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Production of Bioethanol and Xylitol from Sugarcane Bagasse

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1. Introduction

Sugarcane bagasse, a lignocellulosic residue generated from sugar and first generation bioethanol production, is a valuable feedstock for biorefineries, instead of burning it for power generation [1]. This study explores its potential for co-producing second-generation bioethanol and xylitol.

Steam explosion, a well-established pretreatment [2] disrupts the recalcitrant structure of the lignocellulosic matrix, producing two fractions (solid and liquid). A solid fraction that releases fermentable sugars for bioethanol production via enzymatic hydrolysis and subsequent fermentation with *Saccharomyces cerevisiae.* The main challenge in the enzymatic hydrolysis is achieving a concentration of glucose high enough to make an efficient ethanol production [3]. The liquid fraction, or hemicellulosic hydrolysate, has a mixture of oligomers and monomers of the sugars, along with different amounts of degradation compounds.

The production of xylitol using the remaining hemicellulosic hydrolysate implies further valorization of the sugarcane bagasse. Xylitol is a natural sweetener used in the food industry due to its low caloric level [4]. The biotechnological process using microorganisms to produce xylitol has different attractive characteristics [5]. However, detoxification strategies are needed to address inhibitory compounds generated during pretreatment.

This work proposes to study the production of bioethanol and xylitol after steam explosion of sugarcane bagasse. Steam explosion conditions were chosen to produce both products with acceptable efficiency. In the case of xylitol production, an adapted strain of *Whickerhamomyces anomalus* (ALE, Adaptive Laboratory Evolution) was used to tolerate presence of inhibitor compounds.

2. Materials and Methods

2.1. Feedstock

Sugar cane bagasse was provided by ALUR Alcoholes del Uruguay from the Bella Unión (Uruguay) bioethanol and refined sugar production plant. The feedstock was dried at 40 °C by forced convection oven until 10 % moisture, and then milled to an average particle size of 1 cm.

2.2. Steam explosion pretreatment

The milled biomass was pretreated in a semi-continuous pre-pilot reactor. The equipment has an approximate capacity of 10 kg h^{-1} of raw biomass pretreatment. Three conditions were applied to the biomass. Condition 1: 190 °C of temperature and 10 minutes of residence, (i.e. 190.10). Condition 2: 200 °C and 5 minutes (i.e. 200.5); and 200 °C and 10 minutes (i.e. 200.10). After steam explosion pretreatment, the biomass sludge was pressed and filtered to separate the hemicellulosic liquid fraction from the solid fraction. The hemicellulosic liquid fraction, Sugarcane Bagasse Hemicellulosic Hydrolysate (SBHH), was submitted to a post-hydrolysis process to hydrolyze the oligomers to monomers using 4 % (w/w) sulfuric acid in autoclave at 121 °C for 15 minutes.

2.3. Enzymatic Hydrolysis - Solid Fraction

Enzymatic hydrolysis was performed for the three solids obtained after steam explosion. The assays were done according to NREL laboratory procedure 63351 [6]. The enzyme Cellulase Enzyme Blend from Sigma Aldrich was used at 25 FPU/g of substrate. The saccharification was performed in an incubator INNOVA 44R (New Brunswick) at 50 °C and 200 rpm. The reaction was left for 96 hours.

2.4. Glucose Fermentation

The hydrolysate obtained after enzymatic hydrolysis was recovered and 1,5 g/L of yeast extract, 5 g/L of $(NH_4)_2HPO_4$ y 0,5 g/L of $MgSO_4.7H_2O$ were added. The solution was sterilized by filtration and was inoculated with *Saccharomyces cerevisiae*. The fermentation was done in incubator at 32 °C and 150 rpm. **Hydrolysate detoxification**

A liquid-liquid extraction detoxification process using ethyl acetate was applied to reduce the concentration of organic acids in the SBHH. The acetic acid content in the SBHH was analyzed before and after the detoxification process using HPLC. To be used as fermentation medium, the hydrolysates had the pH adjusted with $CaCO_3$ to 5.5 and filtered with polyethersulfone membrane and pore size of 0.2 µm for sterilization.

2.5. Fermentation of detoxified hydrolysates by *Wickerhamomyces anomalus* ALE.

Precultured *Wickerhamomyces anomalus* ALE cells were inoculated into 25 mL Erlenmeyer flasks containing 10 mL of detoxified SBHH at 75 % concentration in Yeast Nitrogen Base Without Amino Acids (YNB).

2.6. Analytical methods

The composition of the sugarcane bagasse hydrolysates was determined by high performance liquid chromatography using a Dionex Ultimate 3000 UHPLC. Total phenolics were quantified by colorimetric method using gallic acid as standard, adapted from [7]. The absorbance was measured at 760 nm using a Mettler Toledo UV-Vis Excellence spectrophotometer.

During fermentation assays glucose, ethanol, xylose, xylitol, and acetic acid concentrations were determined by high performance liquid chromatography (HPLC) equipped with a refractive index detector.

3. Results and Discussion

3.1. Biomass characterization

The composition of the sugarcane bagasse was similar to other studies [2]. As shown in Table 1, the sugarcane bagasse composition had significant presence of xylan, which made it suitable to produce xylitol. Cellulose content was appropriate to produce bioethanol.

Table 1. Sugarcane bagasse composition

Component	*(%)*
Ash	1,3 ± 0,1
Extractives (water and ethanol)	5,7 ± 0,1
Lignin (Insoluble and soluble)	20,2 ± 0,3
Glucan (cellulose)	36,5 ± 0,1
Xylan	19,5 ± 0,1
Arabinan	0,7 ± 0,1
Acetyl groups	5,5 ± 0,1

3.2. Steam explosion pretreatment

3.2.1. Solid fraction characterization

Figure 1 shows the composition of the solid fractions obtained after steam explosion. As expected, cellulose content was increased compared to the original biomass, due mostly to hemicellulose solubilization. As can be seen, all three conditions did not differ significatively (after performing a Tukey test) in the cellulose content. In the case of hemicellulose -mainly xylan- and lignin content, conditions 190.10 and 200.5 were different to condition 200.10. The more severe conditions applied to the biomass in condition 200.10 resulted in more hemicellulose hydrolysis, which will be considered with liquid fraction analysis (section 3.2.2).

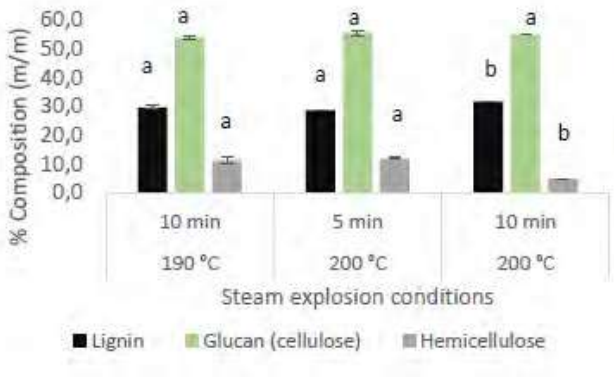

Figure 1. Solid fraction composition after steam explosion (letters indicate level of significance for the same compound).

3.2.2. Liquid fraction characterization

The Table 2 presents the composition of the hemicellulosic hydrolysates after steam explosion, and after the acid hydrolysis. As expected from the previous section, the liquid fraction of the condition 200.10 had higher content of xylose. However, considering the post-hydrolysis characterization, the severe conditions also implied degradation of the xylose oligomers. As can be seen the condition 190.10 gave the highest concentration of xylose after hydrolysis, indicating a high content of xylose oligomers after steam explosion. This aspect made the steam explosion condition 190.10 the most appropriate to produce xylitol.

Table 2. Composition of Sugarcane Bagasse Hemicellulosic Hydrolysates (SHCC) obtained by steam explosion pretreatment.

Compound	Concentration in the hydrolysate (g/L)					
	Original Hydrolysate			Post-hydrolysis		
	Condition 190.10	Condition 200.5	Condition 200.10	Condition 190.10	Condition 200.5	Condition 200.10
Cellobiose	9.4 ± 0.2	7.7 ± 0.4	5.4 ± 0.2	Not detectable	Not detectable	Not detectable
Glucose	0.7 ± 0.1	0.8 ± 0.2	1.0 ± 0.2	6.2 ± 0.3	6.3 ± 0.6	7.0 ± 0.6
Xylose	11.3 ± 0.3	10.4 ± 0.3	14.5 ± 0.2	69.5 ± 0.7	58.5 ± 0.5	33.4 ± 0.5
Arabinose	1.5 ± 0.1	1.3 ± 0.2	3.1 ± 0.1	3.0 ± 0.1	3.1 ± 0.3	1.5 ± 0.1
Acetic acid	6.9 ± 0.1	4.1 ± 0.1	9.6 ± 0.2	16.3 ± 0.2	14.2 ± 0.1	13.1 ± 0.1
Formic acid	4.5 ± 0.2	2.2 ± 0.3	4.3 ± 0.2	1.6 ± 0.1	1.1 ± 0.3	2.2 ± 0.2
5-HMF	0.2 ± 0.1	0.2 ± 0.1	0.7 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
Furfural	0.9 ± 0.1	0.9 ± 0.1	1.9 ± 0.2	4.3 ± 0.2	4.2 ± 0.2	4.5 ± 0.4
Phenolic compounds	7.9 ± 0.2	6.0 ± 0.2	8.0 ± 0.2	3.0 ± 0.2	2.7 ± 0.2	3.6 ± 0.1

3.3. Bioethanol production

3.3.1. Enzymatic Hydrolysis

Figure 2 presents the saccharification yield of the solid fraction after different steam explosion conditions. Almost 100 % saccharification yield was achieved for condition 200.10, 95 % for the condition 190.10 and 90

% for the 200.5 condition. The different yields are explained by the accessibility of the cellulases to the pretreated biomass, being the 200.5 the least deconstructed. On the other hand, the high yield for the condition 200.10, together with the solid and liquid fraction characterizations indicated high degradation of the biomass, with a loss in the process efficiency.

harification yield

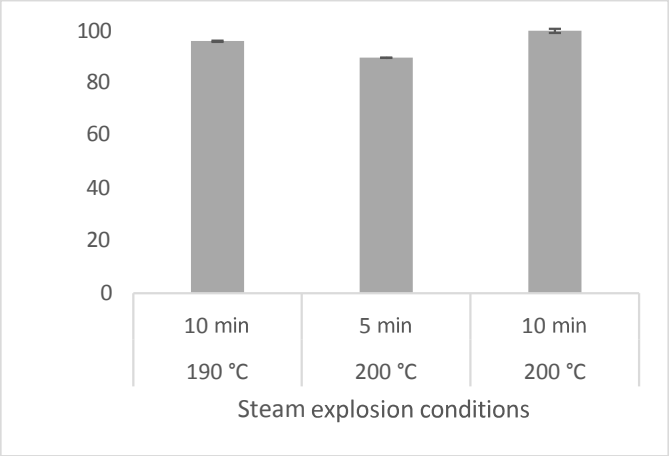

Figure 2. Saccharification yield of the solid fraction after the different steam explosion conditions.

3.3.2. Fermentation

The fermentation profile for the glucose obtained after enzymatic hydrolysis of the solid fraction pretreated at 190 °C and 10 minutes is shown in Figure 3. As can be seen, the fermentation of the glucose was complete after 24 hours, with no glucose left. The yeast was not inhibited, therefore assuming a complete removal of the inhibitors created after steam explosion.

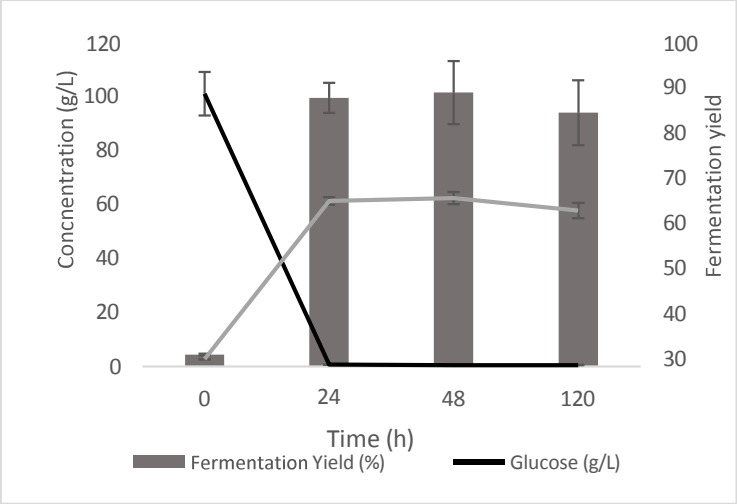

Figure 3. Fermentation profile with Saccharomyces cerevisiae for solid fraction pretreated at 190 °C and 10 minutes.

3.4. Xylitol production

After a screening of different microorganisms, the yeast *Wickerhamomyces anomalus* was found to be a promising xylitol producer. A laboratory adaptive evolution strategy was performed, obtaining a *W. anomalus* strain able to grow on media containing the inhibitors produced xylitol after pretreatment of biomass with steam explosion. The results of the xylitol production with this strain are shown in Table 3. The screening, laboratory adaptive evolution strategy and the different tests to produce xylitol will be published in the article “Xylitol production by a *Wickerhamomyces anomalus* strain adapted for enhanced tolerance to sugarcane bagasse hemicellulosic hydrolysate with high content of fermentation inhibitors”, authors Bonfiglio, Cagno, Nuñez, Castro, Botto and Rodriguez Bonnecarrere, in printing process.

Table 3. Xylitol production in this study ($Y_{p/s}$: Xylitol yield; Q_p: Xylitol productivity)

Pretreatment	Microorganism	Media	Time of fermentation (hr)	Initial xylose (g/L)	Xylitol (g/L)	$Y_{p/s}$ (g/g)	Q_p (g/Lh)
Steam explosion - 190 °C and 10 minutes- followed by acid hydrolysis	*W. anomalus* ALE	75% SCHH detoxified by acetic acid extraction and supplemented with YNB	48	48	13.41	0.44	0.28

3.5. Mass balance

The biomass balance for the condition 190 °C and 10 minutes of steam explosion is presented in Figure 4. As can be seen, one major point of losses in the process is the phase separation after steam explosion. In particular, the utilisation of a press to separate phases was not the most suitable. A different filter mechanism could improve these values and increase the final production of ethanol and xylitol.

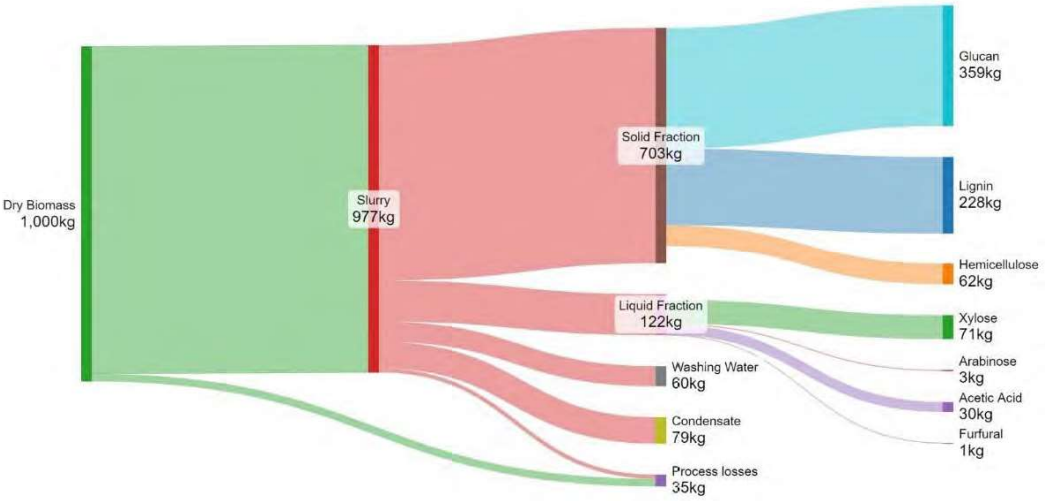

Figure 4. Sankey diagram for the biomass balance for condition of steam explosion 190°C and 10 minutes.

4. Conclusions

Sugarcane bagasse can be industrialized to produce second generation ethanol and xylitol; the former by the common yeast *Saccharomyces cerevisiae,* while the latter with an adapted strain of *Whickerhamomyces anomalous*. A steam explosion condition was identified that effectively disrupts biomass and solubilize xylose oligomers while minimizing extensive degradation.

This work highlights the potential of sugarcane bagasse as a feedstock for the production of valuable bioproducts within a biorefinery framework. Furthermore, the industrialization of sugarcane in Uruguay could stimulate local production, contributing to social and economic development in rural areas.

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