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Alternative Lime Pretreatment of Corn Stover for Second-Generation Bioethanol Production

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Abstract: In this work, a delignification process, using lime (Ca(OH)₂) as an alternative alkali, was evaluated to improve enzymatic saccharification of corn stover cellulose, with the final goal of obtaining second-generation bioethanol. For that, an experimental design was conducted in order to assay the effect of temperature, lime loading, and time on the corn stover fractionation and enzymatic susceptibility of cellulose. Under conditions evaluated, lime pretreatment was selective for the recovery of cellulose (average of 91%) and xylan (average of 75.3%) in the solid phase. In addition, operating in mild conditions, a delignification up to 40% was also attained. On the other hand, a maximal cellulose-to-glucose conversion (CGC_{MAX}) of 89.5% was achieved using the solid, resulting from the treatment carried out at 90 °C for 5 h and lime loading of 0.4 g of Ca(OH)₂/g of corn stover. Finally, under selected conditions of pretreatment, 28.7 g/L (or 3.6% v/v) of bioethanol was produced (corresponding to 72.4% of ethanol conversion) by simultaneous saccharification and fermentation. Hence, the process, based on an alternative alkali proposed in this work, allowed the successful production of biofuel from the important and abundant agro-industrial residue of corn stover.



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Keywords: corn stover; biorefinery; lime pretreatment; delignification; bioethanol

1. Introduction

A sustainable future relies on an increased share of eco-friendly energy, particularly in developing countries. Consequently, biofuels are considered important substitutes for oil-based combustibles, solving the depletion issue of non-renewable sources and decreasing the ecological problems related to them [1,2]. One of the most employed biofuels is bioethanol, which is an alternative for gasoline in vehicles and can be obtained from sources of sugar (namely sucrose and starch) via fermentation. The ethanol obtained from different food-related sources, such as sugarcane, sugar beet, or maize (sources of sucrose and starch, respectively) is commonly known as first-generation bioethanol [3–5].

Among sugars sources, corn or maize (scientific name: *Zea mays* L.) is considered a yearly crop, which is generated worldwide in enormous amounts to obtain feed, food, and material for applications in the industry [6]. However, the use of crops as feedstock may lead to some conflicts, augmenting the demand and price of foods [7–9]. In this way, the use of alternative, ubiquitous, and renewable sources is fundamental for the production of biofuels under a more profitable and sustainable point of view [10–14]. Lignocellulosic materials (LCMs) fulfill these characteristics, including attractive choices for the partial replacement of fossil fuels, like remarkable availability, ubiquity, enhancement of local economy resulting from cultivation, carbon neutrality, and appropriateness for the manufacture of LCM-derived ethanol [15,16].

Regarding the different LCMs, corn stover is an agricultural residue, a by-product from the grain harvesting of maize, and is considered an appealing feedstock for the manufacture of advanced biofuels or second-generation ethanol, due to its availability in

large amounts while being cost-effective [17–19]. Moreover, the reported residue/grain ratio for maize equals 1, which means that the same quantity of grain and corn stover is produced [6]—about 1060 million tons of corn stover per year in the world, resulting in the most extensive by-product globally that can be employed for bioenergy purposes [20]. In order to optimize the exploitation of corn stover, several studies have evaluated the feasibility of ethanol production from this renewable by-product [21–23].

In this sense, the production of ethanol from LCM implies three main steps: (i) pretreatment of the raw material pretreatment, (ii) enzymatic saccharification to transform the cellulose into glucose, and (iii) biological conversion of glucose to ethanol via fermentation the two latter processes can be carried out at the same time through simultaneous saccharification and fermentation (SSF). The selection of a suitable pretreatment is required to enhance the enzymatic saccharification of cellulose, since factors related to LCM structure (such as cellulose crystallinity, moisture content, available surface area, degree of polymerization and lignin content) hinder the enzymes' action [24,25]. Furthermore, the high cost associated with biomass pretreatment still represents a significant drawback for the biorefinery development. The techno-economic evaluation of individual pretreatments, such as autohydrolysis and organosolv, has been performed in several studies, representing close to 20% of the cost in biofuel production [26]. Taking all of the previous into account, widespread research has been carried out on different pretreatment methods, such as hydrothermal pretreatments, acidic pretreatments, or alkali pretreatments [27,28]. Among the latter, the most widely employed alkalis are ammonia (NH₃), sodium hydroxide (NaOH), and lime (Ca(OH)₂) [13].

Accordingly, lime pretreatment is being again considered as a favorable and alternative pretreatment [29–31]. It is considered a low-budget choice for lignin and acetyl group removal at alkali pH, enhancing the subsequent enzymatic hydrolysis rates of cellulose by means of opening up the structure of the LCM and decreasing non-productive cellulase binding [26]. In this way, lime pretreatment is cost-effective (\$0.06/kg), widely available, safely manageable, and can be effortlessly recovered and recycled by washing the biomass with water [26,32]. In addition, few works have evaluated the feasibility of lime pretreatment for the production of bioethanol on different LCMs, e.g., rice straw [33], Bermudagrass [34], or oat straw [30].

Therefore, the purpose of this study was the assessment of lime processing as low-cost biomass processing for bioethanol production. For that, the effect of temperature (90–121 °C), residence time (1–5 h), and lime loading (0.1–0.4 g lime/g raw material) on corn stover fractionation, as well as cellulose susceptibility to enzymatic saccharification, was evaluated by an experimental, central composite design. In addition, results from enzymatic saccharification of lime-pretreated corn stover were fitted to an empirical model to determine kinetic parameters namely maximum cellulose to glucose conversion (CGC_{MAX}) and time to achieve $CGC_{MAX}/2$ ($t_{1/2}$). Finally, lime-pretreated corn stover was evaluated with simultaneous saccharification and fermentation (SSF) for the manufacture of ethanol.

2. Materials and Methods

2.1. Raw Material

Corn stover feedstock was collected in A Bola (Galicia, Spain) and milled at an appropriate size for the pretreatment step (<8 mm). Subsequently, the samples were air-dried in a homogeneous lot, avoiding distinct compositions between aliquots, and kept in a cool, dark, and dry site until used.

2.2. Analysis of the Raw Material

For analytical purposes, corn stover was milled at 0.5 mm, and following standard NREL methods for the determination of moisture [35], ashes [36], extractives [37], and quantitative acid hydrolysis (QAH) with 72% *w/w* sulfuric acid [38]. The resulting liquid phase from QAH was measured via HPLC for the quantitation of units of glucose, xylose, arabinose, and acetic acid using an Agilent chromatograph; a BioRad Aminex HPX-87H

column at 60 °C, with a flow of 0.6 mL/min of 0.01 M H₂SO₄ mobile phase; and a refractive index detector at a temperature of 40 °C. The results obtained allowed the calculation of the polymers (namely cellulose, xylan, arabinan, and acetyl groups) contents, respectively. The acid insoluble solid residue from this process was measured as Klason lignin.

2.3. Lime Pretreatment of Corn Stover

Raw corn stover at liquid solid ratio of 12 g/g was mixed with lime (0.1–0.4 g lime/g biomass) and distilled water in a 500 mL flask, resulting in a slurry of biomass and lime milk. Once it was blended, the mixture was pretreated at different times (1–5 h) and temperatures (90–121 °C). The conditions were set based on previous experiments. The experiments carried out in this work are listed in Table 1.

Table 1. Operational conditions of lime pretreatment (expressed as dimensional and dimensionless independent variables).

Run	Dimensional, Independent Variables			Dimensionless Normalized Independent Variables		
	Temperature (°C)	Time (h)	Lime Loading (g Lime/g r.m)	x_1	x_2	x_3
1	121.0	5	0.40	1	1	1
2	121.0	5	0.10	1	1	−1
3	121.0	1	0.40	1	−1	1
4	121.0	1	0.10	1	−1	−1
5	90.0	5	0.40	−1	1	1
6	90.0	1	0.40	−1	−1	1
7	90.0	1	0.10	−1	−1	−1
8	90.0	5	0.10	−1	1	−1
9	121.0	3	0.25	1	0	0
10	90.0	3	0.25	−1	0	0
11	105.5	5	0.25	0	1	0
12	105.5	1	0.25	0	−1	0
13	105.5	3	0.40	0	0	1
14	105.5	3	0.10	0	0	−1
15	105.5	3	0.25	0	0	0
16	105.5	3	0.25	0	0	0
17	105.5	3	0.25	0	0	0

After the treatment, the resulting slurry was filtered to recover the solid phase, which was then washed with distilled water (until reaching neutral pH) to eliminate the excess alkali. The pretreated LCM was weighted to gravimetrically determine the solid yield in the lime pretreatment stage (abbreviated as SY and quantified as g biomass/100 g raw material, on an oven-dry basis (o.d.b.)).

Pretreated biomass samples were chemically analyzed, employing the methods specified in Section 2.2 for the raw material. The percentage of delignification was quantified as

$$\% \text{ Delignification } (D) = 100 \times \frac{KL_{CS} - KL_{DS} \cdot \frac{SY}{100}}{KL_{CS}} \quad (1)$$

where KL_{CS} represents the amount of Klason lignin contained in the raw material (corn stover), KL_{DS} represents the percentage of Klason lignin in delignified solids, and SY represents the solid yield.

2.4. Enzymatic Hydrolysis of Solids from Lime Pretreatments

Enzymatic saccharification experiments, performed at optimal conditions for the enzymes employed (Celluclast 1.5 L cellulases and 188 Novozyme β -glucosidase, kindly provided by Novozymes), were assessed at 48.5 °C and pH 4.85 (employing 0.05 N citric acid–sodium citrate buffer) with orbital agitation of 150 rpm. The cellulase activity of Celluclast 1.5 L was quantified via the filter paper assay [39], with a value, in filter paper

units (FPU), of 70.1 FPU/mL. In contrast, β -glucosidase activity of 188 Novozyme was quantified by means of international units (IU) [40], with a value of 630 IU/mL.

With the aim of examining the influence of lime processing on cellulose digestibility, enzymatic saccharification experiments were performed at the following conditions, based on literature [41,42]: liquid-to-solid ratio (LSR) of 20 g liquid/g oven-dry solid, enzyme-to-substrate ratio (ESR) of 15 FPU/g substrate, and β -glucosidase-to-cellulase ratio (IU/FPU) of 5 IU/FPU. At the desired times (between 0–96 h), samples were withdrawn, separating the supernatant by centrifugation, which was filtered by a 0.45 μ m membrane prior to the HPLC analysis for monosaccharides and acetic acid quantitation. The experimental data of the glucose concentration profiles was fitted to the subsequent equation [43]:

$$CGC_t = CGC_{MAX} \times \frac{t}{t + t_{1/2}} \quad (2)$$

where CGC_t represents the cellulose-to-glucose conversion reached at a time t , CGC_{MAX} represents the cellulose-to-glucose conversion when the reaction time is infinite, t represents the enzymatic hydrolysis time (h) and $t_{1/2}$ (h) represents the time necessary to accomplish half of the CGC_{MAX} . Additionally, CGC_t was quantified as:

$$CGC_t = 100 \times \frac{G_t - G_{t=0}}{G_{POT}} \quad (3)$$

where G_t represents the concentration of glucose (g/L) reached at a time t , $G_{t=0}$ represents the glucose concentration when time equals 0, and G_{POT} is the potential glucose concentration (which corresponds to the total conversion of the substrate's cellulose into glucose). G_{POT} was quantified as

$$G_{POT} = \frac{Gn}{100} \times \frac{180}{162} \times \frac{\rho}{LSR + 1 - \frac{KL}{100}} \quad (4)$$

where Gn represents the glucan content in the pretreated solid (g glucan/100 g pretreated solid, o.d.b.), 180/162 denotes the stoichiometric factor for cellulose hydration upon hydrolysis, ρ represents the symbol for the density of the reaction medium (representing an average value of 1005 g/L), LSR is the liquid-to-solid ratio (in this case, corresponds to 20 g liquid/g pretreated solid, o.d.b.), and KL is the Klason lignin content in the pretreated solid (g Klason lignin/100 g pretreated solid, o.d.b.).

2.5. Yeast Cultivation and Inoculum Preparation

Saccharomyces cerevisiae CECT-1170 was acquired from the Spanish Collection of Type Cultures (Valencia, Spain) and used for fermentation assays to produce bioethanol. Cells were grown as specified by the provider, in a medium of 10, 5, 3, and 3 g/L of glucose, peptone, yeast extract, and malt extract, respectively. The orbital shaker was set at 32 °C for 24 h and with agitation at 200 rpm. The biomass concentration in the reaction media was quantified by dry cell weight.

2.6. Simultaneous Saccharification and Fermentation (SSF)

Lime-pretreated corn stover, under the two best conditions to achieve higher enzymatic hydrolysis, were employed for SSF assays.

The enzymes used in SSF experiments were the same as those utilized in the enzymatic hydrolysis. Assays were assayed in an temperature-controlled orbital shaker (120 rpm) at a compromise temperature (for the enzymes and yeast) of 35 °C [44,45] for 96 h (pH = 5). SSF media was prepared mixing the pretreated solid and water (at a desired LSR) and sterilized in an autoclave at 121 °C for 15 min. Separately, nutrients were prepared (to reach a final concentration in the SSF medium of 5, 3, and 3 g/L of peptone, yeast extract, and malt extract, respectively) and autoclaved at the same temperature and time than the SSF media. Once the enzymes the medium was cooled, the SSF assays were begun by addition of the

nutrients, enzymes (at desired enzyme-to-substrate ratio), and yeast inoculum (at a final cell concentration of 2.45 g/L, in the medium).

The SSF medium was sampled at preset times (0, 3, 12, 24, 48, 72, or 96 h), centrifuged to recover the supernatant, filtered by 0.22 µm membranes, and subjected to HPLC for the determination of the ethanol and sugars content. The conversion of glucan to ethanol at time t (EC_t ; g ethanol/100 g potential ethanol) was quantified as

$$EC_t = 100 \times \frac{E_t}{G_{POT} \cdot \frac{92}{180}} \quad (5)$$

where E_t represents the ethanol concentration (g/L) at time t , 92/180 exemplifies the stoichiometric factor that relates the conversion from glucose to ethanol, and G_{POT} is the potential glucose, quantified using the same equation indicated in Section 2.4 for enzymatic hydrolysis.

2.7. Fitting Data

A commercial software (Microsoft Excel, Redmon, WA, USA) was employed to fit the experimental data to the proposed models. Optimization was performed via response surface methodology (RSM).

3. Results and Discussion

3.1. Raw Material and Lime Pretreatment

Corn stover was chemically analyzed to determine its content in polysaccharides and Klason lignin. The resulting composition of corn stover is as follows, and is displayed in g of component per 100 g of raw material o.d.b ± standard deviation, regarding three sample replicas: 37.57 ± 0.46 of cellulose (measured as glucan), 21.41 ± 0.05 of xylan, 2.96 ± 0.10 of arabinan, 2.59 ± 0.12 of acetyl groups, 17.99 ± 0.34 of Klason lignin, 5.28 ± 0.06 of ashes, and 8.20 ± 0.18 of extracts. Cellulose represented the major component, followed by hemicellulose (made up of xylan, acetyl groups, and arabinan) and Klason lignin. Hemicellulose (27.0%) content was similar to that reported in literature for corn stover [46]. On the other hand, cellulose was slightly superior than what has been reported previously for different maize genotypes [47].

Lime pretreatment was selected as alternative delignification process to take apart the recalcitrance of corn stover's structure. The experimental design matrix carried out in this work is displayed in Table 1, including operational conditions employed for each lime treatment of corn stover. A range of independent variables (lime loading, temperature, and time) was selected, relying on prior experiments (data not shown).

After lime pretreatment, the solid residue was recovered by filtration to determine solid yield (SY), and was chemically analyzed for composition quantitation, following the procedures defined in Section 2.2. The main results of chemical composition after lime pretreatment (including glucan, xylan, and Klason lignin) are collected in Table 2. As seen in Table 2, slight variations of SY were observed after lime pretreatment, which varied in the range of 71.2–78.8 g of lime-pretreated corn stover/100 g of raw material. With regards to the composition of corn stover after lime pretreatment, glucan content was similar for all conditions evaluated, achieving the highest percentage of glucan (47.4 g/100 g lime pretreated corn stover) at conditions of run 4 (121 °C, 1 h, and 0.1 g/g of lime loading). For the most part, the lime pretreatment evaluated in this work allowed the increase of 22.0% of glucan content with respect to glucan in raw corn stover, due to the removal of hemicelluloses and lignin, as observed in other works [30]. Glucan recovery after treatment is also shown in Table 2, and varied in the range of 86.6–97.7% for runs 13 and 4, respectively. As a general trend, glucan recovery lower than 90% was obtained for pretreatment times >3 h. In contrast, the xylan content in corn stover after lime pretreatment varied in a narrower range from 20.9 to 22.3 g/100 g lime pretreated corn stover. These data revealed that lime treatment was less selective for xylan recovery than glucan, yielding an average of 75.3% regarding the initial xylan. These experimental data are in accordance with those

reported in other scientific works concerning alkali treatments, in which cellulose remained almost quantitatively in the pretreated solid [48]

Table 2. Main results obtained from chemical composition of lime-pretreated corn stover and enzymatic hydrolysis.

Run	SY (g/100 g)	Glucan (g/100 g)	Xylan (g/100 g)	Klason Lignin (g/100 g)	Glucan Recovery (g/100 g)	Xylan Recovery (g/100 g)	Delignification (g/100 g)	CGC _{MAX} (g/100 g)
1	77.4	46.4	20.9	16.0	95.6	75.7	31.4	79.9
2	78.8	44.3	22.3	17.4	92.9	82.0	23.8	71.1
3	73.2	45.4	21.1	16.9	88.5	72.2	31.3	83.8
4	77.4	47.4	22.1	17.9	97.7	79.7	22.9	83.3
5	74.9	45.6	21.5	15.3	90.8	75.2	36.4	89.5
6	76.3	45.2	21.6	15.5	91.9	77.1	34.4	87.0
7	76.7	45.3	22.0	16.3	92.4	78.8	30.6	77.5
8	74.6	45.0	21.3	16.3	89.4	74.4	32.4	78.5
9	71.2	47.5	21.5	15.5	90.1	71.6	38.5	86.9
10	73.0	46.1	22.0	15.6	89.7	75.0	36.6	81.9
11	75.2	46.2	21.3	15.7	92.4	74.6	34.4	72.7
12	73.7	46.1	21.8	15.7	90.4	75.1	35.9	83.8
13	73	45.0	20.8	14.7	87.6	71.1	40.2	82.4
14	74.7	45.1	21.8	16.0	89.7	76.1	33.6	62.8
15	71.9	46.4	21.7	14.9	88.9	72.9	40.7	77.0
16	74.0	45.9	21.4	15.0	90.3	74.1	38.4	79.2
17	73.1	45.8	21.7	14.6	89.1	74.2	40.7	82.7

As expected, lime pretreatment, as alternative alkali delignification, displayed a more selective behavior towards the solubilization of lignin than to polysaccharides. In this regard, the delignification rate (g of solubilized Klason lignin/100 g of Klason lignin in raw material) was determined (see Table 2). In this context, the delignification of corn stover increased, with lime loading >0.25 g/g and residence times of 3 h. In this work, the highest delignification (almost 41%) was achieved at the central points, with conditions of 105.5 °C for 3 h and lime loading of 0.25 g of lime/g of corn stover. A similar percentage of delignification of corn stover was reported by Tan et al. [46] using 3% (*v/v*) of Ca(OH)₂ at 121 °C and 1 h of treatment. Several studies in literature have reported that alkaline pretreatment (using NaOH as alkali) can effectively remove lignin [49,50]. On the other hand, other authors have demonstrated a limited capacity of lime for lignocellulosic biomass delignification [51]. This fact could be related to the interplay among the calcium ions and the lignin in the LCM structure [52,53].

3.2. Evaluation of Enzymatic Susceptibility of Lime-Pretreated Corn Stover

With the purpose of estimating the influence of lime processing on the enzymatic susceptibility of cellulose, solid phases resulted from runs 1–17 (registered in Table 1) were used as the substrates in enzymatic hydrolysis assays under fixed conditions (LSR = 20 g liquid/g oven-dry solid, ESR = 15 FPU/g substrate, and β-glucosidase/cellulase ratio = 5 IU/FPU). Glucose data obtained from these experiments were fitted to empirical equation defined by Holtzapple et al. to define the kinetic parameters of enzymatic hydrolysis [43]. Figure 1 displays the time course of glucose production of enzymatic saccharification assays and the good adjustment of experimental data, with *R*² ranging from 0.958 to 0.989 (corresponding to runs 3 and 4, respectively). As seen, lime pretreatment improved the saccharification of cellulose, achieving glucose concentration higher than 15 g/L within the first 24 h. In order to compare results obtained from different operational conditions of pretreatment, the enzymatic hydrolysis results were expressed as cellulose-to-glucose conversion (CGC, %) (Figure 1). The kinetic parameter CGC_{MAX}, calculated by Equation (2), was also included in Table 2, which allows the correlation of these results with the evaluated lime processing conditions (temperature, time, and lime loading). Maximal cellulose-to-

glucose conversion (CGC_{MAX} , %) varied from 62.8 (run 14, conditions of 105.5 °C, 3 h and 0.1 g/g) to 89.5% (run 5, conditions of 90 °C, 5 h, and 0.4 g/g). In almost all of the enzymatic hydrolysis assays, the cellulose-to-glucose conversions were higher than 80%, showing the positive effect of pretreatment on glucose production. Interestingly, the values of $t_{1/2}$ calculated for all experiments were lower than 3.65 h, indicating a high saccharification rate throughout the experimental domain studied. These results reflect positive behavior compared with other examples reported in the scientific literature, where $t_{1/2} > 10$ h was obtained using hydrothermally treated or auto-hydrolyzed corn cob [41]. Alternatively, similar results for cellulose conversion and $t_{1/2}$ were obtained from lime-pretreated oat straw [30].

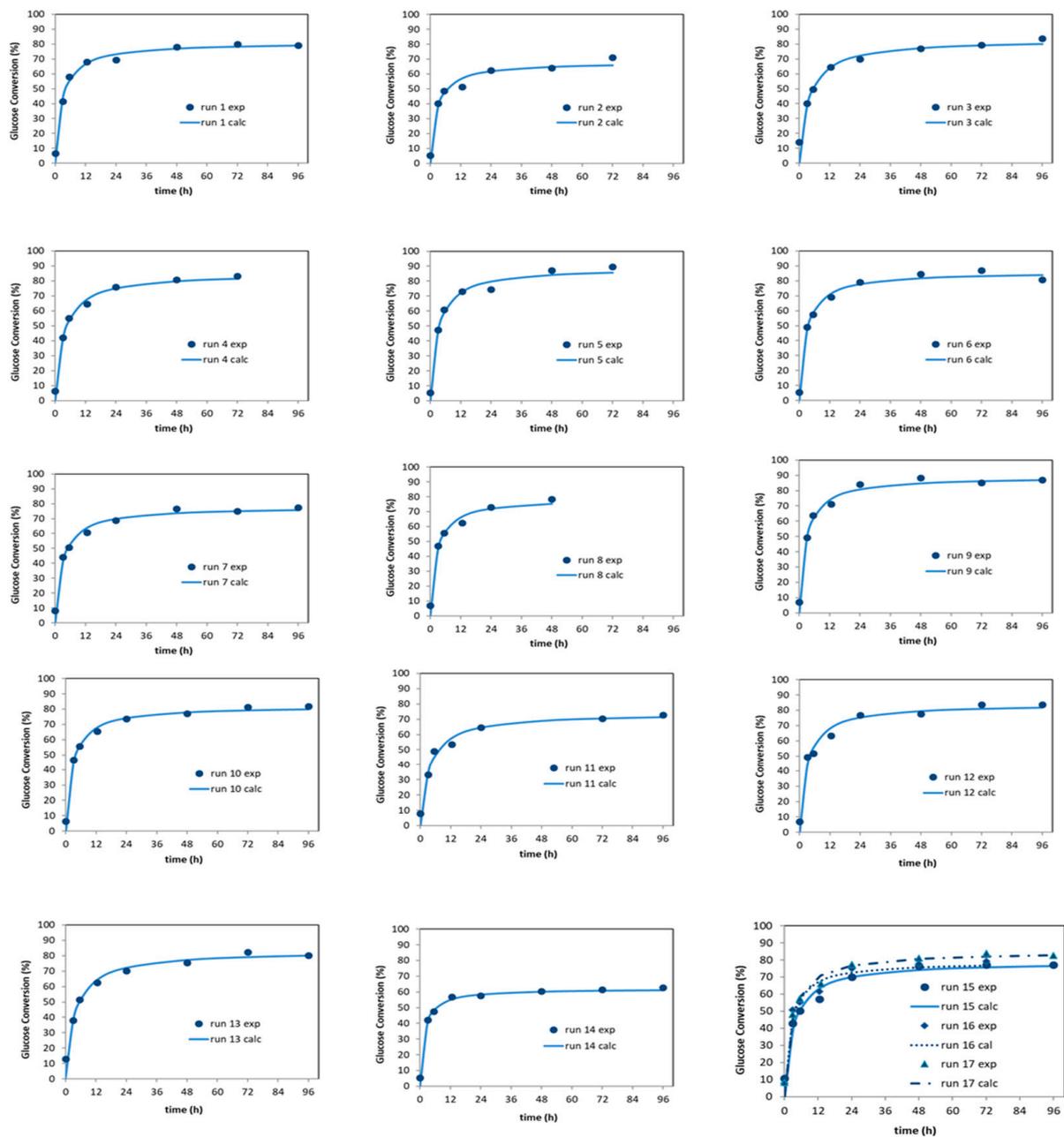


Figure 1. Time course of cellulose-to-glucose conversion (%) from enzymatic saccharification assays of lime-pretreated corn stover (conditions listed in Table 1).

3.3. Response Surface Methodology (RSM) Assessment

In order to get a better understanding of pretreatment conditions' effect on results obtained from the corn stover fractionation and enzymatic susceptibility of pretreated corn stover, experimental data (listed in Table 2) or dependent variables (namely, SY or y_1 , glucan recovery or y_2 , xylan recovery or y_3 , delignification or y_4 , and CGC_{MAX} or y_5) were correlated to independent variables (Table 1) following the expression

$$y_j = b_{0j} + \sum_i b_{ij} x_i + \sum_i \sum_k b_{ikj} x_i x_k \quad (6)$$

where y_j represents the dependent variable considered ($j = 1-5$), x_i or x_k (i or $k = 1-3$, $k \geq i$) are the independent variables (dimensionless) established in Equation (6), and $b_{0j} \dots b_{ikj}$ represent the regression coefficients measured in via the experimental data by multiple regression employing the least-squares method. Table 2 lists the experimental values reached for the experimental variables, while Table 3 displays the regression coefficients $b_{0j} \dots b_{ikj}$ and their significance (according to the Student's t -test), along with the statistical parameters quantifying the correlation (R^2) and significance of the models (according to the Fisher's F -test).

Table 3. Main regression coefficients and significance and statistical parameters measuring the correlation and significance of models obtained for variables y_1 to y_5 .

Coefficients	SY or y_1 (g/100 g)	Glucan Recovery or y_2 (g/100 g)	Xylan Recovery or y_3 (g/100 g)	Delignification or y_4 (g/100 g)	CGC_{MAX} or y_5 (g/100 g)
b_0	72.607	89.124	73.161	39.783	78.430
b_1	0.235	1.043 ^c	0.074	-2.250 ^b	-0.959
b_2	0.360	0.025	-0.091	0.330	-2.371
b_3	-0.748 ^c	-0.786	-1.975 ^a	3.040 ^a	4.936 ^b
b_{11}	1.131 ^b	0.778	1.489 ^b	-0.350	-2.447
b_{22}	-0.691 ^c	-0.922	-1.624 ^b	1.025	-1.399
b_{33}	0.434	1.740 ^b	0.462	-0.075	1.226
b_{12}	-0.188	0.996	0.553	-2.120	6.860 ^b
b_{13}	2.110 ^b	2.489 ^b	2.152 ^b	-4.520 ^b	0.756
b_{23}	1.553 ^b	-0.263	0.903	-2.770 ^c	-4.942
R^2	0.900	0.830	0.910	0.930	0.790
F	6.660	3.690	7.640	9.730	2.880
Significance level (%)	>98	>95	>99	>99	>91

Coefficients significant regarding the Student's t -test with a confidence level of ^a 99%; ^b 95%; ^c 90%.

The fitting parameters (see Table 3) show the adequacy of proposed models. The coefficient of determination (R^2) of the models was greater than 0.9 for the variables (SY, xylan recovery, and delignification) and 0.8 for glucan recovery and CGC_{MAX} .

Figure 2 shows the dependence of selected variables (solid yield, glucan and xylan recoveries, and percentage of delignification) on independent variables studied. As seen, SY decreased with a rise of temperature and time (Figure 2a). In contrast, the maximal glucan recovery was obtained for the highest temperature studied in this work and a lime loading of 0.4 g/g (Figure 2b). For xylan recovery, a maximum was obtained for intermediate conditions of pretreatment (time and temperature), as displayed in Figure 2c. Nevertheless, the delignification of corn stover (Figure 2d) was enhanced using low temperatures (<100 °C) and high lime loadings (>0.3 g/g).

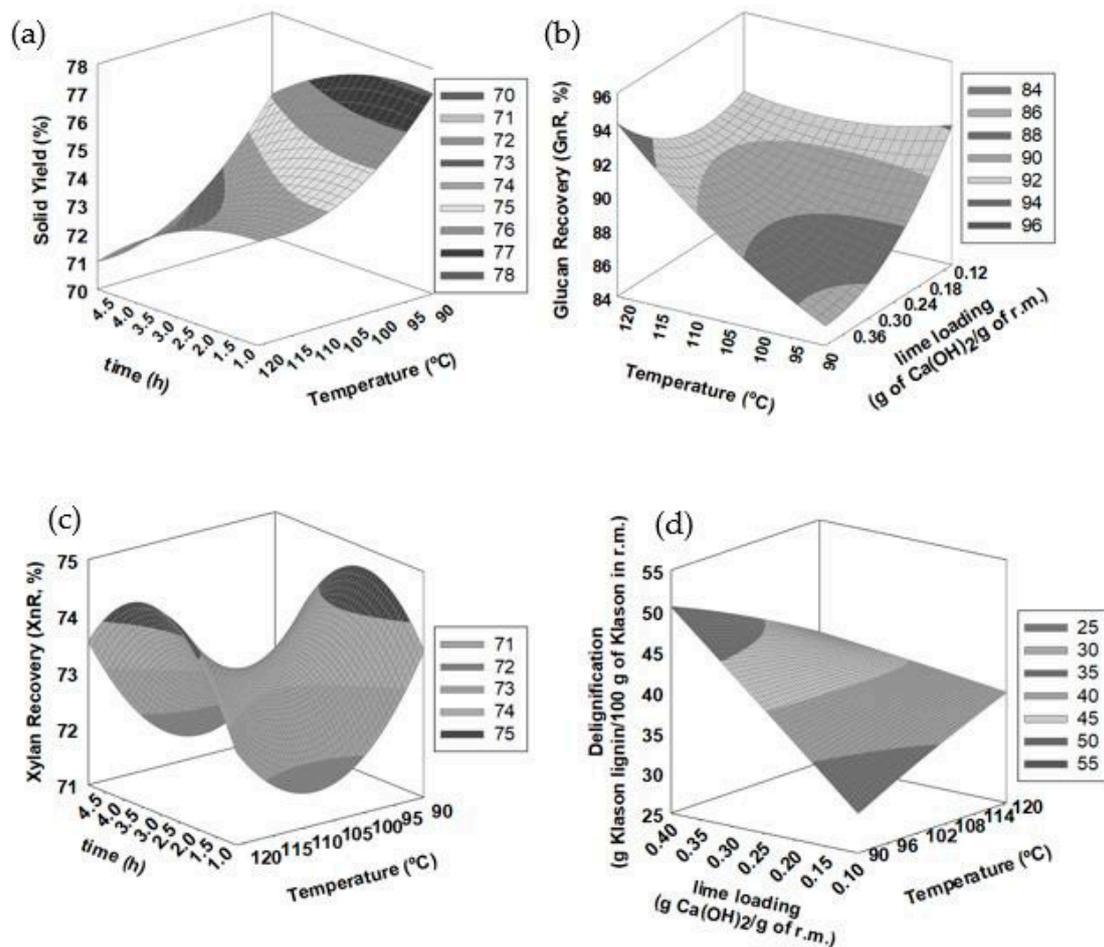


Figure 2. Response surface of calculated (a) dependence of solid yield (%) on temperature ($^{\circ}\text{C}$) and time (h), fixed lime loading = 0.4 g/g; (b) dependence of glucan recovery (%) on temperature and lime loading, fixed time = 5 h; (c) dependence of xylan recovery (%) on temperature and time (h), fixed lime loading = 0.25 g/g; and (d) dependence of delignification on temperature and lime loading, fixed time = 1 h.

3.4. Simultaneous Saccharification and Fermentation (SSF) for Bioethanol Production

Based on the results obtained from experimental design, and taking into account the enzymatic susceptibility of pretreated corn stover, conditions carried out in runs 4 and 6 were selected to evaluate the manufacture of bioethanol by simultaneous saccharification and fermentation (SSF) strategy. The biggest glucan recovery (97.7 g glucan/100 g glucan in raw material) was obtained at $T = 121^{\circ}\text{C}$, $t = 1\text{ h}$, and lime loading = 0.1 g lime/g biomass (run 4). Alternatively, high CGC in enzymatic hydrolysis, according to the model predictions (87.0 g glucose/100 g potential glucose), was obtained at $T = 90^{\circ}\text{C}$, lime loading of 0.4 g lime/g biomass, and the shortest time studied (1 h), corresponding to run 6. Table 4 shows the SSF assays proposed for ethanol production from this raw material, including the main results acquired (maximal ethanol concentration (E_{MAX}) in g/L and ethanol conversion (EC_{MAX}) in %).

Figure 3 shows the profiles of ethanol production using the lime-pretreated corn stover at different substrate loadings and enzyme loadings. SSF assays at high substrate concentration (LSR = 4 and 5 g/g) and low enzyme loading of 5 FPU/g (experiments A3 and B3) achieved the lowest ethanol concentrations (20.72 and 16.90 g/L, respectively). The intensification of enzyme loading up to 15 FPU/g (experiments A5 and B5) significantly improved ethanol conversion, achieving 72.4% and 66.0% of EC_{MAX} , respectively. As general trend, higher ethanol conversions were obtained using lime-pretreated corn stover at the conditions of run 4, compared to the results obtained from the substrate resulting

from run 6 (Table 4). In fact, the combination of temperature and time reflected a significant effect on cellulose-to-glucose conversion (as shown in Table 3), which could be justified due to a higher structural alteration of the lignocellulosic matrix.

Table 4. Operational conditions employed in the simultaneous saccharification and fermentation (SSF) and results (E_{MAX} : maximum ethanol concentration, g/L; EC_{MAX} : maximum ethanol conversion, g ethanol/100 g potential ethanol).

Substrate	Exp.	LSR (g/g)	ESR (FPU/g)	E_{MAX} (g/L)	EC_{MAX} (g E/100 g E_{POT})
Lime-pretreated corn stover (121 °C, 0.1 g lime/g r.m., 1 h)	A1	4	15	27.48	49.0
	A2	5	15	30.61	65.9
	A3	6	5	20.72	52.2
	A4	6	10	23.88	60.2
	A5	6	15	28.73	72.4
Lime-pretreated corn stover (90 °C; 0.4 g lime/g r.m.; 1 h)	B1	4	15	23.76	44.6
	B2	5	15	24.44	55.4
	B3	6	5	16.90	44.8
	B4	6	10	21.90	58.1
	B5	6	15	24.87	66.0

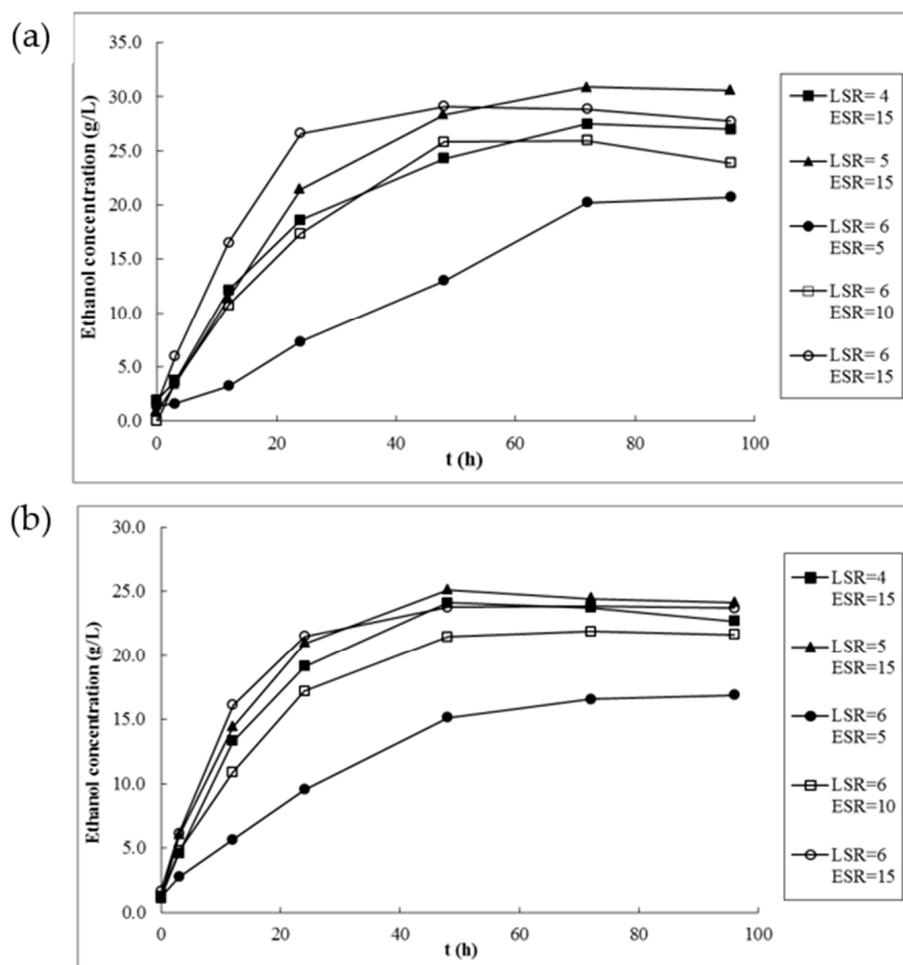


Figure 3. Simultaneous saccharification and fermentation assays carried out using corn stover by lime pretreatment at (a) a temperature of 121 °C, 0.1 g of lime loading/g of corn stover, and 1 h; and (b) a temperature of 90 °C, 0.4 g of lime loading/g of corn stover, and 1 h.

As shown in Figure 3, the ethanol concentrations augmented distinctly during the first fermentation stages, giving rise to ethanol concentrations after 12 h from 25.1% of EC_{MAX} (in experiment B3) up to 43.0% of EC_{MAX} (in experiment B5). After 24 h, the ethanol conversion ranged from 33.2% of EC_{MAX} (in experiment A3) up to 67.1% of EC_{MAX} (in experiment A5). As a general trend, there was a rapid surge in the ethanol concentrations, reaching half of the maximum ethanol concentrations in only 13 h on average.

Similar results of ethanol concentration and yield were obtained by sugarcane hydrolysate (90 h, 90 °C, and 0.47 g lime/g bagasse) [31] and bermudagrass [34].

4. Conclusions

In light of the resulting data obtained in this work, the suitable use of lime processing for the improvement of enzymatic saccharification of cellulose from corn stover using low temperatures, pressures, and low-cost chemicals (such as water and lime) is noteworthy. Under selected conditions (121 °C, 1 h, and 0.1 g of lime/g of corn stover), cellulosic ethanol was successfully produced, reaching an ethanol conversion of 72.4% by SSF. Overall, this study underlines the proper valorization of a residue as widely spread as corn stover via a pretreatment employing a cost-effective reagent like lime, which is an alternative alkali that provides promising results.

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