PRELIMINARY STUDY ON SACCHARIFICATION OF UNTREATED SOYBEAN HULL TO PRODUCE FERMENTABLE SUGARS

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ABSTRACT

Saccharification is a critical process that transforms lignocellulosic biomass, such as soybean hulls, into fermentable sugars, which are needed to produce biofuels. Enzymes such as cellulases and hemicellulases collaborate in this process to decompose the biomass complex structures. Using soybean hulls, a substantial and formerly underutilized byproduct of the food industry, helps reduce waste and promote sustainable agricultural resource management. In this study, the direct saccharification of raw soybean hulls is assessed through an enzyme mixture consisting of cellulases, proteases, pectinases, and oxidoreductases. To maximize the enzymatic conversion of lignocellulosic biomass without the requirement for pretreatment, the primary aim was to produce fermentable sugars. While the biomass was not pretreated prior to enzymatic hydrolysis, the testing results were considered promising. The procedure demonstrated the potential of raw soybean hull as a workable feedstock by producing 19.8 ± 4.2 g L^{-1} of glucose and 9.8 ± 2.0 g L^{-1} of xylose in two days, a respectable amount of dissolved cellulose and hemicellulose. These findings suggest that the saccharification method can provide enough fermentable sugars, even in the absence of pretreatment, to be used in several added-value products.

Keywords: Enzymatic hydrolysis. Saccharification. Lignocellulosic biomass.

1 INTRODUCTION

The search for alternative energy sources has been fueled in recent years by increased concern over environmental sustainability and the depletion of fossil fuels. In this case, biofuels show promise as a substitute for fuels generated from petroleum, either entirely or in part [1]. Utilizing biofuels, which come from renewable resources, helps to mitigate greenhouse gas emissions while also reducing reliance on fossil fuels [2].

Soybean hulls are one of the most plentiful and inexpensive residues from the food sector that can be used as a biomass source [3]. This agricultural byproduct has a large amount of unrealized potential for conversion into biofuels but is often ignored or used insufficiently. This allows to change the perception of what was previously seen to be a waste product with little economic worth, increasing its value and sustainability in the larger industrial landscape [4]. 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 representing 5-8 % of total soybean mass. Another important component is pectin, which makes up 4.2% of soybean hulls [5].

Usually after pretreatment, lignocellulosic biomass is converted into fermentable sugars by enzymatic hydrolysis, which becomes a crucial step in the manufacture of biofuels [3]. Cellulases and hemicellulases are two of the enzymes that cooperate in this process to facilitate the breakdown of biomass's complex structural components into their most basic elements. The main polymer in plant cell walls, cellulose, is specially hydrolyzed by cellulases to produce glucose. Conversely, hemicelluloses, another significant part of lignocellulosic biomass, are broken down into sugars like xylose, arabinose, and mannose by hemicellulases [6]. Because of its efficiency and selectivity, this enzymatic method stands out for producing a mixture of sugars that can be immediately used for biofuel synthesis by microorganisms that ferment [7].Considering this, the current work aims to investigate enzymatic saccharification, which produces sugar from soybean husk, without the need for pretreatment using a combination of enzymes.

2 MATERIAL & METHODS

The soybean hull was characterized using protocols from the National Renewable Energy Laboratory (NREL), assessing proteins, extractives, cellulose, hemicellulose, soluble and insoluble lignin, as well as ash content. Initially, the lignocellulosic composition of the solid samples was determined through sequential extraction with ethanol and water on the raw material to assess extractives content, in accordance with NREL/TP-510-42619. Additionally, the protein content was determined using the Kjeldahl method, following NREL/TP-510-42625. Cellulose, hemicellulose, and lignin were quantified following protocols NREL/TP-510-42623 and NREL/TP-510-42618, respectively. Ash content was determined according to NREL/TP-510-42622.

Soybean hull without pretreatment was used in this study. The biomass was ground and homogenized to ensure uniform distribution during the experiments (from 0.425 to 0.850 mm), and finally, the sample was desiccated to remove any excess moisture and standardize the analysis conditions. The experimental conditions were carried out in two sets of experiments, each in triplicate, using different enzyme pools.

For each replicate, 4 grams of desiccated and ground biomass were added to 80 mL of buffer (liquid to solid ratio of 20). Subsequently, the enzyme pool designated as "Mix 1" and "Mix 2" was added (Table 1). The entire content was transferred to a previously sterilized and sealed serum bottle flask. The flasks were placed on a shaker at 50°C and continuously shaken for 13 days at 200 rpm and pH 4.8.

Table 1 Enzyme pool composition

Mix 1	Quantities	Mix 2	Quantities
Celic Cetec 2	15 FPU. $G_{biomass}^{-1}$	Celluclast	15 FPU. $G_{biomass}^{-1}$
Pectinex	59.56 $mg.mL^{-1}$	Pectinex	59.56 $mg.mL^{-1}$
Pec SP	59.54 $mg.mL^{-1}$	Pec SP	59.54 $mg.mL^{-1}$
Bromelain	16 mg	Bromelain	16 mg
Papain	16 mg	Papain	16 mg
Lacase	10 mg	Lacasse	10 mg

During the incubation period, sample points were taken at time intervals of 0, 2, 6, 10, and 13 days. All these steps were carried out under aseptic conditions within a laminar flow hood to avoid microbial contamination. Temperature and agitation conditions were monitored and kept constant throughout the experiment to ensure result reproducibility. The samples were centrifuged to separate insoluble solids, and the supernatant was analyzed by High-Performance Liquid Chromatography (HPLC) to determine the concentration of fermentable sugars, such as glucose and xylose.

Quantification of arabinose, glucose, and xylose was performed using High-Performance Liquid Chromatography (HPLC) (LC-2050C 3D Shimadzu, Japan) equipped with a RID-20A detector and an Aminex HPX 87H column (300 mm × 7.8 mm; Biorad, USA). A 15 µL injection was eluted with a mobile phase of 5 mM H_2SO_4 at a flow rate of 0.6 $mL\cdot min^{-1}$ at 45°C. Sugar concentrations were determined from standard curves of glucose, xylose, arabinose, and acetic acid.

3 RESULTS & DISCUSSION

The characterization of soybean hull biomass revealed significant compositional attributes, the results will be presented first, as shown in the Table 2.

Table 2 Soybean hull characterization.

Composition	Percentage %
Cellulose	40.4 ± 1.91
Hemicellulose	20.81 ± 0.46
Soluble lignin	13.48 ± 0.54
Insoluble lignin	2.83 ± 0.44
Extractive	15.48 ± 1.71
Protein	10.93 ± 0.08
Ashes	4.8 ± 0.05

The average protein content was found to be 10.93± 0.08%, while the mean extractive content was measured at 15.48 ± 1.71%. Soluble lignin content averaged 13.48 ± 0.54% and ash content averaged 4.80 ± 0.05%. Notably, the focus was placed on hemicellulose and cellulose contents, as they are key components for biomass conversion. As observed in Table 2, the average hemicellulose content was 20.81± 0.46%. Similarly, cellulose content averaged at 40.40 ± 1.91%. Our results are generally consistent with the reported ranges in the literature, the observed cellulose content of 40.40 ± 1.91% aligns closely with the middle of the reported range (28.6–52.3%), suggesting a significant polysaccharide presence, crucial for fermentable sugar production. Similarly, the hemicellulose content of 20.81 ± 0.46% is within the range of 18.5–33.8%, indicating a robust hemicellulosic fraction.[1] Regarding biomass conversion, the enzymatic saccharification process demonstrated remarkable efficiency in converting glucose from cellulose and xylose from hemicellulose. This process exhibited a distinct kinetic profile, with the majority of conversion occurring within the initial 48 hours (Figure 1). After this period, while conversion rates of glucose and xylose showed low increase.

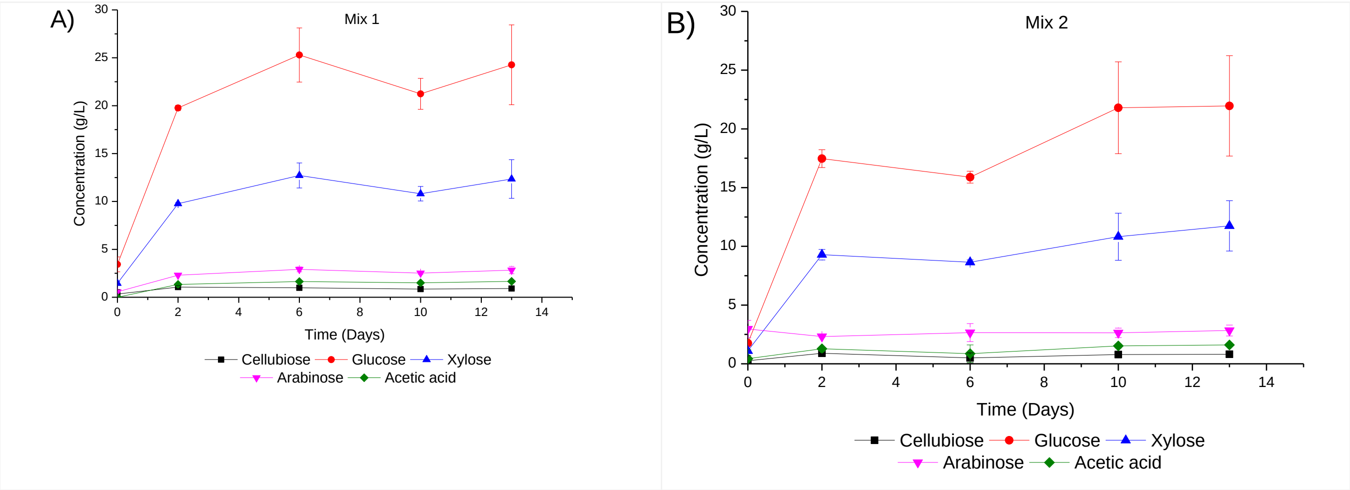

Figure 1 Sugar production overt time using A) Mix 1 and B) Mix 2.

Quantitative analysis reveals that during the initial period, sugar conversion rates were notably high for both enzyme mixes. Glucose and xylose reached concentrations of 19.77 and 9.8 g/L, respectively, for Mix 1 (Figure 1 A) and 17.5 of glucose and 9.3 g/L of xylose for Mix 2 (Figure 1 B), representing significant conversion percentages of 76.19% for glucose and 70.01% for xylose in Mix 1 and of 73.01% for glucose and 69.00% for xylose in Mix 2. However, over the subsequent 13 days, although there were further increases in sugar concentrations, the conversion percentages for glucose and xylose only experienced minimal changes, suggesting a plateau effect in enzymatic activity for both enzyme mixes.

4 CONCLUSION

The results obtained emphasize the soybean hull biomass suitability for saccharification processes without the requirement for earlier pretreatment, establishing it as a promising renewable feedstock for the manufacture of biofuel and biochemicals. Soybean hull biomass's reliable composition and effective sugar conversion rates demonstrate its promise as a useful resource for biorefinery applications. Using soybean hulls in bioenergy technologies allows us to make a positive impact on the development of environmentally friendly energy solutions by promoting the sustainable use of agricultural residue. Furthermore, the effective extraction of sugars from soybean hull biomass highlights its potential to have a major influence on the biofuel and biochemical fields.

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