BIORREFINERY, BIOECONOMY AND CIRCULARITY

PRODUCTION OF XYLITOL FROM SOYBEAN HULLS

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ABSTRACT

Brazil is currently the largest soybean producer in the world, with the production of about 162.5 thousand tons in the 2023/2024 harvest. The industrial soybean processing produces 42 million tons of waste annually, and the hulls are the main by-product. According to the circular economy model, the use of soy hulls promotes economic development, optimizes the stages of the production process, promoting maximum use. The biotechnological production of xylitol, using enzymes and/or microorganisms that convert xylose into xylitol, is an alternative more efficient and economical to the traditional method, as it does not require the initial purification of xylose, using enzymes and/or microorganisms that convert xylose into xylitol. Soybean hulls is a plant biomass formed mainly by cellulose, hemicellulose and lignin. Thus, the hemicellulose fraction, which is mainly formed by xylose monomers, could be used to produce xylitol. The main objective of this work is to evaluate the biotechnological potential of soybean hulls to produce xylitol. The soybean hulls were subjected to different chemical treatments in order to solubilize the hemicellulose fraction and release xylose. Finally, xylose was fermented to xylitol by *Debaryomyces hansenii*. The biotechnological process for producing xylitol shows promise, as it seeks to optimize the soybean biorefinery, generating a value-added product that offers numerous benefits for human health.

Keywords: biotechnology, fermentation, soybean hulls, xylose, xylitol.

1 INTRODUCTION

Soybean, recognized as a primary source in the food, animal feed, cosmetics, soaps, varnishes and biodiesel industries, constitutes an important raw material. Brazil is currently the world leader in soybean production, recording production of 162 thousand tons in the 2023/2024 harvest.[1]

The industrial processing of soy generates a significant amount of waste, with the hulls representing 7 - 8% of the weight of the grain, and constituting the main co-product.[2] Vegetable biomass residues, consisting of cellulose, hemicelluloses and lignin appear as a promising option for sustainable development, through the creation of new products with greater added value, from its constituents.[3]

The biotechnological process of producing xylitol, from soybean hulls, appears as an alternative to the conventional chemical method, eliminating the need for initial purification of xylose (converted into xylitol in the hydrolyzate itself) and employing specific enzymes or microorganisms to convert xylose into xylitol.[4] Therefore, the main objective of this work is to evaluate the biotechnological potential of soybean hulls for xylitol production.

2 MATERIAL & METHODS

Pre-treatment of soybean hulls. The soybean hulls were donated by CJ Selecta located in Industrial District, Araguari – MG. Different pre-treatments: hydrothermal, using water; acid using H_2SO_4 in concentrations from 0.5% to 5.0%; and alkaline, using 1.5% NaOH, under time conditions of 30 and 60 minutes and total solids concentration from 10% to 50% w/v were carried out in an autoclave at 121 °C, to establish the process with the best yield of xylose. All pre-treatments were carried out in triplicate. After these treatments, the samples were filtered and the solid fraction was washed with distilled water and dried to determine the composition. The liquid fraction was cooled for later analysis.[5,6]

Composition of soybean hulls. The soybean hulls were initially ground until particles smaller than 20 mesh and the moisture content was measured using the Ohaus MB25 Moisture Analyzer (Cheeselab, São Paulo). The composition of *in nature* and pretreated soybean hulls were determined by National Renewable Energy Laboratory (NREL)–LAP standard analytical procedures. Chemical characterization was carried out in triplicate.[7,8,9]

Xylose fermentation to Xylitol. The pretreated soybean hulls samples had their pH adjusted to 5.0 and were fermented at 30 °C, 150 rpm for 24 h, at final solid concentration of 7% w/v, using the yeast *Debaryomyces hansenii.* These fermentation assays were carried out in triplicate. At the end of this process, the fermentation products were analyzed by HPLC and the yield of xylitol produced was calculated based on the initial substrate and based on the substrate consumed.[10]

Analytical methods. Saccharide, organic acids, furfural, 5-hydroxymethylfurfural (5-HMF), xylitol and ethanol concentrations were determined by high performance liquid chromatography (HPLC Shimadzu). The HPLC was equipped with an Aminex ion exclusion HPX-87H (300mm × 7.8 mm) cation-exchange column (BioRad Labs, Hercules, CA) 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.[11]

3 RESULTS & DISCUSSION

The relative concentration of cellulose, hemicellulose and lignin of soybean hulls *in natura* and after the different pretreatments, and the mass yield after the pretreatments are shown in Table 1.

Table 1. Composition (% w/w) of soybean hulls before and after pretreatments carried out for 60 min, at 121°C – with a solid load of 25 % (w/v) and the mass yield after the pretreatments.

Treatment	Cellulose	Hemicellulose	Lignin	Total	Mass yield (%)
Soybean hulls *in nature*	36.26 ± 0.86	24.08 ± 0.50	15.41 ± 2,57	75.75	100,00
Hydrothermal	35.20 ± 0.53	24.05 ± 1.05	13.20 ± 1.26	72.45	85,78
H_2SO_4 0,5%	30.51 ± 2.81	15.52 ± 1.50	16.51 ± 2.61	62.54	84,89
H_2SO_4 1,0%	31.66 ± 1.60	16.03 ± 0.88	20.21 ± 2.13	67.90	80,37
H_2SO_4 1,5%	31.97 ± 1.60	12.47 ± 1.45	18.51 ± 2.35	62.95	78,00
H_2SO_4 5,0%	53.29 ± 1.44	1.77 ± 0.30	24.70 ± 0.90	79.76	52,32
NaOH 1,5%	45.80 ± 2.18	29.89 ± 1.64	21.07 ± 1.94	96.76	92,98

No furanic derivatives such as furfural, hydroxymethylfurfural (HMF) were detected in the liquid fractions of soybean hulls regardless of pretreatment. However, alkaline pretreatment produced a small concentration of formic acid (1.09 g/L) and acetic acid (< 0.1 g/L), which at low concentrations does not inhibit fermenting microorganisms. xylose to xylitol. Furans are among the most potent fermentation-inhibiting compounds.[12] These substances formed during the pre-treatment of lignocellulosic raw material inhibit the microbial fermentation stages, impairing the ethanol yield.[13] Therefore, the absence of furans in the liquid fractions analyzed is a desirable characteristic for the fermentation process. Pretreatment with 5.0% H_2SO_4 was selected as it showed greater efficiency in breaking the hemicellulosic structure, promoting the release of around 60% of xylose and showed low levels of inhibitor formation. This pretreatment did not require the enzymatic hydrolysis step for the fermentation since the liquid fraction resulting from this condition is already rich in xylose (10.6 g/L), arabinose (4.1 g/L) and glucose (1.2 g/L). So, the soybean hulls sample, pretreated with 5.0%(v/v) H_2SO_4 was fermented using the yeast *D. hansenii* and the results are showed in Figure 1.

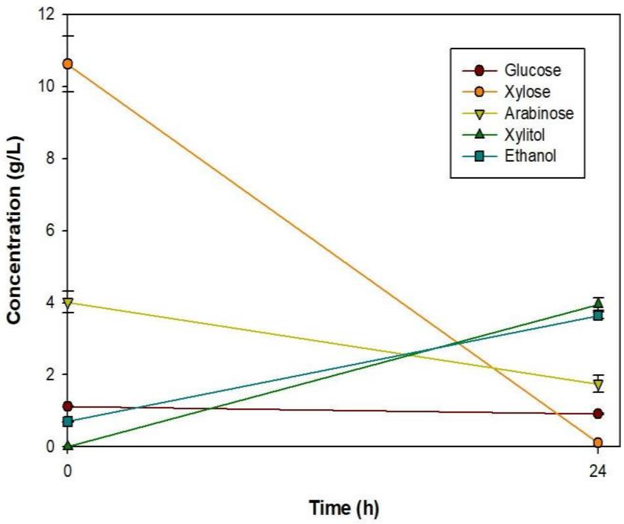

Figure 1. Evaluation of the products formed after 24 hours of pre-treated soybean hulls fermentation with *D. hansenii* pre-treated soybean hulls.

ferment to xylitol (4 g/L) representing a conversion of 41%. However, xylose was also converting to ethanol since the ethanol concentration (3.5 g/L) was observed at the end of fermentation. No glucose consumption was observed and about 2.3 g/L of arabinose was consumed which probably contributed to final ethanol concentration. The fermentative parameters are shown in Table 2.

Table 2. Evaluation of the maximum concentration of xylitol (P max (g/L) and the fermentative parameters of hydrolyzed soybean hull.

Sample	Pmax (g/L)	(Y%)	($Y P/S$)	η(%)
Soybean hulls hydrolyzate	9.67	100	0.38	40.99

Where:
Pmax (g/L): Maximum xylitol concentration;
($Y P/S$): Xylose to xylitol conversion factor. Yield of xylitol production based on xylose consumption (g xylitol/g xylose);
(%): Efficiency in converting xylose to xylitol;
(Y%): Percentage of xylose consumption.

The xylitol yield obtained was 0.38 g xylitol / g xylose ($Y P/S$). It is important observed that all xylose was consumed (Y=100%), but only part of this monosaccharide was convert to xylitol (η=41%). The results indicate a good fermentative performance of the yeast *Debaryomyces hansenii*, however, these yeast parameters can still be optimized to achieve higher yields.

4 CONCLUSION

The pretreatment of soybean hulls with 5%(v/v) sulfuric acid combined with fermentation by the yeast *Debaryomyces hansenii*, demonstrated positive results for the xylitol production. Thus, the bioconversion of soybean hulls into xylitol shows promise, being capable of generating a product with high added value.

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