I COBBIND
I CONGRESSO BRASILEIRO DE BIOTECNOLOGIA INDUSTRIAL
XXIV SINAFERM ** XV SHEB ** XV ENZITEC FLORIANÓPOLIS ** 2024
Creating connections between biotechnology and industrial sustainability
August 25 to 28, 2024
Costão do Santinho Resort, Florianópolis, SC, Brazil
BIOPROCESS ENGINEERING

EFFECT OF AUTOHYDROLYSIS PRETREATMENT OF SOYBEAN HULLS: A PRELIMINARY STUDY

João G. Cabral[1*], Damázio B. S. Júnior[1], José E. A. Mendonça[1], Gabriela A. T. M. Correa[1], Maikon Kelbert[1], Pedro H. H. Araújo[1] & Cristiano J. Andrade[1]

[1] Chemical and Food Engineering Department, Federal University of Santa Catarina, Florianopolis, Brazil.
** Corresponding author's email address: joaocabraleq@gmail.com*

ABSTRACT

Lignocellulosic biomasses have been drawing attention as sustainable and renewable culture medium, in particular carbon source. They can represent an alternative to fossil resources. In this sense, soybean hulls are a promising alternative due to their abundance and high cellulose and hemicellulose content and low lignin content, making their chemical composition favorable. However, a pretreatment step is necessary to obtain the compounds of interest, mainly fermentable sugar. In this work, soybean hulls were pretreated: isothermal autohydrolysis pretreatment at 200 ºC and liquid/solid ratio of 10. The results indicated the main composition of the biomass, demonstrated a high mass loss combined with the increase in cellulose crystallinity, in addition to showing a considerable increase in the glucose concentration in the saccharification stage, indicating that the pretreatment was efficient, that is, an interesting alternative source of fermentable sugars.

Keywords: Soybean hulls. Hydrothermal pretreatment. Lignocellulosic biomass. Fermentable sugars. Saccharification.

1 INTRODUCTION

Lignocellulosic biomass has become a renewable carbon source, offering an alternative for obtaining compounds typically derived from fossil resources. This biomass primarily comprises cellulose, hemicellulose, and lignin, making it a good alternative for producing fermentable sugars (Kucharska et al., 2018). Among these biomass sources, soybeans stand out. In 2020/2021, global production reached 362.1 million metric tons, underscoring its significance as a major crop worldwide (US Department of Agriculture, 2020). The leading soybean producers are the United States, Brazil, and Argentina (Abdulkhani et al., 2017). In Brazil, soybean is the most extensively cultivated crop, with an average production of 124.8 million tons in 2019/2020 (CONAB, 2020). Consequently, the soybean production chain generates various residues, including flour, molasses, and hulls. Soybean hulls are a lignocellulosic residue rich in polysaccharides, composed of 28.6–52.3% cellulose, 18.5–33.8% hemicellulose, and 2.3–13.1% lignin (Bittencourt et al., 2021), representing 5-8% out of the total soybean mass. However, due to the recalcitrance of lignocellulosic biomass, pretreatments are essential to promote the release of fermentable sugars and other usable compounds. (Sindhu et al., 2016).

Autohydrolysis pretreatment lays on water thermal-pressurized system. During this process, water is heated above its boiling point, enhancing the solubility of compounds (Torres-Mayanga et al., 2019). Lignocellulosic biomass is exposed to saturated steam under high pressure, causing the materials to decompress, disintegrating the lignocellulosic matrix, removing and partially redistributing the lignin, facilitating access to the cellulose (Jatoi et al., 2022). Thus, the objective of this study was to characterize pretreated and raw soybean hulls and analyze the effects of autohydrolysis pretreatment on lignocellulosic biomass, evaluating the recovered mass, changes in composition, crystallinity, and saccharification.

2 MATERIAL & METHODS

The soybean hulls used in the experiments were purchased from a local company located in Mafra (Santa Catarina). Consecutively, a washing step was carried out with distilled water, followed by drying to contain moisture. Then, the material was crushed and sieved to obtain particles in the size range of 0.425 to 0.850 mm.

For the soybean hull characterization step, NREL analytical methods were used for standard biomass analysis (NREL 2014). The extractives were determined in cartridges subjected to extraction cycles in a Soxhlet apparatus, using water and ethanol as solvents. The dried samples were calcined in a muffle furnace at 575 °C for 4 h to determine the ash content. A 300 mg sample of soybean hulls was initially subjected to hydrolysis with 3 mL of 72% (v/v) sulfuric acid (H_2SO_4) for one hour at 30 °C. Then, 84 mL of distilled water was added to the reaction mixture, followed by autoclaving at 120 °C for one hour and filtration in crucibles. The acid-insoluble lignin content was determined with the retained material, while the soluble lignin content was measured spectrophotometrically at 280 nm using the permeate. Sugars in the hydrolyzed liquor were quantified using an Aminex HPX 87H column in an HPLC setup equipped with an RI detector, operating at 45 °C with a mobile phase of 0.005 M H_2SO_4 at a flow rate of 0.6 $mL \cdot min^{-1}$.

The autohydrolysis pretreatment process was carried out using a hydrothermal reactor with a liquid-solid ratio (LSR) of 10 $kg_{water}/kg_{biomass}$, and the reactor volume was 200 mL. The reactor temperature was raised to 200 °C and then cooled to 50 °C with stirring at 500 rpm. The temperature was monitored from heating to cooling, every 2 minutes for 3.6 h. After the process, the pretreated mixture was subjected to vacuum filtration to separate the solid and liquid fractions. The liquid fraction was analyzed

by HPLC, according to the methodology described above. Then, the solid fraction was dried in an oven at 105 °C and characterized regarding chemical composition, as previously described. All experiments were performed in triplicate.

The pretreatment severity factor was calculated using equation 1, where R_0 is the severity factor, T is the temperature in °C, T_0 is the initial temperature, t is the time in minutes, and ω is the hydrothermal reactor constant and is equivalent to 14.75.

Equation 1 Equation severity factor.

$$\log R_0 = [R_0\ Heating] + [R_0\ Isothermal\ process] + [R_0\ Cooling]$$

$$\log R_0 = \left[\int_{T_{0Heating}}^{T_{Heating}} \frac{T(t) - 100}{\omega} dt\right] + \left[\int_{ctrl}^{ctr\,f} \exp\left(\frac{T(t) - 100}{\omega}\right) dt\right] + \left[\int_{T_{0Cooling}}^{T_{Cooling}} \frac{T(t) - 100}{\omega} dt\right]$$

The saccharification was performed using the LSR of 10 at pH 4.8 using citrate buffer (50 mM). The enzyme (Celic® Cetec 2, from Novozymes) was added at 15 $FPU{\cdot}g_{biomass}^{-1}$. The flasks were placed on a shaker at 50 °C and continuously shaken for 6 days at 200 rpm. Samples were taken for 6 days and centrifuged to separate insoluble solids. The supernatant was analyzed by High-Performance Liquid Chromatography (HPLC) to determine the concentration of fermentable sugars, as described above.

3 RESULTS & DISCUSSION

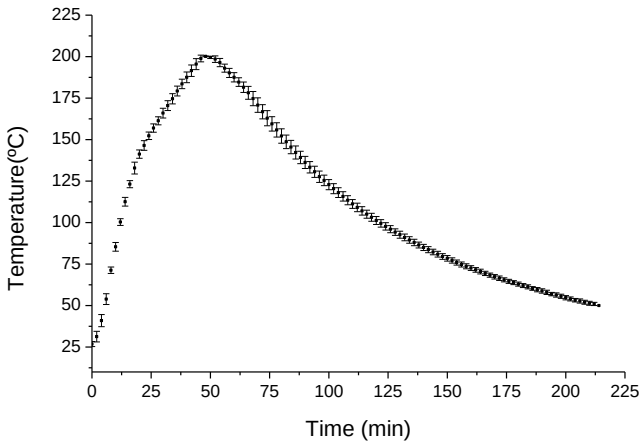
The chemical composition of the soybean hulls was determined using the applied methods, as shown in Table 1.

Table 1 Composition of soybean hulls.

Composition	This work	(Barros et al., 2020)	(Rojas et al., 2014)	(Qing et al., 2017)
Cellulose (%)	37.85 ± 2.83	40.6	35.8	28.6
Hemicellulose (%)	20.37 ± 1.53	33.8	23.1	20.0
Lignin (%)	7.44 ± 0.61	7.8	9.1	13.1
Extractives (%)	14.49 ± 3.31	4.8	15.4	0.2

The percentages of cellulose, hemicellulose, and lignin (37.8%, 20.4%, and 7.44%, respectively) were within the ranges reported in previous studies (Rojas et al., 2014; Barros et al., 2020; Qing et al., 2017). During the pretreatment, the temperature was monitored. Figure 1 shows how the temperature behaved during heating and cooling. For the pretreatment carried out, a severity factor was obtained, based on equation 1, of 2.46 ± 0.10.

Figure 1 Temperature monitoring during pretreatment.



Pedersen-Meyer *et al.* (2010), applied a pretreatment with diluted acid hydrolysis of agricultural residues at 140 ºC and obtained a value of 2.7 for the severity factor. In the same study, a pretreatment with a steam explosion was also applied to wheat straw and wood at 160 ºC and obtained a value of 2.8 for the severity factor. Gonzales e*t al.* (2016), used pretreatment with diluted acid hydrolysis of palm fruit bunch at 121 ºC and obtained a value of 2.4 for the severity factor. Table 2 shows the mass loss and change in the composition after autohydrolysis pretreatment.

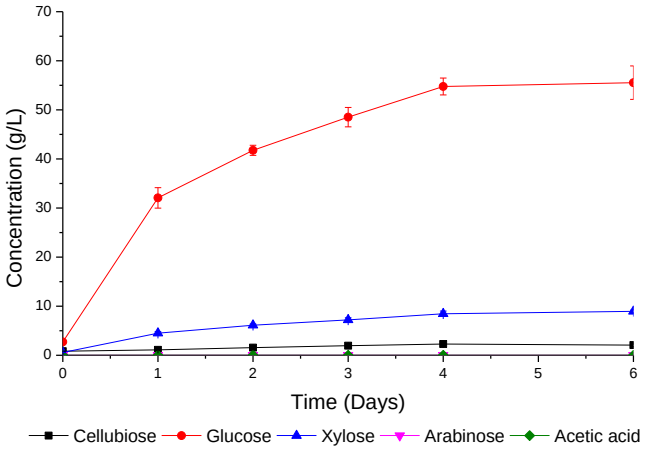
Table 2 Composition befere and after autohydrolysis pretreatment and mass loss resulted by the pretreatment.

Composition	In natura	Pretreated soybean hulls
Mass loss (%)	-	45.65 ± 0.23
Cellulose (%)	37.85 ± 2.82	40.90 ± 2.83
Hemicellulose (%)	20.37 ± 1.53	23.30 ± 1.75
Lignin (%)	7.44 ± 0.61	5.57 ± 0.52
Extractives (%)	14.49 ± 3.31	9.47 ± 1.70
Crystallinity index (%)	32.54 ± 0.73	55.75 ± 2.86

After pretreatment, the mass loss was significant, attributed to the elimination of extractives as shown in Table 3. This is due to the large amount of energy invested in the biomass from autohydrolysis pretreatment, disintegrating the chemical bonds present in the structure. The autohydrolysis pretreatment contributed to the delignification of biomass, in addition to proving to be highly efficient in increasing cellulose crystallinity. The increase in crystallinity is due to the removal of lignin and extractives and can be considered a relevant factor in the efficiency of pretreatment (Qing et al., 2017).

The saccharification carried out after pretreatment resulted in a large release of fermentable sugar, as shown in Figure 2. Glucose and xylose levels reached 55.4 $g \cdot L^{-1}$ and 8.95 $g \cdot L^{-1}$, respectively, in 4 days of saccharification, in the same range as other studies in the literature.

Figure 2 Fermentable sugar concentrations.



Bittencourt *et al.* (2022), obtained a concentration of 36.52 $g \cdot L^{-1}$ of glucose using 10 $FPU \cdot g_{biomass}^{-1}$ after 4 days using 10% solids content. Roesler *et al.* (2024), obtained a concentration of 40.4 $g \cdot L^{-1}$ of reducing sugars for an enzyme/substrate ratio of 20 $FPU \cdot g_{biomass}^{-1}$. Therefore, it is concluded that the values of sugar levels achieved by this work were higher than those of the studies mentioned above.

4 CONCLUSION

The autohydrolysis pretreatment is efficient pretreatment to soybean hulls. Pretreatment modified several components of the biomass, increasing crystallinity, promoting delignification, and reducing extractive levels, totaling a mass loss of 45.65%. Furthermore, producing 55.4 $g \cdot L^{-1}$ of glucose after saccharification step. The sugars produced by saccharification can be applied in various biotechnological processes, such as the production of acids, biofuels, and other value-added products.

REFERENCES

[1] KUCHARSKA, Karolina et al. Pretreatment of lignocellulosic materials as substrates for fermentation processes. Molecules, v. 23, n. 11, p. 2937, 2018.

[2] ABDULKHANI, Ali et al. Potential of Soya as a raw material for a whole crop biorefinery. Renewable and sustainable energy reviews, v. 75, p. 1269-1280, 2017.

[3] ROJAS, M. J., SIQUEIRA, P. F., MIRANDA, L. C., TARDIOLI, P. W., & Giordano, R. L. Sequential proteolysis and cellulolytic hydrolysis of soybean hulls for oligopeptides and ethanol production. Industrial Crops and Products, v. 61, p. 202-210, 2014.

[4] BITTENCOURT, Gustavo Amaro et al. Soybean hull valorization for sugar production through the optimization of citric acid pretreatment and enzymatic hydrolysis. Industrial Crops and Products, v. 186, p. 115178, 2022.

[5] SINDHU, Raveendran; BINOD, Parameswaran; PANDEY, Ashok. Biological pretreatment of lignocellulosic biomass–An overview. Bioresource technology, v. 199, p. 76-82, 2016.

[6] TORRES-MAYANGA, Paulo C. et al. Production of biofuel precursors and value-added chemicals from hydrolysates resulting from hydrothermal processing of biomass: a review. Biomass and Bioenergy, v. 130, p. 105397, 2019.

[7] JATOI, Abdul Sattar et al. Hydrothermal liquefaction of lignocellulosic and protein-containing biomass: a comprehensive review. Catalysts, v. 12, n. 12, p. 1621, 2022.

[8] NREL. 2014. “Standard Procedures for Biomass Compositional Analysis.” National Renewable Energy Laboratory (NREL).

[9] QING, Qing et al. Comparison of alkaline and acid pretreatments for enzymatic hydrolysis of soybean hull and soybean straw to produce fermentable sugars. Industrial Crops and Products, v. 109, p. 391-397, 2017.

[10] PEDERSEN, Mads; MEYER, Anne S. Lignocellulose pretreatment severity–relating pH to biomatrix opening. New biotechnology, v. 27, n. 6, p. 739-750, 2010.

[11] GONZALES, Ralph Rolly; SIVAGURUNATHAN, Periyasamy; KIM, Sang-Hyoun. Effect of severity on dilute acid pretreatment of lignocellulosic biomass and the following hydrogen fermentation. International Journal of Hydrogen Energy, v. 41, n. 46, p. 21678-21684, 2016.

[12] ROESLER, Belkis Chalup Silveira et al. Enzymatic hydrolysis of soybean hulls to produce a potential carbon source for cultivation of exopolysaccharide-producing diazotrophic bacteria. Biocatalysis and Agricultural Biotechnology, v. 57, p. 103112, 2024.

ACKNOWLEDGEMENTS

The authors are grateful to Supergasbras/SHV Energy, CAPES (Federal Agency for Support and Evaluation of Graduate Education) and CNPq (National Council for Scientific and Technological Development) for the financial support and scholarship.