Papers of the 32nd European Biomass Conference

Setting the course for a biobased economy

Extracted from the Proceedings of the International Conference held in Marseille, France

24 - 27 June 2024

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EVALUATION OF LIGNOCELLULOSIC BIOMASSES AVAILABLE IN URUGUAY FOR GLUCOSE PRODUCTION BY ENZYMATIC CELLULOSE HYDROLYSIS

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ABSTRACT: Lignocellulosic biomass can be converted into sugars for sustainable production of biofuels and chemicals. Prior to enzymatic hydrolysis of cellulose, a pretreatment must be applied to increase its digestibility. The objective was to evaluate different biomasses from Uruguay, analyzing their potential to be processed in a biorefinery based on a glucose platform obtained through enzymatic hydrolysis of cellulose. The chemical characterization of *Arundo donax L.*, sugarcane bagasse, and Eucalyptus grandis was conducted. Steam explosion pretreatment (Advance Bio Systems, S1401-D2011) was used, evaluating different conditions (temperature, time, acid impregnation). Solids were enzymatically hydrolyzed (CellicCtec2) at concentrations of ≈ 15 % w/w. At 72 hours, the glucose concentration ranged between 62.0 and 81.8 g/L, and the efficiency ranged between 53.7 and 83.5 %. The glucose overall yield was 384 ± 167 kg/ton, 253 ± 95 kg/ton, and 225 ± 31 kg/ton, for *A. donax*, bagasse, and *E. grandis*, respectively. *A. donax* achieved the highest glucose yield per ton of raw material. Steam explosion was an appropriate pretreatment for obtaining glucose from all materials studied. For valorization at an industrial scale, it will be necessary to evaluate other technical and economic aspects.
Keywords: biorefinery, lignocellulosic sources, steam explosion, enzymatic hydrolysis, cellulose.

1 INTRODUCTION

The processing of lignocellulosic biomass offers alternatives for sustainable production of biofuels and chemicals within a transitioning economy with low use of fossil carbon [1,2]. Many valuable products can be obtained from glucose, derived from enzymatic hydrolysis of cellulose, such as ethanol, butanol, lactic acid, succinic acid, etc. Many of these products serve as precursors for biobased polymers. To achieve this, pretreatments aimed at increasing the enzymatic digestibility of cellulose must be carried out, preserving other components such as hemicellulose and lignin for other applications, within a biorefinery context [3,4]. During steam explosion pretreatment, biomass is subjected to high temperature and pressure for a specific period and then the pressure is suddenly released. Uruguay has various biomass resources that could be utilized, some of which have not been tested to evaluate their processing potential in a biorefinery [5].

This study aims to conduct a comparative evaluation of available biomasses in Uruguay, analyzing their potential to be processed in a biorefinery based on a glucose platform obtained through enzymatic hydrolysis of cellulose.

2 METHODOLOGY

2.1 Raw material

The studied biomasses were: *Arundo donax L.* harvested from a wild-growing site, sugarcane bagasse from a fuel and sugar production plant, and *Eucalyptus grandis* timber from forest plantations intended for plywood production.

2.2 Chemical characterization

The three substrates were used with a particle size below 700 µm and moisture content below 10 %. Chemical characterization was performed using NREL analytical protocols [6,7].

2.3 Steam explosion

Steam explosion was used as a pretreatment producing a hemicellulose hydrolysate and a solid with a high glucan content. The pretreatment was carried out in a semi-continuous pilot reactor (Advance Bio Systems, S1401-D2011). Different pretreatment conditions were evaluated for each biomass based on preliminary assays aiming for high glucose concentrations and enzymatic hydrolysis yields with minimal glucan losses [8 -10].

2.4 Enzymatic hydrolysis

The pretreated solids were hydrolyzed taking as reference the NREL protocol [11]. The reactions were carried out using the CellicCtec2 enzymatic complex, at 48 °C, pH 4.8, with a solid content of approximately 15 % w/w, and enzyme dosage of 22 - 25 FPU/g glucan, for 72 hours, with periodic sampling for glucose determination using HPLC.

To evaluate the performance of the saccharification reactions, the concentrations of glucose obtained in the hydrolysate and the theoretical content contained in the pretreated solid fraction were considered [11].

2.5 Glucose yield per ton of raw material

To calculate the glucose yield per ton of raw material, the following experimental values were considered: mass of the slurry obtained after pretreatment (data not shown), mass of the pretreated solid fraction (data not shown), glucan content in the pretreated solid fraction and enzymatic hydrolysis efficiency.

3 RESULTS AND DISCUSSIONS

3.1 Chemical characterization of raw material

The chemical composition of the studied biomasses is presented in Table I. As can be seen, *A. donax* and *E. grandis* had the highest content of glucan (mainly cellulose), whereas sugarcane bagasse had the lowest amount of glucan. However, in the case of *E. grandis*, it also had the highest amount of lignin, indicating a more recalcitrant matrix than the other two biomasses, as will be seen in the other results. Also, the high content of acetyl groups in the A. *donax* can be pointing to a better autohydrolysis during the steam explosion pretreatment (Table I).

Table I: Chemical composition of the tested biomasses

Component (% w/w)	*Arundo donax L.*	Sugarcane bagasse	*Eucalyptus grandis* waste
Glucan	45.7 ± 3,6	36.5 ± 0.1	46.1 ± 1.2
Xilan	19.0 ± 1.8	19.5 ± 0.1	14.2 ± 1.1
Arabinan	Un-determined	0.7 ± 0.1	< 0.1
Lignin	21.6 ± 0.7	20.2 ± 0.3	30.2 ± 0.3
Acetyl groups	9.1 ± 0.1	5.5 ± 0.1	1.9 ± 0.5
Extractives	10.8 ± 0.8	5.7 ± 0.1	3.7 ± 0.9
Ash	1.7 ± 0.2	1.3 ± 0.1	0.3 ± 0.1

Values are on a dry basis.

3.2 Steam explosion and enzymatic hydrolysis

During the steam explosion pretreatment, the biomass is deconstructed allowing a better performance of the cellulases in the enzymatic hydrolysis. Also, other reactions of depolymerization, relocation and degradation may occur. The more severe conditions (i.e. temperature, time, and acid addition) can favor desired but also undesired reactions.

Table II presents the glucan content in the solid fraction recovered after steam explosion pretreatment.

Table II: Glucan content in pretreated solid fraction

Raw Material	Pretreatment Conditions	Glucan content in pretreated solid fraction (%)
Arundo donax L.	195 °C - 15 min	68.1 ± 3.5
Sugarcane bagasse	190 °C - 10 min	51.0 ± 1.1
Eucalyptus grandis waste	180°C - 10 min	62.3 ± 5.6
	180°C - 10 min. - 0.25% H_2SO_4(*)	64.1 ± 3.8
	180°C - 10 min.- 0.50% H_2SO_4(*)	59.4 ± 0.8

(*) Pretreatment involves prior impregnation of the material with sulfuric acid, at 0.25 or 0.50 g per 100 g of dry raw material. Average values of duplicate or triplicate experiments

For the *E grandis*, the addition of acid previous to the steam explosion pretreatment allowed to use lower temperatures than the other biomasses. The higher content of glucan in the *A donax* solid fraction after pretreatment is in concordance with the chemical composition of the raw biomass (Table I and Table II).

3.3 Enzymatic hydrolysis of the pretreated solids

Subsequently, enzymatic hydrolysis of the pretreated solids was carried out. As described in the methodological section, the reactions were carried out with high solids content.

In this case, the sugarcane bagasse presented the highest efficiency (Table III). This aspect can be explained by the process of sugarcane bagasse itself, where an extrusion process is applied to the sugarcane to extract the juices enriched in sugar. This process can be seen as a pretreatment, where the biomass is already pre-deconstructed. The higher conversion efficiency of herbaceous biomasses compared to *E. grandis* may be due to a lower resistance of the lignocellulosic structure.

Table III: Experimental results of glucose concentration and hydrolysis efficiency at 72 hours for trials with high solids content (≈ 15% w/w).

Raw Material	Pretreatment Conditions	Glucose concentration (g/L)	Hydrolysis efficiency (%)
Arundo donax L.	195 °C - 15 min	64.6 ± 2.6	73.2 ± 2.0
Sugarcane bagasse	190 °C - 10 min	80.8 ± 1.0	82.4 ± 1.1
Euca-lyptus grandis waste	180°C - 10 min	27.0 ± 0.8	26.8 ± 0.6
	180°C - 10 min. - 0.25% H_2SO_4	68.4 ± 5.4	45.0 ± 1.0
	180°C - 10 min. - 0.50% H_2SO_4	78.5 ± 3.1	55.8 ± 2.1

Average values of duplicate or triplicate experiments

To achieve an efficient fermentation of the glucose in the hydrolysate for bioethanol production, it is necessary to consider its concentration. It is considered that distillation is economically viable with a minimum ethanol concentration value of 4 % w/w, which corresponds to a minimum value of 80 g/L of sugars prior to fermentation [12]. For bagasse and *E. grandis*, the values obtained are around this critical value. However, for *A donax*, the value is below this limit. This requires new tests for *A. donax* at higher solids concentrations to achieve this value. An explanation of this relatively low value of glucose concentration can be found in the hydrolysis efficiency, with 73 % efficiency for *A. donax* and 82 % efficiency for the sugarcane bagasse. Aspects like cellulose crystallinity should also be investigated.

3.4 Glucose yield per ton

Finally, glucose yield per ton of each studied raw material were calculated (Table IV).

Table IV: Glucose yield per ton of raw material

Raw Material	Pretreatment Conditions	Yield (kg $_{glucose}$/ ton)
Arundo donax L.	195 °C - 15 min	384 ± 167(**)
Sugarcane bagasse	190 °C – 10 min	253 ± 95
Eucalyptus grandis waste	180°C - 10 min	133 ± 47
	180°C - 10 min. - 0.25% H_2SO_4	207 ± 62
	180°C - 10 min. - 0.50% H_2SO_4	225 ± 31

Yield expressed on a dry basis of raw material. (**) Value estimated based on the yield of slurry on dry biomass of *E. grandis*. Average values of duplicate or triplicate experiments.

As it is shown in Table III, *A. donax* reached the highest glucose yield of glucose in the raw material which is consistent with a higher glucan concentration in the untreated raw material and in the pretreated solid fraction. Also, as previously discussed, the higher amount of acetyl groups in the *A. donax* may be beneficial to the rupture of the lignocellulosic matrix (Table I and Table II).

Finally, it is important to highlight that a great dispersion was observed in the measurement of the mass of the solid fraction obtained in the duplicates of the steam explosions, resulting in a greater error in the determination of the glucose yield per ton of dry raw material. More studies are needed to determine the origin of this dispersion and minimize it.

4 CONCLUSIONS

This study demonstrates that steam explosion is an appropriate pretreatment for obtaining glucose from low-cost lignocellulosic materials available in our country. The sugars obtained could be used to produce biofuels and other high-value chemicals. In particular, *A. donax* showed the best glucose yield per ton of dry raw material, although other technical and economic aspects need to be evaluated. In the case of *E. grandis*, pretreatment with acid impregnation before steam explosion would improve the performance in enzymatic hydrolysis. The results found in this study are promising for the valorization of lignocellulosic biomass on an industrial scale.

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6 ACKNOWLEDGEMENTS

- The authors acknowledge the Department of Bioengineering (Faculty of Engineering, UdelaR) for their support in the execution of experiments with E. grandis, analysis, and discussion of results