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SOYBEAN HULLS AS CARBOHYDRATE FEEDSTOCK FOR ETHANOL PRODUCTION

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ABSTRACT

Due to the large amounts generates worldwide, and its low lignin content in comparison to other biomasses, soybean hulls have great potential as raw material for high performance processes on lignocellulosic biorefineries. In the present study, a process including pretreatment, enzymatic hydrolysis and fermentation steps was proposed aiming the ethanol production from soybean hulls. The results suggest that the use of liquid hot water as pretreatment in fact improves cellulases and hemicellulases action due to the change in the structure of SH, providing 87% and 67% of glucan and xylan conversion, respectively. Moreover, the fermentation of hydrolyzed broth by commercial yeast achieved a yield beyond the theoretical maximum of 0.51 g ethanol/ g glucose, generating ethanol concentration of 3.5^{o} GL. The utilization of this process resulted in satisfactory rates of cellulose and hemicellulose conversion at enzymatic hydrolysis step providing a hydrolyzed broth rich in sugars which have been efficiently fermented to ethanol, a high-value product.

Keywords: Biorefinery. Soybean hulls. Pretreament. Enzymes. Biofuels.

1 INTRODUCTION

Soybean is one of the major world crops, with an annual production of 359 million tons. Each ton of processed soybean generates 50–80 kg of soybean hulls, representing 5–8% of the whole seed. Due to environmental concerns and great economic potential, the search of soybean hulls (SH) re-use solutions are deeply discussed. The lignocellulosic composition of it has attracted the attention of the scientific and productive sector, mainly related to production of medium to high value-added molecules, with potential applications in food and feed, agriculture, bioenergy, and other segments.[1] Bioethanol production fits into the strategy of a circular economy and zero waste plans, and using ethanol as an alternative fuel gives the world economy a chance to become independent of the petrochemical industry, providing energy security and environmental safety. [2] The aim of this work was to evaluate the pretreatment, enzymatic hydrolysis and fermentation of soybean hulls in view of ethanol production.

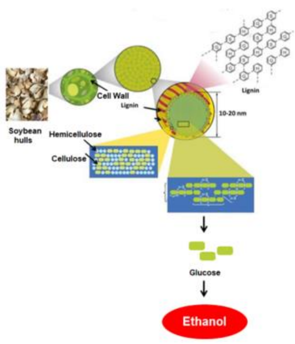

Figure 1 Soybean hulls (SH) as carbohydrate feedstock for conversion into ethanol.

2 MATERIAL & METHODS

Chemical composition of soybean hulls. Soybean hulls were supplied by CJ Selecta Company located in Araguari, Minas Gerais, Brazil. The hydrothermal pretreatment was performed on soybean hulls *in natura* using only distilled water, at a solid loading of 25% (w/v) in an autoclave at 121°C, for 30 minutes. The composition of biomass solids was determined by National Renewable Energy Laboratory (NREL)–LAP standard analytical procedures. [3] Chemical characterization was carried out in triplicate.

Enzymatic hydrolysis. Enzymatic hydrolysis was performed in 125 mL Erlenmeyer flasks containing 15% soybean hulls dry matter (w/v) in 100mM sodium citrate buffer at pH 4.8 in a final volume of 20 mL. Enzyme loading (Cellic CTec3®) was specified as 8 FPase units per gram of biomass (13 g protein/ 100 g glucan, respectively). The reaction was carried out in an orbital shaker at 200 rpm and 50°C for 72 hr. Aliquots of 1 mL was being immediately heated to 100°C to denature the enzymes, centrifuged for 5 min at 15,000 g, and then cooled until use.

Fermentation conditions. Commercial strain of the yeast *Saccharomyces cerevisiae* was used for fermentation in a 250 mL Erlenmeyer flask at 28 ^{o}C with an agitation speed of 150 rpm using the hydrothermal pretreated soybean hulls enzymatically hydrolyzed at 15% loading (w/v). Aliquots were taken at 0, 24, 48 and 60h and frozen for later analysis. All tests were conducted with technical triplicate replicates.

Analytical methods. Glucose, xylose and ethanol concentrations and were analyzed by high-performance liquid chromatography (HPLC - Shimadzu) equipped with an Aminex ion exclusion HPX-87H (Bio-Rad) and refractive index detector. The column was eluted with a mobile phase (5 mmol/L H_2SO_4) at a flow rate of 0.6 mL/min and it operated at 65°C. All analytical values were calculated from triplicates.

3 RESULTS & DISCUSSION

The results observed in Table 1 are in accordance with the literature. High variations in composition are mainly due to different seed origin and dehulling methods, but in general SH have high cellulose (28.6–52.3%) and hemicellulose contents (18.5–33.8%). This biomass also presents lower lignin content (2.3–13.1%) when compared to other commonly studied lignocellulosic materials. In addition, there is also the presence of pectin (4.2%) and protein (9.4%). [4.5]

Table 1 Chemical composition of Soybean hulls (SH).

Sample	Component (g / 100 g)							
	Cellulose	Hemicellulose	Lignin	Protein	Oil	Pectin	Ash	Extractives
SH in natura	37.1 ± 0.7	18.6 ± 0.9	9.6 ± 0.3	11.6 ± 0.5	1.8 ± 0.3	2.4 ± 0.2	4.1 ± 0.7	12.0 ± 0.8
SH + LHW	38.4 ± 0.4	17.3± 0.4	8.2 ± 0.5	9.5 ± 0.3	1.4 ± 0.3	2.0 ± 0.2	3.7 ± 0.7	12.3 ± 0.7

The hydrothermal pretreatment can infiltrate the biomass and moisturize cellulose. This enhances its accessible and susceptible surface area, and improves its accessibility to the hydrolytic enzymes, consequently facilitating the removal of hemicellulose and lignin. [6] Liquid hot water (LHW) is a type of hydrothermal pretreatment that improves enzymatic hydrolysis of cellulose by solubilizing xylan, increasing porosity and decreasing particle size.

The enzymatic hydrolysis of pretreated soybean hulls at 15% (w/v) using the commercial enzymes (8 FPU/g SH) was able to convert about 87% and 66% of glucan and xylan, respectively, as observed in Figure 2. Despite the excellent hydrolysis yield, it is important to highlight that this experiment was not intended to optimize the solids and enzyme loadings, but rather to ensure the obtaining of hydrolyzed broth rich in fermentable sugars such as glucose and xylose to next fermentation step.

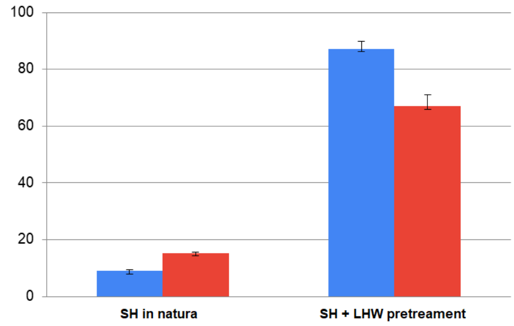

Figure 2 Enzymatic hydrolysis of soybean hulls (SH) using 15%(m/v) solid load and 8 FPU/g biomass enzyme load. (■) Glucan and (■) Xylan conversion.

These results suggest that the use of hot water in fact improves cellulases and hemicellulases action due to the change in the structure of SH, even under mild conditions and without drastically affecting the chemical composition of the raw biomass.

Soybean hulls is a hygroscopic material, making it challenging to ferment a hydrolyzed broth from saccharification containing high solids load. However, as can be seen in the Figure 3, the yeast was able to consume practically all glucose in just 24 h of fermentation. Xylose consumption is also observed in the first 24h. At 48h, ethanol production was 27.5 g/L, which represent a yield beyond the theoretical maximum of 0.51 g ethanol/ g glucose. This may indicate that the xylose consumption observed is also due to the yeast's ability to convert xylose to ethanol. So, this ethanol concentration represent about 3.5^{o} GL.

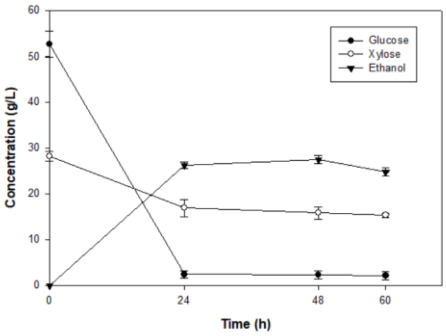

Figure 3. Fermentation profile

4 CONCLUSION

The process of soybean hulls conversion into ethanol proposed in this study proved to be promising. The hydrothermal pretreatment, even in mild temperature conditions, is an environmentally correct alternative with the potential to increase the competitiveness of second-generation ethanol. The utilization of this method resulted in satisfactory rates of cellulose and hemicellulose conversion at enzymatic hydrolysis step providing a hydrolyzed broth rich in fermentable sugars. Moreover, the yeast used was able to convert practically all glucose and also some xylose into ethanol achieving interesting fermentation yield.

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