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Pretreatment of Corn Stover Fractions Using Urea for the Obtention of Fermentable Sugars

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Abstract

Objectives: The current research uses stems and leaves from corn stover for the application of urea pretreatment, in order to evaluate the most favorable conditions for the obtention of reducing sugars. **Methods**: Biomass particles with sizes of 0.5 and 2.0 mm were subjected to urea pretreatment (2.0 %w/v and 5.0 %w/v). Quantification of reducing sugars was performed using the DNS method and a calibration curve. **Findings**: The maximum value of total reducing sugars for the stems fraction (35.76 g/L) was reported when the particle size and the urea concentration were 0.5 mm and 5.0 %w/v, respectively. Corn stover leaves fraction obtained its highest result (59.65 g/L) using 0.5 mm particles size and 2.0 %w/v urea. **Application/Improvements:** This research contributes to the studies about urea pretreatment and its effect in different corn stover fractions.

Keywords: Corn Stover, Lignocellulosic, Pretreatment, Reducing Sugars, Urea

1. Introduction

Cob is the main product of corn crops; the remaining parts of the plant are considered waste. Open burning is used as disposal medium for these residues[1,2] which generates negative effects in the soil[3] and harmful emissions into the environment[4]. Different alternatives have been discussed over the years with the purpose of making the most out of this residues[5–9]. The cellulose content in corn stover, along with its abundance and relatively low cost has drawn international attention towards its application as biomass feedstock in recent researches[10–13]. Cellulose can be converted into fermentable sugars and be used as source for obtaining value-added products including biofuels[1,14]. In addition to cellulose, corn stover content consists of a matrix of hemicellulose (15-35%) and lignin (10-20%)[15]. This complex constitutes a strong barrier, which generates mechanic resistance when trying to release cellulose for its subsequent conversions[13]. Lignin function is to give support to the plant, being an important part of the vegetal cell walls.

Milling and cutting are mechanical pretreatments that disrupt the structure of crystalline lignocellulose[16]. When these techniques are combined with chemical pretreatments there can be an increase in cellulose accessibility and porosity of biomass, along with alterations of the lignin arrange and decrease of polymerization degree[17]. Different chemical agents have been used to degrade lignin and hemicellulose from lignocellulosic residues. Some pretreatments have used substances ranging from alkali[12] dilute acid[10], organic solvents[18] and so on. Between these chemicals, alkaline pretreatments have desirable

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features such as non-corrosive, non-polluting and mild conditions[19]. They also require lower temperatures and pressures, causing less sugar degradation when compared to other chemical pretreatment technologies[20]. This is the reason why some authors have studied aqueous urea[21,14] and urea in combination with other substances including choline chloride/urea[22] and NaOH/urea[23]. These reports have shown urea pretreatment has the potential of increasing cellulose conversion into reducing sugars from lignocellulosic material. However, urea has not been sufficiently studied as an agent of pretreatment.

Most of the existing literature on pretreatment of corn residues has considered them as a whole and just few authors have reported results from different parts of lignocellulosic biomass. The differences between chemical composition and characteristics of the structure in the parts of the corn stover have proved to modify the response and behavior of pretreatments[24,25]. Their fibers might behave differently when undergoing depolymerization under similar pretreatment conditions[10]. Some authors have proved that separating corn fractions might be helpful for pretreatments[26].

Analyses of the effect of urea pretreatment on different parts of stover have not been made before. In this research, stems and leaves of corn (0.5 and 2.0 mm) were pretreated separately with different urea concentrations (2.0 and 5.0 %w/v) for the obtention of Total Reducing Sugars (TRS). This was performed with the purpose of determining the most favorable factors for TRS production (urea concentration, particle size and corn stover fraction used).

2. Materials and Methods

2.1 Materials

Corn stems and leaves were obtained from Maria La Baja, in Bolivar Region (Colombia) (9°58′52″N 75°17′55″O). The biomass was air-dried, milled and sieved through the Standard Taylor Sieves, obtaining 0.5 mm and 2.0 mm particle sizes. The processed biomass was stored in re-sealable bags for its later use. For chemical pretreatment was used urea (PanReac, urea > 99%) and the sulfuric acid (97% v/v H2SO4) was used for acid hydrolysis. Characterization of the raw materials was executed through High-Performance Liquid Chromatographic (HPLC) (Table 1) according with the NREL/TP-510-42618 rule[27].

2.2 Pretreatment and Acid Hydrolysis

500 mL Erlenmeyer flasks were used to prepare samples of 10 g of biomass (stem and leaves in 0.5 and 2.0 mm

Table 1. Chemical composition of corn stover (dry basis)

Component	Leaves (%)	Stems (%)
Cellulose	19.02	28.38
Hemicelluloses	19.54	20.89
Acid insoluble lignin	3.90	2.16
Lignin	13.31	13.07
Ash	12.83	4.53
Extractives	31.92	22.63

sizes) and aqueous urea (2.0 %w/v and 5.0 %w/v) in a solid-liquid ratio of 1:25 at 80°C. The pretreatment was performed using a Shaker (LABCONDO) for 20 h; all the experiments were carried out in duplicated. After the pretreatment time was finished, the liquid phase was collected by filtration and its pH was regulated. The solid phase from the pretreatment was washed using distilled water until remained liquid reached a pH of 7.0[26]. Once the pretreated biomass was washed, it was also dried during two days using a solar dryer. The pretreated biomass was subjected to acid hydrolysis in an autoclave (SA-300H) using a solid-liquid ratio of 1:10 at 121 °C during 1 h. Once the hydrolysis was complete TRS were calculated from liquid phase using the dinitrosalicylic acid (DNS) method. In order to compare the results from the pretreated biomass, a control sample for each particle size was defined. These experiments were done exposing the biomass to an acid hydrolysis without carrying out the pretreatment stage.

2.3 TRS Measurement

The DNS method was performed with the purpose of calculating the Total Reducing Sugars (TRS) of the samples. Samples were collected from the liquid fractions of the pretreatment processes with urea and acid hydrolysis. The DNS method consists in the reduction of 3.5-dinitrosalicylic acid (DNS) to 3-amino-5-nitrosalicylic acid by the aldehyde groups of the reducing sugars[28]. Different amounts of reducing sugars have a correspondence with values of absorbance that can be determined in a UV-Vis spectrophotometer (UV-2650). Absorbance lectures are an indication of the amount of TRS in some samples. In this case the relation between TRS and absorbance is given by a calibration curve previously made (Figure 1).

3. Results and Discussion

3.1 Effect of Urea Concentration in Corn Stems

The effect of aqueous urea concentration on the yield of Total Reducing Sugar from corn stems was studied using two arrangements: 2.0 %w/v and 5.0 %w/v of urea concentrations. The results are shown in Figure 2. As it can be seen, the amount of TRS increases when the concentration of aqueous urea is higher with both particle sizes. The

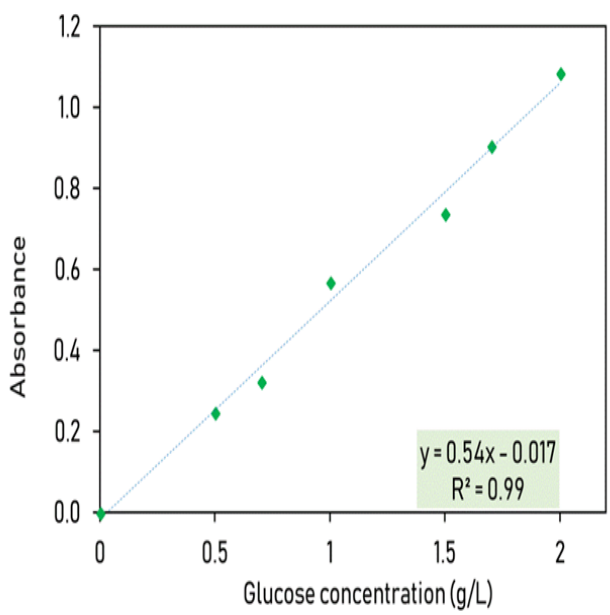

Figure 1. Calibration curve.

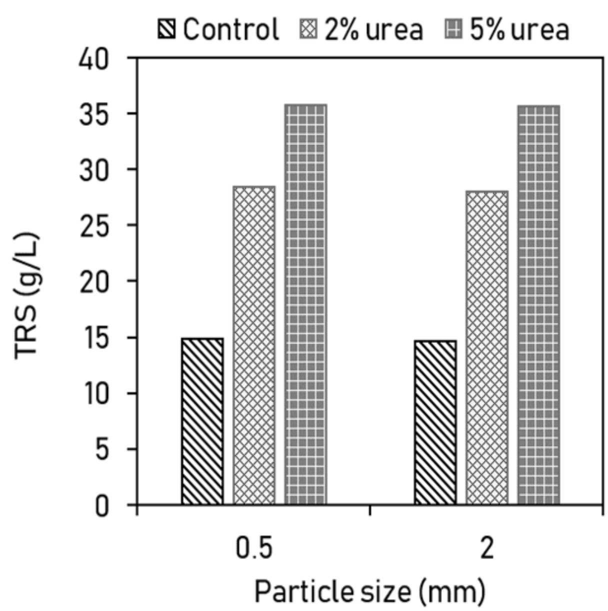

Figure 2. Total Reducing Sugars (TRS) for different particle sizes obtained from stems.

amount of TRS was increased by 26% using a particle size of 0.5 mm and 5.0 %w/v aqueous urea, when compared to the samples that were pretreated with 2.0 %w/v aqueous urea and the same particle size (from 28.43 g/L to 35.76 g/L). In comparison with non-treated biomass, the content of TRS was duplicated (from 14.8 g/L to 35.76 g/L). A similar behavior was found in the 2.0 mm samples, with reported Total Reducing Sugars of 35.69 g/L and 28.02 g/L when using concentrations of 5.0 %w/v and 2.0 %w/v, respectively.

The previous findings suggest that higher concentrations of aqueous urea promote the formation of TRS. Hence, aqueous urea contributes to the degradation of lignocellulosic biomass, making it easier for cellulose to be accessed and transformed into monosaccharides[11].

3.2 Effect of Urea Concentration in Corn Leaves

The influence of aqueous urea concentration on the total reducing sugars using corn leaves was studied in the same conditions of corn stems fraction. As it can be seen in Figure 3, there is an increasing in the amount of TRS when the chemical pretreatment is applied to both particle sizes (0.5 mm and 2 mm). The highest amount of fermentable sugars was obtained from the samples pretreated with 2.0 %w/v aqueous urea, followed by 5.0 %w/v urea. The highest amount of TRS was observed in the sample with 2.0 %w/v urea pretreatment and particle size of 0.5 mm (59.65 g/L) with an increasing of 60%, when it's compared with the control sample (37.36 g/L). This demonstrates the effectiveness of urea pretreatment for the degradation of the cell wall. The results reported in this study are similar to the obtained by[22], in which was studied a mixture of Urea/CHCl (choline chloride) to break the structure of rice straw, the solvent allowed to increase the solubization rate, degree of crystalline and α-cellulose availability, also it helped to decrease lignin content of the samples. These results lead to a better performance of hydrolysis and more TRS at the end of the process.

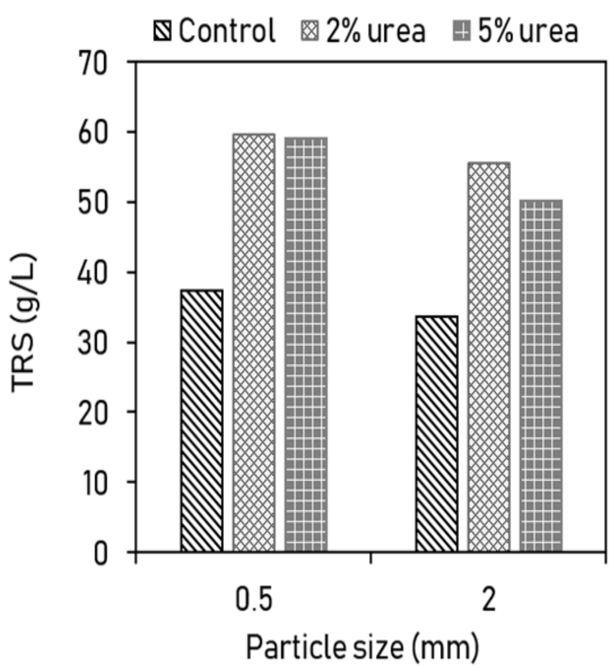

Figure 3. Total Reducing Sugars (TRS) for different particle sizes obtained from leaves.

However, the final results obtained in the present research using corn leaves, behave differently than the ones observed in corn stems. In the case of leaves, when the aqueous urea concentration was 5.0 %w/v, the resulting TRS were not higher in relation with the 2.0 %w/v solutions. A similar situation was reported in the study of [21], in that case was found that lower urea concentrations (1.0 %w/v – 3.0 %w/v) leads to a better performance in terms of biomass degradation. Nevertheless, analysis showed that using higher concentrations of urea (5.0 %w/v), a series of spherical structures were present in the surface of the biomass, and at the same time this structure contains a lignin-like compound. Such phenomenon can happen under extreme pretreatment conditions and these types of structures represent an additional barrier for the acid hydrolysis[21], and therefore, for obtaining better results in terms of total reducing sugars.

3.3 Comparisons of Leaves and Stems Results

Figures 4-6 show the TRS obtained for each pretreatment condition (control sample, 2.0 and 5.0 %w/v urea), these

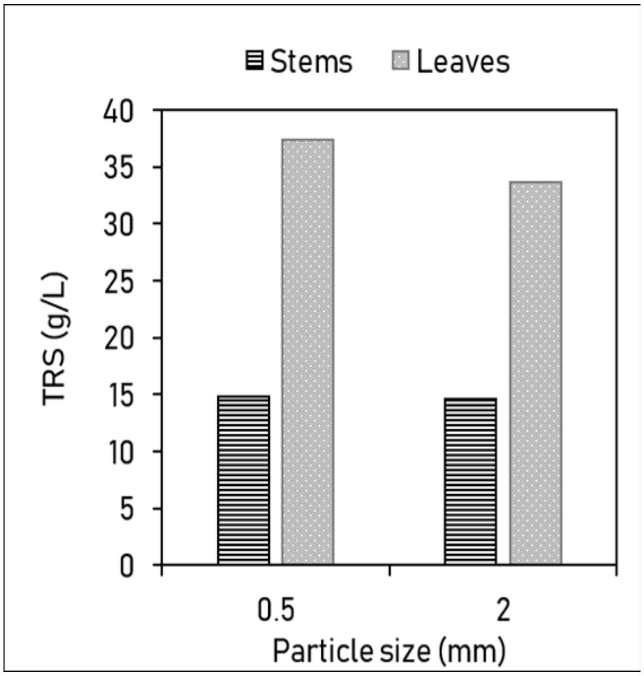

Figure 4. Total Reducing Sugars (TRS) in the fractions for control samples.

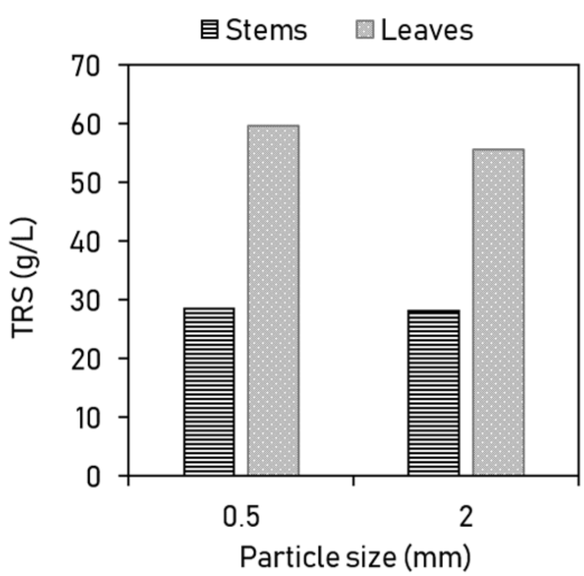

Figure 5. Total Reducing Sugars (TRS) in the fractions for control samples.

figures are used in order to compare effect of urea pretreatment of stems and leaves. In the three casesit can be

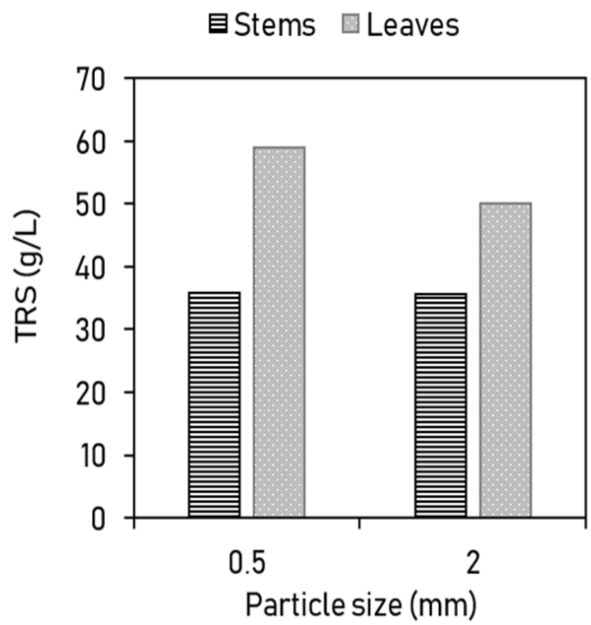

Figure 6. Total Reducing Sugars (TRS) in the fractions for 5.0 %w/v urea.

seen that leaves present a better performance compared with stems, this kind of results could be explained if it is taken into account that structure of lignocellulosic biomass is different for every part of it[10,24,25]. It has been reported[26] that leaf corn fractions are easier to hydrolyze than stems, thus higher reducing sugars can be obtained.

Therefore, leaves are a greater potential as a source of fermentable sugars, this is comparable with the study done by[10,24]. When using NaOH pretreatment the author'sreported[24] that leaves have more potential as source of TRSin comparison with other corn stover fractions (steam, leaf, flowerand husk). However, different results are reported when an acid (H_2SO_4) is used in the pretreatment[10], in this case stems have better performance in terms of ethanol production. The results reported in previous studies and the obtained in this research, have showed that the TRS produced from different fractions of the corn stover could vary, depending on the type of chemical used for pretreatment and the conditions to which this is carried out.

4. Conclusions

The combined use of urea and mechanical pretreatment has confirmed to be favorable for the obtention of fermentable sugars, delignification of biomass and crystallinity reduction of polysaccharides. As a result, conversion of cellulose into sugars was feasible. Different results were obtained under the same conditions when using stems and leaves. It was observed that physical structure and chemical characteristics of each fraction might interfere in the amount of sugars that are converted. In general, leaves exhibited the higher TRS concentrations than stems, with 59.65 g/L as the highest yield at 0.5 mm and urea concentration of 2.0 %w/v. The maximum TRS value achieved with stem fractions (35.76 g/L) was reported with 0.5 mm particle size and 5.0 %w/v urea. Stems and leaves as separately fractions have shown to have a great potential as a source of fermentable sugars for the obtention of value-added products.

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