# PHYSICAL AND CHEMICAL METHODS OF PRETREATMENT OF LIGNOCELULOSIC BIOMASS

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*In this paper we presented the results obtained by physical and chemical pretreatment of wheat straw.In the current context of increasing environmental pollution and depletion of fossil fuels, researchers are turning their attention to alternative sources of energy. Lignocellulosic biomass is a source of renewable energy used to obtain biofuels. Biofuels can be produced from biomass through various pretreatment techniques.*

*The increased demand for fuel, the increase in pollution worldwide, has driven the demand for a clean source of fuel. Thus, in the work we used the lignocellulosic biomass available ubiquitously to obtain biofuel. Through the mechanical pretreatment of lignocellulosic biomass, we aim to accelerate the disintegration into individual components: cellulose, hemicellulose and lignin, increasing the yield of obtaining fermentable sugars from cellulose or hemicellulose.*

*In the present paper we aimed to demonstrate the variation of glucose and xylose concentrations obtained from different biomass pretreatment methods. We applied both physical and chemical treatment, following their effect not only from the perspective of sugar concentration but also from the perspective of environmental pollution.*

**Keywords**: mechanical pretreatment, lignocellulosic biomass, biofuel

## 1. Introduction

In the current context of increasing environmental pollution, depletion of fossil fuels, researchers are turning their attention to alternative sources of energy [1-4]. Lignocellulosic biomass is a source of renewable energy used to obtain

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biofuels [3,4]. Biofuels can be produced from biomass through various pretreatment techniques.

Bioethanol is the second largest renewable energy source used in the transport sector and 20% of total biofuel consumption [1].

Mechanical pretreatment of cellulosic biomass from agriculture leads to a biofuel yield of approximately 90%, compared to a yield of approximately 20% when no pretreatment is applied [5]. Through mechanical pretreatment, lignocellulosic biomass is fractionated into biopolymers, enzymatic attack is facilitated, lignin and other non-fermentable constituents are easily recovered, with a view to their subsequent use for the synthesis of other useful chemical compounds [6,7]. During the first stage of the bioconversion of lignocellulose to bioethanol, an increase in the size of the biomass is obtained. The purpose of this step is to improve the speed of enzyme hydrolysis, to destroy or remove structural and compositional obstacles that may be involved in the hydrolysis process and to increase the yield of obtaining fermentable sugars from cellulose or hemicellulose [8]. The pretreatment alters the cellulosic structure of the biomass to facilitate the access of the enzyme (cellulases) to the conversion of polymeric carbohydrates into fermentable sugars [2,8].

The pretreatment of wheat straw has the following objective: [9]

- avoiding or minimizing substrate degradation or carbohydrate loss;
-decomposition of hemicellulose by damaging the crystalline structure of cellulose [15, 16];
- improving the formation of sugars through hydrolysis;
- avoiding the formation of by-products that can inhibit further hydrolysis and fermentation processes [1,2];
- the arrangement of damaged cellulose crystals can facilitate the disintegration of cellulose into glucose and breaks down hemicellulose into simple sugars [10];
- the compounds of these simple sugars can then be fermented with the help of microorganisms to produce bioethanol [11-13];
- obtaining bioethanol as a result of the utilization of lignocellulosic biomass considered to be vegetable waste;
- cost efficiency [14].

Dissolved lignin under certain pretreatment conditions can redeposit in cellulose, creating a barrier for cellulose hydrolysis, reducing sugar yield.
Through chemical, physical or biological methods, the total or partial solubilization of lignin and hemicellulose takes place [1-9].

Physical methods of pretreatment of lignocellulosic biomass include pressure, chopping, shredding, grinding, stirring, compression, expansion or other types of mechanical processes for cleaning, comminution of biomass and destruction of cellular structure, to facilitate subsequent chemical and biological treatments. Steam expansion is a method of biomass pretreatment, which is carried out at high temperatures and pressures, in sealed enclosures [10-18]. The lignocellulosic material is heated for a few minutes at a temperature of 210-290°C, with the help of steam at a pressure of 20-50 bar. After an interval of 1-5 minutes, through decompression at atmospheric pressure, steam is formed, which causes the lignocellulosic material to expand [5,6].

The introduction of a catalyst has favorable effects on the removal and hydrolysis of hemicellulose [5].

Another mechanical pretreatment is the treatment with hot water under pressure, to hydrolyze the hemicellulose, to obtain a high yield of xylose recovery (88-98%). With this treatment, no acids or chemical catalysts are used and no waste is produced [5].

## 2. Materials and methods

Three batches of wheat straw were analyzed from the perspective of the content of reducing sugars (glucose, fructose, xylose, arabinose) after the application of different pretreatments. Thus, the following tests were performed: Steam blast pretreatment of biomass (Table 2); Hydrothermal pretreatment of biomass (Table 3); Chemical pretreatment with 25% sodium hydroxide solution (Table 4). The batches of straw analyzed were samples from closed, semi-open and open warehouses: lot 1 - open warehouse; lot 2 - semi-open warehouse; Lot 3 - Closed Deposit. For the batches used in the experiments, humidity was determined, an important parameter to obtain the optimal dimensions for cutting.

Determination of moisture content and temperature in wheat straw is carried out using a temperature and moisture probe for hay and straw, Pfeuffer HFM. Lignocellulosic biomass is composed of individual plant cells. Each cell consists of three main components: cellulose, hemicellulose and lignin. These are sources of carbohydrates that can be converted into sugars through various processes. Lignocellulosic biomass can be seen as plant stems, leaves or other fibrous structures. It consists of a complex arrangement of plant cells and their associated components. The composition of wheat straw for obtaining bioethanol is shown in table 1.

*Table 1*

**Composition of wheat straw**

| Parameter | Min [%] | Max [%] |
|---|---|---|
| Cellulose | 32 | 37 |
| Xylan | 20.5 | 23.7 |
| Dry substance<br>Ash content | | 15<br>10 |

Wheat straw is first subjected to a pretreatment step to increase the accessibility of cellulose and hemicellulose and to increase the surface area of the biomass. The method used is grinding and steam treatment of the ground material. Grinding is fully automated, first the bales are separated one by one, the tightening straps of each bale are cut on a cutting machine and stored in a container. Sequentially, the bales are transported to a guillotine, which cuts approximately 10 cm thick/slice. The mills cut the straw into pieces ≤ 50 mm. The wheat straws are cut to the size of about 5 cm according to figure 1.

Fig. 1. Sectioning of wheat straw

**Steam explosion pretreatment of biomass**

This method is a thermophysical-chemical process, which facilitates the transformation of lignocellulose into sugars. In this process, the biomass is subjected to saturated steam of high pressure in the short term (minutes). Then, the pressure is suddenly released, causing a breakdown of the cell wall structure and solubilization mainly of hemicellulose and lignin fractions. Steam blasting is one of the most commonly used methods with a high potential for removing hemicellulose and lignin from the biomass structure. The steam explosion can affect the physicochemical properties of lignocellulose by increasing the crystallinity of cellulose and changes in the structure of lignin. Fiber release and low environmental impact as well as high energy efficiency are the main advantages of the method. However, the incomplete lignin released as well as the elimination and production of toxic chemicals (furfural, hydroxymethylfurfural) are the disadvantages of this method.

**Hydrothermal pretreatment of biomass**

The hydrothermal hot water method works on the basis of the use of high-temperature water to destroy lignocellulose to facilitate the production of sugars. In this method, hot water applied at high pressure to maintain its liquid form to increase the degradation of the lignocellulosic matrix at a temperature between 160°C and 200°C. It is considered a physicochemical pretreatment and is widely accepted as a green technology that does not require the use of chemical reagents. This is a remarkable advantage in terms of the costs of the entire production process, while the disadvantage of this method is the high consumption of energy and water, low concentrations of sugars, as well as fermentation inhibitors. The mechanism of this method is the removal of most of the hemicellulose and part of the lignin by degrading them into soluble fractions. This happens by transferring the structural components from the water-insoluble phase to the soluble phase. The results indicated that the pretreatments destroyed lignin with the alteration of the cell wall structure.

**Chemical pretreatment with sodium hydroxide solution**

This method is one of the most reliable pretreatments due to its strong effect and relatively simple process. Selective transformation of lignin without losing sugar and carbohydrate reduction, increasing porosity and biomass surface, and therefore improving enzymatic hydrolysis can be considered the most important advantages of the alkaline method. However, the main disadvantage of this method is the longer reaction time (several hours to a day) as well as the impact on environmental pollution. The major role of this method is to improve the enzymatic digestibility by delignifying the biomass. Different types of alkaline solutions were used, such as sulfite, sodium hydroxide, ammonium hydroxide, and lime. Based on the literature, sodium hydroxide is the most widely used alkaline solution because it is effective for delignification and works in different conditions and concentrations.

The substrate obtained after the applied pretreatments (fig 2) was subjected to enzymatic hydrolysis using the same strain of Trichoderma Reesei. The sugar concentrations obtained were measured using the ThermoFisher HPLC equipment (high-performance liquid chromatography).


Fig. 2. Wheat straw Substrate samples

## 3. Experimental results

3 batches of wheat straw were analyzed from the perspective of the content of reducing sugars (glucose, fructose, xylose, arabinose) after the application of different pretreatments. Thus, the following tests were performed:

a. Steam blast pretreatment of biomass (Table 2)
b. Hydrothermal pretreatment of biomass (Table 3)
c. Chemical pretreatment with 25% sodium hydroxide solution (Table 4).

Table 2 shows the results obtained by steam explosion pretreatment of lignocellulosic biomass. The 3 batches of straw were subjected to analysis. After pretreatment, the obtained substrate was subjected to enzymatic hydrolysis. The hydrolysate obtained was analyzed to determine the concentration of sugars. The results show that a treatment at 120°C;15min led to a high yield of sugars.

*Table 2*

**Steam blast pretreatment of lignocellulosic biomass for bioethanol production**

| Lot of straw | Raw material | Pretreatment condition | Reducing sugars (%) |
|---|---|---|---|
| 1 | Wheat straw | $160^0$C;10min | Glucose:38<br>Xylose:24 |
| 2 | Wheat straw | $151^0$C;16min | Glucose:59<br>Xylose:18 |
| 3 | Wheat straw | $120^0$C;15min | Glucose:57<br>Xylose:28 |

*Table 3*

**Hydrothermal pretreatment of lignocellulosic biomass for bioethanol production**

| Lot of straw | Raw material | Pretreatment condition | Reducing sugars (%) |
|---|---|---|---|
| 1 | Wheat straw | $180^0$C; 40min | Glucose:35<br>Xylose:18 |
| 2 | Wheat straw | $200^0$C; 15min | Glucose:20<br>Xylose:9 |
| 3 | Wheat straw | $160^0$C; 30min | Glucose:56<br>Xylose:26 |

Table 3 shows the results obtained by hydrothermal pretreatment of lignocellulosic biomass. The 3 batches of straw were subjected to analysis. After pretreatment, the obtained substrate was subjected to enzymatic hydrolysis. The hydrolysate obtained was analyzed to determine the concentration of sugars. The results show that a hydrothermal treatment at 160°C; 30min led to a high yield of sugars.

*Table 4*

**Alkaline solution (NaOH 25% concentration) pretreatment**

| Lot of straw | Raw material | Pretreatment condition | Reducing sugars (%) |
|---|---|---|---|
| 1 | Wheat straw | NaOH 25%;<br>30min | Glucose:25<br>Xylose:10 |
| 2 | Wheat straw | NaOH 25%;<br>40min | Glucose:15<br>Xylose:9 |
| 3 | Wheat straw | NaOH 25%;<br>45min | Glucose:44<br>Xylose:23 |

Table 4 shows the results obtained by alkaline chemical pretreatment with same concentration of NaOH (sodium hydroxide) solutions, 25%, on lignocellulosic biomass from 3 different straw batches. The 3 different batches of straw were subjected to analysis. After pretreatment, the obtained substrate was subjected to enzymatic hydrolysis. The hydrolysate obtained was analyzed to determine the concentration of sugars. The results show that the use of a 25%NaOH solution with a concentration of 25% for 45 minutes led to a high yield of sugars.

## 4. Conclusions

This paper proves the importance of pretreatment, physical or chemical, applied to wheat straw in order to release the lignocellulosic material to obtain the optimal yield of sugars, with low impact on environmental pollution and production costs specific to the use of steam, hot water or chemical substances.

In this document, we presented the impact of the mechanical pretreatment and steam treatment on the straw for obtaining maximum yield in sugar content.

It was observed that, for certain straw dimensions (40-50 mm), the accessibility of cellulose and hemicellulose increased during mechanical pretreatment. The heat treatment with steam explosion applied to the 3 batches of straw stored differently, proved optimal for the 3rd batch subjected to steam treatment at 120°C; 15min, for which, following enzymatic hydrolysis, a high content of fermentable sugars was obtained: Glucose :57% and Xylose:28%.

The hydrothermal treatment with water applied to the 3 batches of straw stored differently, proved optimal for the 3rd batch subjected to 160°C; 30min, for which, following enzymatic hydrolysis, a high content of fermentable sugars was obtained: Glucose: 56% and Xylose: 26%. The chemical treatment with alkaline solution of NaOH 25% concentration, applied to the 3 batches of straw stored differently, proved optimal for batch 3 subjected to treatment for 45 minutes, for which a high content of fermentable sugars was obtained, following enzymatic hydrolysis: Glucose: 44% and Xylose: 23%.

Among the pretreatments experienced, it was proven that the batch of straw from the closed warehouse (batch 3), subjected to thermal explosion pretreatment with steam, hydrothermal with water and chemical with alkaline solution 25% NaOH released, during enzymatic hydrolysis, the largest amount of fermentable sugars.

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