THE INFLUENCE OF PRETREATMENT METHODS ON SACCHARIFICATION OF SOYBEAN HULLS

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ABSTRACT

Biomass enzymatic hydrolysis depends on the pretreatment methods employed, the composition of initial feedstock and the enzyme cocktail used to release sugars for subsequent fermentation into ethanol. In this study, Box–Behnken experimental design was used to evaluate the effect of LHW (Liquid Hot Water) and Steam exploded pretreatment on the enzymatic hydrolysis yield of soybean hulls (SH), investigating the independent variables time (t) and temperature (T). For the LHW pretreatment, the conditions tested varied from 30–45 min at 160–190°C in a fluidized glycerin bath. In turn to Steam exploded, the pretreatment conditions tested were 5-35 min of residence time at 120-172.5 °C. The biomass saccharification was performed with 10 and 20% solids loading using 4 and 8 FPase units/g SH of the commercial enzyme cocktails for a comparative study. Overall, the best glucose and xylose releases were obtained from hydrothermal pretreated SH. The commercial enzyme promoted higher glucan conversion (100%) and xylan conversion (83.8%) on the biomass pretreated by LHW at 160ºC, 30 min.

Keywords: lignocellulosic biomass, LHW pretreatment, steam exploded pretreatment, enzymatic hydrolysis, ethanol.

1 INTRODUCTION

The conversion of agroindustrial wastes into value-added products, such as second-generation ethanol, using lignocellulosic biomass can be processed in consecutive steps, which include: (physico) chemical pretreatment, enzymatic saccharification and alcoholic fermentation. Due to the high level of structural complexity, the raw lignocellulosic biomass is resistant to the direct enzymatic saccharification and therefore the first step is a pretreatment step, which is typically performed for the fractionation of biomass components and reduction of its recalcitrance. [1] Pretreatment still represents a significant portion of the process cost and it is necessary to find a technology that is efficient in the partial or total separation of lignocellulosic components, with a low generation of hydrolysis and fermentation inhibitors, low energetic cost, and minimum sugar loss.[2, 3]
The water-based pretreatments, like hydrothermal and steam exploded pretreatment, is one of the most promising technologies for processing agroindustrial wastes and the development of lignocellulosic feedstock biorefinery, since it does not require chemical inputs other than heat and water (or steam). They promote the solubilization of hemicelluloses (mainly xylans) and redistribution of lignin, making the cellulose fraction more susceptible to the action of enzymes, also positively contributing to the efficiency of hydrolytic processes.[4]

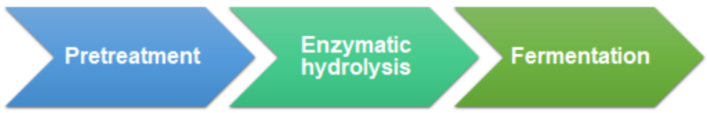

Figure 1. Main steps for biomass lignocellulose conversion into value-added products.

2 MATERIAL & METHODS

Chemical composition of soybean huls *in* natura. The composition of biomass solids was determined by National Renewable Energy Laboratory (NREL)–LAP standard analytical Procedures [5]. Chemical characterization was carried out in triplicate.

Pretreatment conditions. Doehlert experimental design was used to evaluate the effect of LHW (Liquid Hot Water) and Steam exploded pretreatment on the enzymatic hydrolysis yield of soybean hulls (SH), investigating the independent variables time (t) and temperature (T). For LHW pretreatment, stainless steel tubes were filled with 50 g of SH (moisture content = 99% w/w) and 100 ml of distilled water for a final concentration of 33% (w/v) dry solids slurry. After a heat-up time for 10 min, the tube was held for 30–45 min at 160–190°C in a fluidized glycerin bath. The pretreated tubes were then immediately placed in water at room temperature for mild quenching. The hydrothermal pretreated biomasses were stored at 4° C and used entirely in saccharification. In the steam exploded pretreatment, a pilot scale continuous system reactor was fed SH (20% w/v), with a residence time of 5-35 minutes, injecting saturated steam at 15 barr with flow of 25-30 $kg.h^{-1}$ at 120-172.5 °C. At the end the biomass was discharged, centrifuged for separation of the liquid fraction and stored at 4° C until use.

Enzymatic hydrolysis. Enzymatic hydrolysis was performed in 125 mL Erlenmeyer flasks containing 10-20% soybean hulls dry matter (w/v) in 100mM sodium citrate buffer at pH 4.8 in a final volume of 20 mL. Enzyme loading (Cellic CTec3®) was specified as 4.0 and 8.0 FPase units per gram of biomass (corresponding to 6.7 and 13.5 g protein/100 g cellulose, respectively). The reaction was carried out in an orbital shaker at 200 rpm and 50°C for 120 hr. This was followed by the samples being immediately heated to 100°C to denature the enzymes, centrifuged for 5 min at 15,000 g, and then cooled until use.

Analytical methods. Saccharide concentrations, organic acids and furfural, 5-hydroxymethylfurfural(5-HMF) were analyzed by high-performance liquid chromatography (HPLC Shimadzu). The HPLC was equipped with an Aminex ion exclusion HPX-87H (300mm × 7.8 mm) cation-exchange column (Bio-Rad Labs, Hercules, CA) and refractive index detector. The column was eluted with a mobile phase (5 mmol/L H_2SO_4) at a flow rate of 0.6 mL/min and it operated at 65°C. All analytical values were calculated from triplicates.

3 RESULTS & DISCUSSION

As it can be seen in Table 1, SH has lower lignin content when compared to other commonly studied lignocellulosic materials, e. g. sugarcane bagasse (23.1–27.6%), corn stover (16.7–21.8%) and switchgrass (13.2–22.5%),[6] which makes the exploitation of its carbohydrates cheaper due to milder pretreatment conditions. SH have higher cellulose and hemicellulose contents. In addition, there is also the presence of protein, which could be an important differential of this biomass. Several studies have reported that the use of soybean protein as a cost-effective lignin-blocking additive during high-solids processing of pretreated sugarcane bagasse, for example, results in remarkable improvements in both the enzymatic hydrolysis and fermentation steps.[7,8]

Table 1 Chemical composition of Soybean hulls (SH) in natura.

Component (g / 100 g)							
Cellulose	Hemicellulose	Lignin	Protein	Oil	Pectin	Ash	Extractives
37.1 ± 0.7	18.6 ± 0.9	9.6 ± 0.3	11.6 ± 0.5	1.8 ± 0.3	2.4 ± 0.2	4.1 ± 0.7	12.0 ± 08

From SH, hydrothermal and steam exploded pretreatments were performed acording to described previoulsy and the respective liquid fractions generated were evaluated. It is important evaluate the generation of possible inhibitors after each pretreatment, since the compounds produced from biomass dissolution could be inhibit the enzymes and the fermentation microrganims. [9] No signficant concentrations of inhibitors (formic, levulinic, acetic acids and furfurals) were observed in the liquid fraction resulting from the pretreatment conditons applied on soybean hulls.

In relation to enzymatic hydrolysis of pretreated biomass, the commercial enzymes used was able to convert 72.6% of glucan and 66.4% of xylan after saccharification of hydrothermal pretreated SH at 160° C, 30 min at 20%(w/v) solid loading and 8 FPU/g SH (Figure 2). The best result for the hydrolysis of steam exploded pretreated SH was observed at 172.5° C, 5 min, when the enzymes promoted only 54.6 and 43.9% of glucan and xylan conversions, respectively (Figure 3).

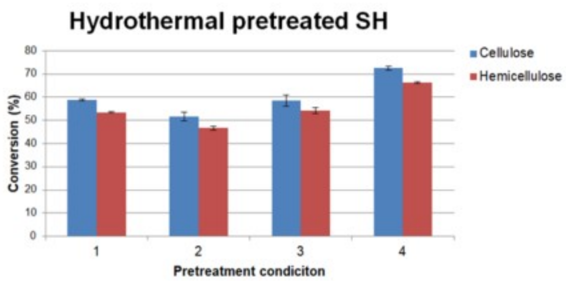

Figure 2 Enzymatic hydrolysis of liquid hot water pretreated solids. Hydrolysis conditions: 20% (w/v) solid load, 8 FPU/ g biomass, pH 4.8, 50°C, 120 hr, 200 rpm. Pretreatment conditions: (1) 190° C, 30 min; (2) 175° C, 45 min; (3) 145° C, 45 min and (4)160° C, 30 min. Experiments run in triplicate.

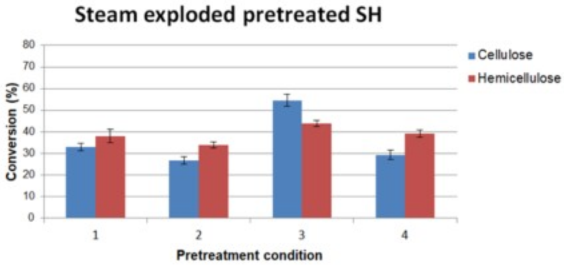

Figure 3 Enzymatic hydrolysis of steam exploded pretreated solids. Hydrolysis conditions: 10% (w/v) solid load, 4 FPU/ g biomass, pH 4.8, 50°C, 120 hr, 200 rpm. Pretreatment conditions: (1) 120° C, 20 min; (2) 137.5° C, 5 min; (3) 172.5° C, 5 min and (4)137.5° C, 35 min. Experiments run in triplicate.

However, to define the best pre-treatment among the tested conditions, the hydrolysis of hydrothermal pretreated biomass at 160° C, 30 min was also carried out at 10% (m/v) and 4 FPU/g SH to compare with steam exploded condition. These results indicated that 100 and 83.8% of glucan and xylan, respectively, were converted into fermentable sugars.

4 CONCLUSION

The different pretreatment methods can be only compared with respect to efficiency and solids loading used in the saccharification step. For the soybean hulls studied in this work, the hydrothermal pretreatment at 160° C, 30 min promoted the best saccharification yields (glucose and xylose release). Since it is a mild pretreatment, it does not generates inhibitors for enzymes and fermention microrganisms, which make its favorable pretreatment condition for soybean hulls conversion into ethanol.

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ACKNOWLEDGEMENTS

The authors are grateful to the CJ Selecta ® Company for providing soybean hulls. This study was financed by Minas Gerais State Research Funding Agency (FAPEMIG grant numbers APQ-0124-22 and BPD-00791-22). The authors would also like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) and the Brazilian National Council for Scientific and Technological Development (CNPq)) for funding this research.