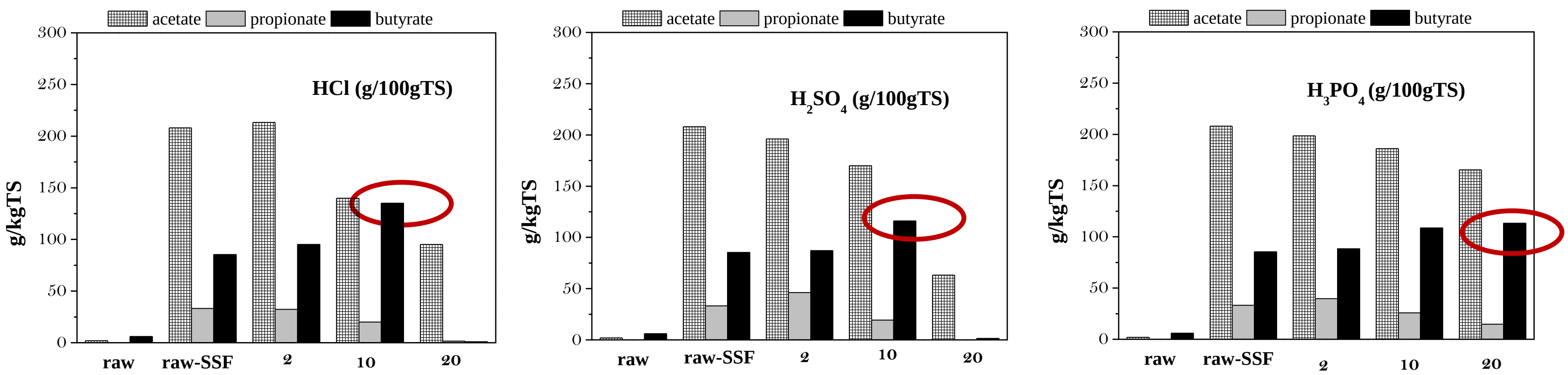






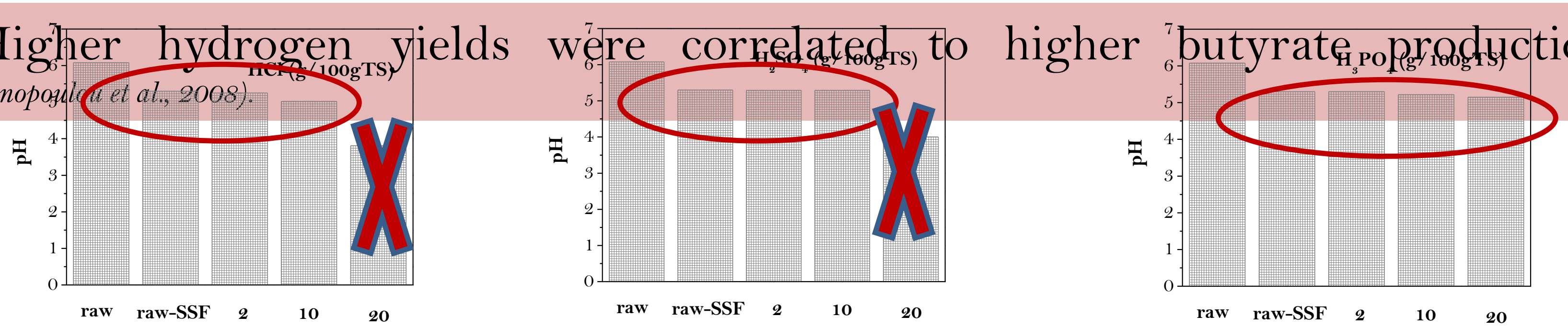
✓Treatment with HCl and H_2SO_4 of 10g/100gTS and H_3PO_4 of 20g/100gTS led to **higher hydrogen yields**.
✓Treatment with HCl 10g/100gTS caused a **159% increase** when compared with the untreated sample (in a SSF concept- **62.4L/kg TS**).
✓Concentration of HCl and H_2SO_4 of 20g/100gTS caused an inhibition to hydrogen production microorganisms.

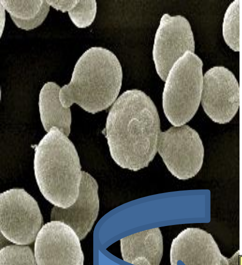
Fermentative hydrogen production



- $C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$

✓Higher hydrogen yields were correlated to higher butyrate production *(Antonopoulou et al., 2008)*.





Ethanol fermentation experiments

Pichia Stipitis (strain CECT 1922)

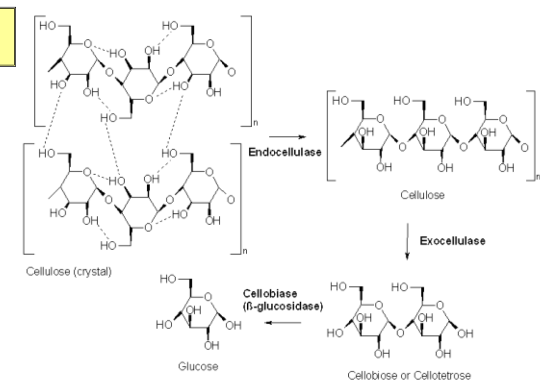
- Solution (g/L): KH_2PO_4, (1) $MgCl_2*6H_2O$, (1) $(NH_4)_2SO_4$, (1)
- **C5 and C6 sugars uptake**
- **Main metabolic products: Ethanol -xylitol**

The whole pretreated biomass (0.75gTS SS + 15 mL of each chemical agent)→5% organic load

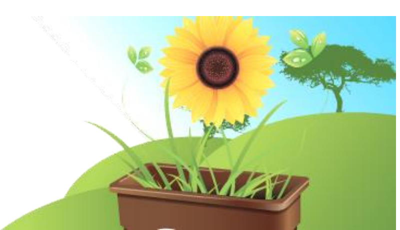
- **Mixture of Celluclast 1.5L + Novozyme at a ratio of 3:1, (30FPU Celluclast/gTS)**

Cellulase from *Trichoderma reesei*, ATCC 26921

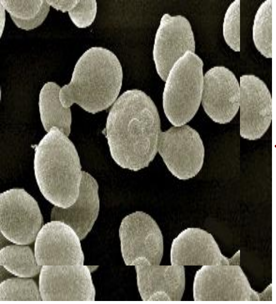
Cellobiase from *Aspergillus niger*

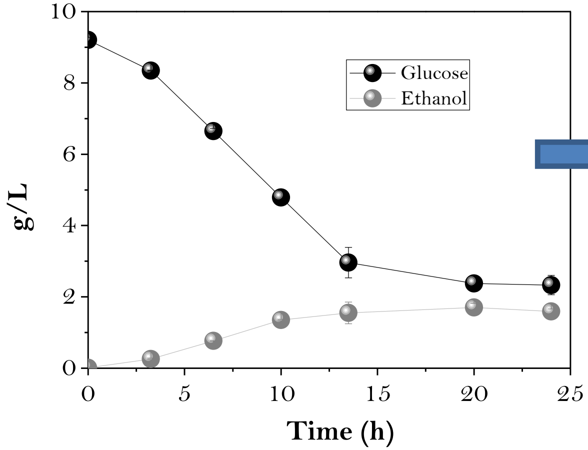


- **Adjustment of initial pH at 5**
- **Agitation at 100rpm at 30°C**
- **Fermentation for 48h**



Ethanol fermentation experiments



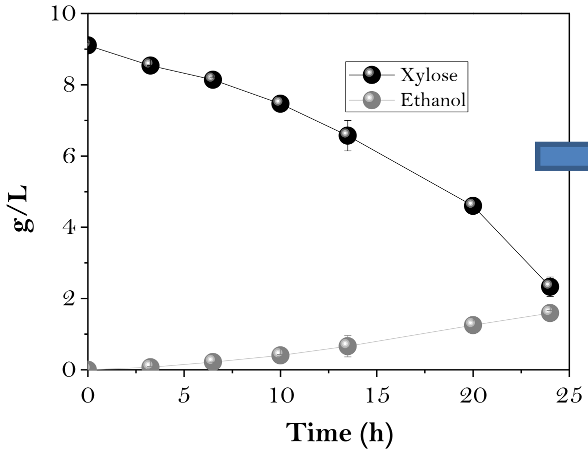


0.28 g ethanol/g glucose

1.08 mol ethanol /mol glucose

- $C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$

54 % of maximum theoretical

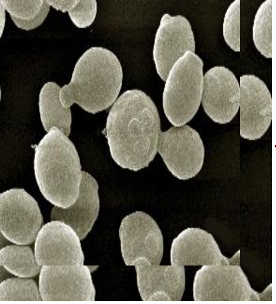


0.28 g ethanol/g xylose

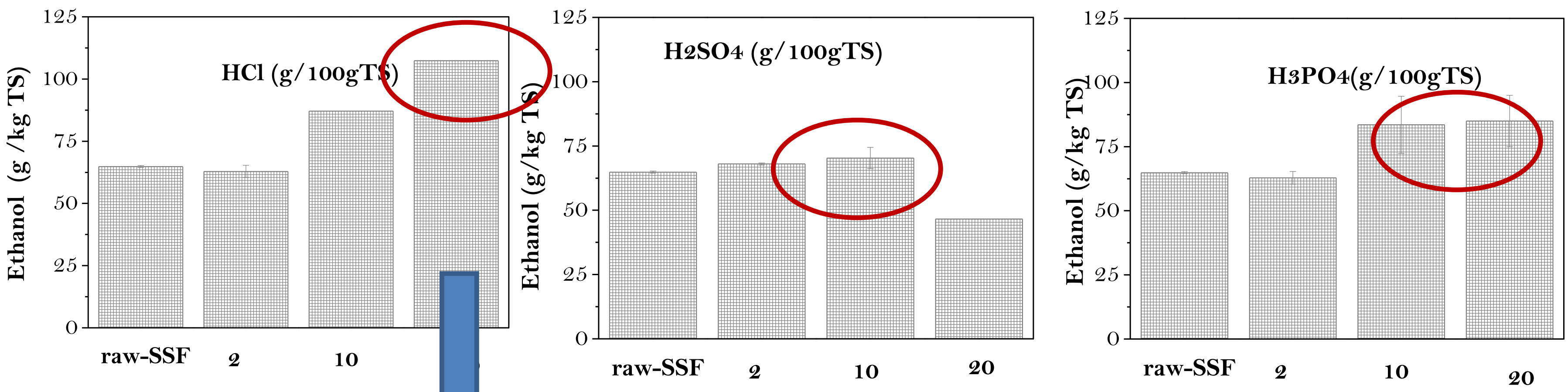
0.91 mol ethanol /mol xylose

- $3C_5H_{10}O_5 \rightarrow 5CH_3CH_2OH + 5CO_2 + 5 H2O$

55 % of maximum theoretical

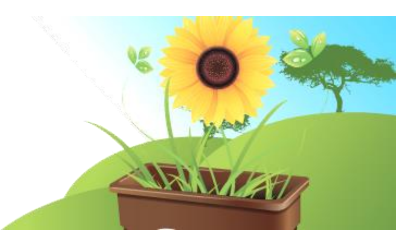


Ethanol fermentation experiments



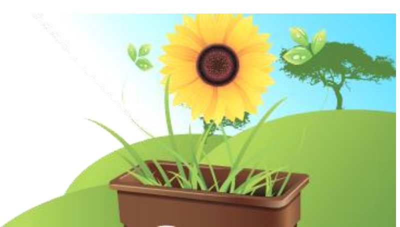
- ✓Treatment with HCl and H_3PO_4 of 20g/100gTS led to **higher ethanol yields**.
- ✓Concentration of H_2SO_4 of 20g/100gTS caused an inhibition to *P.stipitis.*

78% of the maximum obtained



Conclusions

- Acid pretreatment could be used to enhance hydrogen and ethanol production from SS
- The optimum, for hydrogen production, pretreatment method, was not the optimum for ethanol production, implying that the same pretreatment method is not appropriate for all subsequent bio-conversion processes.
- Pretreatment with HCl seems to be more effective. HCl at 10g/100gTS led to 161.35 LH_2/kg TS while HCl at 20g/100gTS led to 107 g ethanol/kg TS.
- Higher hydrogen yields were correlated to higher butyrate production
- Pretreatment with H_2SO_4 at 20g/100gTS led to higher furfural and HMF concentration and led to lower ethanol and hydrogen production yields.



Thank you very much!

General Secretariat for Research and Technology
"Supporting Postdoctoral Researchers Projects" - Pretreatment of lignocellulosic wastes for 2nd generation biofuels (POSTDOC _ PE8(1756)) (post-doc fellowship of Dr. G. Antonopoulou).