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CHARACTERIZATION AND EFFECT OF ULTRASOUND PRETREATMENT OF SOYBEAN HULLS

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ABSTRACT

Lignocellulosic biomass is a renewable carbon source that stands out as a viable alternative for producing chemical compounds in place of fossil fuels due to its abundance and chemical composition, with soybean hulls being of great interest in recent studies. However, pretreatments are required to produce fermentable sugars and other compounds of interest. Conventional methods, including acid and base pretreatments might not be environmentally sustainable and lead to the formation of inhibitory compounds. In this work, a chemical characterization of soybean hulls and a preliminary study of ultrasound pretreatment were performed with the following parameters: LSR 10:1, 15:1 and 20:1, particle size between 0.425 and 0.850 mm, amplitude (30%). The results indicated the composition of the main components, 35% cellulose, 21% hemicellulose, and 9% lignin. Ultrasound pretreatment resulted in mass loss and alteration in composition, notably increasing cellulose crystallinity. The pretreatment proved to be a promising alternative for obtaining fermentable sugars.

Keywords: Soybean hulls. Ultrasound pretreatment. Lignocellulosic biomass. Fermentable sugars.

1 INTRODUCTION

The depletion of non-renewable fossil fuels and the escalating environmental consciousness have spurred the adoption of renewable and eco-friendly sources for generating energy and chemicals, aiming for solutions that are both environmentally sustainable and economically viable.[1] Biomass has emerged as a viable substitute for traditional carbon sources like coal and petroleum, owing to its widespread availability and renewable nature.[2] Among the options, lignocellulosic biomass stands out for its abundance, sustainability, and chemical composition.[3]

Lignocellulosic biomass is composed, predominantly, of cellulose, hemicellulose, and lignin; and minority by components such as chlorophyll, proteins, resins, pectin, ashes, terpenoids, and various extracts.[4] Derived from organic matter and agro-industrial remnants, these biomass primarily originate from agricultural or forestry byproducts, containing an abundance of carbohydrates like hexoses, pentoses, xylose, and arabinose.[5]

Soybean hulls represent a lignocellulosic residue garnering considerable attention in recent research, renowned for its polysaccharide richness and other biotechnologically pertinent polymers. Constituting roughly 5-8% of the total soybean mass, soybean hulls are ubiquitous in industrial facilities dedicated to soybean processing.[6,7,8] Nonetheless, owing to the inherent recalcitrance of lignocellulosic biomass, pretreatments become imperative to facilitate the liberation of fermentable sugars and other exploitable compounds.[9]

Several strategies for pretreating lignocellulosic biomass can be classified as chemical, physical, biological, or hybrid methods.[10] Traditional pretreatment techniques, relying on aggressive chemicals and elevated temperatures, are notorious for their energy intensiveness and environmental ramifications.[11] Additionally, these methods may engender the formation of undesirable byproducts like aliphatic acids, vanillic acid, uronic acid, 4-hydroxybenzoic acid, phenol, furan aldehydes, cinnamaldehyde, and formaldehyde, which could impede the growth of fermentative microorganisms during subsequent fermentation processes.[12]

Ultrasound technology generates elevated temperatures, pressures, and shear forces, facilitating the disruption of lignin and xylan networks.[13] This disruption enhances the accessibility of sugars within the biomass, rendering them more amenable to conversion into desired products.[14] Thus, the aim of this study was to characterize soybean hulls raw and after pretreatment and to carry out an investigation into the effects of ultrasound pretreatment, monitoring temperature and pressure variations and evaluating the recovered mass and crystallinity.

2 MATERIAL & METHODS

The soybean hulls utilized in the experiments were procured in a singular batch from a local establishment in the city of Mafra, (Santa Catarina). Subsequently, they underwent a series of preparatory steps, including washing with distilled water, drying to eliminate moisture, grinding, and sieving to attain particles within the size range of 0.425 to 0.850 mm. The processed material was then stored in polyethylene bags at ambient temperature.

For the compositional analysis of soybean hulls, NREL analytical methods for standard biomass analysis were employed.[15] Extractives were estimated by subjecting soybean hulls to sequential extraction using a cellulose cartridge in a Soxhlet apparatus, employing water and ethanol as solvents. The ash content was determined by calcining the dried samples in a muffle oven at 575°C for 4 hours. A 300 mg portion of soybean hulls was initially hydrolyzed with 3 mL of 72% (v/v) H_2SO_4 acid for 1 hour at 30°C. Subsequently, 84 mL of distilled water was added to the reaction mixture, followed by autoclaving at 120°C for 1 hour and filtration through crucibles. The acid-insoluble lignin content was ascertained using the retentate, while the soluble lignin content was determined by spectrophotometry at 280 nm using the permeate. Structural sugars present in the hydrolyzed liquor were quantified using an Agilent Hi-Plex H column in an HPLC setup (Agilent 1260 Infinity LC) equipped with an RI detector, operating at 55°C with a mobile phase consisting of 0.005 M H_2SO_4 at a flow rate of 0.7 mL/min. Monomeric sugars served as standards for the structural carbohydrate analysis conducted via HPLC.

The ultrasound pretreatment process was executed utilizing an ultrasound probe (VCX750, SONICS Vibra Cell™) operating at a frequency of 20 kHz and a nominal power of 750 W. The experiments were conducted in 50 mL beakers, each containing a total reaction mixture of 50 g (comprising soybean hulls and distilled water in various proportions). The operational parameters included an amplitude set at 30%, a liquid-solid ratio (LSR) of 10, 15 e 20 $kg_{water}/ kg_{biomass}$, particle sizes ranging from 0.425 to 0.850 mm, and a sonication duration of 15 minutes. Before to the commencement of each test, all samples underwent a 30-minutes hydration period. Temperature and power were monitored at 30-second intervals throughout the process. Following sonication, the pretreated mixture was subjected to vacuum filtration to separate the solid and liquid fractions. A 10 mL aliquot of the liquid fraction was collected and preserved at -4°C for subsequent analysis. Subsequently, the solid fraction was desiccated in an oven at 105°C, and the dried soybean hull samples were stored at 25°C. All experiments were conducted in triplicate.

The ultrasound pretreatment was performed in an ultrasound probe (VCX750, SONICS Vibra Cell™) operating at 20 kHz and nominal power of 750 W. The assays were carried out in a 50 mL beaker containing 50 g of total reaction amount (soybean hulls and distilled water in different proportions). The operating parameters were: amplitude of 30%, liquid-solid ratio (LSR) of 20 $kg_{water}/kg_{biomass}$, particle size (between 0.425 and 0.850 mm), sonication time of 15 min, all samples were 30 min in hydration before starting the test, temperature, and power were monitored every 30 seconds. After sonication, each sample was vacuum filtered to separate the pretreated liquor into solid and liquid fractions. 10 mL of the liquid fraction was collected and stored at -4 °C for further analysis. Finally, the solid fraction was dried in an oven at 105 °C, and the dried soybean hull samples were dried and stored at 25 °C. All experiments were performed in triplicate.

3 RESULTS & DISCUSSION

From the conducted analyses, it was possible to determine the chemical composition of soybean hulls. Typically, the composition depends on the characteristics of the crop, genetic factors, and the type of grain processing. Table 1 shows the chemical composition of soybean hulls.

Table 1 Main components of soybean hulls.

Composition	This work	Barros et al, 2020	Rojas et al, 2014	Qing et al, 2017
Cellulose (%)	34.56 ± 2.79	40.6	35.8	28.6
Hemicellulose (%)	20.76 ± 2.15	33.8	23.1	20.0
Lignin (%)	9.41 ± 0.56	7.8	9.1	13.1
Extractives+Ashes (%)	22.04 ± 0.08	4.8	15.4	0.2
Protein (%)	13.22 ± 1.06	9.4	9	-

The percentages of cellulose, hemicellulose and lignin (34.56%, 20.76% and 9.41% respectively) were within the ranges reported in previous studies.[8,16,17] Soybean hulls present a lower lignin content compared to sugarcane bagasse (25-28%), which is the main biomass studied in Brazil. [18,19] The lower lignin content in soybean hulls may allow enzymatic hydrolysis of cellulose to proceed without generating degradation products (such as aromatic compounds) that could inhibit yeast during the fermentation phase.[20] Soybean hulls also have a high protein concentration (14.04%), distinguishing them from other lignocellulosic materials such as woody materials and sugarcane bagasse. The recovered proteins can be used as a nitrogen source in culture media. [8,21]

A preliminary study was conducted to assess the effects caused by ultrasound pretreatment on the biomass. Table 2 shows the mass loss, composition alteration, and crystallinity after ultrasound pretreatment.

Table 2 Soybean hull pretreated with ultrasound.

Composition	*In natura*	LSR 10 - 30%	LSR 15 - 30%	LSR 20 - 30%
Mass Loss (%)	-	7.28 ± 0.58	11.58 ± 0.09	11.40 ± 1.15
Cellulose (%)	34.56 ± 2.79	38.47 ± 0.27	40.80 ± 2.65	43.03 ± 1.62
Hemicellulose (%)	20.76 ± 2.15	20.21 ± 0.34	20.50 ± 1.96	19.94 ± 0.70
Lignin (%)	9.41 ± 0.56	9.33 ± 0.42	10.44 ± 0.17	9.47 ± 0.65
Extractives (%)	22.04 ± 0.08	19.45 ± 0.34	17.00 ± 0.68	16.80 ± 0.64
Protein (%)	13.22 ± 1.06	12.54 ± 0.46	11.27 ± 0.63	10.76 ± 0.70
Crystallinity index (%)	31.51 ± 0.80	41.68 ± 1.54	43.64 ± 0.08	47.62 ± 0.75

In all the tests, there was a reduction in mass after pre-treating the biomass with ultrasound. This result can be attributed to the release of extractives and a small portion of proteins, as shown in Table 2. This can be attributed to ultrasound causing cavitation, which generates the implosion of bubbles, releasing a large amount of energy and causing high temperatures and pressures in the surrounding area. This generates shear forces that destabilize the cell wall, contributing to the disintegration of the chemical bonds present in the structure of lignocellulosic biomass. In addition, cleavage reactions are also enhanced by the radicals produced by ultrasound. [13,22]

The ultrasound pretreatment using water as a solvent did not prove to be efficient in delignification, maintaining a composition similar to the raw material, and demonstrated to be highly effective in increasing cellulose crystallinity. Considering the raw material, there was an increase in crystallinity of 41.68 ± 1.54%, 43.64 ± 0.08%, and 47.62 ± 0.75% under the respective conditions. The increase in crystallinity in soybean husk can be attributed to the removal of amorphous components such as lignin, hemicellulose, and extractives. The crystallinity index and crystalline structure of cellulose are considered relevant factors in the efficiency of enzymatic hydrolysis of lignocellulose.[17]

4 CONCLUSION

This preliminary study indicates that pretreatment with ultrasound is effective in solubilizing a fraction of soybean hulls. This result was attributed to the synergistic effect between cavitation waves and sonochemistry, which affected the recalcitrant structure of the biomass, resulting in a reduction in extractives and other compounds. Additionally, pretreatment favored an increase in the crystallinity of the biomass by up to 47.62 ± 0.75% after pretreatment. Ultrasound-pretreated soybean hulls demonstrate potential for saccharification and fermentation stage.

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