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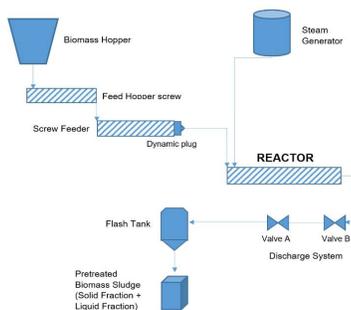
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1. Aim & Background

To investigate the fermentative capacity of the hydrolysate generated by **steam explosion** of two feedstocks, **switchgrass** (*Panicum virgatum*) and ***Eucalyptus globulus***, to produce coproducts of value. A evolved strain of *Kluyveromyces marxianus* was the yeast strain used in the experiments.

The production of biofuels from lignocellulosic biomass has environmental and social advantages. Several strategies, feedstocks and pretreatments are being studied to overcome the obstacles of this process and increase the process efficiency and economic viability. Using all the fractions for the production of industrially relevant compounds is one of the strategies to improve the competitiveness of ethanol production.

2. Steam explosion continuous pre-pilot plant



- ❖ Approximate capacity of 10 kg/h, depending on conditions and biomass
- ❖ Maximum working pressure 15 bar, maximum temperature 200 °C, controlled by a PLC that also regulates and sets different screw speeds (hopper screw, feeding screw and reactor screw).



Sludge

Filtration by pressing machine

Solid Fraction (Lignin + Cellulose)

Enzymatic hydrolysis (glucose + solid residue of lignin)

Production of bioethanol with *Saccharomyces cerevisiae*

Liquid Fraction Hemicellulose Phenolic compounds

Hydrolysis with sulfuric acid

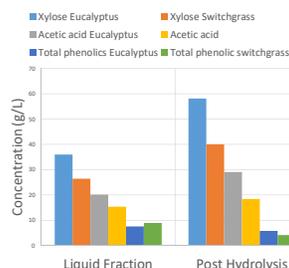
Fermentation with *Kluyveromyces marxianus* (Xylitol production)

3. Hydrolysate composition: pre and post hydrolysis

- ❖ Analysis of the liquid fraction showed that there was significant amount of sugar oligomers.
- ❖ Post – hydrolysis with sulfuric acid to 4 % and 1 h in autoclave (according to NREL Standard Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples) increased concentration of xylose by approximately 50% in both liquid fractions.
- ❖ Increased acetic acid content, more remarkable in the case of the Eucalyptus, due to xylan composition
- ❖ Precipitation of phenolic compounds

Hydrolysates composition

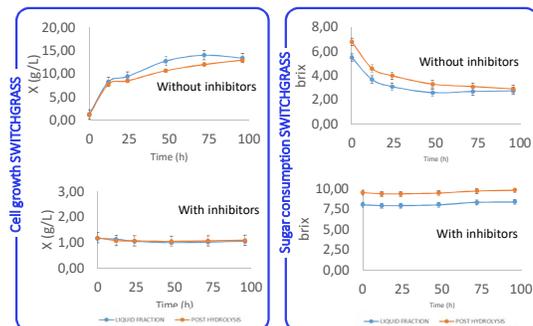
Component	<i>Eucalyptus</i> LIQUID FRACTION (g/L)	<i>Eucalyptus</i> post Hydrolysis (g/L)	Switchgrass LIQUID FRACTION (g/L)	Switchgrass post Hydrolysis (g/L)
Xylose	36.01	58.06	26.46	39.99
Glucose	7.82	14.02	5.30	14.96
Arabinose	6.78	3.50	7.81	3.47
Cellulobiose	11.89	Nd	3.59	nd
Acetic acid	20.12	29.03	15.39	18.44
Formic acid	5.93	6.96	8.82	9.87
5-HMF	2.74	1.28	3.08	1.75
Furfural	3.42	4.82	3.11	3.23
Total phenolic	7.64	5.82	9.01	4.21



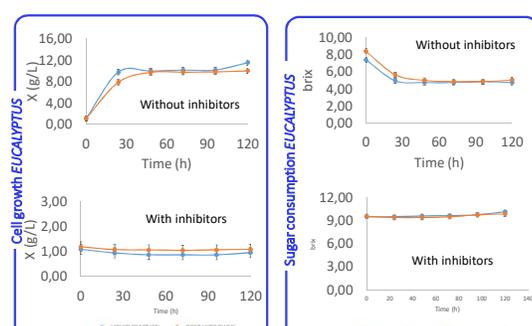
Phenolic compounds in hydrolysates

Component	Switchgrass LIQUID FRACTION (g/L)	Switchgrass Post-Hydrolysis (g/L)	<i>Eucalyptus</i> LIQUID FRACTION (g/L)	EUCA Post-hydrolysis (g/L)
Vanillin (G)	0,23	0,12	0,08	0,05
Vanillic acid (G)	0,15	0,14	0,03	0,05
Coumaric acid (H)	0,15	0,04	0,00	0,00
Syringaldehyde (S)	0,10	0,03	0,21	0,14
Syringic acid (S)	0,09	0,09	0,09	0,10
Hydroxybenzaldehyde (H)	0,12	0,08	0,01	0,02

4. Synthetic media fermentation



Kluyveromyces marxianus



- ❖ Chemically defined media simulating the composition of the hydrolysates (original and hydrolysed without inhibitors, and original and hydrolysed with inhibitors).

- ❖ Evolved strain of *Kluyveromyces marxianus*
- ❖ Conditions: 40 °C, 250 rpm, X₀ = 1 g/L

5. Conclusions

- ❖ The post hydrolysis process was successful increasing the xylose concentration in the hydrolysate. The oligomers were successfully converted to monomers. However, it also increased the concentrations of acetic acid, phenolic compounds and furfural, which are inhibitors of the cell growth.
- ❖ Synthetic media fermentation revealed that a detoxification of the liquid fraction after the chemical hydrolysis is needed to improve the media fermentability.

Acknowledgements

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