

Extruded corn cob organosolv optimization into high glucose conversion and high lignin precipitation

José A. H. M¹ & Rodrigues R. C. L. B¹.

¹ Laboratory of Applied Microbiology and Bioprocesses, Department of Biotechnology, Lorena School of Engineering, University of São Paulo, Brazil.

* Author's e-mail for correspondence: alvaro_he@usp.br

ABSTRACT

In the concept of a biorefinery, lignocellulosic biomass, such as corn cobs, can be used as raw material to produce innumerable products, including biofuels, base products for the chemical industry, lignin, among others. The combination of different pretreatments must be used to enable the use of the cellulose, hemicellulose, and lignin fractions. The twin-extrusion pretreatment facilitated the production of a defibrillated corn cob. The study of the impact of different variables in the organosolv treatment showed that time is the most relevant, and on its optimization study, together with the concentration of solids, 60 minutes of organosolv treatment together with 20.6 % w/w of concentration of solids presented the best lignin recovery and cellulose conversion to glucose on subsequent enzymatic hydrolysis.

Keywords: Corn cob. Twin-screw extrusion. Organosolv. Lignin precipitation.

INTRODUCTION

Based on a platform for biorefinery, lignocellulosic biomass, such as corn cobs, can serve as a source of renewable biomass to produce soluble sugars or phenolic compounds. Through chemical, enzymatic, and fermentative processes, these can provide liquid fuels and a variety of chemicals, with value chains like those of petroleum derivatives but with a lower environmental impact ^{1,2}.

The twin-screw extrusion technology is commonly used in the polymer and food industries and can be a viable pretreatment method within the biorefinery concept. According to Zheng et al. (2014)³, the extrusion process has advantages over other pretreatments by providing high shear, rapid heat transfer, mixing, moderate temperature, short residence time, and ease of process modification. This process can operate in continuous flow with a large amount of material (40-60 % w/w) and is easily scalable. Extrusion provides high shear force on the material, increasing its surface area and thus facilitating enzyme action ⁴. However, for biomass to be extruded, the use of additives, such as glycerol is necessary to facilitate its transport within the extruder ^{4,5}.

Despite its advantages, the extrusion process does not alter the chemical structure of lignocellulosic biomass ⁶, making it beneficial to use other pretreatments that remove the lignin and hemicellulose present in this material. In this regard, the solvent fractionation process, also known as organosolv, is a pretreatment method in which lignin from lignocellulosic biomass is extracted by different organic solvents ⁷. After extraction, the solid fraction consists of cellulose, which has high enzymatic digestibility. The liquid fraction consists of lignin and hemicellulose removed during the solvent fractionation process ⁴.

Combining twin-screw extrusion pretreatment with solvent fractionation (organosolv) can separate fractions with high purity and low effluent generation ⁸. This method can extract lignin from lignocellulosic biomass using various organic solvents. Furthermore, combining pretreatment with enzymatic hydrolysis of cellulose is a key step in obtaining fermentable sugars for the subsequent production of ethanol or other fermentation products by microorganisms ⁹.

MATERIAL & METHODS

The corn cob, crushed, dried, and bagged (20 kg), sourced from RASUL - Indústria e Comércio de Rações Ltda – PR, was processed in a knife mill and sieved into sizes between 200 and 710 µm, then stored at room temperature until pretreatment extrusion tests were conducted. The twin-screw extrusion process of the corn cob was carried out at the company Afinko at the city of São Carlos (SP-Brazil) with screws rotation speed of 200 rpm and 144 °C. A 1:1 w/w solution of distilled water and commercial glycerin was added until a corn cob mixture with 25% water by mass, 25% glycerin by mass, and 50% raw corn cob by mass was obtained ⁴.

The solvent fractionation process was divided into two phases, the variable screening phase, and the optimization phase.

The screening phase was carried out in a high-pressure reactor with a capacity of 250 mL, equipped with temperature and pressure control and magnetic stirring. Six variables were studied using the Plackett-Burman model and their ranges are present at Table 1. The response variables studied were the conversion of cellulose to glucose and the glucose productivity in the enzymatic hydrolysis process. The solvent ratio was based on the study by Brudecki et al. (2013) ¹⁰, where Solvent A consists of 30% water, 61% ethanol, and 9% methyl isobutyl ketone (MIBK), and Solution B consists of 30% water and 70% ethanol.

Table 1 Factors studied at the Plackett-Burman model.

Item	Variable A (unit)	Variable B (unit)
Temperature (°C)	110	140
Stirring speed (rpm)	100	300
Sulfuric acid concentration (mg g ⁻¹)	0	10
Solvent	A	B
Time (min)	15	45
Glycerol (% w/w)	0	45

According to the results obtained in the last section, it was determined that the treatment time variable had the greatest impact on the solvent fractionation process of corn cob. Therefore, a 2² factorial design with central face and 4 replications at the central point was carried out to evaluate the effect of treatment time and the concentration of solids present in the reaction. The levels of the independent variables studied were determined based on other works (temperature from 20 to 60 minutes and concentration of solids from 5 to 20 % w/w), aiming to reduce the treatment time and increase the solid load ^{11,12}. The response variables used were the conversion of cellulose to glucose after enzymatic hydrolysis evaluated in section, as well as the yield of precipitated lignin.

The enzymatic hydrolysis, conducted to evaluate the efficiency of the solvent fractionation, was carried out in 125 mL Erlenmeyer flasks under the following conditions: 50 mmol L⁻¹ sodium citrate buffer, pH 4.8, agitation at 200 rpm in a rotary shaker incubator, 50 °C, and a solid load of 10 % w/w with a total liquid volume of 50 mL. The enzymatic load used was 31 FPU g⁻¹ of dry lignocellulosic material for the Cellic CTec2 enzyme complex kindly provided by Novozymes®. Sodium azide was also added to the reaction medium at a concentration of 0.3 % w/w to inhibit microbial contamination. After 57 hours, the enzymatic hydrolysate was centrifuged at 2000 x g for 20 minutes, and the supernatant was collected for subsequent sugar analysis by high-performance liquid chromatography ⁴.

RESULTS & DISCUSSION

Table 2 presents the condition used on each experiment of the Plackett-Burman model and the response variables results.

Table 2 Conditions and results of the Plackett-Burman model experiments

	T (°C)	Agitation (rpm)	Acid (mg g ⁻¹)	Solvent	Time (min)	Glycerol (% w/w)	Cellulose into glucose conversion (% w/w)	Productivity (g L ⁻¹ h ⁻¹)
1	-1 (110)	1 (300)	-1(0)	-1 (A)	-1 (15)	1 (0)	78.5	0.48
2	1 (140)	1 (300)	1(10)	-1 (A)	1 (45)	1 (45)	95.9	0.71
3	1 (140)	1 (300)	-1(0)	1 (B)	1 (45)	-1 (0)	74.5	0.50
4	-1 (110)	-1 (100)	-1(0)	-1 (A)	-1 (15)	-1 (0)	73.1	0.46
5	-1 (110)	-1 (100)	1(10)	1 (B)	1 (45)	-1 (0)	96.5	0.64
6	1 (140)	-1 (100)	-1(0)	-1 (A)	1 (45)	1 (45)	79.0	0.52
7	1 (140)	-1 (100)	1(10)	-1 (A)	-1 (15)	-1 (0)	68.7	0.58
8	-1 (110)	1 (300)	1(10)	-1 (A)	1 (45)	-1 (0)	70.9	0.54
9	-1 (110)	1 (300)	1(10)	1 (B)	-1 (15)	1 (45)	70.3	0.41
10	1 (140)	-1 (100)	1(10)	1 (B)	-1 (15)	1 (45)	65.2	0.59
11	1 (140)	1 (300)	-1(0)	1 (B)	-1 (15)	-1 (0)	90.1	0.55
12	-1 (110)	-1 (100)	-1(0)	1 (B)	1 (45)	1 (45)	85.1	0.59

The ANOVA analyses were performed for both response variables studied (conversion of cellulose to glucose and the glucose productivity in the enzymatic hydrolysis process) and the pareto chart obtained for both variables are present at figure 1.

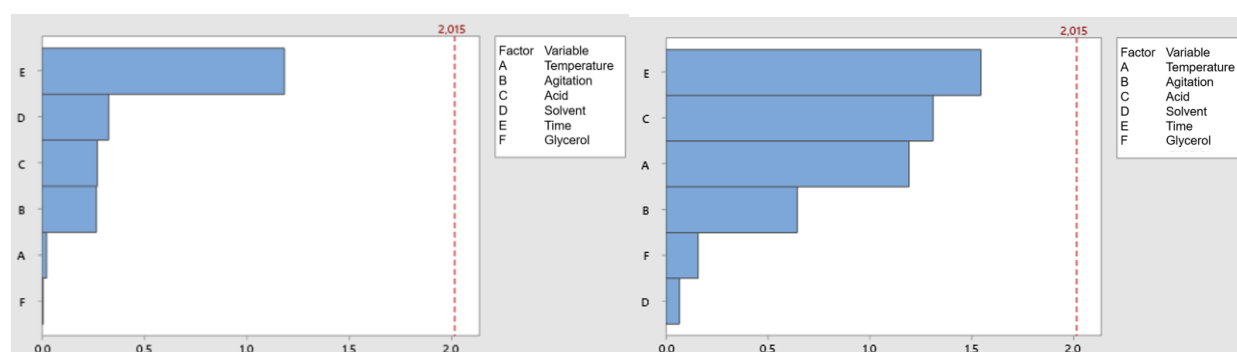


Figure 1 Pareto chart for conversion of cellulose to glucose and the glucose productivity, from left to right.

The variable with most impact in the conversion of cellulose to glucose and the glucose productivity was the temperature, indicating that between all variables studied and the ranges selected, changing the temperature of the organosolv treatment might impact the results the most. On that matter, that variable was selected to be studied and the rest were fixed at: 140 °C, Solvent B, Agitation at 100 rpm, 10 mg g⁻¹ sulfuric acid and 45 % w/w glycerol.

Table 3 Conditions and results of the 2² factorial design experiments

	Time (min)	Concentration of solids (% w/w)	Cellulose into glucose conversion (%)	Recovered lignin (g)
1	20	5.1	63.72	1.26
2	20	20.6	65.25	3.72
3	60	5.1	71.65	1.73
4	60	20.6	92.61	5.67
5	20	12.9	77.48	3.25
6	60	12.9	83.07	3.71
7	40	5.1	70.01	1.31
8	40	20.6	96.49	4.86
9	40	12.9	87.45	3.49
10	40	12.9	84.14	3.31
11	40	12.9	84.99	3.42
12	40	12.9	86.75	3.31

Analyzing the table 3, the highest cellulose into glucose conversion was obtained at the 8th experiment and the highest recovered lignin was obtained at the 4th experiment, indicating a region of optimization for both variables. After calculating the ANOVA for both response variables, the solids concentration on its maximum condition pointed to the higher values for both response variables. However, time presented as an area of optimization for the cellulose conversion into glucose, from 50 to 60 minutes, but it got the highest value of recovered lignin using the maximum value used in this study, 60 min. With that in mind, the optimal values of time and concentration of solids in the organosolv optimization study was their maximum values, 60 minutes and 20.6 % w/w.

To validate the model obtained in this study, three experiments in the optimal condition were realized, obtaining $93,01 \pm 1,2$ % w/w of cellulose conversion and $5,73 \pm 0,27$ g of recovered lignin, like the predicted values from the model, 93,86 % w/w and 5,61 g.

CONCLUSION

The time present greater influence on the cellulose into glucose conversion than other variables analyzed in this study. The highest concentration of solids presented the highest recovered lignin, and when combined with high reaction time, it also presented high cellulose into glucose conversion, indicating that potentially an even higher concentration might be used to obtain higher recovered lignin values.

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