



Integrated twin-screw extrusion and organosolv pretreatment efficiently enhances cellulose-to-glucose conversion and lignin recovery from corn cobs

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ABSTRACT

Efficient conversion of lignocellulosic biomass into bioproducts depends on optimized pretreatment strategies that enhance the accessibility of structural macromolecules. However, individual methods often face limitations regarding cellulose-to-glucose conversion and lignin recovery. This study evaluated an integrated approach combining twin-screw extrusion, organosolv fractionation, and enzymatic hydrolysis using corn cobs as feedstock. A factorial design applied to the extrusion process identified optimal conditions (144 °C, 11 rpm), resulting in a cellulose-to-glucose conversion of 92.6 wt% after enzymatic hydrolysis. In the subsequent organosolv step, treatment time was the most influential factor for improving cellulose digestibility. A second factorial design identified optimal conditions (60 min, 20.1 wt% solids), yielding the highest glucose conversion (93.0 ± 1.2 wt%) and enabling recovery of 45.4% of the solubilized lignin. Additionally, increasing solids loading from 10 to 15 wt% during enzymatic hydrolysis improved glucose productivity by 23.7%. These findings demonstrate that integrating twin-screw extrusion and organosolv pretreatment effectively enhances sustainable valorization of corn cobs.

1. Introduction

Lignocellulosic biomass plays a central role in emerging biorefinery concepts, enabling the production of biofuels, renewable chemicals, and functional biomaterials through integrated fractionation and conversion strategies [1,2]. Efficient biomass valorization relies heavily on pretreatment, which disrupts plant cell wall recalcitrance and enhances the accessibility of cellulose and hemicellulose. Recent advances highlight the use of biphasic solvent systems, organosolv technologies, ionic liquids, deep eutectic solvents, and mechanical–chemical hybrid approaches to selectively fractionate lignocellulose and improve downstream processing [3,4]. Corn cob, a widely available agricultural residue with substantial polysaccharide content, is particularly suitable for integrated mechanical–chemical pretreatment strategies due to its recalcitrant lignin–carbohydrate complex [5].

Twin-screw extrusion has emerged as a promising pretreatment method, offering advantages such as high shear, efficient heat and mass transfer, easy scale-up, and relatively short residence times, which may be beneficial or limiting depending on the process requirements [6]. Additionally, it allows continuous processing at high solid loads (40–60% w/w) and may preserve hemicellulose due to the absence of chemical addition and the relatively short residence time, which can contribute to limiting the formation of fermentation inhibitors, as generally reported for extrusion-based pretreatments [7]; however, inhibitor generation is governed by the overall pretreatment severity, which depends on the combined effects of temperature, residence time, and chemical conditions, and may still be significant depending on the operating conditions.

Nonetheless, extrusion typically requires the addition of plasticizers such as glycerol, Tween 80, or ethylene glycol to facilitate biomass

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transport within the extruder [6,8]. Among these, glycerol stands out for its low cost and high boiling point, being a by-product of biodiesel production. To optimize extrusion efficiency, process variables such as compression rate, temperature, and screw speed must be evaluated [9], and statistical tools like response surface methodology can aid in identifying optimal conditions, since response surface designs outperform basic factorials because they capture curvature, allowing true optimization rather than just identifying significant factors [6].

Although extrusion improves physical structure and digestibility, it does not significantly alter the chemical structure of lignocellulosic biomass [10]. Therefore, additional pretreatments aimed at lignin removal are necessary. Organosolv fractionation is a broad class of solvent-based pretreatment methods that employ organic solvents to fractionate lignocellulosic biomass, where lignin solubilization and selectivity depend strongly on the solvent system and operating conditions [11]. The resulting liquid fraction contains solubilized lignin and hemicellulose, which can be recovered and valorized separately [12]. This method enables the fractionation of lignocellulosic biomass using organic solvents, typically resulting in a cellulose-rich solid fraction and a liquid fraction containing solubilized lignin and hemicellulose, which can be further processed depending on the selected solvent system and recovery strategy [13].

Despite advances in chemical pretreatments such as organosolv, the intrinsic recalcitrance of raw lignocellulosic biomass limits enzyme accessibility and overall sugar yield [14]. By applying mechanical disruption via twin-screw extrusion prior to the organosolv stage, the biomass structure is physically deconstructed, increasing surface area, reducing crystallinity, and improving mass-transfer [6]. This enhanced accessibility significantly improves the efficiency of subsequent enzymatic hydrolysis of cellulose, leading to higher glucose yields under milder chemical conditions. In contrast, reversing the sequence (organosolv first, then extrusion) would require harsher solvent or reaction conditions to overcome intact cell-wall recalcitrance and risks damaging or losing partially delignified material during mechanical processing [15].

However, enzymatic hydrolysis remains one of the costliest steps in lignocellulosic ethanol production due to the high enzyme load required [16–18]. Therefore, reducing enzyme usage while maintaining high conversion yields is a key objective.

Furthermore, techno-economic assessments show that operating enzymatic hydrolysis at high solids loading (>15 wt%) is crucial for commercial viability, as it reduces water usage and lowers capital and operational costs [19]. Despite these advantages, high solids loading can pose technical challenges, including limited mass transfer and lower sugar yields, potentially affecting overall process economics [20,21].

In this context, this study aims to evaluate an integrated strategy combining twin-screw extrusion and organosolv pretreatment, followed by enzymatic hydrolysis of corn cob biomass, focusing on maximizing glucose release and enabling lignin recovery under optimized process conditions.

2. Material and methods

A schematic representation of the experimental workflow used in this study is presented in Fig. S1 (Supplementary Material), where the operational parameters correspond to the optimized conditions determined in this work.

2.1. Biomass preparation

The corn cob, crushed, dried, and bagged (20 kg), originating from RASUL - Indústria e Comércio de Rações Ltda – Andirá, Paraná, Brazil, was processed in a knife mill and sieved to particle sizes between 200 and 710 μm . This fraction represented approximately 40% of the total milled material and was selected to ensure better experimental reproducibility during the extrusion pretreatment tests. The material was subsequently

stored at room temperature until use.

2.2. Extrusion pretreatment

The corn cob extrusion process was conducted at the Center for Chemistry and Environment of the Institute of Energetic and Nuclear Research – IPEN using a twin-screw extruder (American Maplan Corporation – AMC). The operational parameters evaluated in this study included barrel temperature and screw rotation, which were controlled during the pretreatment experiments. Although torque and specific mechanical energy were not available, the selected operational parameters allowed a consistent evaluation of the thermomechanical effects of extrusion on biomass structure. A 1:1 (w/w) solution of distilled water and commercial glycerol was prepared and gradually added to the natural corn cob to obtain a final mixture containing 25 wt% water, 25 wt% glycerol, and 50 wt% untreated corn cob, based on the total mass of the mixture. The required amounts of water and glycerol were first mixed to form a homogeneous solution, which was then slowly incorporated into the corn cob under manual mixing to ensure uniform moisture distribution before the extrusion step [6].

A 2^2 star factorial design with $\alpha = 1.66$ and 4 repetitions at the central point was conducted to assess the effect of barrel temperature and screw angular velocity of the extruder. The levels of the independent variables studied were empirically determined according to the upper and lower limits within which the extruder could operate safely. The temperature range studied varied from 110 to 180 $^{\circ}\text{C}$, the screw angular velocity varied from 5 to 35 rpm and the response variable used was the conversion of cellulose to glucose through enzymatic hydrolysis. The software Minitab 19 (2019) was used to model and analyze.

2.3. Organosolv pretreatment

2.3.1. Experimental strategy and reactor setup

The organosolv solvent fractionation was carried out in a high-pressure batch reactor with a working volume of 250 mL, equipped with temperature and pressure control systems. Heating was provided by an external metal jacket, cooling was achieved through an internal coil, and agitation was maintained by magnetic stirring.

A sequential experimental design strategy was adopted to investigate and optimize the organosolv pretreatment of extruded corn cob. In the first stage, a Plackett–Burman experimental design was employed to evaluate the influence of six independent process variables and to identify the variable exerting the greatest impact on enzymatic hydrolysis performance. In the second stage, the most influential variable identified in the screening step was combined with solids concentration and further evaluated using a response surface methodology based on a 2^2 factorial design. This approach enabled a focused optimization of the organosolv fractionation process while minimizing the number of experimental runs.

The performance of the organosolv pretreatment was assessed based on cellulose-to-glucose conversion and glucose productivity after enzymatic hydrolysis, as well as lignin recovery yield in the optimization stage.

2.3.2. Screening of organosolv process variables (Plackett–Burman design)

The influence of six independent variables on the organosolv pretreatment was evaluated using a Plackett–Burman experimental design, implemented and analyzed using Minitab® 19 software (2019). The variables investigated were temperature, stirring speed, sulfuric acid concentration, solvent composition, treatment time, and glycerol concentration. The factor levels investigated are presented in Table 1.

The glycerol concentration was defined according to the residual glycerol content of the extruded biomass. A glycerol content of 0 wt% corresponds to extruded corn cob washed with distilled water, with no residual glycerol, while 45 wt% corresponds to extruded corn cob containing 45 wt% glycerol and 55 wt% structural biomass.

Table 1
Factors studied at the Plackett-Burman model.

Item	Inferior limit	Superior limit
Temperature (°C)	110	140
Stirring speed (rpm)	100	300
Sulfuric acid concentration (mg g ⁻¹)	0	10
Solution	A	B
Time (min)	15	45
Glycerol (wt.%)	0	45

The biomass used in all experiments consisted of extruded corn cob obtained under previously optimized extrusion conditions, using a screw speed of 20 rpm and a barrel temperature of 145 °C. The response variables evaluated were the cellulose-to-glucose conversion and glucose productivity after enzymatic hydrolysis.

The solvent composition was selected based on the study by Brudecki et al. (2013) [22]. Solution A consisted of 30 wt% water, 61 wt% ethanol, and 9 wt% methyl isobutyl ketone (MIBK), while Solution B consisted of 30 wt% water and 70 wt% ethanol. The water content was kept constant to allow the removal of MIBK, following the approach described by Zhou et al. (2018) [23].

For each solvent fractionation assay, a solid-to-liquid ratio of 1:20 was employed. The solid phase corresponded to the previously extruded corn cob, while the liquid phase consisted of the selected solvent mixture (Solution A or B) with or without sulfuric acid as a catalyst. The experimental matrix is summarized in Table 1.

At the end of each treatment, heating and stirring were stopped, and the reaction mixture was cooled to room temperature using ambient-temperature water circulated through the internal cooling coil. After cooling to approximately 50 °C for safe handling, the slurry was transferred to a 250 mL Pyrex bottle and stored under refrigeration.

The treated material was vacuum-filtered at room temperature using cellulose filter paper to separate the liquid fraction, containing solubilized hemicellulose and lignin, from the solid fraction, mainly composed of cellulose with residual hemicellulose and lignin. The solid fraction was washed with distilled water (2 L) to remove residual solvents, collected, weighed, and stored frozen until further chemical composition analysis and enzymatic hydrolysis. The liquid fraction was discarded at this stage.

2.3.3. Optimization of organosolv pretreatment by response surface methodology

Based on the results obtained in the exploratory study (Section 2.3.2), treatment time was identified as the variable with the greatest influence on the organosolv fractionation of corn cob. Therefore, a 2² factorial design with face-centered axial points and four replicates at the central point was conducted to evaluate the combined effects of treatment time and solids concentration on the process performance.

The levels of the independent variables were defined based on previous studies, with treatment time ranging from 20 to 60 min and solids concentration ranging from 5 to 20 wt%, aiming to reduce reaction time while increasing solids loading [24,25]. The response variables evaluated were the cellulose-to-glucose conversion after enzymatic hydrolysis, as described in Section 2.4, and the yield of recovered lignin, as described in Section 2.3.4.

2.3.4. Lignin recovery procedure

After completion of the solvent fractionation experiments described in Section 2.3.3, the entire reaction mixture was collected in a 1 L flask and were centrifuged at 2000 rpm (≈626×g) for 10 min at room temperature. The supernatant was separated and reserved.

To recover lignin deposited on the surface of the solid fraction, the solid residue was mixed with a solution containing 70 wt% ethanol and 1 wt% sulfuric acid at room temperature and magnetically stirred for 10 min. For each gram of solid initially used in the solvent fractionation

process, 2 mL of the washing solution was applied. The mixture was then centrifuged at 2000 rpm for 20 min (≈626×g) for 10 min at room temperature, and the resulting liquid phase was combined with the previously collected supernatant.

The solid residue was washed twice more following the same procedure to ensure complete removal of surface-deposited lignin; however, the supernatants obtained from these additional washing steps were discarded.

The combined supernatant was transferred to an open tray and dried in an oven at 40 °C for 24 h to remove residual water and ethanol. The remaining solid was resuspended in 500 mL of acidified water (pH 4, adjusted with H₂SO₄) and stirred for 60 min to solubilize water-soluble compounds and improve lignin purity. The suspension was then filtered, and the recovered lignin was washed twice with distilled water.

Finally, the lignin was dried in an oven at 90 °C for 3 h, weighed, and returned to the oven until constant mass was achieved. The final mass was recorded for lignin yield determination.

It should be noted that the solvent ratios and washing steps applied in this procedure were selected for laboratory-scale operation to ensure effective fractionation and accurate lignin recovery. These conditions are not intended to represent optimized process parameters from a techno-economic perspective. In industrial applications, solvent recovery and recycling, as well as reduced liquid-to-solid ratios, would be essential to minimize solvent consumption and effluent generation.

2.4. Corn cob enzymatic saccharification

2.4.1. Evaluation of the extrusion and organosolv processes

The enzymatic saccharification, or enzymatic hydrolysis, conducted to evaluate the efficiency of the pretreatments by extrusion and by 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 shaker incubator, 50 °C, and a solid load of 10 wt% 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 wt% to inhibit microbial contamination. After 57 h, the enzymatic hydrolysate was centrifuged at 2000×g for 20 min, and the supernatant was collected and stored at -20 °C for subsequent sugar analysis by high-performance liquid chromatography [6].

2.4.2. Study of different solid contents in the treated corn cob enzymatic saccharification

The enzymatic saccharification, or enzymatic hydrolysis, conducted to evaluate the solid content in the enzymatic hydrolysis process using the solid obtained under the best condition studied in item 2.3, 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 shaker incubator, 50 °C, and solid contents of 10, 15, and 20 wt% with a total liquid volume of 50 mL. The enzymatic load used was 31 FPU/g of dry lignocellulosic material for the Cellic CTec2 enzyme complex. Sodium azide was also added to the reaction medium at a concentration of 0.3 wt% to inhibit microbial contamination. After 57 h, the enzymatic hydrolysate was centrifuged at 2000×g for 20 min, and the supernatant was collected and stored at -20 °C for subsequent sugar analysis by high-performance liquid chromatography [6].

2.5. Analytical methods

2.5.1. Moisture content determination

The moisture content of the corn cob, both in its natural state and after different pretreatments, was determined using a semi-analytical balance (MARTE ID50) equipped with an infrared dryer, at 105 °C. Approximately 1.0 g of the wet corn cob was used for this determination.

2.5.2. Compositional analysis of solids

The solids obtained in this work, including corn cobs pretreated by extrusion and solvent fractionation, as well as the untreated corn cob, were analyzed for their cellulose, hemicellulose, soluble lignin, and insoluble lignin contents according to the methodology used by the National Renewable Energy Laboratory (NREL) [26]. The samples for compositional analysis, corresponding to the hydrolysis filtrate (before washing the residue), were properly diluted and filtered using a Sep Pak C18 filter (Millipore). The concentrations of glucose, xylose, arabinose, and acetic acid were determined by High-Performance Liquid Chromatography (HPLC) according to item 2.6.3. To determine the percentage (wt.%) of hemicellulose and cellulose in the corn cob, the conversion factors of 0.88 and 0.90, respectively, were used.

2.5.3. Determination of sugars and glycerol

The concentrations of sugars (glucose, xylose, cellobiose) and glycerol were determined by High-Performance Liquid Chromatography (HPLC) under the following conditions: Bio Rad Aminex HPX-87H column (300 × 7.8 mm); column temperature, 45 °C; detector temperature, 35 °C; Waters 410 refractive index detector; eluent, 0.005 mol L⁻¹ H₂SO₄ solution, flow rate of 0.6 mL min⁻¹; injected sample volume, 20 µL. The samples, after proper dilution, were filtered using a Sep Pak C18 filter (Millipore). The eluent was filtered under vacuum using a cellulose ester membrane with a 0.45 µm pore size and a 47 mm diameter (Millipore) and simultaneously degassed in an ultrasonic bath (Thornton) for 25 min [27].

3. Results and discussion

3.1. Extrusion pretreatment: influence of the extruder's temperature and screw speed in the conversion of cellulose in glucose after enzymatic saccharification

Chemical composition analysis was performed on the corn cob in its natural state (before extrusion pretreatment) and on the 12 samples studied in the statistical design, resulting in Tables 2 and 3. Photographs of each sample are presented in Appendix A (Figure A.1).

In Table 2, the mass percentages of the main components of the corn cob after the extrusion process under different conditions are presented. For comparison with other studies and with untreated corncob, the glycerol fraction was excluded from the reported totals. However, glycerol remained present in the material during characterization. It can be observed that there was not a significant alteration in the chemical composition of the corn cob under different extrusion conditions [10]. This is because the extrusion pretreatment only acts on biomass defibrillation through intense shear, increasing enzyme accessibility, with little to no alteration in the biomass's chemical composition (Pérez-Rodríguez et al., 2017).

Table 2
Chemical composition of each sample after extrusion – without glycerol.

Sample	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Extractives (%)	Ashes (%)	Total (%)
1	45.0 ± 3.3	36.8 ± 2.0	15.9 ± 2.1	2.8 ± 0.7	2.3 ± 0.3	102.9 ± 4.4
2	44.1 ± 2.1	35.2 ± 3.4	16.2 ± 2.2	3.2 ± 0.2	2.3 ± 0.3	101.0 ± 4.6
3	43.2 ± 1.2	36.0 ± 3.6	15.8 ± 1.8	2.5 ± 1.3	2.4 ± 0.2	99.9 ± 4.4
4	42.3 ± 1.5	35.6 ± 3.0	16.9 ± 0.1	1.6 ± 1.1	2.9 ± 0.6	99.3 ± 3.6
5	43.5 ± 2.7	38.0 ± 1.5	16.8 ± 0.1	1.7 ± 1.2	2.8 ± 0.6	102.8 ± 3.4
6	44.6 ± 1.3	37.3 ± 2.5	15.8 ± 1.7	1.9 ± 1.1	3.0 ± 0.5	102.6 ± 3.5
7	44.7 ± 1.2	37.6 ± 2.5	16.6 ± 2.9	1.6 ± 1.5	2.0 ± 1.2	102.5 ± 4.5
8	44.5 ± 1.4	36.4 ± 1.6	16.4 ± 2.9	1.5 ± 1.2	1.4 ± 1.2	100.2 ± 4.0
9	43.5 ± 0.5	37.0 ± 1.1	16.7 ± 2.5	1.8 ± 1.4	0.7 ± 0.1	99.7 ± 3.1
10	42.3 ± 1.9	36.4 ± 0.8	15.8 ± 0.8	2.7 ± 0.0	0.8 ± 0.0	98.0 ± 2.2
11	42.4 ± 1.8	37.3 ± 0.8	15.3 ± 0.6	2.5 ± 0.3	0.9 ± 0.3	98.4 ± 2.1
12	42.2 ± 1.8	36.7 ± 1.2	15.9 ± 0.5	2.4 ± 0.3	1.6 ± 0.9	98.8 ± 2.4
UN	43.1 ± 1.3	39.2 ± 3.1	15.2 ± 1.2	2.8 ± 0.3	1.4 ± 0.1	101.7 ± 6.0

*UN: untreated corn cob (before extrusion).

Table 3

Cellulose conversion in glucose in the extrusion processes.

Sample	Screw speed (rpm)	Barrel temperature (°C)	Cellulose conversion in glucose wt. %
1	11.0	124.0	84.0
2	29.0	124.0	73.8
3	11.0	166.0	88.8
4	29.0	166.0	83.1
5	5.0	145.0	95.4
6	35.0	145.0	74.8
7	20.0	110.0	65.7
8	20.0	180.0	88.9
9	20.0	145.0	87.2
10	20.0	145.0	89.2
11	20.0	145.0	91.0
12	20.0	145.0	85.7
UN	-	-	43.2 ± 1.2

*UN: untreated corn cob (before extrusion).

Subsequently, enzymatic hydrolysis was performed on each of the samples after the extrusion to assess the influence of the factors studied in the extrusion on the conversion of cellulose present in the corn cob into glucose. The results are presented in Table 3.

According to Table 3, the highest cellulose conversion (95.4 wt%) was obtained in trial 5 at the lowest screw rotation speed evaluated (5 rpm), which increased the residence time of the material in the barrel at 145 °C. It should be noted that this value corresponds to the experimental range investigated in this study (5–35 rpm) using a laboratory-scale extruder. The improved conversion is therefore associated with the longer residence time during thermomechanical pretreatment. However, a reduction in cellulose conversion to 65.7 wt% was achieved by reducing the temperature to 110 °C in the extruder barrel (trial 7). Nevertheless, increasing the extrusion temperature of the corn cob to 124 °C resulted in a higher cellulose conversion, reaching only up to 84 wt%. Therefore, it can be said that lower temperature values in the barrel at 124 °C have little impact on the cellulose conversion into glucose in the subsequent enzymatic hydrolysis process of the biomass.

The data presented in Table 3 were analyzed statistically for the response variable conversion of cellulose to glucose using the software Statistica, and the full Analysis of Variance (ANOVA) table and figures are present in Appendix A.

According to the pareto graph and the ANOVA table, the effects correspondent to the interaction between the variables, and the quadratic effect of the screw speed were not significant up to 90 % of confidence level, so both effects were removed and equation (1) represents the variation of conversion of cellulose to glucose after enzymatic saccharification according to the temperature and screw speed in the extrusion process.

$$y_1 \left(\frac{w}{w} \right) = 0,087 - 0,056 \cdot x_1 + 0,059 \cdot x_1 - 0,049 \cdot x_2^2 \quad (1)$$

Where: y_1 = conversion of cellulose to glucose (wt.%); x_1 = Extruder's screw speed; x_2 = Barrel's temperature.

According to equation (1), it is observed that the higher the screw speed, the greater the negative impact on cellulose conversion to glucose. This is due to the reduction in biomass residence time inside the extruder, which reduces the impact exerted by the extrusion pretreatment on the corn cob and thus contributes simultaneously to the reduction of biomass defibrillation and enzyme accessibility. The influence of temperature in this process can be considered complex, as it presents a quadratic factor. By partially differentiating equation (1) with respect to temperature (x_2), and setting equation (1) equal to 0, the encoded temperature value that maximizes cellulose conversion to glucose is 0.59, which decoded is 157 °C.

In order to maximize cellulose conversion to glucose, the optimization tool of the software was used, and the following results were obtained: screw speed of 5 rpm and extruder barrel temperature of 157 °C.

Fig. 1 shows the contour plot of cellulose conversion to glucose for the factors studied in the factorial design. An optimization region is observed near the central point of the barrel temperature, as well as a region corresponding to lower screw rotation. Temperature values between 135 °C and 158 °C indicate a region where cellulose conversion above 90 wt% is achieved at a screw speed of 5 rpm.

Fig. 1 allowed the identification of a region where glucose conversion is maximized. Although the condition of 144 °C and 5 rpm yields a slightly higher predicted conversion (96.3 wt%), this operating point reduces the screw speed by 55% compared to 11 rpm. In practical terms, this corresponds to a proportional 55% decrease in mass flow and, consequently, theoretical productivity. For the subsequent experimental stages, the objective was not only to maximize conversion but also to select a condition that is more realistic for scale-up, where throughput becomes a critical factor. Therefore, the condition of 144 °C and 11 rpm was chosen as a compromise: it still lies within the maximization region and provides a high conversion (92.6 wt%), while maintaining a

substantially higher material throughput. This trade-off reflects a more industrially relevant balance between efficiency and productivity.

Although extrusion pretreatment significantly improved enzymatic digestibility, it does not enable selective fractionation of biomass components. The subsequent organosolv step was therefore essential for lignin solubilization and recovery, enabling the separation of biomass fractions and supporting lignin valorization within an integrated bio-refinery concept.

3.2. Organosolv pretreatment: influence of six parameters in the cellulose conversion to glucose

The solvent fractionation process of the extruded material was studied as described in item 2.3.1. For this stage, an initial study was conducted on the possible independent variables that could impact the organosolv process, which are: temperature, agitation, sulfuric acid concentration, solvent compositions, treatment time, and glycerol concentration, varying according to the values presented in Table 1.

Table 4 shows the percentage of cellulose, hemicellulose, and lignin in the corn cob after each experiment in the Plackett-Burman design. For these tests, the mass yield was also calculated, referring to the yield of solids collected in the filtration process.

The recovered solids yield ranged from 64 to 92.3 wt% for the different experiments conducted. The organosolv process affects lignocellulosic biomass in various ways, solubilizing part of its lignin into the medium, thereby reducing the mass of solids at the end of the reaction, i. e., reducing the process yield [28,29]. A chemical catalyst can be used in the solvent fractionation process to catalyze the breaking of bonds present in hemicellulose [30]. In this case, with the addition of acid and the presence of other chemical solvents, a fraction of cellulose may be solubilized in the medium [31].

After the chemical characterization of each sample from this factorial design, enzymatic hydrolysis of each sample was performed. The conversion of cellulose to glucose and glucose productivity are presented in Table 5.

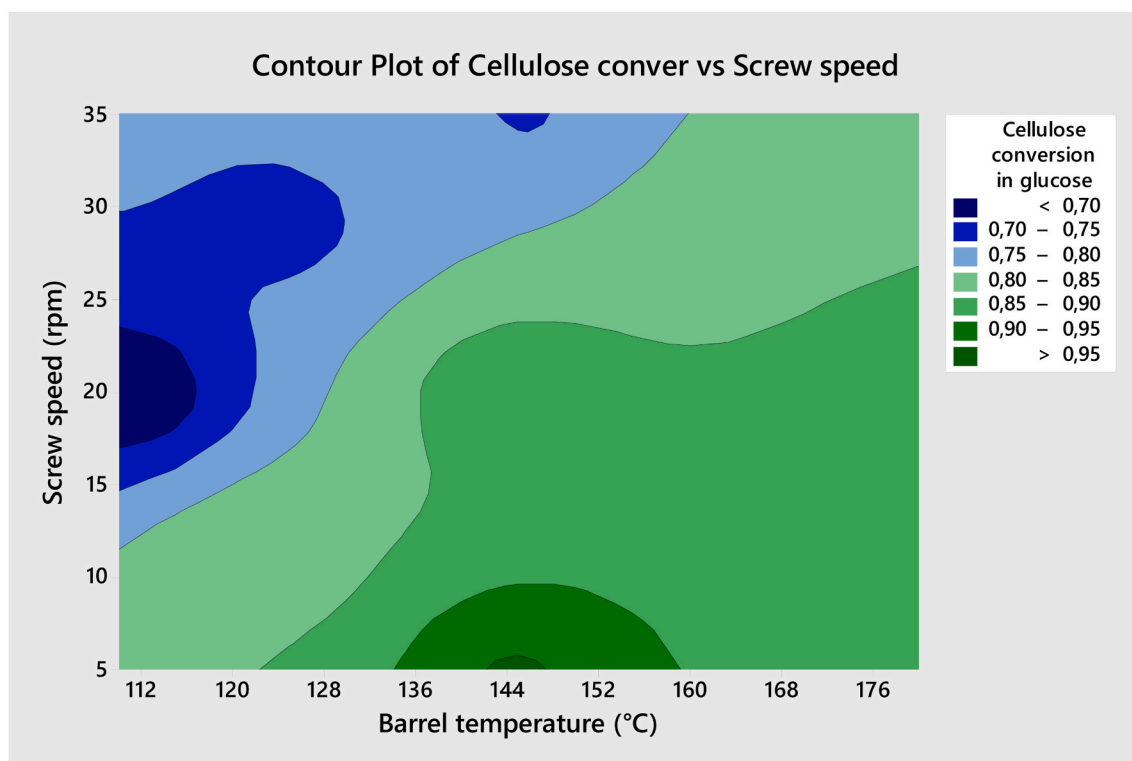


Fig. 1. Contour graph of the cellulose conversion to glucose in relation to screw speed and barrel temperature.

Table 4

Chemical composition of the samples after the solvent fractionation process in the Plackett-Burman factorial design.

Sample	Cellulose (%)	Lignin (%)	Hemicellulose (%)	Total (%)	Recovered yield (% m/m)
1	45.6 ± 1.3	17.2 ± 0.8	34.4 ± 3.5	97.2 ± 5.6	64.3
2	53.2 ± 0.7	13.8 ± 0.8	31.7 ± 1.1	98.7 ± 2.6	88.2
3	46.6 ± 2.1	12.9 ± 3.2	33.5 ± 4.9	93 ± 10.2	69.7
4	46.9 ± 3.1	13.6 ± 1.5	38.2 ± 1.7	98.7 ± 6.3	86.5
5	46.8 ± 2.2	13.2 ± 0.8	37.5 ± 1.2	97.5 ± 4.2	92.3
6	45.1 ± 1.8	12.0 ± 1.2	35.6 ± 2.5	92.7 ± 5.5	75.6
7	68.6 ± 4.7	5.0 ± 1.7	24.6 ± 0.7	98.2 ± 6.1	67.4
8	54.2 ± 1.5	11.6 ± 1.4	31.2 ± 2.4	97.0 ± 5.3	64.0
9	41.5 ± 4.1	10.2 ± 2.4	43.6 ± 7.2	95.3 ± 13.7	85.7
10	63.8 ± 5.4	11.9 ± 1.6	25.1 ± 3.6	100.8 ± 10.6	83.8
11	44.6 ± 4.9	16.3 ± 0.5	31.8 ± 2.9	92.7 ± 8.3	67.2
12	49.7 ± 1.1	12.3 ± 1.7	36.6 ± 0.4	98.6 ± 3.2	73.5

Table 5

Conditions and results of the Plackett-Burman model experiments.

	T (°C)	Agitation (rpm)	Acid (mg g ⁻¹)	Solution	Time (min)	Glycerol (wt.%)	Cellulose into glucose conversion (wt.%)	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

Note: 1: inferior limit; 1: superior limit. Solution A: 30 wt% water, 61 wt% ethanol and 9 wt% MIBK; Solution B: 30 wt% water and 70 wt% ethanol.

Tests 2 and 5 showed the highest cellulose to glucose conversion values, above 95 wt%, and also the highest productivity values, above 0.64 g L⁻¹ h⁻¹. These tests were both conducted with the independent variables time and acid under the same conditions, indicating a possible influence of these factors on cellulose to glucose conversion and glucose productivity.

Next, the tables and figures related to the analysis of this Plackett-Burman factorial design is provided in Appendix A, where the two response variables were cellulose conversion to glucose and glucose productivity.

The analysis of variance (ANOVA) indicates that none of the factors studied were statistically significant at a 90% confidence level, once all p-values were greater than 0.1. Therefore, this model proposed by Plackett-Burman is not quantitative and suggests an exploratory study of many independent variables and the intensity of their influence on the process [32].

The Pareto chart, also present in Appendix A, shows that the independent time had the greatest impact on the response variable of cellulose conversion to glucose.

This same approach was applied to the response variable, glucose productivity, for the solvent fractionation process using a design proposed by Plackett-Burman. In this case, it was also observed that none of the independent variables studied were statistically significant at the 90% confidence level, meaning all terms had p-values greater than 0.1.

However, for glucose productivity, it can be observed that, in addition to treatment time, as found for the response variable conversion of cellulose to glucose, the independent factors temperature and sulfuric acid concentration also had a greater impact. Thus, the independent variable under study, time, was more influential in the organosolv process for cellulose-to-glucose conversion and glucose productivity, being one of the terms to be evaluated in the next stage of this work.

The response variables, conversion of cellulose to glucose and glucose productivity, were maximized under the experimental conditions of trials 2 and 5 (Table 5), which employed a maximum

temperature of 140 °C and were conducted in the presence of sulfuric acid in the process. The next experiments were conducted under these experimental conditions. Other authors use temperatures close to it, and the presence of acid in the organosolv process [23].

Regarding the solvent composition factor, it was decided to use the solvent reaction composition without the addition of the MIBK reagent. The use of this reagent necessitates its extraction by distillation at the end of the solvent fractionation process for reuse, besides having toxic characteristics [33]. Furthermore, other authors mention good yields in the solvent fractionation step using only water:ethanol solutions [23].

Subsequently, it was observed that glycerol and agitation had small impact among the studied variables in the solvent fractionation process. This indicated that for the next stage of studies, minimized values of these factors should be employed. The influence of glycerol was evaluated by comparing experiments conducted with and without its presence in the organosolv stage, the latter achieved by washing the extruded corncob prior to pretreatment. The results showed no significant differences in cellulose-to-glucose conversion, indicating that glycerol carryover does not negatively affect enzymatic hydrolysis under the evaluated conditions. Moreover, no detrimental effects on the overall pretreatment performance were observed, including lignin recovery. Therefore, to avoid additional processing steps, water consumption, and effluent generation, the washing step was not included in subsequent experiments, and glycerol was maintained in the reaction medium. In their study, Pascal et al. (2019) conducted the solvent fractionation process using only water and glycerol, achieving cellulose-to-glucose conversion values of 70 to 98 wt% [31]. Furthermore, its presence under the evaluated conditions is compatible with the proposed process configuration. Regarding the independent variable, agitation, its minimized value ensures adequate homogeneity of the reaction medium.

Thus, after evaluating the most significant independent variables in the solvent fractionation process using the Plackett-Burman design, the following conditions were set: 140 °C, water:ethanol ratio of 3:7, glycerol (45 wt%), agitation at 100 rpm, and sulfuric acid concentration of

10 mg per gram of dry corn cob. With these fixed independent variables, the treatment time (20 and 60 min) and the solids load in the reaction medium, between 1:20 and 1:5 (5 and 20 wt%, respectively), were studied using a 2² factorial design with center points.

3.3. Influence of solid concentration and treatment time in the solvent fractionation process of extruded corn cob

With the conditions established in item 3.2, the influence of solid concentration and temperature in the solvent fractionation process of the extruded material was studied. For this purpose, a 2² experimental design was conducted with the independent variables being solid concentration and temperature. The response factors studied were conversion of cellulose to glucose and lignin recovery.

According to Table 6, experiment 8 achieved the highest conversion of cellulose to glucose value, with a total conversion of 96.49 wt%, using the highest solid concentration studied and an intermediate treatment time. However, the recovered lignin mass was not the highest. The highest lignin recovery was observed in experiment 4, where the cellulose-to-glucose conversion was also above 90 wt%.

To obtain a local maximum for the response variables studied (conversion of cellulose to and recovered lignin mass), the software Statistica was used to perform the statistical calculations for this study using the experimental design methodology. The tables containing the ANOVA table and the pareto figures are present in Appendix B.

Initially, the p-value of each linear and quadratic variable were analyzed, as well as the interaction between the variables. The p-value is less than 0.1 for the linear variables treatment time and solid concentration, as well as for the quadratic variable of Time and for the interaction of the variables Concentration*Time. These p-value results indicate that these variables have statistical significance at a 90 % confidence level and can be considered relevant to this study. The quadratic variable of Concentration has a p-value of 0.164, indicating a statistical significance of up to 83%. Therefore, a confidence level of up to 83% was chosen for this study.

Upon analyzing the pareto graph for the response variable conversion of cellulose to glucose, it was observed that the linear effect of solid concentration is the most impactful factor in the process, followed by the linear effect of time, the quadratic effect of time, the interaction between concentration and time, and finally, the least impactful factor in the process, the quadratic effect of solid concentration.

After considering all the studied effects and with the assistance of the Statistica software, equation (2) was generated that represents the studied process in up to 88.26% of the cases, as this is the R² value obtained in this model. equation (2) is described below, where z = conversion of cellulose to glucose, x = treatment time, and y = solid concentration. The values used are not coded.

$$z \text{ (wt. \%)} = 0.3125 + 0.0149.x - 0.00019.x^2 + 1.8631.y - 8.033.y^2 + 0.0315.x.y \quad (2)$$

After obtaining equation (2), a response surface was generated for the response variable conversion of cellulose to glucose, and it is available in Fig. 2.

According to Fig. 2, it can be observed that the highest possible values for cellulose-to-glucose conversion would be between a treatment time of 50 to 60 min and a solid concentration between 0.18 and 0.22, resulting in conversion values greater than 0.93, or 93 wt%, which can be considered a local maximum.

After obtaining the local maximum for the conversion of cellulose to glucose, statistical calculations were performed for this study using the Statistica software to find the local maximum for maximizing the recovered lignin mass. The ANOVA table for the response variable recovered lignin mass and the pareto graph is present in Appendix B.

As in the analysis related to the lignin recovery, the p-value of each linear and quadratic variable, as well as the interaction between variables, was initially analyzed. The p-value is less than 0.05 for the linear variables treatment time and solid concentration, as well as for the quadratic variable of solids concentration and for the interaction of the variables solids concentration * Time. These p-value results indicate that these variables have statistical significance at a 95% confidence level and can be considered relevant to this study. The quadratic variable time has a p-value of 0.686, indicating a statistical significance of up to 31%, a value too low to be considered relevant for this study. Therefore, the quadratic variable of time was removed from this study, and a new statistical analysis of this study was conducted, and the ANOVA table is present in Appendix C.

With the removal of the quadratic variable Time*Time, it was possible to obtain a model in which all independent variables have a p-value less than 0.05, meaning they have statistical significance greater than 95%; therefore, these studied variables were retained. To assess the impact of each studied independent variable, analyzing the pareto chart for the lignin recovered, the linear variable of solid concentration is the most impactful, followed by the linear effect of the variable time, the interaction between solids concentration and time, and finally, the least impactful factor in the process, the quadratic effect of solids concentration. The linear variable solid concentration has a three times greater impact on the lignin mass recovery process than treatment time. This superior impact may be due to the fact that the higher the initial mass of solids in the medium, the greater the amount of lignin available in the solid, and consequently, the greater the amount of recovered lignin.

After retaining the effects listed in the Pareto chart, Equation (3) was generated using the Statistica software that represents the studied process in up to 98.61% of cases, as this is the R² value obtained in this model. Equation (3) is described below, where z = Recovered lignin mass, x = Treatment time, and y = Solid concentration. The values used are not coded.

$$z \text{ (g)} = 0.0263 - 0.0068 + 25.8867.y - 54.3796.y^2 + 0.2399.x.y \quad (3)$$

According to Fig. 3, it can be observed that the highest possible value for the recovered lignin mass would be at the maximum reaction time (60 min) and also at the maximum solid concentration (20.6 wt%), resulting in a recovered lignin mass value of 5.61 g according to Equation (3). An experimental value of 5.67 g was obtained for the recovered lignin mass according to Table 6, indicating that the response surface obtained is a model close to reality.

In order to obtain a process where both the conversion of cellulose to glucose and recovered lignin mass are maximized, the two mathematical models obtained were analyzed. The maximum conversion of cellulose to glucose can be obtained under various conditions of solid concentration and treatment time, being a local maximum. However, for lignin recovery, a local maximum was not obtained, but rather a maximum point within the studied conditions. Therefore, the conditions for

Table 6

Conversion of cellulose to glucose and recovered lignin mass from samples obtained under different solvent fractionation conditions.

	Time (min)	Concentration of solids (wt.%)	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

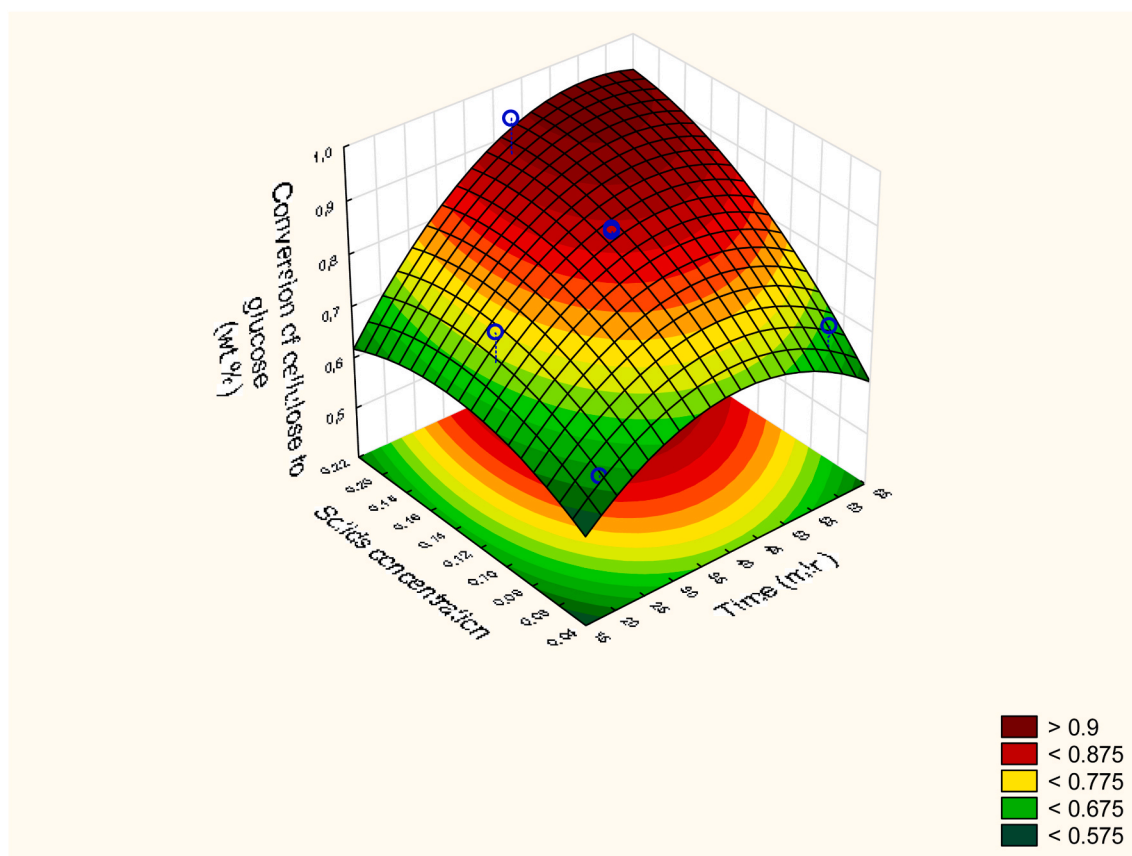


Fig. 2. Response surface of conversion of cellulose to glucose as a function of solids concentration and time.

treatment time and solid concentration were selected where lignin recovery is maximized, since under these conditions, conversion of cellulose to glucose is also maximized.

The condition selected as the best for conversion of cellulose to glucose as well as lignin recovery was a treatment time of 60 min and a solid concentration of 20.6 wt%. The experiment was repeated under the conditions described above to confirm the results obtained by the model. The predict value for conversion of cellulose to glucose and lignin recovered were 93.86 wt% and 5.61 g, respectively, and the results obtained experimentally were 93.01 ± 1.2 wt% and 5.73 ± 0.27 g, respectively. The experimental results for conversion of cellulose to glucose and recovered lignin mass closely resembled the predicted results. Despite the existence of deviations between the experimental results and the predicted values, it can be considered that the results obtained in the validation test were satisfactory. Therefore, this condition was used for the next steps of this work.

Appendix C contains a photo of the solids obtained at each stage of the processes studied in this work. Item 1 is the lignin recovered by the lignin recovery process described in section 2.3.4, item 2 is the washed and dried solid recovered from the organosolv process under the optimized condition, item 3 is the corn cob after the extrusion process, and item 4 is the natural corn cob.

The elemental and physicochemical composition of the corncob at different treatment stages (untreated, after twin-screw extrusion and after both pretreatments twin-screw extrusion and organosolv) is provided in the Supplementary Material (Table S1). Elemental composition (C, H, and N) was determined using an elemental analyzer according to ASTM D5373, while ash content was determined following ASTM E1755. Higher and lower heating values were measured using a bomb calorimeter according to ASTM D5865.

3.4. Evaluation of different solids contents in enzymatic hydrolysis of extruded and organosolv corn cob

The solid fraction obtained from the solvent fractionation process under the conditions established by the study in section 3.3 was subjected to enzymatic hydrolysis at three different solid concentrations aiming to increase the solid load, consequently increasing glucose productivity in the enzymatic hydrolysis process. The results in terms of cellulose-to-glucose conversion and productivity are reported in Table 7.

It is known that there is a difficulty in the process of increasing solid load in the enzymatic hydrolysis process, as plant biomass is highly hygroscopic, significantly increasing the viscosity of the medium [34]. At this stage, reactions with the lowest solid concentrations (10 and 15 wt%) appeared as a solid-liquid mixture, showing low apparent viscosity compared to other conditions. The step using 20 wt% solids appeared as a very viscous mixture and was difficult to homogenize during the first 3 h of enzymatic hydrolysis. After 3 h of enzymatic hydrolysis, it was observed that the viscosity appeared to have reduced, as the mixture appeared to be homogeneous.

It can be observed from Table 7 that the highest value for cellulose-to-glucose conversion in mass was obtained in the experiment using the lowest solid concentration in this study, obtaining a value of 91.12 wt%. As the initial solid concentration used in the enzymatic hydrolysis process increased, the cellulose-to-glucose conversion obtained decreased, with a cellulose-to-glucose conversion value of 64.8 wt% when using 20 wt% of solids. When analyzing glucose productivity, the highest value was obtained when using the highest solid concentration in the process, obtaining concentration values of up to 123.62 g per liter in each batch. Xu et al. (2019) obtained 122 g L^{-1} of glucose productivity per batch using pre-treated sugarcane bagasse with an alkaline treatment and using 22% solids in its enzymatic hydrolysis, with up to 80% glucose yield using a fed-batch process [35].

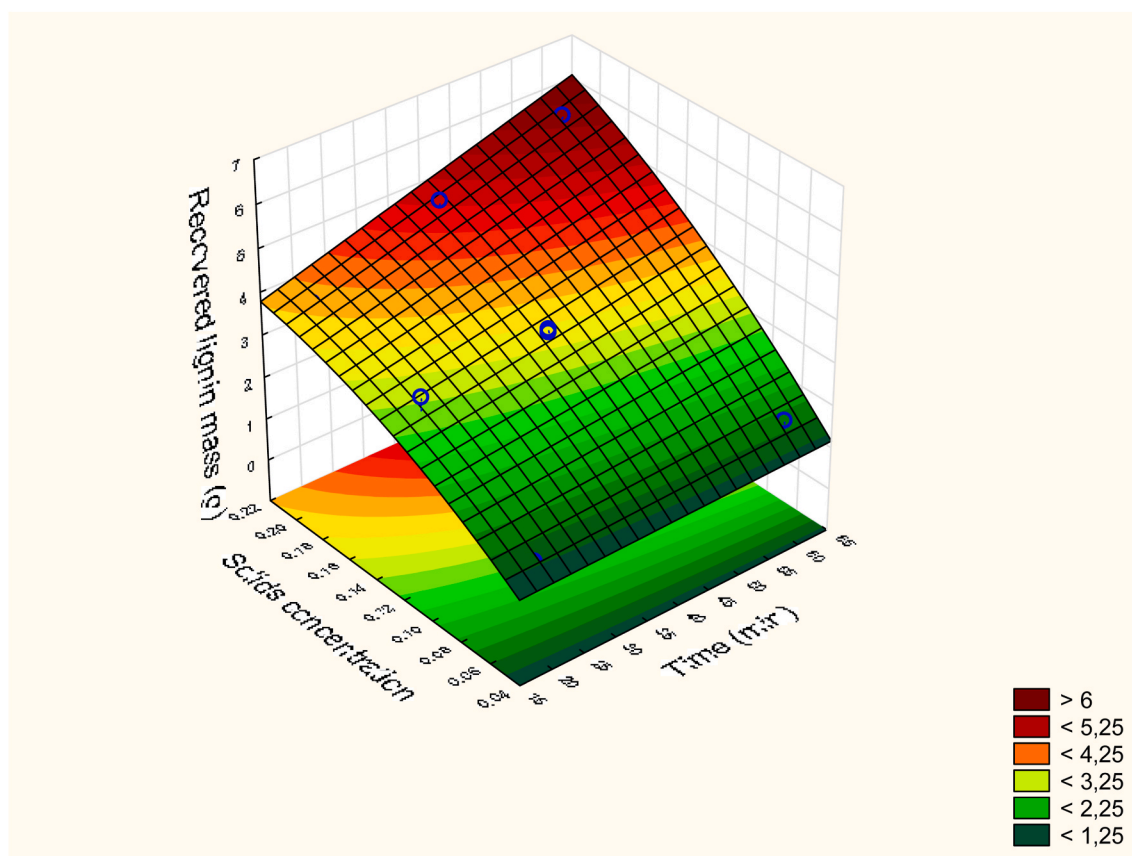


Fig. 3. Response surface of recovered lignin mass as a function of solids concentration and time.

Table 7

Values of cellulose conversion to glucose and glucose productivity for experiments using different initial solid concentration values.

Solids concentration (wt.%)	Cellulose conversion in glucose (wt.%)	Glucose productivity ($\text{g L}^{-1} \text{batch}^{-1}$)
10	91.12 ± 3.20	83.19 ± 3.00
15	75.80 ± 3.40	102.93 ± 3.96
20	64.80 ± 2.80	123.62 ± 3.62

Increased productivity allows for the production of a greater quantity of products in less time and with less resource utilization [36]. High conversion of cellulose to glucose increases resource utilization efficiency, minimizing waste and maximizing efficient resource utilization [35,36]. Therefore, it is necessary to find a value that maintains both variables, glucose productivity and cellulose-to-glucose conversion, at acceptable values. When analyzing cellulose-to-glucose conversion of materials containing 10% and 15% solids, it can be observed that the solid load increased by 50%, but productivity increased by only 23.7%, as conversion had a reduction of 16.9%. When comparing the results of cellulose-to-glucose conversion of materials containing 10% and 20% solids, it was observed that the solid load increased by 100%, with an increase in productivity of only 48.6% and a reduction in conversion of 28.9%. To further increase cellulose-to-glucose conversion and overall glucose productivity at high solids loadings, fed-batch strategies could be implemented to gradually introduce biomass and reduce inhibitory effects. This approach helps maintain optimal mixing and enzyme accessibility, potentially improving yields even under concentrated conditions [35,37].

To obtain more precise results regarding the best solid concentration to be used to achieve high productivity with a small loss in cellulose-to-glucose conversion, a study with more studied conditions and possibly a

technical-economic analysis of this process is necessary. Therefore, the intermediate condition was selected, with 15 wt% solids in the medium. This condition was selected since this solution presents the highest productivity value within the studied conditions that presents in its liquid state from the beginning of the enzymatic hydrolysis reaction, maintaining a productivity above $100 \text{ g L}^{-1} \text{batch}^{-1}$, which provides a high glucose concentration.

Table 8 contains the data regarding the chemical characterization in terms of cellulose, hemicellulose, and lignin of the corn cob after the extrusion process, solvent fractionation under the condition chosen by item 3.3, and after enzymatic hydrolysis using different solid concentrations. All materials were washed and dried prior to characterization.

The extruded corn cob presents a concentration of cellulose, hemicellulose, and lignin similar to the corn cob *in natura* [5], since the extrusion process does not have a relevant impact on the material's chemical structure [6].

After the solvent fractionation process, the lignin content in the solid fraction decreased by 34 wt%, confirming the efficiency of this method in removing lignin from lignocellulosic biomass [30]. Based on the mass balance, approximately 86% of the total solids were recovered after

Table 8

Chemical composition of the studied fractions of extruded corn cob, after the solvent fractionation process, and after enzymatic hydrolysis under different conditions.

	Cellulose wt. %	Hemicellulose wt. %	Lignin wt. %	Total wt. %
Extruded	40.47 ± 0.52	37.21 ± 0.43	19.85 ± 0.89	97.54 ± 1.45
Organosolv	74.90 ± 5.03	16.32 ± 0.47	13.17 ± 1.31	104.39 ± 4.14
10%	24.53 ± 3.60	3.21 ± 0.30	66.80 ± 2.79	94.55 ± 1.10
15%	28.08 ± 4.10	7.67 ± 0.53	62.65 ± 1.90	98.40 ± 6.53
20%	32.57 ± 0.11	5.31 ± 0.47	58.24 ± 4.17	96.11 ± 4.14

organosolv pretreatment, resulting in a lignin removal (solubilization) of 42.37% from the extruded corncob, in agreement with findings from other studies under similar conditions [24,38].

A total of 150 g of dry extruded corncob, containing 29.78 g of lignin, was subjected to organosolv treatment. This process solubilized approximately 12.62 g of lignin (42.37% from the total lignin), from which 45.4% was successfully recovered in the form of dry lignin powder, corresponding to 5.73 ± 0.27 g. This amount represents $19.24 \pm 0.9\%$ of the initial lignin content. Consequently, approximately 6.89 g of lignin (54.6% of the solubilized fraction) were not recovered during the precipitation and purification stages. These losses are likely associated with lignin remaining dissolved in the organosolv liquor, as well as minor losses during washing, centrifugation, and drying procedures. The remaining lignin fraction (17.16 g, corresponding to 57.63% of the initial lignin) remained in the solid residue after organosolv pretreatment.

Previous studies indicate that recovering lignin after organosolv treatment is often challenging and typically requires additional chemicals or solvents to improve yield [39]. In this study, however, only acidified water at pH 4.0 was used, highlighting the simplicity and sustainability of the method.

For context, extrusion-only pretreatment has been shown to achieve high saccharification: José et al. (2022) reported 90.12% cellulose-to-glucose conversion for corncob after only twin-screw extrusion [6]. Other authors reported ~50 % lignin removal under organosolv process only [24]. The literature-reported ranges and reviews support that the integrated extrusion and organosolv sequence combines the mechanical enhancement of enzyme accessibility with chemical delignification, explaining the synergistic improvement observed here (higher glucose conversion and meaningful lignin recovery).

Although organosolv efficiently solubilizes lignin, it is not optimal for lignin recovery under the simplified, low-severity conditions used in this integrated process. Because lignin was precipitated using only acidified water (pH 4.0), a substantial portion of the solubilized lignin remained dissolved as solvent-stabilized or hydrophilic species, recoveries that typically require additional solvents or multi-stage fractionation, which we intentionally avoided to keep the process simpler and greener. Importantly, the goal of the integrated extrusion and organosolv sequence was to enhance enzymatic digestibility rather than maximize lignin precipitation. Within these constraints, organosolv was effective as a delignification step, but limited for lignin recovery.

The hemicellulose content in the solid fraction obtained in the organosolv process also decreased by 56 wt%, as acid was added to the solvent fractionation process [31]. Although hemicellulose removal was substantial during organosolv pretreatment, the valorization of the hemicellulose-rich liquid fraction was not investigated in this study, as our focus was on maximizing enzymatic hydrolysis and evaluating lignin recovery; nonetheless, this stream is currently being explored in ongoing research within our group for potential conversion into value-added products.

The enzymatic hydrolysis process using cellulases and hemicellulases aims to catalyze the breakdown of cellulose into glucose, and hemicellulose into its derivatives (mostly xylose for corn cob) [10]. Observing the results in Tables 8 and it can be noted that in all cases involving the enzymatic hydrolysis of previously extruded corn cob and subjected to the solvent fractionation process, the concentration of cellulose and hemicellulose in the resulting solid fraction decreased, while the lignin concentration increased. However, there is a noticeable difference between the concentrations of each fraction since the initial solid concentration was altered, increasing the residual concentration of cellulose and hemicellulose as the solid concentration increased at the beginning of the enzymatic hydrolysis process, causing the lignin concentration in the solid to become increasingly higher proportionally to the other

fractions. This process is due to the saturation of enzymes present in the solution by the product (glucose and xylose) [41], so that the higher the initial solid concentration, the higher the final concentration of glucose and xylose in the enzymatic solution, resulting in greater inhibition of these enzymes, and ultimately a reduction in the final conversion of cellulose and hemicellulose fractions, as demonstrated in Table 7.

Thinking about increasing this process to industrial scale, this integrated strategy requires consideration of energy and solvent management. Extrusion at high-solids loadings increases specific mechanical energy and demands robust equipment, whereas organosolv pretreatment relies on efficient ethanol–water recycling and heat recovery to remain economical. High-solids slurries also pose challenges for mixing and pumping. Nevertheless, the mechanical effects of extrusion reduce the chemical severity needed for subsequent organosolv delignification, partially balancing energy and solvent costs and supporting the feasibility of integrated implementation [39].

4. Conclusion

This study demonstrates that the integration of twin-screw extrusion with organosolv pretreatment represents a promising approach for the efficient fractionation of lignocellulosic biomass. Rather than acting as isolated steps, the combination of mechanical and solvent-based treatments enables a more effective deconstruction of biomass structure, facilitating downstream processing. From a biorefinery perspective, this integrated approach supports the generation of multiple value streams, including a cellulose-rich fraction suitable for enzymatic conversion, as well as lignin and hemicellulose streams with potential for further valorization. Such fractionation strategies are essential for improving the overall efficiency and economic viability of lignocellulosic biorefineries. The results also indicate that process simplification, such as avoiding intermediate washing steps, can be achieved without compromising performance, which is particularly relevant for reducing water consumption and operational complexity. Future work should focus on improving lignin recovery efficiency, assessing solvent recycling strategies, and evaluating the techno-economic and environmental performance of the proposed process at larger scales. Overall, this study contributes to the development of more integrated, efficient, and sustainable lignocellulosic biorefinery systems.

CRedit authorship contribution statement

Álvaro H.M. José: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Bruna G. Rodrigues:** Formal analysis, Writing – review & editing. **Esperidiana A.B. Moura:** Investigation, Methodology, Resources, Writing – review & editing. **João V.S. Motta:** Investigation, Visualization, Writing – review & editing. **Renata Moreira:** Formal analysis, Methodology, Resources, Writing – review & editing. **Rita C.L.B. Rodrigues:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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Appendix. ASupplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biombioe.2026.109349>.

Appendix A - Extrusion

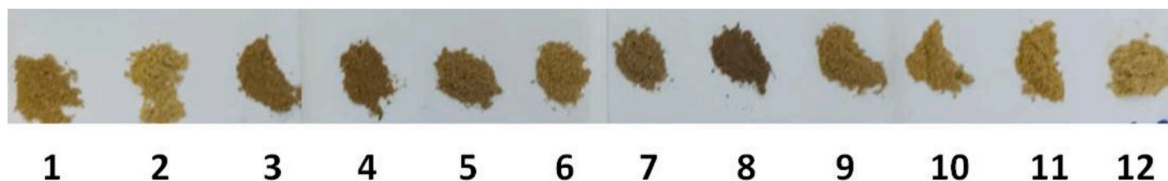


Fig. A.1. Photo of each sample of the factorial design of the extrusion pretreatment.

Table A.1

Analysis of variance (ANOVA) of the data for the extrusion trials with all linear, quadratic, and interaction terms

Source	DF	SS	MS	F-Value	P-Value
Model	5	0.07	0.014	10.03	0.007
Linear	2	0.052	0.026	18.76	0.003
Screw rotation	1	0.026	0.025	18.00	0.005
Temperature	1	0.026	0.027	19.52	0.004
Quadratic	2	0.017	0.009	6.13	0.035
Screw rotation ²	1	0.001	0.001	0.75	0.420
Temperature ²	1	0.017	0.017	12.23	0.013
2 factors interaction	1	0.001	0.001	0.36	0.573
Rotation*temperature	1	0.001	0.001	0.36	0.573
Error	6	0.008	0.001		
Lack of fit	3	0.007	0.002	4.17	0.136
Pure error	3	0.002	0.001		
Total	11	0.079			

Note: DF – Degrees of freedom; SS – Sum of Squares; MS – Mean Squares.

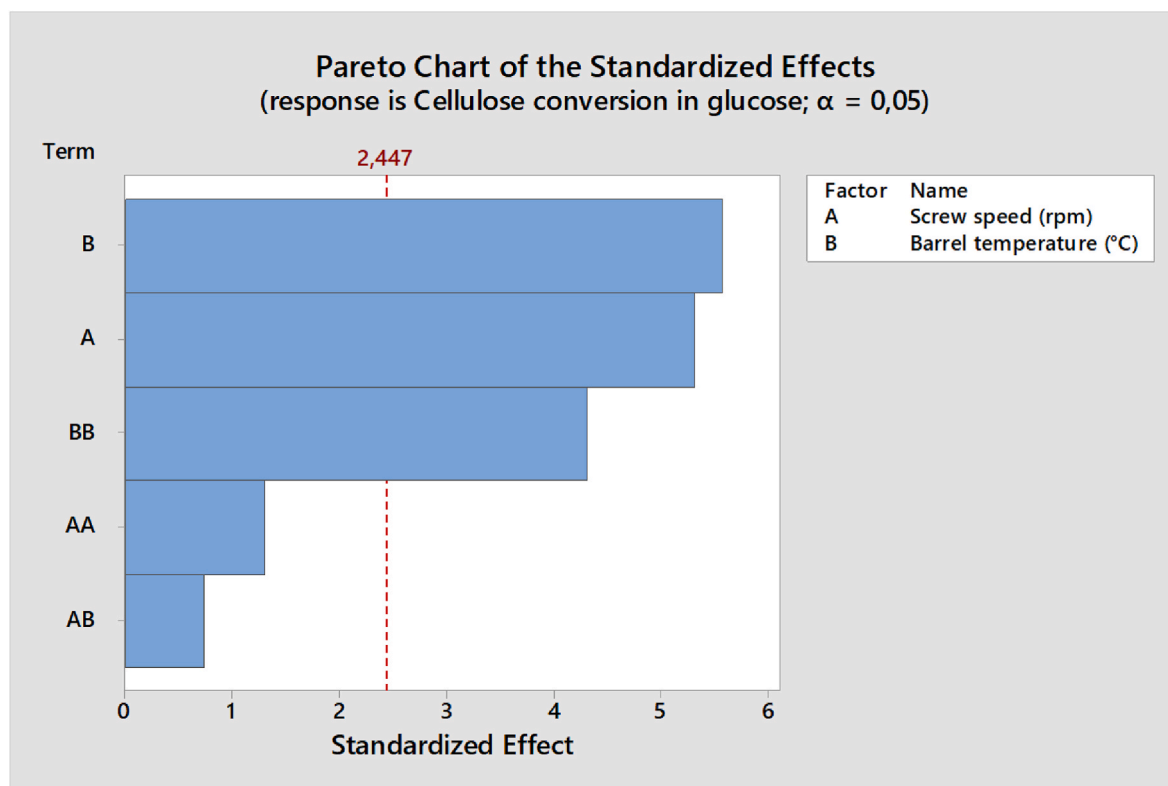


Fig. A.2. Pareto chart with all linear, quadratic, and interaction terms for the extrusion trials.

Table A.2

Analysis of variance (ANOVA) of the data for the extrusion trials with all significant linear, quadratic, and interaction terms at a 90% confidence level for the extrusion trials

Source	DF	SS	MS	F-Value	P-Value
Model	3	0.07	0.02	18.41	0.001
Linear	2	0.05	0.03	21.13	0.001
Screw rotation	1	0.03	0.03	20.27	0.002
Temperature	1	0.03	0.03	21.98	0.002
Quadratic	1	0.02	0.02	12.96	0.007
Temperature²	1	0.02	0.02	12.96	0.007
Error	8	0.01	0.001		
Lack of fit	5	0.01	0.002	3.07	0.192
Pure error	3	0.002	0.001	*	*
Total	11	0.08			

Note: DF – Degrees of freedom; SS – Sum of Squares; MS – Mean Squares.

Appendix B – Organosolv Plackett-Burman

Table B.1

Analysis of variance (ANOVA) of the data for the solvent fractionation process using a Plackett-Burman design including all linear terms for the response variable cellulose conversion to glucose

Source	DF	SS	MS	F-Value	P-Value
Model	6	0.031	0.005	0.28	0.926
Linear	6	0.031	0.005	0.28	0.926
Temperature	1	0.000	0.000	0.00	0.983
Agitation	1	0.001	0.001	0.07	0.800
Acid	1	0.001	0.001	0.07	0.797
Solvent	1	0.002	0.002	0.11	0.758
Time	1	0.026	0.026	1.41	0.289
Glycerol	1	0.000	0.000	0.00	0.996
Error	5	0.093	0.019		
Total	11	0.124			

Note: DF – Degrees of freedom; SS – Sum of Squares; MS – Mean Squares.

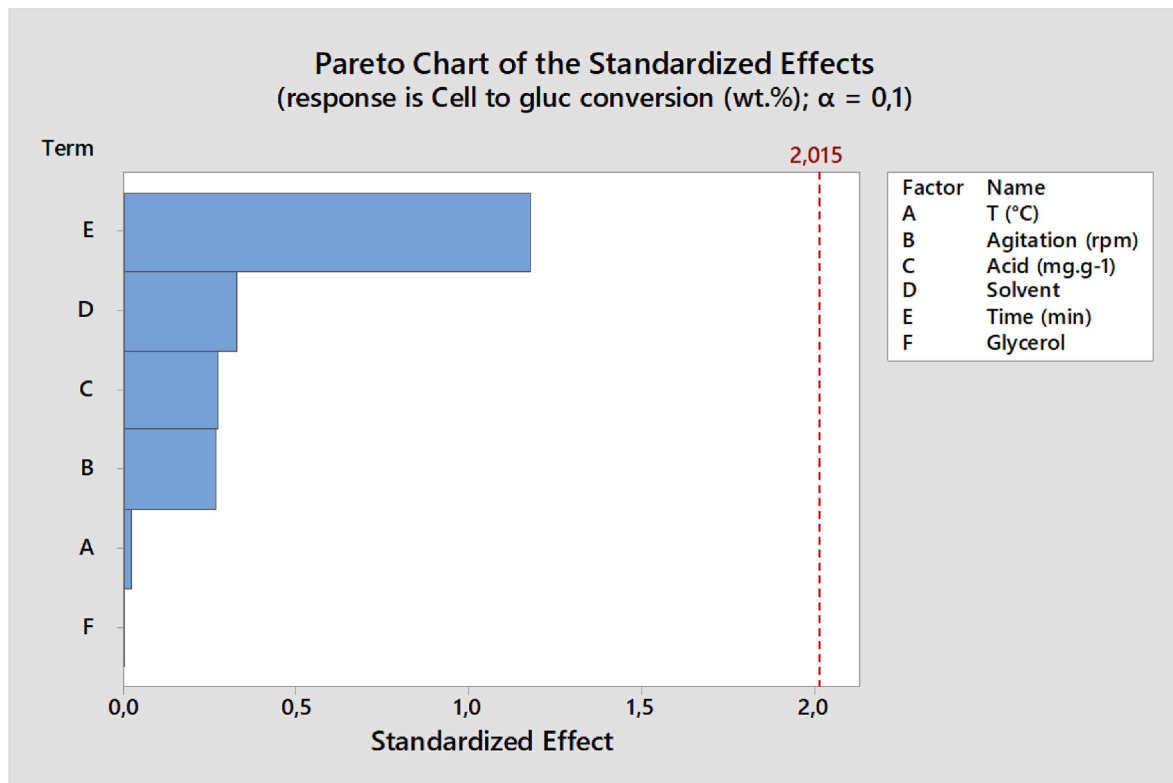


Fig. B.1. Pareto chart for the solvent fractionation process using a Plackett-Burman design including all linear terms for the response variable cellulose conversion to glucose.

Table B.2

Analysis of variance (ANOVA) of the data for the solvent fractionation process using a Plackett-Burman design including all linear terms for the response variable glucose productivity

Source	DF	SS	MS	F-Value	P-Value
Model	6	203.9	33.9	1.00	0.512
Linear	6	203.9	33.9	1.00	0.512
Temperature	1	48.5	48.5	1.42	0.287
Agitation	1	14.1	14.1	0.42	0.548
Acid	1	58.6	58.6	1.72	0.247
Solvent	1	0.1	0.1	0.00	0.949
Time	1	81.6	81.6	2.39	0.183
Glycerol	1	0.8	0.8	0.03	0.880
Error	5	170.5	34.1		
Total	11	374.5			

Note: DF – Degrees of freedom; SS – Sum of Squares; MS – Mean Squares.

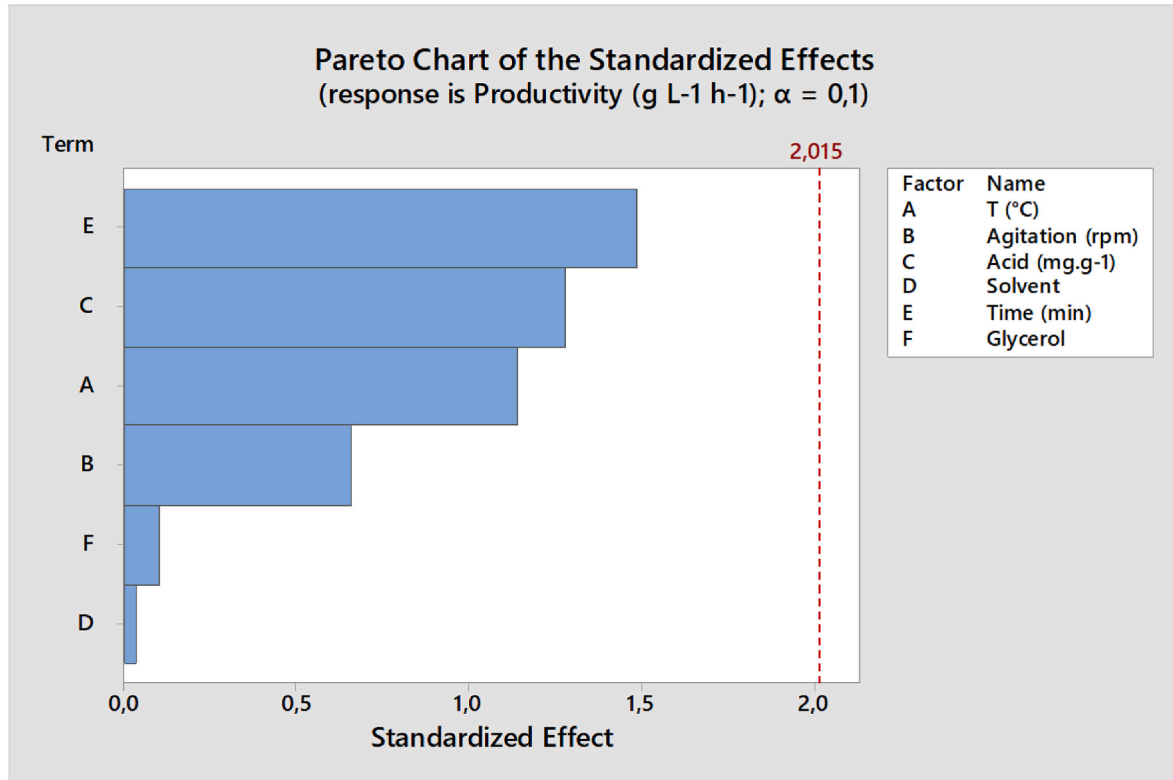


Fig. B.2. Pareto chart for the solvent fractionation process using a Plackett-Burman design including all linear terms for the response variable glucose productivity.

Appendix C – Organosolv factorial fixed

Table C.1

Analysis of variance (ANOVA) including the effect of each variable for the extrusion trials with all linear, quadratic, and interaction terms for the response variable cellulose conversion to glucose

Source	DF	SS	MS	F-Value	P-Value	Source
Time	0.136	1	0.028	0.028	11.48	0.015
Solids concentration	0.163	1	0.040	0.040	16.47	0.007
Time ²	-0.155	1	0.016	0.016	6.60	0.042
Solids concentration ²	-0.096	1	0.006	0.006	2.51	0.164
Solids concentration*Time	0.097	1	0.009	0.009	3.89	0.096
Error		6	0.015	0.002		
Total		11	0.124			

Note: DF – Degrees of freedom; SS – Sum of Squares; MS – Mean Squares.

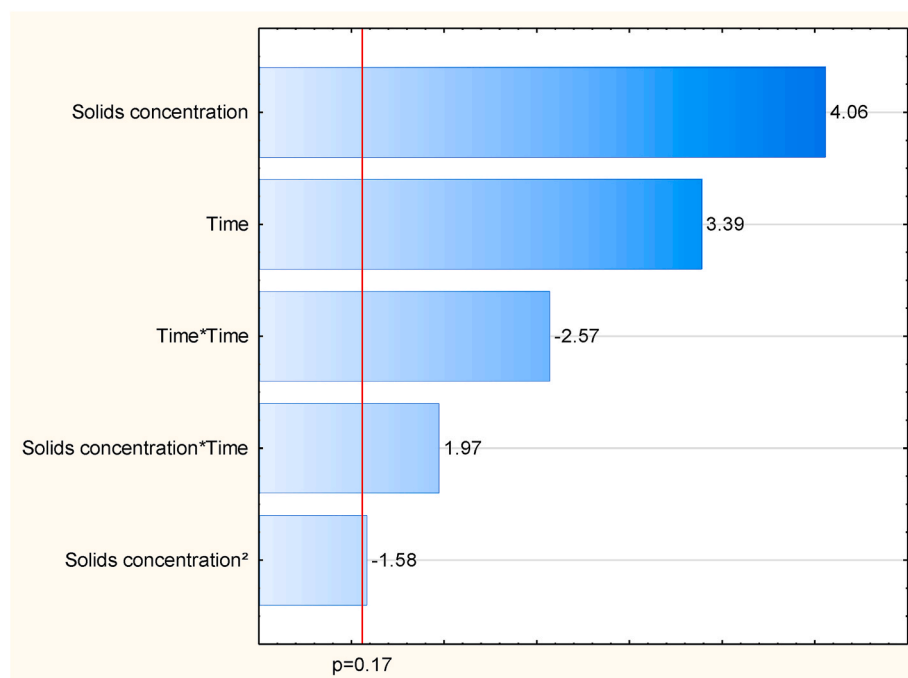


Fig. C.1. Pareto chart with all linear, quadratic, and interaction terms for the organic solvent fractionation trials.

Data availability

Data will be made available on request.

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