



## Comparison of industrially viable pretreatments to enhance soybean straw biodegradability



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### HIGHLIGHTS

- Almost complete polysaccharide hydrolysis with acid pretreatment followed by an enzymatic stage.
- High efficiency of alkaline pretreatment at mild conditions.
- Above an specific value of carbohydrates released, enzyme hydrolysis does not improve.
- High hydrolysis yield of pretreated biomass with low enzyme loading.
- Pectinase enzymes improve soybean straw biodegradability.

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### ABSTRACT

This study explores acid and alkaline pretreatments in order to enhance soybean straw biodegradability. The effects of sulfuric acid and sodium hydroxide for different pretreatment times at 30 °C and 121 °C on biomass dissolution and the subsequent enzymatic hydrolysis were investigated. The highest total conversion to reducing sugars of 93.9% was attained when soybean straw was pretreated with acid (4% H<sub>2</sub>SO<sub>4</sub>, 121 °C, 1 h) and subsequently subjected to the enzymatic process. However, conversion of 86.5%, were reached only with the hydrolysis of the pretreated residue using mild conditions, (0.5% NaOH, 30 °C, 48 h), involving the reduction cost of the process. In addition to this, this result was dramatically decreased when pectinase was removed from the enzyme cocktail. It has been also demonstrated that the reduction of the enzyme loading to less than half allowed obtaining about 96% of the reducing sugars attained with the highest enzyme dose.

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## 1. Introduction

The availability of fermentable sugars from no food source is still a limiting factor for large-scale biological production of fuel ethanol (Singh et al., 2015). Agricultural residues have been regarded for bioethanol production since they are inexpensive, abundant and have high polysaccharide content (Sarkar et al., 2012).

Soybean is an important source of food in many countries. Soybean straw is an agricultural byproduct that remains after soybean harvest. It is comprised mainly by dry leaves, husks and stalks that are mechanically removed during soybean crop. Large

volumes of this material are generally abandoned or burnt in the fields causing environmental pollution. In addition to its high polysaccharide content, soybean straw does not require an extensive grinding process prior to pretreatment as some other lignocellulosic material. Therefore, it is a renewable and low-cost material that can be considered as a potential source for the production of chemicals with economic and social interest. Nevertheless, this residue has received little attention as a feedstock for fuel ethanol production in comparison with soybean hull.

A key issue for utilization of lignocellulosic biomass is the disruption of the complex polymer matrix to liberate the monosaccharides (Mosier et al., 2005). Agricultural residues have shown high resistance to polymeric degradation (Alvira et al., 2010; Behera et al., 2014). In order to break down the structure of those residues and enhance enzymatic action, various physical, chemical and biological pretreatments have been investigated (Galbe and Zacchi, 2012). However, pretreatment is one of the most expensive

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and least technologically mature step in this process (Singh et al., 2015). Although there have been some successes in terms of increased ethanol yield, advances are still needed for efficiency improvement, cost reduction and the satisfaction of environmental requirements to improve the competitiveness (Viikari et al., 2012).

Among chemical pretreatments, acid and alkaline methods under mild conditions have shown high effectiveness on several agricultural residues (Alvira et al., 2010; Lee et al., 2013). Basically, some of the main advantages of these methods are the use of readily available chemicals at low concentrations, and a lower operation cost than other highly effective pretreatments such as ionic liquids and organic solvents (Menon and Rao, 2012). Nevertheless, possible loss of fermentable sugars and production of inhibitory compounds must be taken into consideration mainly at high temperatures (Alvira et al., 2010).

Furthermore, alkaline pretreatment can operate in some cases at mild temperature and atmospheric pressure, not being necessary expensive specialized reactors (Cabrera et al., 2014). The mechanism by which this pretreatment enhances enzymatic saccharification appears to involve a release or redistribution of lignin due to the degradation of ester bonds and cleavage of glycosidic linkages in the cell wall matrix. Partial loss of hemicellulose, decrease in cellulose crystallinity and dramatic increase in cellulose swelling have been also observed as results of solvation and saponification reactions (Hendriks and Zeeman, 2009). Alkaline pretreatment also removes acetyl groups and various uronic acid substitutions on hemicelluloses that increase the accessibility of hemicelluloses as well as cellulose to hydrolytic enzymes (Zheng et al., 2009). Among alkalis, sodium hydroxide has received the greatest attention due to its outstanding delignification capacity, high reaction rate and non-production of any inhibitory material (Singh et al., 2015).

On the other hand, dilute acid prehydrolysis at mild temperatures can be used as a pretreatment method for improving the efficiency of enzymatic hydrolysis (Hernández et al., 2015). These conditions generate lower degradation products than concentrated acid, thus releasing less biomass fermentation inhibitors (Behera et al., 2014). This pretreatment increase cellulose accessibility toward cellulases by removal nearly complete hemicelluloses (Merino and Cherry, 2007). Among all acids, the most widely exploited and tested approach is based on dilute sulfuric acid because it is low-cost, highly active and readily available chemical for industrial applications along with low safety and environmental concerns (Singh et al., 2015).

Whereas soybean straw has already been investigated for ethanol production, no reports on comparison of acid and alkaline method as the most traditional pretreatments were found in the reviewed literature. Furthermore, any information about acid pretreatment application on this residue has not been published either. In this investigation, the efficacy of acid and alkaline pretreatments using sulfuric acid and sodium hydroxide at different conditions was evaluated on the enzymatic hydrolysis of soybean straw. The influence of pectinase on biodegradability was also investigated owing to the fact that this residue is constituted by pectin, which acts as a barrier to enzyme penetration.

## 2. Methods

Soybean straw was provided by local producers from Güira de Melena (La Habana, Cuba). This residue was first milled (Wonder Max blender) and then sieved to collect the fraction of 0.2–1 mm size, which was the one used in all experiments. Next, it was homogenized to avoid compositional differences and stored in plastic bags until use. The native material showed a moisture content of 9.5%.

### 2.1. Pretreatments

Acid and alkaline pretreatments were carried out by mixing 2.5 g of residue with dissolutions of sulfuric acid or sodium hydroxide to reach a solid/liquid ratio of 1:20 (w/v). Sulfuric acid (98% w/w) and sodium hydroxide (99% w/w) were purchased from Panreac (Barcelona, Spain). Suspensions were prepared in 100 mL erlenmeyer flasks, which were incubated in an autoclave (PRESOCLAVE 30 L) at 121 °C for both pretreatments. Moreover, alkaline method was also accomplished in 250 mL erlenmeyer flasks, using a convection oven (Binder FD-53, Germany) at 30 °C, without mechanical motion and pH readjustment during the pretreatment.

Pretreatments were performed at different sulfuric acid (1%, 2% and 4% w/w) and sodium hydroxide (0.5%, 1% and 3% w/w) concentrations for shorter pretreatment times at 121 °C (0.5 and 1 h) and longer pretreatment times at 30 °C (24 and 48 h). Pretreatment at 121 °C was also conducted just in water. The fact to use moderate temperature and low reagent concentration would let reduce the cost of the process and environmental pollution.

After each pretreatment, the slurry was filtered through a Whatman No. 1 filter paper (Sigma–Aldrich, Madrid, Spain) to separate solid and liquid fractions. The liquid was collected to determine the total reducing sugars and carbohydrates released, and the residual solid was washed with distilled water to remove undesired chemicals up to reach pH 7. Subsequently, the washed solid was dried at 40 °C for 24 h in a convection oven (Binder FD-53, Germany) in order to calculate the weight loss after the pretreatment. Finally, the dried solid was subjected to enzymatic hydrolysis to evaluate the efficacy of the pretreatment methods proposed.

### 2.2. Enzymatic hydrolysis

Enzymatic saccharification of pretreated material was evaluated by conversion of cellulose and hemicellulose to monomeric sugars. For the hydrolysis, it was added to each 100 mL erlenmeyer flask the pretreated dried solid, and 25 mL of a liquid fraction constituted by 0.05 mol/L sodium citrate buffer at pH 5 (Panreac, Barcelona, Spain) and a cocktail of enzymes kindly supplied by Novozymes (Novozymes Cellulosic Ethanol Enzyme Kit, Novozymes, Denmark). Regarding enzyme loading, it was used the dose recommended by the manufactures and were used by other authors who work in the pretreatment of lignocellulosic residues (Yang and Wyman, 2008).

The cocktail was a mixture of cellulase, xylanase,  $\beta$ -glucosidase and pectinase with loadings of 19.8 FPU, 20 IU, 14 IU and 550 IU per gram of dry biomass, respectively. Given that Novozyme cocktails contain certain amount of sugars, they were calculated in samples at time zero and subtracted to determine the ones produced exclusively as a result of enzyme hydrolysis.

The buffer dissolution was supplemented with 0.25 mL of 0.2 mg/mL sodium azide (Sigma–Aldrich, Madrid, Spain) to prevent microbial contamination. The hydrolysis mixture was incubated in an orbital shaker (New Brunswick Scientific, Excella E-24R) at 50 °C and 150 rpm for 72 h. After incubation, samples were collected and centrifuged for reducing sugar analysis. Soybean straw without any pretreatment was also subjected to enzymatic hydrolysis as a control.

### 2.3. Analytical methods

The chemical composition of untreated residue was analyzed according to NREL methods (Sluiter et al., 2008). Sugars were quantified using an HPLC (Alliance 2695, Waters, Milford, MA, USA) equipped with a refractive index detector (Waters 2414, Milford,

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