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To cite this version:

Mouna Aouine, Doha Elalami, Abdellatif Haggoud, Saad Ibnsouda Koraichi, Laurent Roumeas, et al.. Dry chemo-mechanical pretreatment of chickpea straw: Effect and optimization of experimental parameters to improve hydrolysis yields. Bioresource Technology Reports, 2022, 18, pp.101011. 10.1016/j.biteb.2022.101011 . hal-03652320

HAL Id: hal-03652320
https://hal.inrae.fr/hal-03652320

Submitted on 22 Jul 2024

Dry chemo-mechanical pretreatment of chickpea straw: effect and optimization of experimental parameters to improve hydrolysis yields

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Abstract

Dry chemo-mechanical (DCM) pretreatment is an interesting eco-friendly approach for bioconversion of lignocellulosic biomass into sugars and other valuable molecules. In this study, different dry DCM pretreatments were developed using a combination of alkaline and vibro-milling “VBM”. High-resolution fractional factorial 2^{k-1} design (FFD) was applied to evaluate statistically the effects of NaOH concentration (2-10 %), impregnation ratio (20-50 %), milling time (10-60 min), milling frequency (15-30 Hz), and ball diameter (1 or 2.5 cm) on reducing sugars release from chickpea straw (CS). The optimal conditions ensuring the maximum concentration of reducing sugars (374.70 mg/g biomass) after 72 h of enzymatic hydrolysis were 10 % of NaOH, impregnation of 50 %, 60 min of VBM, frequency VBM of 30 Hz, and ball diameter of 2.5 cm. Furthermore, an ethanol concentration of 17.81 g/L was obtained after simultaneous saccharification and fermentation of the pretreated CS under the defined optimized conditions.

Keywords: dry chemo-mechanical pretreatment, chickpea straw, reducing sugars, fractional factorial design, ethanol.

Abbreviations

CrI Crystallinity index

CS chickpea straw

DCM dry chemo-mechanical

FFD 2^{5-1} design fractional factorial design

SEM scanning electron microscopy

SSF simultaneous saccharification and fermentation

VBM Vibro-ball milling

1. Introduction

In Morocco, chickpea production tops the list of grain legumes cultivated. Its agriculture occupies more than 65.9 hectares per 1000 cultivated hectares and its production estimated at 440.6 quintals per 1000 quintals (Agricultural companion, 2015- 2016). Along with the production of chickpeas, there is also the generation of straw co-product, which is widely used as an alternative forage in ruminant diet due to its fibers and proteins content. In recent years, agricultural residues such as chickpeas straw are increasingly considered in the production of biomolecules as a sustainable way of managing these by-products. Moreover, when using land for sugarcane production dedicated for bioethanol, accessibility to food will be limited. Hence, the importance of valorizing agro-food residues. Production of carboxylates, biofuels (biogas, bioethanol and biodiesel), energy (syngas via gasification) and valuable chemicals can be carried out using agricultural residues (Barakat et al., 2014c). In addition, the availability of chickpea straw (CS), and its low cost make this feedstock an interesting lignocellulosic biomass for biofuel and chemical production. However, this biomass is not much exploited in literature compared to wheat and rice straw and bagasse.

Lignocellulosic biomass is a complex matrix mainly composed of cellulose, hemicellulose, and lignin (Laurichesse and Avérous, 2014). The bioconversion of this recalcitrant structure into fermentable sugars involves a pretreatment process in order to eliminate lignin and make cellulose and hemicellulose more accessible to chemical or enzymatic attack during saccharification and fermentation. In general, the pretreatment of lignocellulosic biomass for bioethanol production at pilot and industrial scale is the most important expensive compared to other steps and operations in biorefinery (Rocha-Meneses et al., 2019; Rajendran et al., 2018). Therefore, it is essential to optimize this process in function to biomass proprieties and valorization route. Several pretreatment technologies have been developed so far, the most common are thermal, alkaline, dilute acid, steam explosion and organosolv pretreatments (Memon and Memon, 2020; Liu and Chen, 2017;Vargas et al., 2015). However, these technologies present some disadvantages in terms of energy consumption, corrosion of equipments, and generation of inhibiting molecules such as furans and involves some additional steps such as separation and purification (Licari et al., 2016).

Dry chemo-mechanical (DCM) pretreatment is an eco-friendly approach with a combination of alkaline and mechanical pretreatments (Barakat et al., 2014a). Technically, lignocellulosic biomass first undergoes a chemical treatment by alkaline impregnation at high solid loads, followed by a mechanical size reduction (Barakat et al., 2014a). Studies has demonstrated that dry chemical pretreatment increases sugars yield, reduces energy requirements, and decrease effluents generation (Chuetor et al., 2019; Barakat et al., 2014). To take full advantage of this pretreatment procedure, it is necessary to identify and optimize the parameters that affect the efficiency of the pretreatment. Lazuka et al. (2017) investigated the effect of particle size and sodium hydroxide (NaOH) impregnation on the microbial transformation of wheat straw. Their results showed that the highest reducing sugar content was obtained after the treatment with NaOH at 100 µm. These results are inconclusive, which focused only on two factors, NaOH concentration and particle size. Thus, the integration of other factors such as milling time, impregnation time, milling frequency, alkaline concentration…and their interaction effects on biomass proprieties is very importance to develop an efficiency mechanochemical pretreatment.

Up to day, there is no study in literature describing the use experiments design for evaluating the influence of different DCM factors and their interaction on biomass proprieties and accessibility by enzymes. Hence, in this study, a high-resolution fractional factorial 2^{5-1} design (FFD 2^{5-1}) was implemented to evaluate the effects of NaOH concentration, impregnation, milling time and frequency, and ball diameter on biomass proprieties and sugars release from CS biomass. The effectiveness of pretreatment was monitored by measuring reducing sugar concentrations after enzymatic hydrolysis.

2. Materials and methods

2.1. Biomass

CS biomass was kindly harvested from a farm (Fez region, Morocco). CS samples were air-dried and coarsely cut into a particle size of less than 1 mm using a hammer mill (Retsch SM 100, Germany). Raw CS contained 27 % cellulose, 18 % hemicelluloses, and 26 % lignin. The samples were dried to a moisture content of 8- 10 % and sealed in plastic bags until use.

2.2. Pretreatment

Dry mechanochemical (DCM) pretreatment was carried out in two stages according to (Barakat et al., 2014a). Briefly, CS samples were first impregnated with NaOH solution for 5 hours at room temperature and then dried at 50 °C. The dried biomass was ground using a vibrating ball mill (VBM).

2.3. Experimental design

A two-level FFD 2^{5-1} design was used for evaluating the experiments conditions that affect reducing sugars release from CS biomass. As shown in **Table 1**, the selected independent variables and their variation ranges were: NaOH concentration (g/100 g biomass, 2 – 10 % wt), impregnation (water/ biomass, 20- 50 % wt), milling time (10-60 min), milling frequency (15-30 Hz) and ball diameter (1 and 2.5 cm). Those conditions were based on (Barakat et al., 2014a) work. The concentration of reducing sugars released (mg/g biomass) was retained as response variable. Data analysis was performed via Minitab 18 software.

2.4. Enzymatic hydrolysis

The enzymatic hydrolysis was carried out in 50 mM sodium acetate buffer (pH 5) at a solid loading of 10 % (w/v) of the biomass. The reaction mixture contained also a cellulase Cellic CTec2 (Novozymes) with an activity loading of 20 FPU/g and a endo-1,4-xylanase from *Trichoderma longibrachiatum* (Sigma-Aldrich) with an activity loading of 20 U/g. Sodium azide (3 g/L) was added to prevent any microbial contamination. Incubation was done at 50 ° C for 72 h with stirring at 100 rpm. Reducing sugars contents in saccharification liquors were determined colorimetrically using the dinitrosalicylic acid (DNS) method (Miller, 1959) and the absorbance was measured at 540 nm. The experiments were performed in duplicate.

2.5. X-ray diffraction

The crystallinity of raw and pretreated CS samples was analyzed using an X-ray diffractometer (XPERT-PRO) with a Cu tube at an accelerating voltage of 40 kV and a current of 30 mA. Scans were conducted at a 2θ angle, between 8 and 28°, with a step of 0.01°, and at a scan rate of 2°/min. The crystallinity index (CrI) of samples was calculated according to the following equation (Segal et al., 1959):

$$CrI\ (\%)\ = \frac{100*(I002 - I001)}{I002}$$

where I002 is the intensity of the diffraction from the 002 plane at $2\theta = 22°$, and I001 is the peak intensity of the amorphous zone at $2\theta = 16°$, in diffractogram.

2.6. Scanning electron microscopy

The morphological alterations occurred in the surface of CS biomass after the pretreatment conducted at the optimal conditions were evaluated with scanning electron microscopy (SEM) analysis. The analysis of SEM was performed using a scanning electron microscope (JSM-IT500 InTouchScope^{TM}).

2.7. Simultaneous saccharification and fermentation (SSF) of raw and pretreated CS

2.7.1. Microorganism and growth conditions

The fermentative yeast used in this study was *Pichia kudriavzevii,* a thermotolerant strain selected and identified in our laboratory. Yeast cells were grown overnight at 35 °C in 100 mL of YPD liquid medium (10 g/L of yeast extract, 20 g/L of peptone, and 20 g/L of glucose) with an orbital agitation of 150 rpm. After incubation, cells were collected by centrifugation for 10 min at 10,000 rpm, washed twice with sterile deionized water and suspended in 0.9 % NaCl solution with an absorbance at 600 nm.

2.7.2. Simultaneous saccharification and fermentation (SSF)

Raw and pretreated CS was subjected to simultaneous saccharification and fermentation (SSF) under the optimal conditions to produce bioethanol. For SSF experiments, 10% of the biomass were suspended in 50 mM sodium acetate buffer (pH 5). Nutrients such as yeast extract 5 g/L, peptone 5 g/L, K_2HPO_4 1 g/L, and $MgSO_4$ 1 g/L were supplemented. The fermentation medium was sterilized for 15 min at 120 °C. *P. kudriavzevii* (10 %, v/v) and an enzymatic cocktail of Cellic CTec2 (Novozymes, 20 FPU/g) and endo- 1,4-xylanase from *Trichoderma longibrachiatum* (Sigma-Aldrich, 20 U/g) were subsequently added. SSF assays were carried out at 42 °C and 150 rpm. The samples taken after 24, 48 and 72 h were centrifuged for 10 min at 10,000 rpm. The concentration of bioethanol in the supernatant was estimated by the chromic acid method (Caputi et al., 1968) and the absorbance was measured at 600 nm using a spectrophotometer. SSF assays were performed in duplicate.

3. Results and discussion

3.1. Assessment of DCM pretreatment on reducing sugars release and cellulose crystallinity

Figure 1 displays the concentrations of reducing sugars released after 72 h of enzymatic hydrolysis at 50 °C of untreated and DCM pretreated CS, as well as the crystallinity index. The experiment conditions of each run are described in **Table 2**. It' can be seen in **Table 2** and **Figure1** that, the concentration of reducing sugars ranged from 274.61 to 374.70 mg/g biomass. The highest concentration was observed in run 6, in which CS sample was treated with 10 % of NaOH, 50 % of impregnation, 60 min of milling, 30 Hz of frequency, and 2.5 cm as ball diameter. However, the run 15 resulted in the lowest concentration (276.50 mg / g biomass) (**Table 2**). Generally, all the trials improved reducing sugars production over the untreated CS (274.61 mg / g biomass). For the crystallinity measurements, the majority of the pretreated CS samples showed a reduction in the crystallinity index, run 6 provided an important decrease reaching a value of 23.50 % compared to 38.71% obtained with the raw CS.

Previously, sugar release was raised by 5-fold due to NaOH (2%v/v) addition under 60°C, and this increase was further enhanced when increasing temperature to 121°C (McIntosh and Vancov, 2011). Overall, using alkali reagent at high doses (6-20%) can lead to dissolution of nondegraded polysaccharides, while high temperature pretreatment can enhance cellulose hydrolysis. However, under more severe temperature conditions, enzymatic digestibility of lignocellulosic matrix can strongly decrease due to phenolic and furans compounds (Carrere et al., 2016).

DCM pretreatment is based on the combined action of NaOH which solubilize lignin and swells the structural components of lignocellulose and milling which reduces the particle size and decrease cellulose crystallinity (Chuetor et al., 2021). The highest concentration obtained in this study was 374.70 mg/g biomass. These results are in agreement with previous studies reporting a remarkable enhancement in fermentable sugars release from lignocellulosic biomass when DCM was applied (Lazuka et al., 2017; Sambusiti et al., 2015). A significant negative correlation was observed between reducing sugar yields and crystallinity index (R = – 0.51). These findings join Barakat's (2014) statement that DCM pretreatment reduces cellulose crystallinity and solubilize lignin and acetyl groups, which increase saccharification rate. Similarly, Qu et al. (2017) demonstrated that ball mill-assisted alkaline peroxide pretreatment of wheat straw samples changed the crystalline cellulose into an amorphous form, resulting in improved enzymatic hydrolysis due to the higher surface accessibility. However, the only concern is that the solid/liquid ratio can drastically increase the energy consumption during drying before mechanical pretreatment. An optimization of this ratio is therefore necessary to improve the sustainability of this combination (Barakat et al., 2014b).

3.2.DCM efficiency using fractional factorial design

To better understand the influence of each factor on the efficiency of DCM pretreatment, a 2^{5-1} FFD was implemented. The factors evaluated were NaOH concentration, impregnation, milling time, milling frequency, and ball diameter. The concentration of reducing sugars released (mg/g biomass) was retained as response variable (Table 2).

3.2.1. Analysis of the main and interaction effects

Figure 2 represents the Normal plot and the Pareto chart of the standardized effects. The graphical in **Figure 2** indicate that, all significant factors are positive, demonstrating a direct correlation with the release of reducing sugars. In terms of main effects, milling time (C) and ball diameter (E) were the most influencing variables. Rezende et al. (2018) applied an FFD 2^{5-1} to optimize a sequential acid–alkali pretreatment of elephant grass leaves. They found that extending ball milling time before the chemical treatment has a positive significant effect on reducing sugar release. Zhang et al. (2021) also proved that ball milling time factor evaluated in ball mill-assisted alkaline peroxide pretreatment influenced the compositions, particle sizes, morphology, and crystallinity of corn stalks biomass and hence, increased the yield of xylo-oligosaccharides and fermentable sugars. For ball diameter, Khumalo et al. (2006) claimed that larger sized grinding media would break complex matrix faster. In general, when the diameter of the ball is large, the impact and compression of the biomass particles trapped either between the grinding balls or between the grinding ball and the mill casing is important.

In terms of binary interactions, NaOH concentration (A) impregnation (B) had the most significant impact on reducing sugar production from CS biomass. Impregnation (B) is individually insignificant variable, but it had a pronounced effect when is coupled with NaOH concentration. Several researchers have approved that NaOH is very effective at disrupting the lignocellulosic structure (Valles et al., 2021, Mukherjee et al., 2018, Kim et al., 2016). Thus, in DCM pretreatment when the biomass particles are well sprayed with high concentrations of NaOH, more lignin is solubilized, resulting in high fermentable sugar yields. Huang et al. 2019 statically assessed during a wet-ball milling the effect of various concentrations of NaOH on the enzymatic saccharification of bagasse and pennisetum. Their results showed that glucose yields increased with increasing NaOH concentration.

3.2.2. Statistical modelling and ANOVA

Analysis of variance (ANOVA) is reproduced in **Table 3**. Calculations were performed at a confidence level of 95% (p-value < 0.05). The obtained F-value of 26.84 was greater than the tabulated F-value of 3.35, while the p-value of 0.003 was less than 0.05. These results indicate that the model selected in this study is statistically significant. As predicted by the Pareto chart, the most significant influence on reducing sugars release was exerted by milling time (p-value of 0,001), ball diameter (p-value of 0,002), and by the coupled effect of NaOH concentration x impregnation (p-value of 0,002). The model R-square was 0.9866 and the adjusted R-square was 0.9499, ensuring a good fitness of the model. **Figure 3** depicts the analysis of the residuals. For the normal probability plot (**Figure 3a**), the residuals were adjusted to a straight-line. As for the graph comparing the residuals versus fits (**Figure 3b**), no pattern for the residual distribution was recognized. Therefore, the constant variance assumption was satisfied.

3.2.3. Response surface and validation of the optimum conditions for reducing sugars release

Contour and 3D surface graphs were used to visualize the interactions of the significant independent factors (**Figure 4**). The categorical factor (ball diameter) was maintained on 2.5 cm. As observed, a shift to higher values of factors provides higher concentrations of reducing sugars, values exceeding 335 mg/g biomass.

According to the regression equation (1), the predicted maximum concentration of reducing sugars of 391.58 mg/g biomass is attained by adopting the following optimal conditions: NaOH concentration of 10 %, impregnation of 50 %, milling time of 60 min, frequency of 30 Hz, and ball diameter of 2.5 cm. To ensure the model validity, two new experiments were conducted under the optimal conditions given above. The average concentration of reducing sugars after 72 h of saccharification was 376. 68 mg/g biomass. This value is in reasonable agreement with the predicted concentration. It was evident that applying FFD approach was advantageous since the importance of each factor was established. Subsequently, the generation of fermentable sugars from lignocellulosic biomass pretreated by the DCM method can be optimized by fixing the settings of the factors on their maximum.

$$\text{Concentration of reducing sugars (mg/g biomass)} = 363.1 - 5.032\,A - 1.676\,B - 0.818\,C - 1.453\,D - 4.97\,E + 0.1360\,A{*}B + 0.0481\,A{*}C + 0.00943\,B{*}C + 0.0326\,B{*}D + 0.3716\,B{*}E + 0.02622\,C{*}D \quad (1)$$

3.3. Effect of DCM pretreatment on surface morphology of CS biomass

Figure 5 shows the scanning electron microscopy (SEM) images of untreated and processed CS under the optimal conditions identified by the experimental design. As it is clear, DCM pretreatment caused an obvious disruption of the structure morphology of the biomass. In the raw CS (**Figure 5a**), the surface appeared dense, smooth without apparent damage. Conversely, in **Figure 5b** the pretreatment gave an ultrafine powder showing that the fibers were completely fragmented and the surface had cracks, micropores and wrinkles. These observations are in agreement with those reported by Zhang et al. (2021), Chen et al. (2019), and Qu et al. (2017), who indicate that mechanochemical pretreatments generate fibrillated structures and cause a significant breakdown of the plant cell walls, which facilitates the accessibility of enzymes at the hydrolysis stage.

3.4. Simultaneous saccharification and fermentation (SSF) of the pretreated CS for bioethanol production

Figure 6 summarizes the SSF profile of *P. kudriavzevii* using untreated and pretreated CS under optimal conditions. The totality of ethanol was produced after 48 h of fermentation. For pretreated CS, the maximum ethanol concentration was 17.81 g/L, in contrast, the raw CS provided only a concentration in ethanol of 9.54 g/L. In the study of Sambusiti et al. (2015), Alkali-mechanically pretreated sugarcane bagasse yielded an ethanol concentration of up to 7.45 g/L during the SSF process. Another study of Monlau et al. (2019) demonstrated that mechanical fractionation by vibro-ball milling (VBM) of solid separated digestate of agricultural biogas plants resulted in 4.9 g/L of ethanol after the SSF test. In comparison with conventional alkaline pretreatment of agricultural residues, Singh and Kumar (2020) obtained an ethanol concentration of 18.07 g/L from sodium carbonate pretreated rice straw. Likewise, a concentration of 17.26 g/L (0.48g of ethanol/g of glucose and xylose consumed) was generated from alkaline pretreated sugarcane bagasse after SSF (Hilares et al., 2017). Overall, DCM pretreatment followed by SSF technique has given promising results in terms of bioethanol production from lignocellulosic biomass. It is worth noting that this combination allows a reduction in processing time and steps, which is highly recommended in transformation plants.

4. Conclusion

In this study, a high resolution 2^{5-1} FFD allowed simultaneous evaluation of five variables and their coupled interactions in DCM pretreatment for reducing sugars recovery from CS biomass. The results indicate that milling time, and ball diameter and the interaction between NaOH concentration and impregnation had the most significant effect on DCM efficiency. The highest concentration of reducing sugars was obtained during pretreatment under 10 % of NaOH, impregnation of 50 %, milling time of 60 min, frequency of 30 Hz, and ball diameter of 2.5 cm. Moreover, an ethanol yield of 17.81 g/L was obtained through the SSF process using the thermotolerant yeast *P. kudriavzevii* and CS pretreated under the optimized conditions. The statistical optimization of DCM pretreatment enables intelligent eco-valorization of lignocellulosic raw materials, particularly for integrated biorefineries.

Acknowledgements

This research was supported by University Mohamed VI Polytechnic (UM6P) of Benguerir, and Regional University Interface Center (CURI) of Sidi Mohamed Ben Abdellah University.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Figure captions

Figure 1. Reducing sugars (in mg/ g of biomass) released from DCM pretreated CS samples after 72 h of enzymatic hydrolysis, and their crystallinity index according to FFD 2^{5-1} design matrix. Untreated CS was included for comparison.

Figure 2. Normal plot of the standardized effects (a), Pareto chart of the standardized effects (b). Panels were obtained after implementing backward selection using α -value to enter of 0.05.

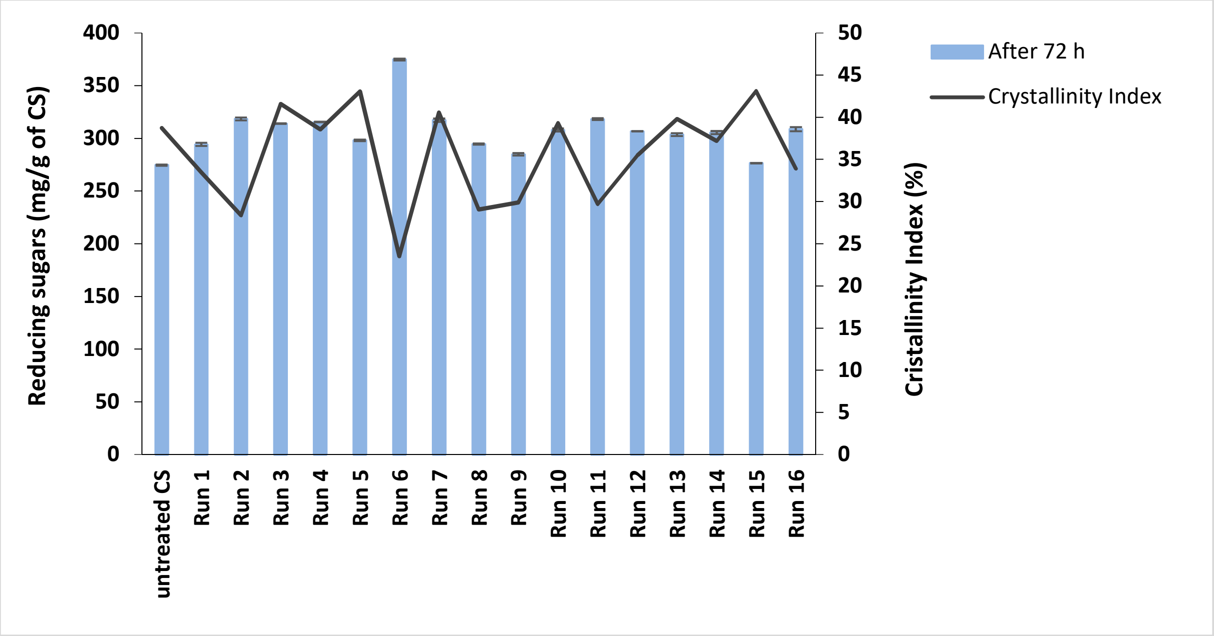
Figure 3. Plots of residuals (a) Normal probability (b) Residuals versus fits.

Figure 4. Contour and surface plots showing the interactions between the significant independent factors.

Figure 5. SEM images of (a) untreated CS, (b) pretreated CS with: NaOH concentration of 10 %, impregnation of 50 %, milling time of 60 min, milling frequency of 30 Hz, and ball diameter of 2.5 cm. The numbers 1-3 in panel names denote images of the same area at increasing magnifications

Figure 6. Ethanol production profile (in g/L) of *P. kudriavzevii* during SSF of untreated and pretreated CS biomass.

Figure 1



Reducing sugars (mg/g of CS)
400
350
300
250
200
150
100
50
0
Cristallinity Index (%)
50
45
40
35
30
25
20
15
10
5
0
untreated CS
Run 1
Run 2
Run 3
Run 4
Run 5
Run 6
Run 7
Run 8
Run 9
Run 10
Run 11
Run 12
Run 13
Run 14
Run 15
Run 16
After 72 h
Crystallinity Index

Figure 2

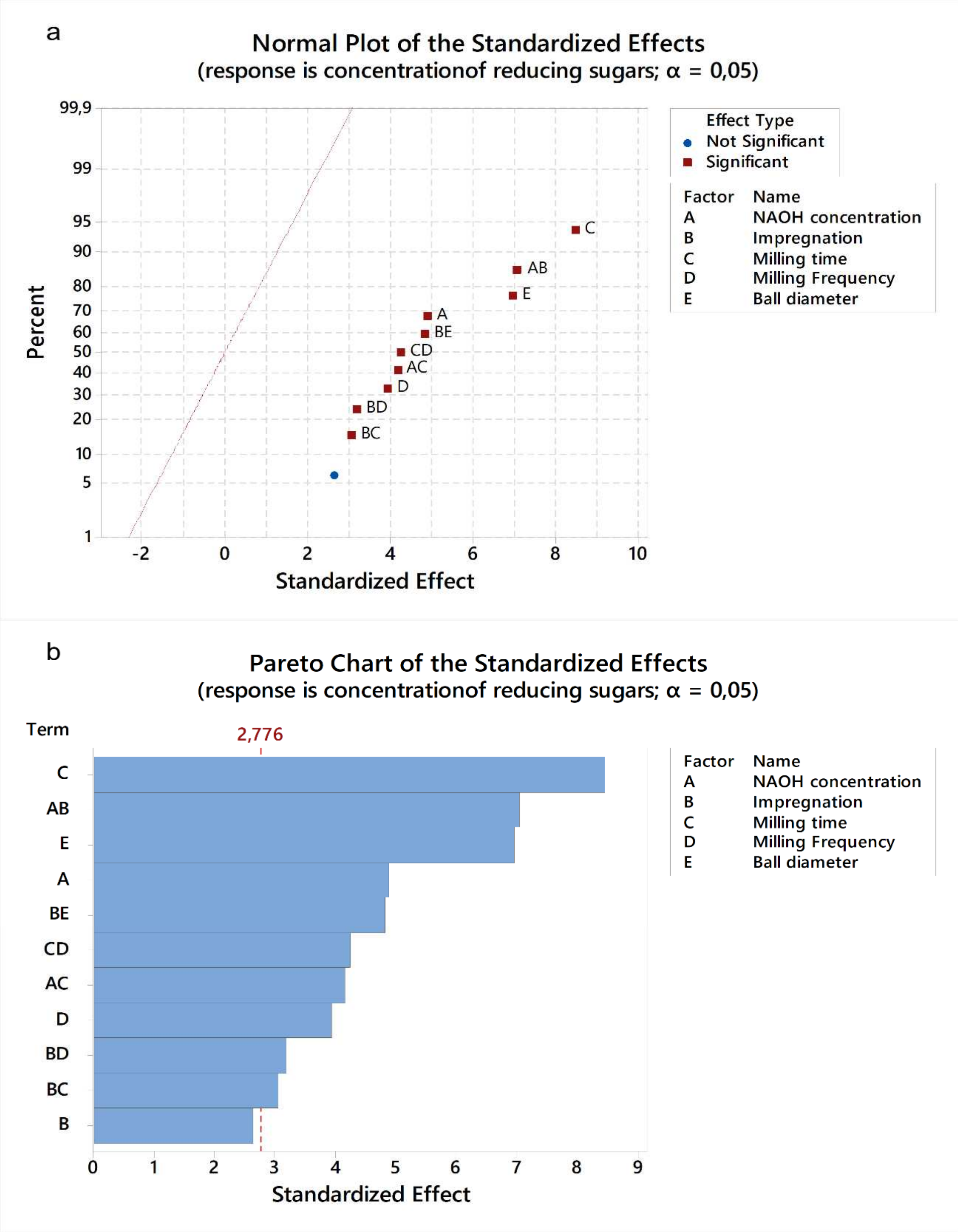

a
Normal Plot of the Standardized Effects
(response is concentrationof reducing sugars; α = 0,05)
Percent
99,9
99
95
90
80
70
60
50
40
30
20
10
5
1
-2
0
2
4
6
8
10
Standardized Effect
C
AB
E
A
BE
CD
AC
D
BD
BC
Effect Type
● Not Significant
■ Significant
Factor Name
A NAOH concentration
B Impregnation
C Milling time
D Milling Frequency
E Ball diameter
b
Pareto Chart of the Standardized Effects
(response is concentrationof reducing sugars; α = 0,05)
Term
2,776
C
AB
E
A
BE
CD
AC
D
BD
BC
B
0
1
2
3
4
5
6
7
8
9
Standardized Effect
Factor Name
A NAOH concentration
B Impregnation
C Milling time
D Milling Frequency
E Ball diameter

Figure 3

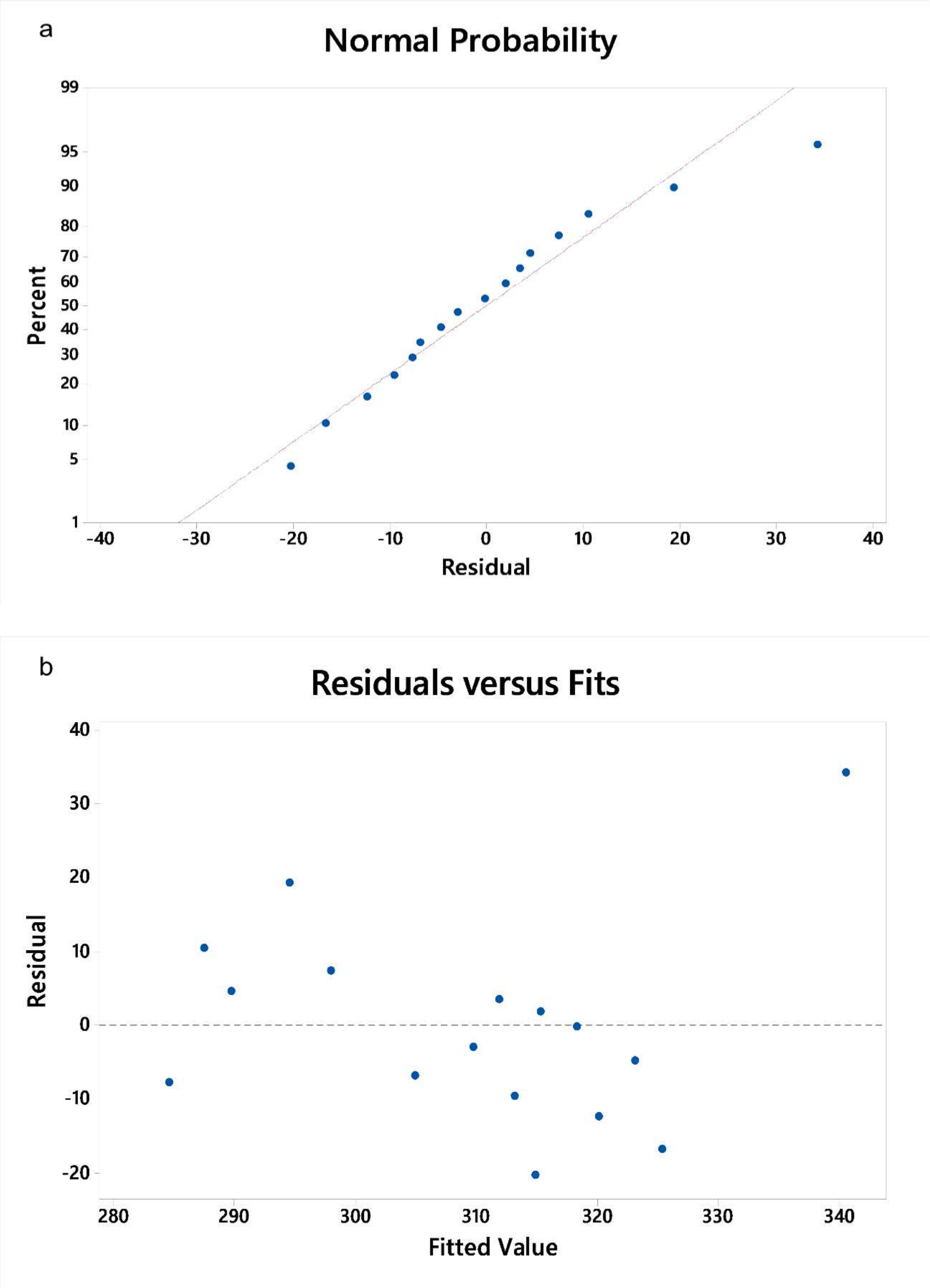

a
Normal Probability
Percent
99
95
90
80
70
60
50
40
30
20
10
5
1
-40
-30
-20
-10
0
10
20
30
40
Residual
b
Residuals versus Fits
Residual
40
30
20
10
0
-10
-20
280
290
300
310
320
330
340
Fitted Value

Figure 4

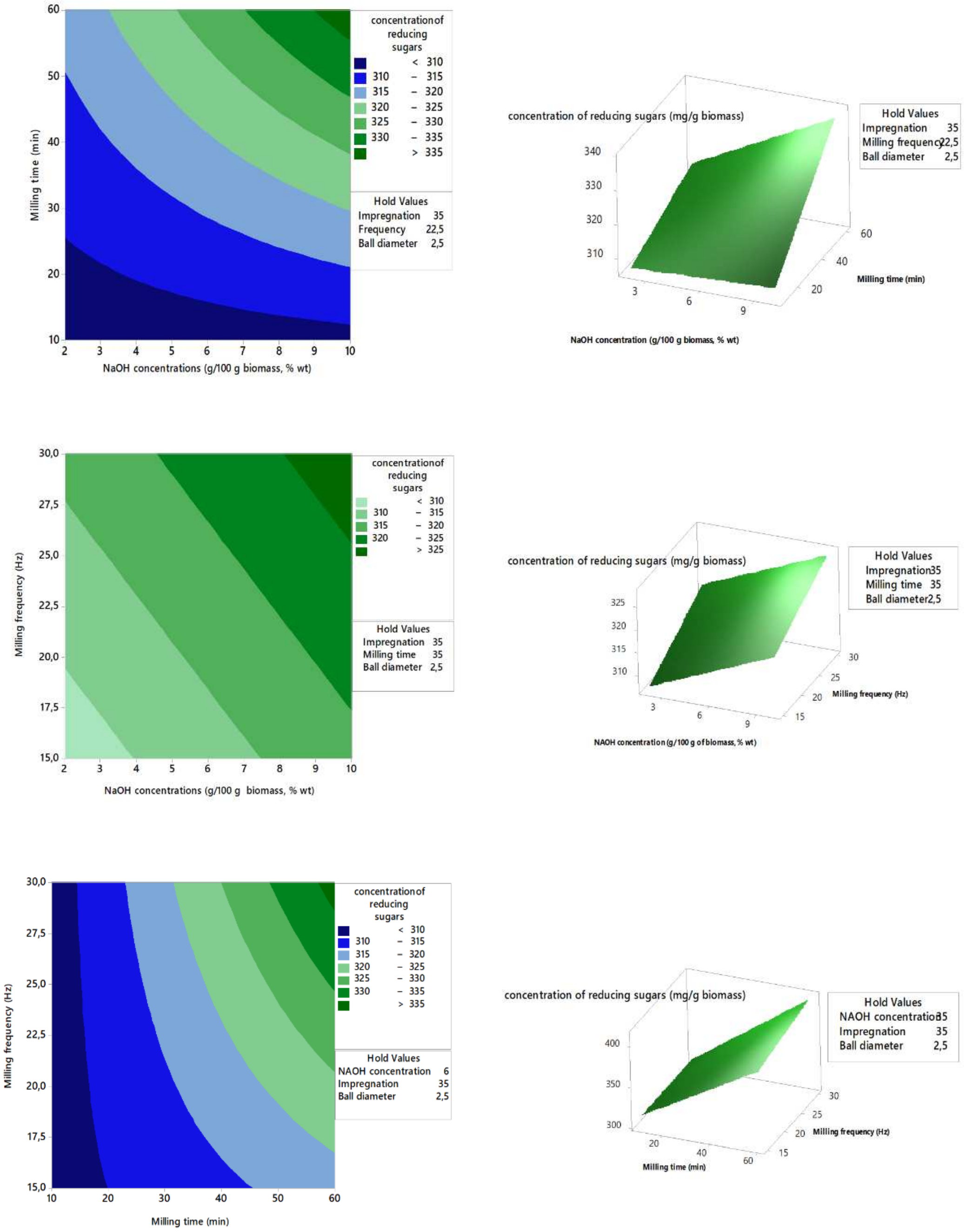

Milling time (min)
NaOH concentrations (g/100 g biomass, % wt)
concentration of reducing sugars
< 310
310 – 315
315 – 320
320 – 325
325 – 330
330 – 335
> 335
Hold Values
Impregnation 35
Frequency 22,5
Ball diameter 2,5
concentration of reducing sugars (mg/g biomass)
Hold Values
Impregnation 35
Milling frequency 22,5
Ball diameter 2,5
Milling time (min)
NaOH concentration (g/100 g biomass, % wt)
Milling frequency (Hz)
NaOH concentrations (g/100 g biomass, % wt)
concentration of reducing sugars
< 310
310 – 315
315 – 320
320 – 325
> 325
Hold Values
Impregnation 35
Milling time 35
Ball diameter 2,5
concentration of reducing sugars (mg/g biomass)
Hold Values
Impregnation 35
Milling time 35
Ball diameter 2,5
Milling frequency (Hz)
NAOH concentration (g/100 g of biomass, % wt)
Milling frequency (Hz)
Milling time (min)
concentration of reducing sugars
< 310
310 – 315
315 – 320
320 – 325
325 – 330
330 – 335
> 335
Hold Values
NAOH concentration 6
Impregnation 35
Ball diameter 2,5
concentration of reducing sugars (mg/g biomass)
Hold Values
NAOH concentration 35
Impregnation 35
Ball diameter 2,5
Milling frequency (Hz)
Milling time (min)

Figure 5

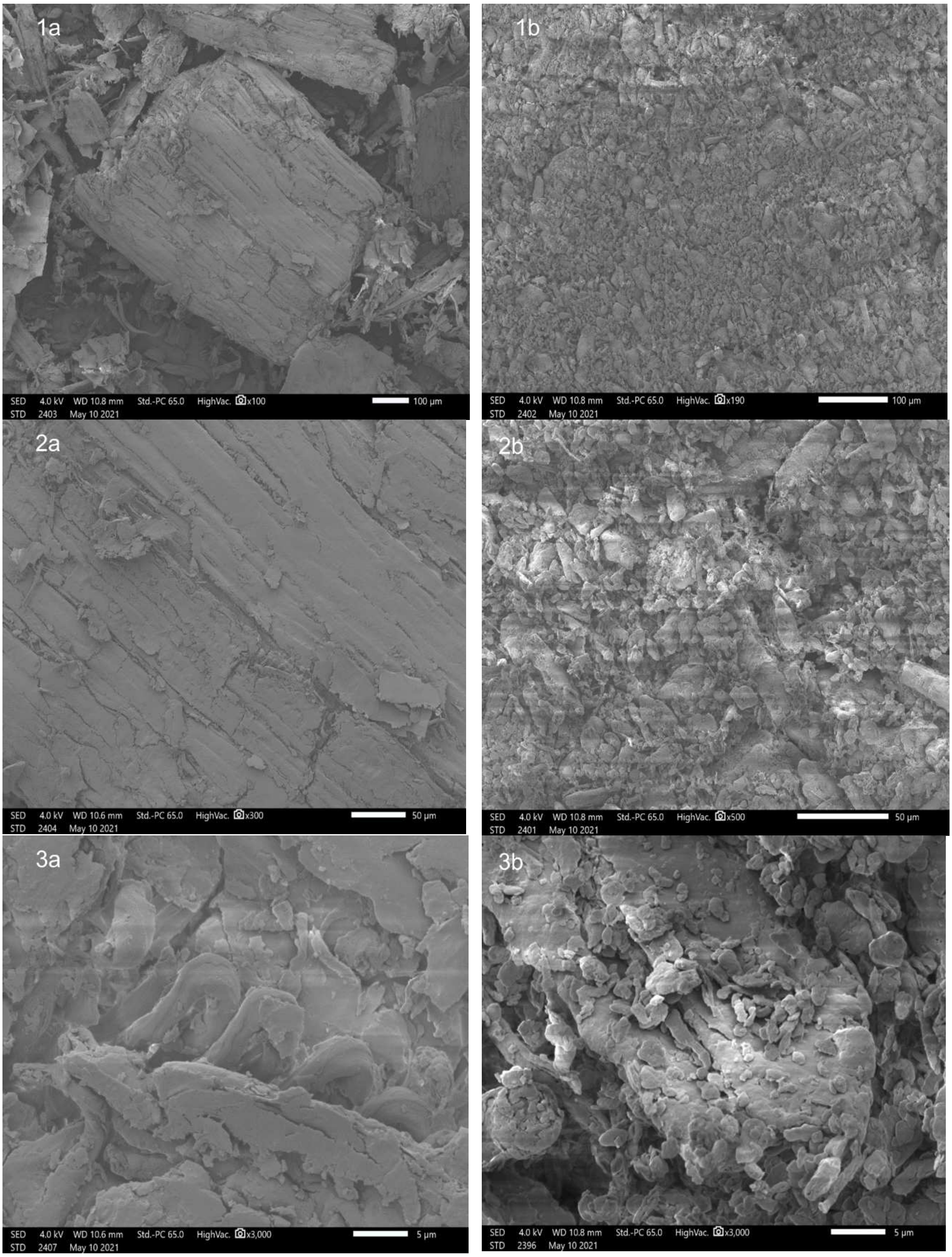
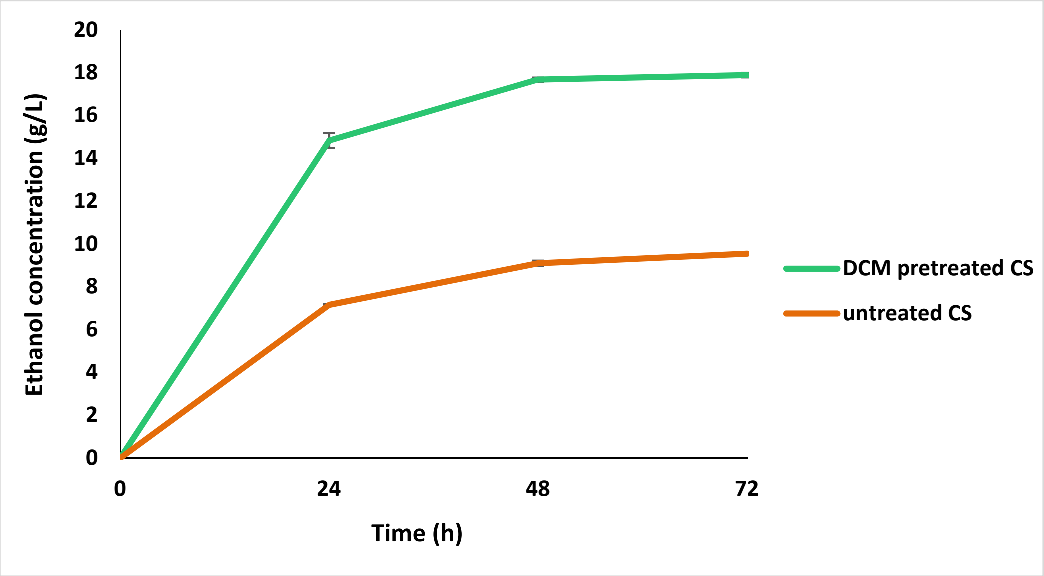

1a
SED 4.0 kV WD 10.8 mm Std.-PC 65.0 HighVac. x100 100 µm
STD 2403 May 10 2021
1b
SED 4.0 kV WD 10.8 mm Std.-PC 65.0 HighVac. x190 100 µm
STD 2402 May 10 2021
2a
SED 4.0 kV WD 10.6 mm Std.-PC 65.0 HighVac. x300 50 µm
STD 2404 May 10 2021
2b
SED 4.0 kV WD 10.8 mm Std.-PC 65.0 HighVac. x500 50 µm
STD 2401 May 10 2021
3a
SED 4.0 kV WD 10.6 mm Std.-PC 65.0 HighVac. x3,000 5 µm
STD 2407 May 10 2021
3b
SED 4.0 kV WD 10.8 mm Std.-PC 65.0 HighVac. x3,000 5 µm
STD 2396 May 10 2021

Figure 6



Ethanol concentration (g/L)
20
18
16
14
12
10
8
6
4
2
0
0
24
48
72
Time (h)
DCM pretreated CS
untreated CS

Tables

Table 1. Levels of the factors evaluated in the 2^{5-1} FFD

Factors	Low level (-)	High level (+)
A- NaOH concentration (g/100 g biomass, % wt)	2	10
B- Impregnation (water/biomass, % wt)	20	50
C- Milling time (min)	10	60
D- Milling frequency (Hz)	15	30
E- Ball diameter (cm)	1	2.5

Table 2. Runs identification with the corresponding experimental conditions and the response of reducing sugars (mg/g biomass) released after 72 h of enzymatic hydrolysis.

Runs	NaOH concentration (% wt)	Impregnation (% wt)	Milling time (min)	Milling frequency (Hz)	Ball diameter (cm)	Concentration of reducing sugars released (mg/g biomass)
1	10	20	10	15	1	294.19
2	2	20	60	30	2.5	318.39
3	2	20	10	15	2.5	313.88
4	10	50	10	15	2.5	315.29
5	2	20	10	30	1	298
6	10	50	60	30	2.5	374.70
7	10	50	60	15	1	317.25
8	10	20	10	30	2.5	294.54
9	10	50	10	30	1	284.78
10	2	50	60	15	2.5	307.80
11	10	20	60	30	1	318.15
12	2	50	10	30	2.5	306.70
13	2	50	60	30	1	303.52
14	2	20	60	15	1	305.37
15	2	50	10	15	1	276.50
16	10	20	60	15	2.5	308.62

Table 3. ANOVA of the 2^{5-1} FFD describing the reducing sugars released after 72 h of enzymatic hydrolysis as a linear function of the selected variables.

Source	Degrees of freedom	Sum of squares	Mean square	F-Value	P-Value
Model	11	6288.44	571.68	26.84	0.003
Linear	5	3553.49	710.70	33.37	0.002
A : NaOH concentration	1	509.51	509.51	23.92	0.008
B: Impregnation	1	149.60	149.60	7.02	0.057
C: milling time	1	1528.87	1528.87	71.78	0.001
D: Frequency	1	331.23	331.23	15.55	0.017
E: Ball diameter	1	1034.28	1034.28	48.56	0.002
2-Way Interactions	6	2734.95	455.83	21.40	0.005
A*B	1	1064.80	1064.80	49.99	0.002
A*C	1	370.59	370.59	17.40	0.014
B*C	1	199.96	199.96	9.39	0.038
B*D	1	215.79	215.79	10.13	0.033
B*E	1	497.17	497.17	23.34	0.008
C*D	1	386.64	386.64	18.15	0.013
Error	4	85.20	21.30		
Total	15	6373.64			

Fractional factorial design 2^{5-1}

16 experiments

Application of alkaline vibro- ball milling treatment

Raw chickpea straw (CS)

Pretreated chickpea straw (CS)

- ✓ **Reducing sugar yield of 374.70 mg/g biomass**
- ✓ **Reduction of cristallinity index to 23.50 %**

Simultaneous saccharification and fermentation

Bioethanol

17.81 g/L