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Research Article

Pre-treatment by Acidification and Freezing on Corncob Polymers and its Enzymatic Hydrolysis

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Abstract

Background and Objective: Corncob contain high percentage of cellulose ($40.95 \pm 0.38\%$), hemicellulose ($38.94 \pm 1.72\%$) and low lignin content ($16.54 \pm 0.38\%$) which has a great potential of bioconversion to bioethanol. The objective of this research was to evaluate the effect of acidification, freezing and the combination of these pre-treatments in corncob compounds and its enzymatic hydrolysis. **Materials and Methods:** It was evaluated the changes occurred in macrostructure and lignocellulosic compounds after pre-treatments. Corncob fiber was pretreated by freezing at -20°C for 12 h; with 0.25 M sulfuric acid, pH 2.0, at 121°C for 20 min and by the combination of both pre-treatments. After pre-treatment, an enzymatic hydrolysis with $150 \text{ U}_{\text{cellulase}}/\text{g}_{\text{substrate}}$ in 10 mL of acetate was performed at 100 rpm, 37°C and pH 5.48 for 72 h. Data were analyzed by one-way analysis of variance using SPSS. **Results:** The combined pre-treatment (acidification and freezing) allowed to obtain the highest amount of cellulose ($64.12 \pm 1.03\%$) and lignin ($19.76 \pm 0.42\%$) and low amount of hemicellulose ($10.16 \pm 1.42\%$) was determined. The optical microscopy analysis of samples with combined pre-treatment show partitioned and less agglomerated structure. **Conclusion:** The higher concentration of glucose ($40.90 \pm 2.11 \text{ g L}^{-1}$) was obtained by combined pre-treatment, which increased the enzyme efficiency with 57.41%, which could be approved for the conversion of high value products, like bioethanol.

Key words: Corncob, combined pre-treatment, lignocellulosic material, substrate conversion, enzymatic hydrolysis

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nowadays, the need for alternative energy sources is increasing due to fossil fuel problems: Their finite supply, greenhouse gasses, emission and global warming and increasing price and unexpected fluctuations¹. All these weaknesses have strengthened the interest in renewable, sustainable and economically viable alternatives of green energy or biofuels such as bioethanol.

The first generation of bioethanol production, starch and sugar are derived from maize and sugar cane which are used as raw material, they are expensive. The usage of second generation materials, lignocellulosic materials are cheap, abundant, renewable and not negatively affect the human food chain by eliminating food in favor bioethanol production²⁻⁴. Materials lignocellulosic are composed of cellulose, hemicelluloses and lignin in an intricate structure, generating a low accessibility of the enzyme due to rigid bonding of cellulose to lignin^{5,1}. For this reason lignocellulosic materials required a pre-treatment to break down the lignin barrier and thus recover the cellulose, which can be subjected to enzymatic hydrolysis for converting in fermentable sugars⁶⁻⁸.

Corn cob have won a lot of attention as a viable feedstock for the production of reducing sugars and some efforts are made in the pre-treatment of corn cob^{9,10}. Corn in terms of production is the main cereal crop worldwide, it is used for the production of a great variety of food, feed, biofuels and other industrial uses¹¹. According to the US Department of Agriculture (USDA)¹², it projects maize production for 2016/2017 at 1025.69 million metric t, contributing approximately 307.7 million t of corn cob⁹. For this reason corn cob have a potential for biofuels production, especially due to its chemical composition: Cellulose (35-45.6%), hemicellulose (36.9-40%) and lignin (5-20%)^{13,14}, which make it one of the best raw materials for products, such as bioethanol⁹. In this study, corn cob fiber (*Zea mays* L.) was subjected to three pre-treatments, which were pre-treatment with 0.25 M H₂SO₄, a slow freezing at -20°C and applying the combination of both pre-treatments. The objective was to evaluate the effect of these pre-treatments on macrostructure changes, lignocellulosic compounds and the enzymatic hydrolysis using *Aspergillus niger* cellulase.

MATERIALS AND METHODS

This study, including raw material collection, experimental analysis and data processing, was carried out from January, 2017-June, 2017.

Raw material: Corn cob was obtained from yellow corn cobs purchased from local farmers in the city of Trujillo (La Libertad, Peru). In order to neutralize and to remove toxic and interference compounds, corn cobs submerged in fresh water for 24 h and then washed with distilled water. Subsequently that was cut into small slices with stainless steel knives and was dried in an oven (Mettler, BE-400, Alemania) at 105°C for 3.5 h. These samples were ground in a Bach manual mill (Corona, Peru) and later were passed on a N° 40 sieve (Tyler, USA) to obtain a homogeneous corn cob fiber (T₀) of about 0.37 mm particle size.

Pre-treatments: T₀ was pretreated as shown in Fig. 1.

Physical : T₀ was mixed with sodium acetate buffer (50 mM, pH 5.48) in a ratio of 40 mg T₀ mL⁻¹ buffer for 1 h and later pretreated by freezing at -20°C for 12 h in a freezer (Coldex CH-10, Peru). Subsequently, T₁ was thawed at room temperature for 1 h and later dried in an oven (Mettler, BE-400, Germany) at 60°C for 12 h¹⁵.

Chemical: To perform the acid pre-treatment a solid to liquid (S/L) ratio of 50 mg T₀ mL⁻¹ 0.25 M H₂SO₄ (analytical grade, 95.0-98.0 %), pH 2.0, (JT BAKER, 7664-93-9, USA) was used. All these experiments were carried out in Erlenmeyer flask kept at 121°C for 20 min in an autoclave (Fravill, IP-010, Peru). Afterwards the residual solid of T₂ was removed by filtration in filter paper (Whatman, No. 1, China) and dried in an oven (Mettler, BE-400, Germany) at 60°C for 12 h¹⁶.

Physicochemical: This pre-treatment was performed by combining the previous pre-treatments. Finally the sample pretreated by both pre-treatments was dried in an oven (Mettler, BE-400, Germany) for 12 h at 60°C (T₃).

Enzymatic hydrolysis: Enzymatic hydrolysis experiments were carried out in 100 mL erlenmeyer flasks. Cellulase of *Aspergillus niger* (Sigma-Aldrich, C1184-5KU, USA) with a concentration of 1.3 U mg⁻¹ for hydrolysis of T₀, T₁, T₂ and T₃ was used. 150 U g⁻¹ cellulase of sample in 10 mL of 50 mM⁻¹ sodium acetate buffer pH 5.48 was mixed. The mixture was incubated at 37°C in an orbital shaker incubator (Biomaker, BS-GS-30, China) at 100 rpm for 72 h^{15,16}.

Analytical methods

Physicochemical analysis of raw material and homogeneous

fiber: Moisture, ash and pH assay were performed according methods described by AOAC¹⁷⁻¹⁹, for raw material and samples corn cob fibers.

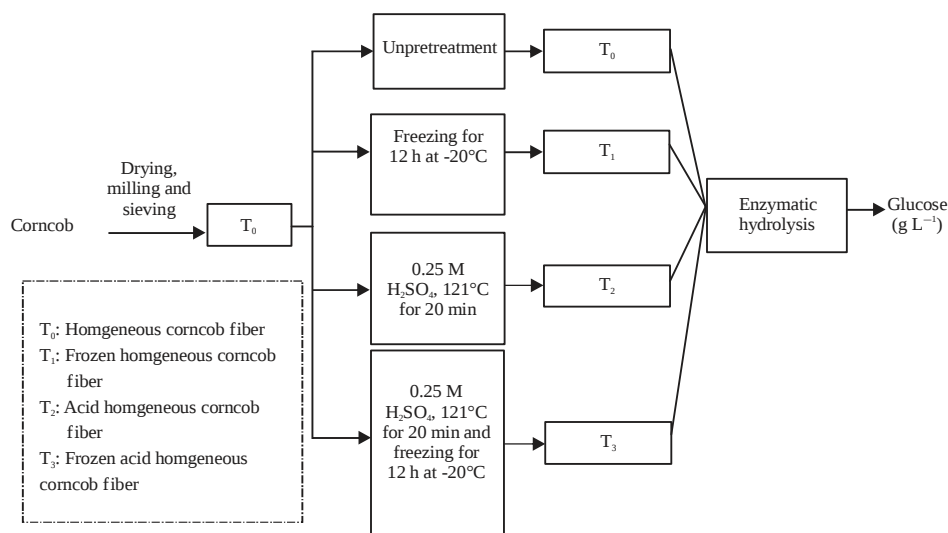


Fig. 1: Methods of the different pre-treatments

Optical microscopy analysis: Samples (T_0 , T_1 , T_2 and T_3) were observed at room temperature, using an optical microscope (Motic, BA310, China) with a 10X objective using Motic Images 2.0 software, which took photographs of sample macrostructure¹⁵.

Determination of lignocellulosic compounds: To the T_0 , T_1 , T_2 and T_3 samples, the following lignocellulosic compounds were determined: Holocellulose, cellulose, lignin and hemicellulose, that was determined by the difference between holocellulose and cellulose²⁰⁻²².

Reducing sugar analysis: Total reducing sugars were determined for each hydrolyzed sample every 12 h during the 72 h of hydrolysis, according to the dinitrosalicylic acid (DNS) method described by Miller²³. Aliquots of 2 mL of hydrolyzate from each sample were taken every 12 h and were centrifuged (Hettich, EBA 20, Germany) to obtain a solvent free of solid samples.

Substrate cellulose conversion: In this study cellulose conversion refers to enzymatic hydrolysis of cellulose to glucose in a substrate, pretreated or not. The glucose yield of the enzymatic hydrolysis is obtained from the glucose concentration in the enzymatic hydrolyzate. Each glucan unit produces one glucose molecule after gaining one water molecule, therefore the ratio of molecular weight between one glucan unit and glucose of 0.9 ($C_6H_{10}O_5/C_6H_{12}O_6$) is used as the conversion factor in the calculation of cellulose conversion. Eq. 1 was used to determine the conversion of cellulose to glucose²⁴:

$$\text{Conversion cellulose (\%)} = \frac{m_{\text{glucose}} \times f_h}{m_{\text{initial}} \times y_i} \times 100 \quad (1)$$

where, m_{glucose} is the mass of glucose in final hydrolyzate (g), m_{initial} is the initial mass of dry substrate (g), y_i is the cellulose in biomass (%) and f_h is the conversion factor (for cellulose is 0.9).

Statistical analysis: One-way analysis of variance (ANOVA), based on a 95% confidence level with a p-value of significance of $p < 0.05$ per each 12 h of hydrolysis, was performed to evaluate the significant differences between pre-treatments. If $p < 0.05$, a test of homogeneity of variances was performed evaluating the Levene statistic, followed by a Tukey test to identify differences between treatments. These analyzes were performed using SPSS (Statistical Program for Social Sciences, SPSS Corporation, IL, USA) version 22.0 for Windows²⁵.

RESULTS

Physicochemical analysis of raw material and homogeneous fiber:

Physicochemical characterization of initial raw material was done, being the moisture, ash and pH, $48.46 \pm 1.65\%$, $1.59 \pm 0.02\%$ and 5.62, respectively. It was found that the physicochemical characteristics of T_0 were as follows: pH: 5.08, moisture: $3.53 \pm 0.57\%$ and ash: $1.47 \pm 0.03\%$.

Lignocellulosic compounds after pre-treatments:

Lignocellulosic compounds of corn cob after the different pre-treatments are shown in Table 1. The major changes in the

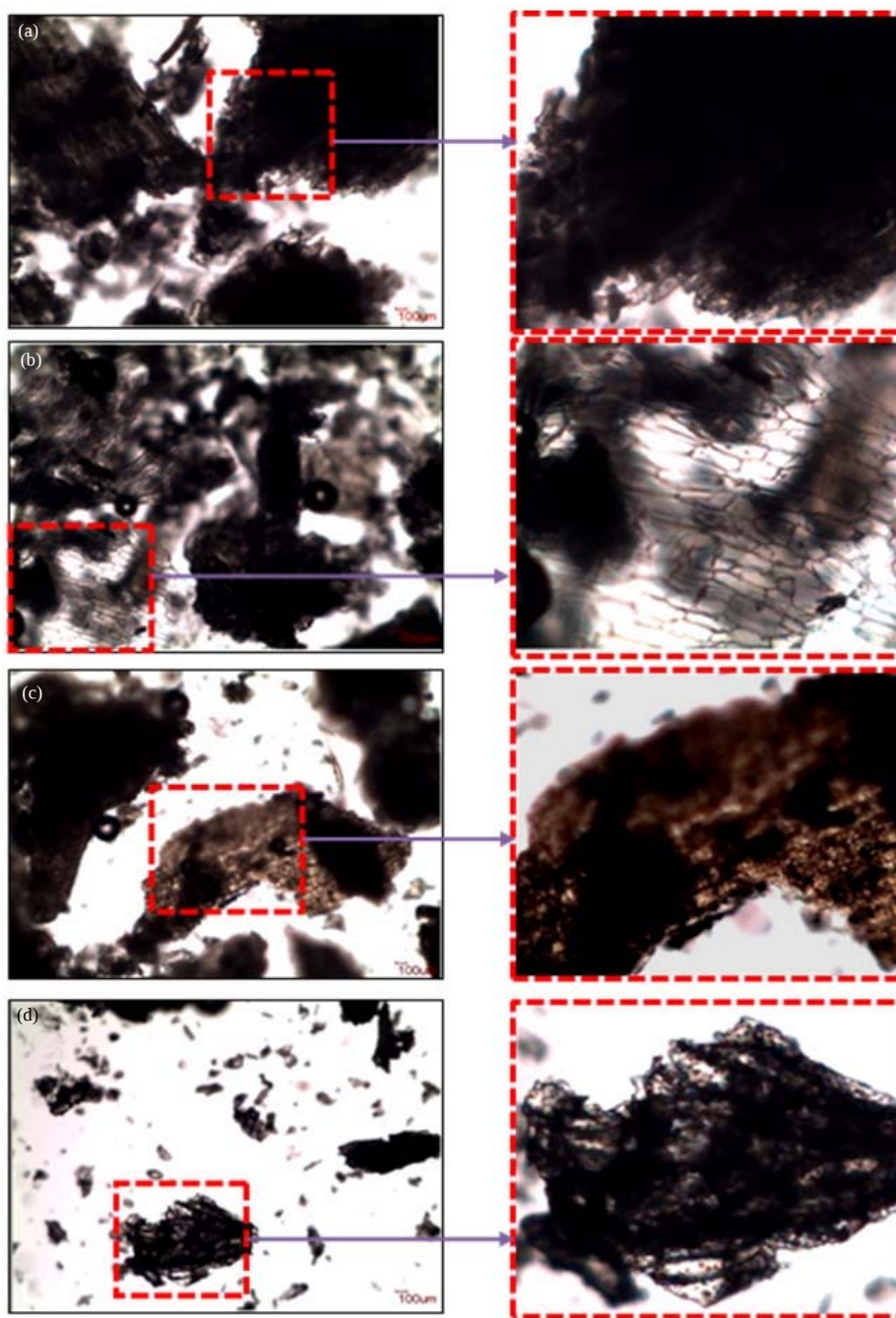


Fig.2(a-d): Images of optical microscope of homogeneous corncob fiber unpre-treatment and after pre-treatments (a) Homogeneous corncob fiber (T_0), (b) Frozen homogeneous corncob fiber (T_1), (c) Acid homogeneous corncob fiber (T_2) and (d) Frozen acid homogeneous corncob fiber (T_3)

lignocellulosic compounds occurred with the combined pre-treatment, which shows a greater presence of cellulose (64.12 ± 1.03), a greater degradation of hemicellulose (10.16 ± 1.42) and a slight increase of lignin (19.76 ± 0.42) when compared with the control (T_0). Statistically, non-significant differences were observed in the

amount of hemicellulose, cellulose and lignin between T_0 and T_1 , likewise for T_2 and T_3 .

Optical microscopy analysis: Corncob macro structure before and after pre-treatments were observed by optical microscopy and are presented in Fig. 2a-d.

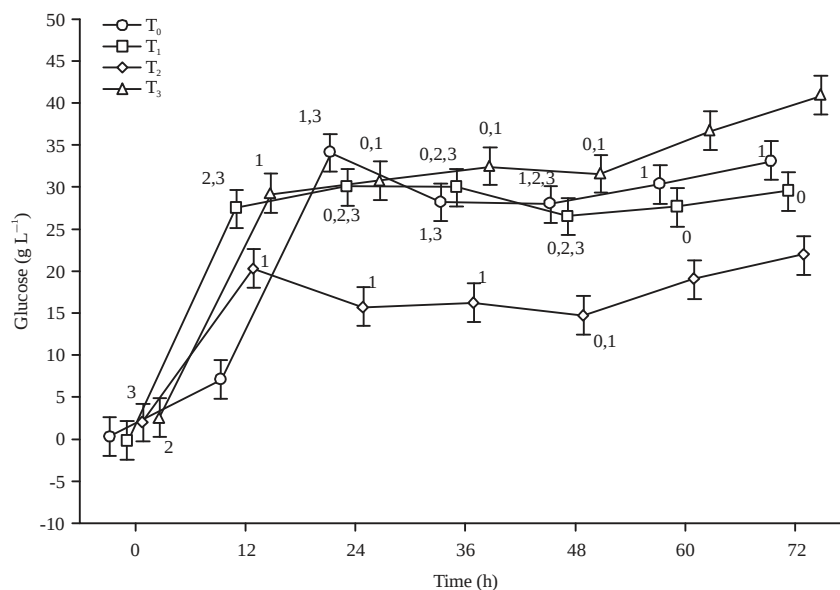


Fig. 3: Glucose concentration produced by enzymatic hydrolysis of corncob with different pre-treatments. (It was coded, T₀: 0, T₁: 1, T₂: 2, T₃: 3, the numbers (0, 1, 2 or 3) for each pre-treatment for every 12 h show that there aren't significant differences between them)

In Fig. 2a, the T₀ structure is shown agglomerated and a little irregular. In Fig. 2b, it is observed that T₁ has a rigid structure in the form a crystalline network and less agglomerated. In Fig. 2c, T₂ shows that 0.25 M H₂SO₄ increased its surface area, because there is a greater partition of its structure, being observed less agglomerated and irregular may be due to the elimination of hemicellulose. Finally in Fig. 2d, the T₃ is observed very partitioned and less agglomerated with a structure in form of a crystalline network.

Enzymatic hydrolysis: Comparing the enzymatic digestibility of samples were observed that T₃ proved to be more susceptible to enzymatic hydrolysis. Figure 3 shows the glucose production (g L⁻¹) of different pretreated samples, through the 72 h of enzymatic hydrolysis. The highest glucose concentration was at 72 h of hydrolysis for the T₃ and at 24 h of hydrolysis for the T₃, which were 40.90±2.11 and 34.11±1.28 g L⁻¹, respectively. In Fig. 3, it can also be observed that the highest glucose concentration was generated between 12 and 24 h of hydrolysis for the samples: T₀, T₁ and T₂, which gradually decreased during the following h of hydrolysis.

Figure 3 shows that at 72 h of hydrolysis the concentration of glucose obtained from T₀ and T₁, 33.17±2.03 and 29.50±1.23 g L⁻¹, respectively, aren't statistically different, however, there were significant differences between the pre-treatments T₀, T₂ and T₃ (p<0.05).

Substrate cellulose conversion: Figure 4 shows the conversion rate of cellulose to glucose for each pre-treatment after 72 h of enzymatic hydrolysis. The T₃ (57.41%) conversion rate of cellulose to glucose was lower than that T₀ (72.90%). Also Fig. 4 shows the production of 409.03 mg_{glucose}/g_{substrate} for T₃, with a positive effect of freezing on samples subjected to acid dilution in enzymatic hydrolysis due to higher glucose generation, which is 219.24-409.03 mg_{glucose}/g_{substrate}, which represented an increase in glucose yield of 86.57%.

DISCUSSION

The initial corncobs contained 48.46±1.65% moisture, indicating that corncobs were obtained within a few weeks of their harvest. After the conditioning was done, it worked with corncob fiber (T₀) with 3.53±0.57% of humidity. It should be noted that chemical composition of agricultural residues varies according to the location, season and methods of harvesting²⁷. Lignocellulosic compounds content was in the range reported by other studies also carried out with corncob as shown in Table 2.

In this Table, it is observed that the primary component is cellulase (40.95±0.38%) and due to a low amount of lignin (16.54±0.38%), corncob fiber is an ideal lignocellulosic material for production of fermentable sugars. After performing different pre-treatments at T₀, it was observed that the T₂ increased cellulose initial content from

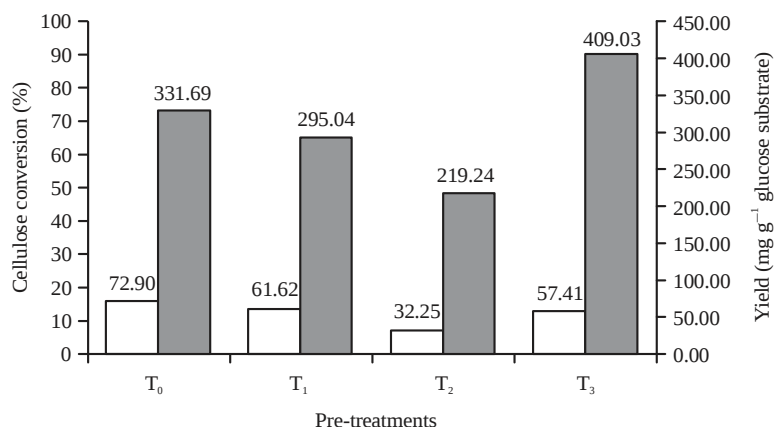


Fig. 4: Substrate cellulose conversion after enzymatic hydrolysis

Table 1: Corncob lignocellulosic compounds pretreated with 0.25 M H₂SO₄

Pre-treatments	Authors	Lignocellulosic compounds		
		Cellulose (%)	Hemicellulose (%)	Lignin (%)
T ₀	This study	40.95±0.38 ^a	38.94±1.72 ^c	16.54±0.38 ^e
T ₁		43.09±2.23 ^a	36.70±1.67 ^c	16.08±1.79 ^e
T ₂ (0.25 M H ₂ SO ₄)		61.18±1.42 ^b	13.05±1.47 ^d	20.22±0.45 ^f
T ₃		64.12±1.03 ^b	10.16±1.42 ^d	19.76±0.42 ^f
T ₀	Liu <i>et al.</i> ²⁶	38.80	36.40	13.10
0.5 M H ₂ SO ₄		62.60	2.40	17.70
T ₀	Baadhe <i>et al.</i> ¹⁶	45.10	34.00	17.20
0.25 M H ₂ SO ₄		59.75	11.63	25.89
T ₀	Gupta <i>et al.</i> ⁵	37.4±4.18	34.2±1.02	19.2±0.83
2% (p/v)H ₂ SO ₄		52.1±2.80	14.6±1.50	22.4±0.50

Mean values±standard deviation is presented. Differences between superscript letters indicate significant difference (p<0.05)

Table 2: Corncob chemical composition

Chemical composition (%)	This study	Cheng <i>et al.</i> ²⁸	Zych ²⁹ and Potumarthi <i>et al.</i> ⁹	Yang <i>et al.</i> ³⁰ and Boonchuay <i>et al.</i> ¹³	Brar <i>et al.</i> ¹⁴	Cheng <i>et al.</i> ³¹
Cellulose	40.95±0.38	43-45	32.3-45.6	35-45	45.6	34.3
Hemicellulose	38.94±1.72	34-35	38.9	40	36.9	21.7
Lignin	16.54±0.38	15-18	6.7-13.9	5-20	15.1	17.6

Mean values±standard deviation is presented

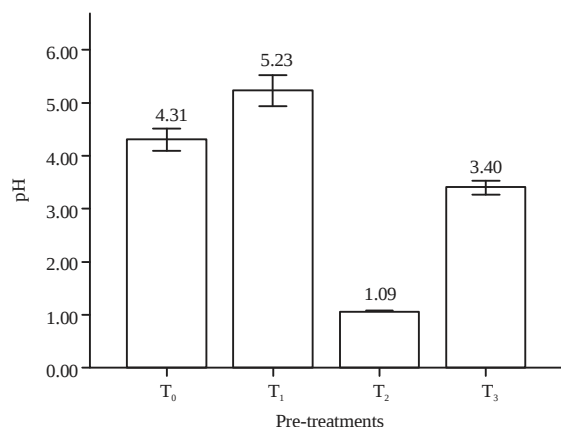


Fig. 5: PH of final hydrolyzate for the different pre-treatments (72 h)

40.95±0.38- 61.18±1.42% making it more accessible to the enzymatic hydrolysis. Several investigations used diluted sulfuric acid as a chemical pre-treatment in order to degrade the hemicellulose and make the cellulose more accessible to enzymes, besides to generating fewer inhibitors of fermentation. In this study that after T₂, hemicellulose decreased from 38.94±1.72-13.05±1.47%. However, depending on the operating temperature, some sugar degradation compounds such as furfural and hemifurales (HMF) and aromatics are generated³². Studies show that pre-treatment with diluted H₂SO₄ (2-5% p/v) of corncob at temperatures of 121-122°C for 20-25 min generated more accessible cellulose sites for cellulase enzymes, improving enzymatic digestion significantly,

obtaining sugar yield of 80% (w/w)^{33,9}. On the other hand lignocellulosic components are in the range by previous studies performed with corncob pretreated with diluted H₂SO₄ as shown in Table 1.

Although a physical pre-treatment reduces the particles size, it increases the accessible surface and pore size leading to decrease the degree of polymerization and decrystallization of lignocellulosic material^{34,35}. In Table 1, it is observed that T₁ generate smaller amount of cellulose and lower degradation of hemicellulose when compared with the other pre-treatments. These results are due to the fact that the complex structure of the lignocellulosic biomass doesn't easily degrade using only storage in freezing³⁶. The increase of lignin after pre-treatments can be explained because of physicochemical changes, such as lignin fusing and condensation or the formation of pseudo-lignin because of combination of carbohydrates generated by the degradation of hemicellulose and lignin degradation products³⁷.

Optical microscopy results determined that there are morphological changes of corncob fiber before and after pre-treatment. Observing irregular, agglomerated and crystalline network structures, affirming the effect of each pre-treatment on fiber structure. In other words, a structure agglomerated and irregular is due to the presence of amorphous hemicelluloses on its surface¹⁴. According to Aachary and Prapulla³⁸, on corncob fiber observed by scanning electron microscopy (SEM), the globular particles represent the lignin component and the fibrils represent the saccharide complex (hemicelluloses and celluloses) component, this may explain the agglomerated and irregular structure of T₀ structure. In particular, freeze pre-treatment utilize the principle of water; the density of water decreases and its volume expands when it freezes³⁹. This property has been mainly studied in the fields of food preservation and storage^{40,41}. In Fig. 2b, it is observed that T₁ has a rigid structure in the form a crystalline network and less agglomerated. Kahar *et al.*³³, conducted studies on corncob fibers morphology observed by SEM pretreated with and without 0.5% H₂SO₄ (%v/v) in an autoclave at 122°C for 20 min, observing that pre-treatment with acid and in autoclave could break the structural rigidity of lignocellulose matrices of corncob fiber and make many micropores in the interior. This meant that the surface of corncob was significantly increased because of the dissociation of hemicellulose matrices from cellulose microfibrils. The most striking effect of pre-treatment with 1% H₂SO₄ (% v/v) is the separation of fibers from cell and the loosening of the fibrous network⁴². Acid concentration of 1.5% v/v or 0.25 M, used in this study increased its surface area, because there is a greater partition of its structure, being

observed less agglomerated and irregular due may to the elimination of hemicellulose, in Fig. 2c, it is showed. Finally Chang *et al.*¹⁵ reported that the process of freezing pretreated rice straw with H₂SO₄ 4% increased surface area and enzymatic accessibility in the pretreated biomass. In Fig. 2d, T₃ is observed very partitioned and less agglomerated with a structure in form of a crystalline network, this could have been generated by removal of hemicellulose and separation of the fibers from the lignocellulosic structure, due first to treatment with acid and in addition to the increase of the volume of pores due to the treatment by freezing. Studies show that the high temperature and pressure during the pre-treatment influence the external surface resulting in an increase of 16 fold in the surface area as well as in the pore volume in 1.69 times^{43,44}. For this reason before to freezing samples pretreated with sulfuric acid, the high temperature and pressure of acid pre-treatment also can be controlled.

The obtained results of glucose concentration by the combined pre-treatment at 72 h of hydrolysis, 40.90±2.11 g L⁻¹ (409.0±21.1 mg g⁻¹ substrate) are within the ranges obtained by different studies performed with different pre-treatments in corncob and using as enzymes cellulases, which are between 350.12-453.0 mg_{TRS}/g_S (TRS: Total Reducing Sugars and S: Substrate)^{45-49,25,9,16,14}. Enzyme hydrolysis yield obtained after of freezing storage to samples pretreat with dilute acid be increased by about 86.57% compared to acid pretreated samples, that is freezing storage improves enzymatic hydrolysis yield to lignocellulosic biomass pretreat with dilute acid. In research carried out, the freezing improves enzymatic hydrolysis yield after a pre-treatment by freezing to lignocellulosic biomass (rice straw, Mongolian oak and halophilous *Juncus maritimus*) pretreated by H₂SO₄ at low concentrations and high temperatures. Smichi *et al.*⁵⁰, found the maximum glucose concentration released from *J. maritimus* (53.78±3.24 g L⁻¹) was by freeze/thaw pre-treatment and enzymatic scarification using Novozymes CellicCTec2 and by pre-treatment with 1% v/v dilute acid (49.14±5.24 g L⁻¹). Freeze pre-treatment improved enzyme hydrolysis yields by about 9.44% compared to acid pretreated samples. Jeong *et al.*³⁶, investigated the effect of freezing storage on the degradation of Mongolian oak hemicellulose by pre-treatment with 1% sulfuric acid, which a glucose production of 36.20 g glucose/100 g substrate. Freeze pre-treatment improved enzyme hydrolysis yields by about 10.27% compared to acid pretreated samples.

At 72 h of T₂ hydrolysis 21.92±1.23 g L⁻¹ of glucose was generated, this result differed of studies carried out with corncob subjected to treatments with diluted H₂SO₄, which are in the range of 39.52-45.30 g L⁻¹^{9,16,14}. Low glucose yield could

be due to the generation of enzymatic hydrolysis inhibitor products which are generated by unwanted compounds from the degradation of hemicelluloses and lignin, which is aliphatic, furfural, HMF and phenolic compounds⁵¹. These compounds could inhibit enzymatic hydrolysis by at least 50%⁵². On the other hand in Fig. 5, it is observed the final pH for T_2 (1.09 ± 0.01) and T_3 (3.40 ± 0.11) were the lowest, although the pH at the beginning of the hydrolysis was 5.48 ± 0.09 , these pH changes are due to a low neutralization of samples before hydrolysis. It is clear that optimum conditions for improved enzymatic activity of *Aspergillus niger* cellulase C1184-5KU is pH 5.0 and 37°C temperature. Variations in pH and temperature are the main factors affecting the yield of the catalytic activity of enzymatic extracts produced by *Aspergillus ficuum* (cellulases, xyloses and phytases)⁵³. The use of 1 M NaOH and 0.05 M H_2SO_4 helps to neutralize the samples of corncob to a pH of 7⁹. For future studies it is recommended to apply a mixture of acid and alkali to neutralize samples before the hydrolysis and obtain better glucose yields.

The low conversion rate of cellulose to glucose may have been due to increased lignin. Lignin limits the rate of enzymatic hydrolysis because of acting as a physical barrier and by preventing the digestible parts of substrate from being hydrolyzed⁵⁴. Cellulases are more affected because of inhibition of lignin compared to xylanases and glucosidases due to the irregular link between cellulolytic enzymes and lignin^{55,56}. Irregular binding of enzymes to lignin is also influenced because of substrate nature.

Glucose fermentation can lead to a maximum theoretical ethanol yield 90%, which is 0.51 g ethanol per g sugar⁵⁷. Therefore, considering the production of $409.03 \text{ mg}_{\text{glucose}}/\text{g}_{\text{substrate}}$ and the ethanol density 0.79 kg L^{-1} , would have a theoretical ethanol yield from corncob of $0.238 \text{ L}_{\text{ethanol}}/\text{Kg}_{\text{substrate}}$. These results clearly show potential use of corncob for the bioethanol production.

The results of the present study contribute to academic knowledge in development of production of sugar fractions fermentation, like glucose and reveal the potential for industrial production of glucose from corncob. On the other hand, this study raises future research questions. It is recommended that to complement this work, future studies should provide new combined pre-treatment approaches using safer dilute acids or alkalis, leading to a pH control during the time of enzymatic hydrolysis, with the aim of increasing the yield of fermentable sugars from different lignocellulosic materials during the hydrolysis.

CONCLUSION

This study concluded that corncob is a lignocellulosic material that can be used in production of fermentable sugars because of its high cellulose content ($40.95 \pm 0.38\%$), hemicellulose ($38.94 \pm 1.72\%$) and low lignin content ($16.54 \pm 0.38\%$). Combined pre-treatment obtained the highest glucose yield ($40.90 \pm 2.11 \text{ g L}^{-1}$), showing freezing to samples with dilute acid had a positive effect on the hydrolysis, that is a production of $219.24\text{--}409.03 \text{ mg}_{\text{glucose}}/\text{g}_{\text{substrate}}$ which represented an increase in glucose yield of 86.57%. Interest in renewable, sustainable and economically viable alternatives biofuels such as bioethanol, they make the corncob glucose production is a potential alternative.

SIGNIFICANCE STATEMENTS

This study determined that the lignocellulosic compounds of corncob, make it a biomass with a great potential for the bioconversion to glucose. The results provided that the combined pre-treatment increases the direct yield of glucose, suggesting that corncob has suitable characteristics to be used in the production of bioethanol, which could be beneficial from academic and industrial point of view. This study will act as a basis for future studies in this field and also raises the possibility of elaborating a process for obtaining bioethanol, giving an industrial added value to the residue of one of the main crops of the world.

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