*Bioenergy - II: Fuels and Chemicals from Renewable Resources*
*March 8-13, 2009 – Rio de Janeiro, Brazil*

# THERMOPHILIC ENZYMES FOR BIOMASS CONVERSION

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**THERMOPHILIC ENZYMES FOR BIOMASS CONVERSION**
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# STATE OF THE ART

The search for alternative energy sources for the development of sustainable fuels is an environmental and economic priority. During the last decades, considerable attention has been addressed to the "*second generation*" production of bioethanol that derives from the utilization of cheap substrates.

Agronomic residues, such as corn stover, wheat or rice straw, barley spent grains, forestry discards, collectively termed "lignocellulosic biomass", represent renewable supplies of fermentable sugars for bioethanol production.

**Lignocellulosic biomasses** are an attractive feedstock for producing liquid fuels and chemicals because they are the most abundant source of carbohydrates on Earth and are renewable and not in competition with food sources.

Moreover, their exploitation could solve the problem of the elimination of wastes that represent a source of pollution.

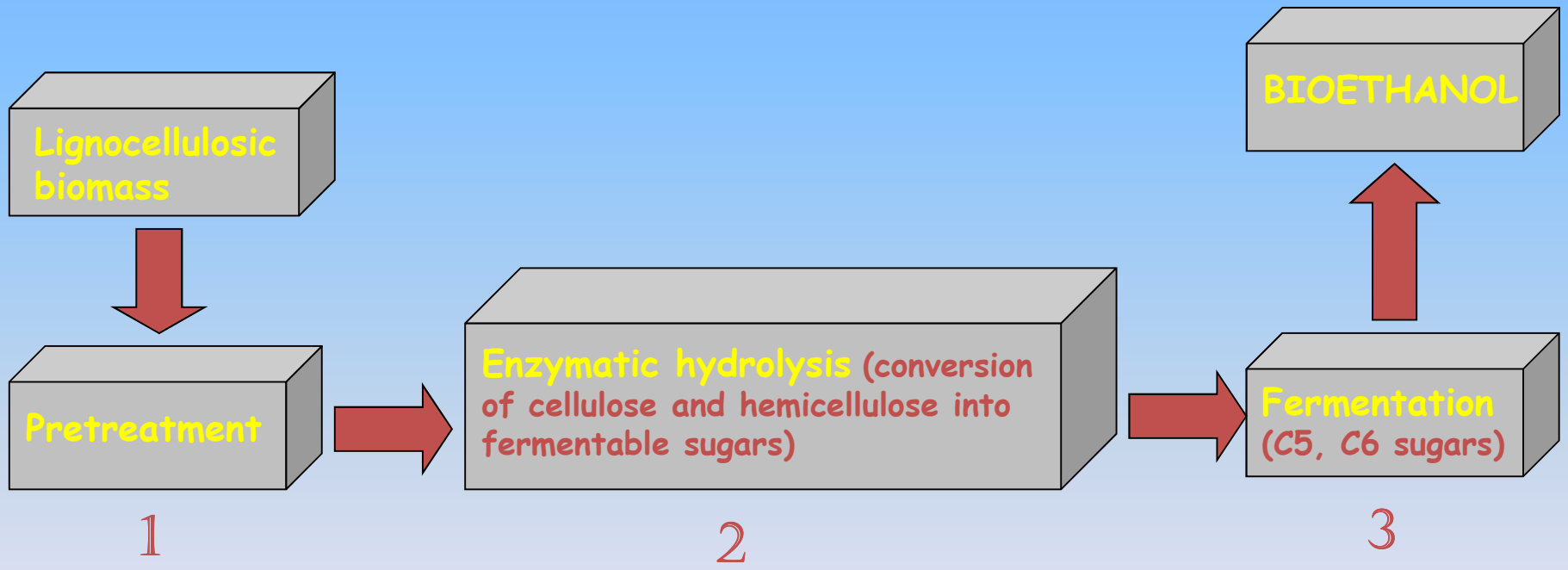
**However, the commercial development of bioethanol from lignocellulosic biomass is hindered by several technical restrictions.**

**Processing costs are high and mainly due to the incidence of the biomass and enzyme costs.**

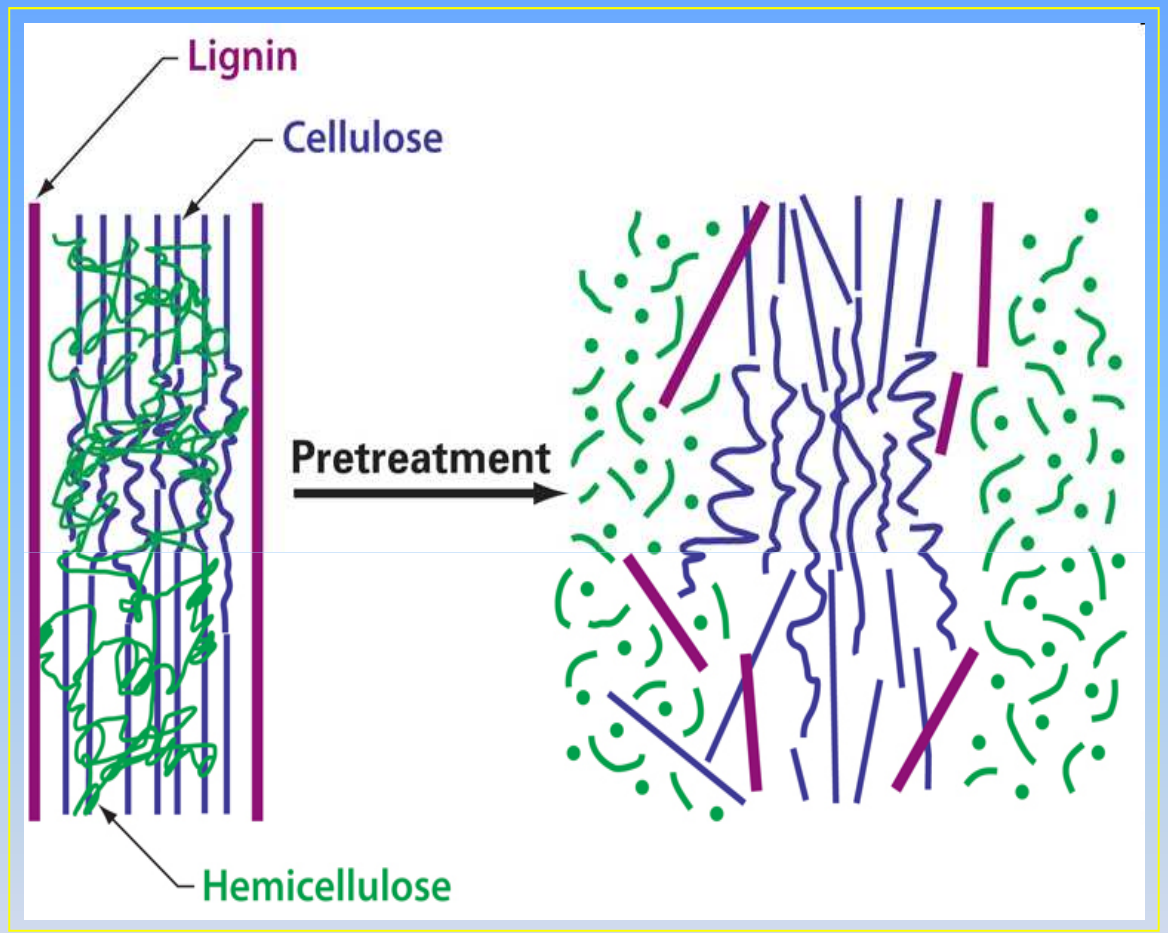
**The technological solutions must be focused on two major points:**

- ★ **to maximize the exploitation of all the biomass components;**
- ★ **to achieve high yields in each process step.**

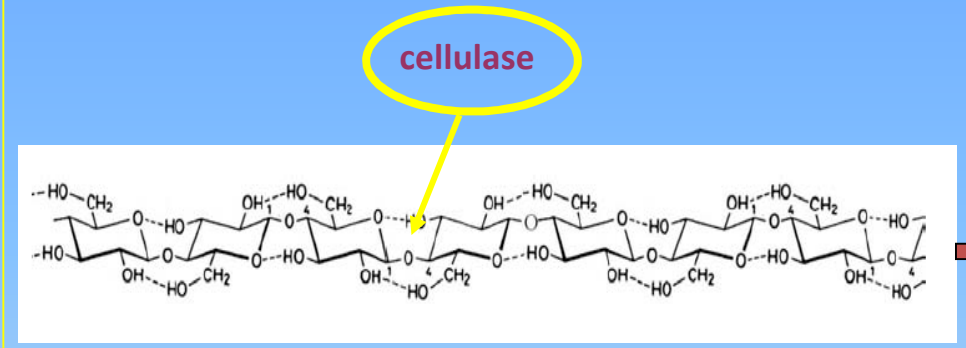
## The bioconversion process necessary to produce bioethanol from lignocellulosic biomass requires the following steps:



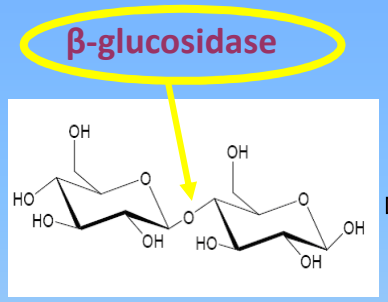
**1) Pretreatment:** the delignification process to release cellulose and hemicellulose from their complex with lignin making the polysaccharides more accessible to the enzymes that convert them into fermentable sugars.



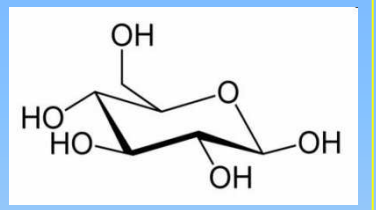
# 2) Saccharification: hydrolysis of the polysaccharide chains into fermentable sugars.



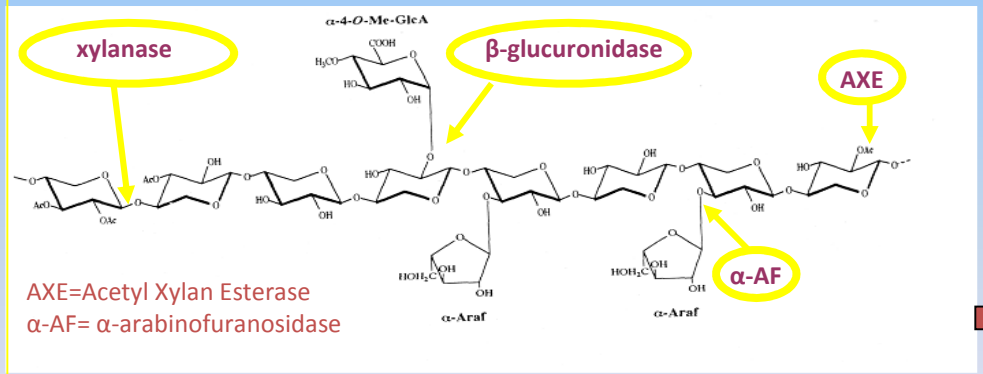

CELLULOSE



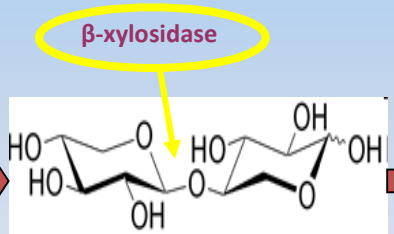

CELLOBIOSE



GLUCOSE



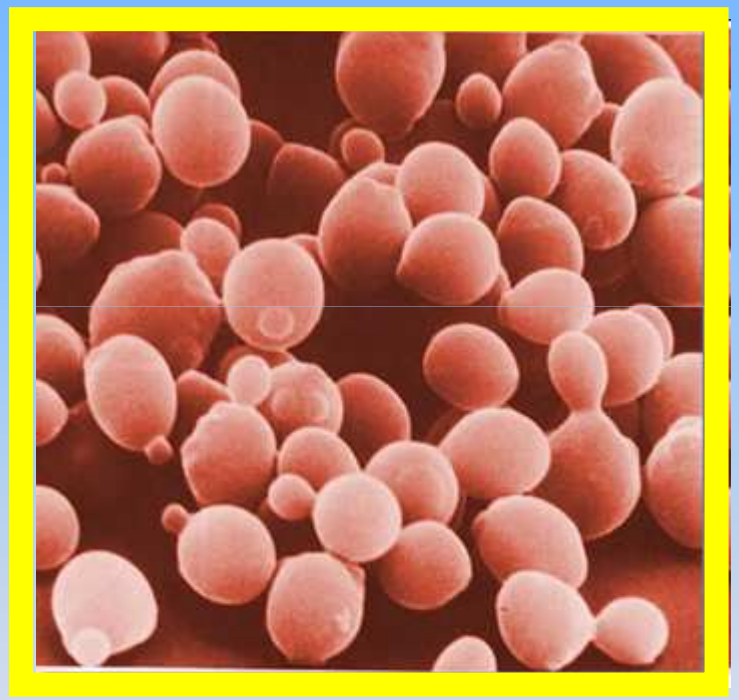

XYLAN




XYLOBIOSE

XYLOSE

**3) Fermentation:** fermentation of the resulting mixture of hexose and pentose sugars to produce ethanol.



Yeast cells

→ **ETHANOL**

**Physical, chemical and biochemical pretreatments have been proposed, but the harsh conditions required by many of them, such as high temperatures and low pH, give rise to problems in the subsequent saccharification step when using mesophilic enzymes.**

**In order to overcome this difficulty, micro-organisms living in extreme habitats characterized by high temperatures must be taken under investigation as potential source of polysaccharide-degrading enzymes, because they allow to perform biotransformation reactions at *non-conventional conditions*.**

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# THERMOPHILIC ENZYMES FROM *SULFOLOBUS SOLFATARICUS*

Enzymes isolated from micro-organisms growing at elevated temperatures are thermostable, active at high temperatures (thermophilic) and resistant to solvents and detergents. These unusual properties make them a valuable resource for industrial applications and promising candidates for the development of an efficient saccharification process at high temperature of the lignocellulosic biomass.

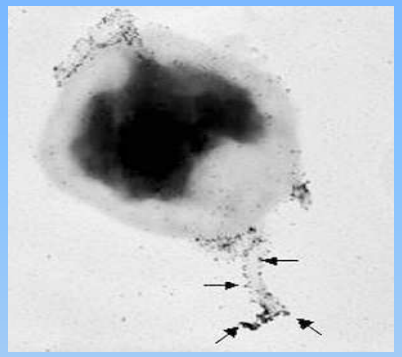
Thermostable cellulolytic and hemicellulolytic activities of potential interest for lignocellulosic biomass conversion were isolated from the hyperthermophilic aerobic archaeon *Sulfolobus solfataricus*.

IBP Istituto Biochimica Proteine

**This micro-organism, originally isolated from a solfataric field in the area of Naples (Italy) thrives at acidic pH (2.0-5.0) and high temperatures (80-87°C).**

*Solfatara* in Pozzuoli, Naples, Italy.

a



b


Micrographs of *S. solfataricus*, strain Oα.

**Enzymes from *S. solfataricus* strain Oα, previously isolated for its ability to grow on xylan as unique carbon source, were used to degrade at high temperature the polysaccharidic fractions contained in brewer's spent grains (BSG), the main by-product deriving from breweries after the extraction of wort.**

The enzyme cocktail contains the main glycolytic activities (namely cellulase, β-D-glucosidase, xylanase and β-D-xylosidase) required to hydrolyze cellulose and hemicellulose into fermentable sugars.

**Table 1. Glycosyl-hydrolases from *S. solfataricus* strain Oα contained in the enzyme cocktail used for BSG saccharification.**

| Enzyme | Activity (mU/ml enzyme cocktail) | Optimal temperature (°C) | Optimal pH |
|---|---|---|---|
| Cellulase | 29 | 95 | 3.5 |
| Xylanase | 63 | 90 | 4.0 |
| β-Glucosidase | 158 | >80 | 6.5 |
| β-Xylosidase | 609 | 85 | 6.0 |

# PRETREATMENT OF BREWER'S SPENT GRAINS

**Milled brewer's spent grains were subjected to the following pretreatment methods:**

1) **biomass (1% w/v) was suspended in de-ionized water and treated at 700 Watt in microwave oven for 1 min;**

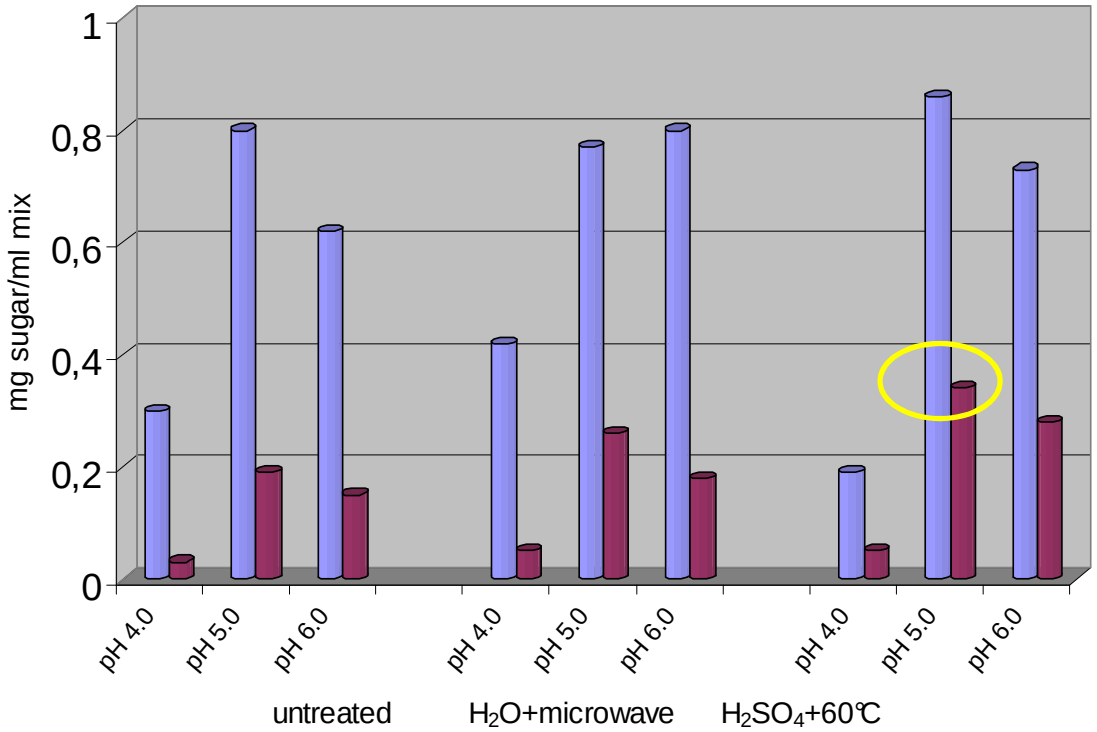
2) **biomass (1% w/v) was suspended in 1% sulphuric acid and heated at 60°C for 3 h;**

3) **biomass (1 or 5% w/v) was suspended in 0.45% sulphuric acid at 121°C.**

# ENZYMATIC SACCHARIFICATION OF BREWER'S SPENT GRAINS AT HIGH TEMPERATURE

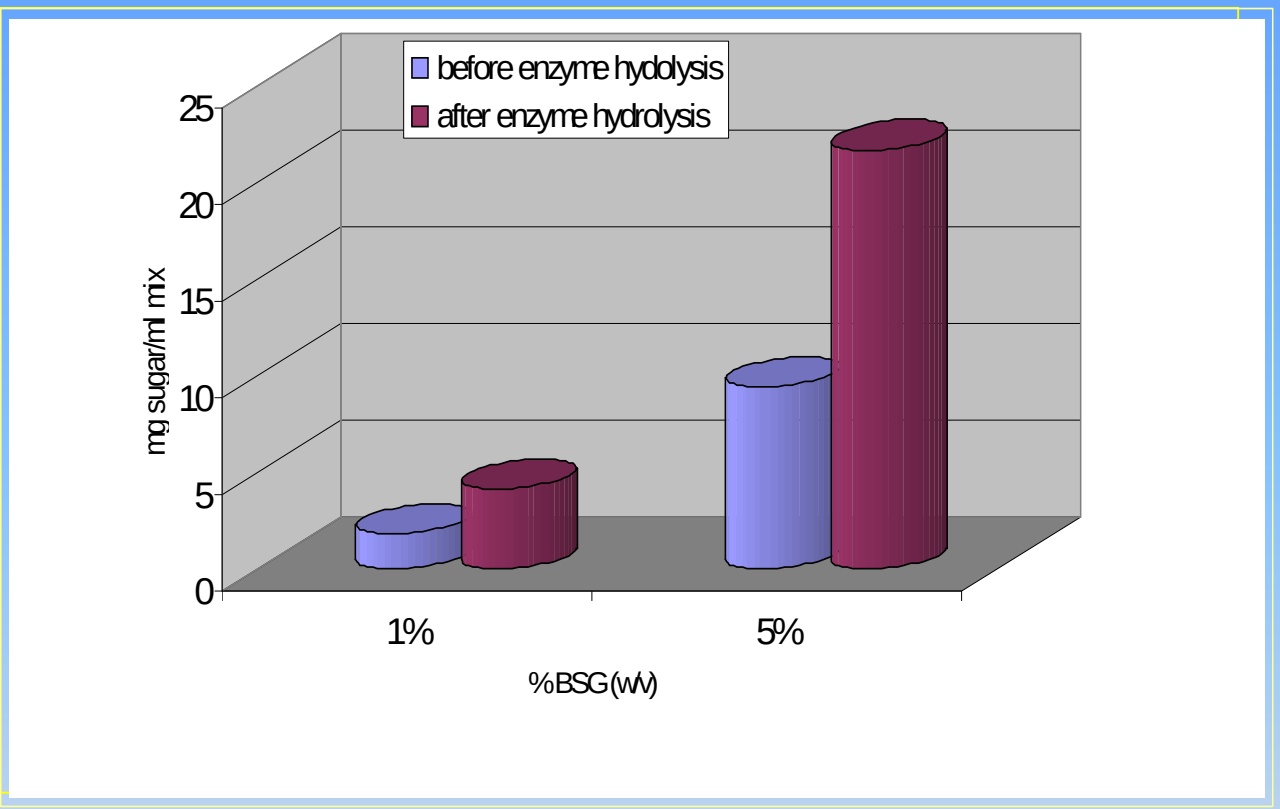
Brewer's spen grains, untreated or after pretreatment, were subjected to enzymatic saccharification at 80°C by addition of the enzyme cocktail from *S. Solfataricus* (pH 4.0, 5.0 or 6.0).

The incubation mixtures were analysed by anionic exchange high-performance liquid chromatography. Monosaccharides were separated with 16 mM sodium hydroxide at a flow rate of 0.25 ml/min, and identified by the respective standards (glucose and xylose).




Glucose( ) and xylose ( ) yields after enzyme saccharification of differently pretreated (1%) BSG samples in comparison with untreated (1%) BSG.

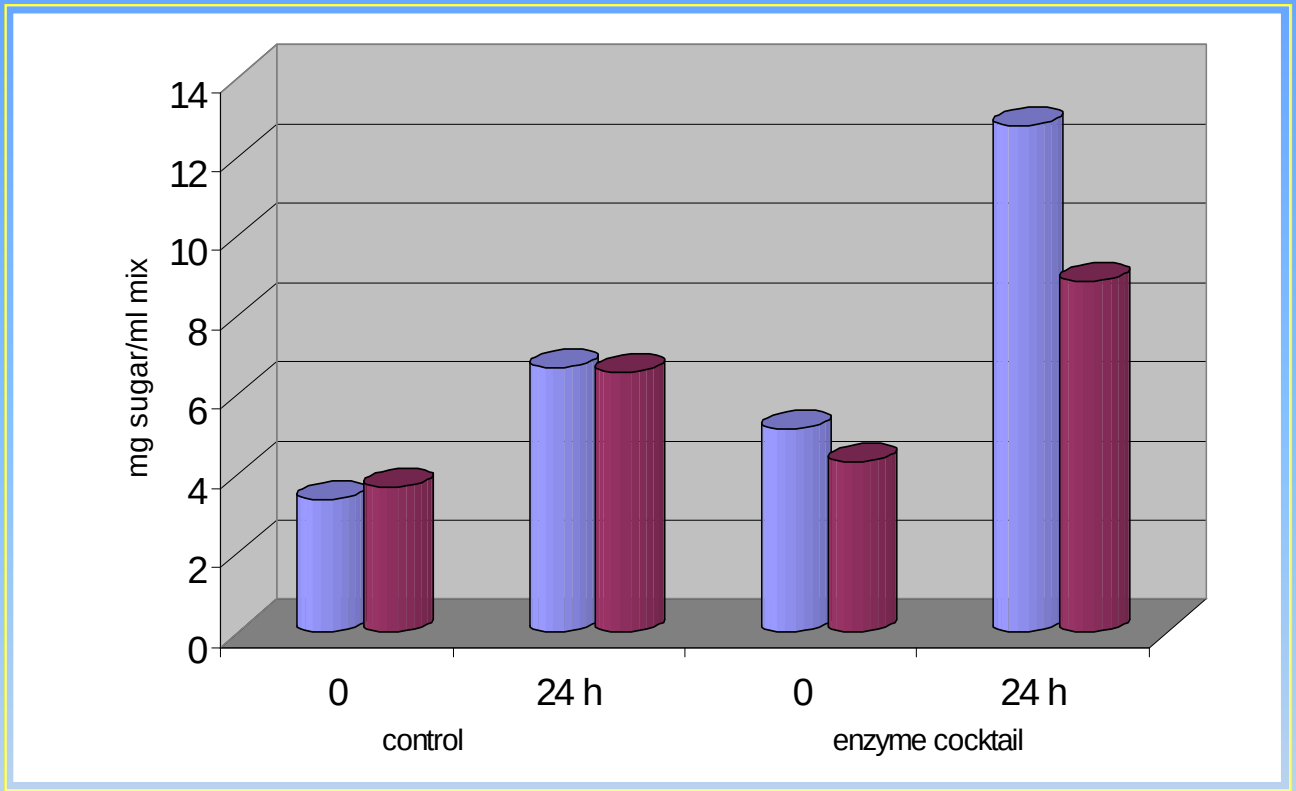
The highest amount of sugar release was obtained at pH 5.0 from BSG pretreated with $H_2SO_4$ at 60°C. However, glucose and xylose amounts were very far from the theoretical full release (Theoretical content: glucose 39.8% and xylose 27.5 % of the dry material, respectively).




**Sugars release from BSG at different concentrations**

**BSG was pretreated with 0.45% $H_2SO_4$ at 121°C for 15 min, and then subjected to enzymatic saccharification at 80°C and pH 5.0 for 24h. Sugar content was estimated before and after enzymatic hydrolysis.**

**The BSG concentration was increased with the aim of obtaining an hydrolysate with high content of fermentable sugars. Glucose and xylose yields from 1 and 5% BSG were 3.4 and 18-folds higher than the previous trials. Moreover we have confirmed the importance of the high temperature in destroying the compactness of the lignocellulosic biomass for an easier action of the enzymes.**




**Glucose (■) and xylose (■) composition of hydrolysate from 5% BSG pretreated with 0.45% $H_2SO_4$ at 121°C for 15 min, and then subjected to enzymatic saccharification.**

**To avoid any doubt regarding a possible thermal hydrolysis of the polysaccharides, a control was incubated with the enzyme sample. After 24h, glucose in the enzyme sample was almost doubled in comparison to the control at the same time. Smaller increase was observed for xylose, but both sugars quantities represented 64% of their theoretical full release.**

# CONCLUSIONS

Very encouraging results were obtained after the enzymatic saccharification with thermophilic enzymes of brewer's spent grains, pretreated at high temperature with sulphuric acid.

Further studies are in progress to select the best operative parameters (e.g. substrate and enzyme cocktail concentrations) in order to maximize the release of fermentable sugars.

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# Thank you for the attention

# and....