XII Seminário Brasileiro de Tecnologia Enzimática

ENZITEC 2016

The effect of phenolic compounds from different pretreatment on enzymatic hydrolysis of sugarcane bagasse

Túlio Morgan [1], Rafaela Inês de Souza Ladeira Ázar [1] e Valéria Monteze Guimarães [1]

[1]Universidade Federal de Viçosa – Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO)
CEP 36570-900 Viçosa – MG - E-mail: tulio.morgan@ufv.br

ABSTRACT

The pretreatment is critical for enzymatic hydrolysis of biomasses, whereas is facilitates the access of enzymes to polysaccharides. However, when act on biomass, can lead inhibitors to downstream processes. In this study, sugarcane bagasse was pretreated with alkali, acid or water. After pretreatments, the bagasses were washed with water or kept without wash in order to obtain biomasses with and without by-products that can act as enzyme inhibitors. Phenolic compounds (PC), most prominent inhibitors, were quantified in saccharification medium. The results revealed that the unwashed and washed alkaline bagasses showed greatest and lowest concentration of PC, respectively. This accounts, partly, the difference observed in release of glucose and xylose from these bagasses. The acid and hydrothermal pretreated bagasses did not have significant differences of PC concentration in saccharification medium. Nevertheless, the washed acid bagasse was more hydrolyzed than the unwashed, suggesting the presence of other classes of (hemi)cellulase inhibitors.

Keywords: pretreatment, phenolic compounds, enzymatic inhibitors, sugarcane bagasse saccharification

INTRODUCTION

The biorefinery concept are wide and covers the conversion of lignocellulosic materials in many high-value products such chemicals, biofuels, animal food and biofertilizers. Sugarcane bagasse is one of the most abundant biomass in Brazil and often subject of studies related to ethanol (E2G) production. A well-established E2G production process involves the steps of pretreatment, followed by enzymatic saccharification, fermentation and distillation. Pretreatment is a key step to break down the recalcitrant nature of biomass, and makes the biomass easily hydrolyzed into fermentable sugars. However, pretreatment of lignocellulosic materials may result in the release of inhibitors and deactivators of cellulose enzyme hydrolysis and fermentative microorganism. Because of that, this process are being intensely studied in order to get a balance between parameters as low cost, ease to operate, avoid the formation of enzymatic inhibitors and consequently minimize the use of enzymes during hydrolysis. Among these inhibitors, phenolic compounds (PC) are the most prominent. The amounts of these soluble inhibitors and their distribution depend on type and severity of pretreatment, concentration of lignocellulosic solids during the pretreatment and biomass type. Washing the biomass after the pretreatment may improve enzymatic digestibility due to removal of potential inhibitors. The quantification of PC from pretreatment and evaluation of its possible effects on hydrolysis may help a better understanding of applicability of pretreatment.

The purpose of this study was to determine phenols released after three common water-based pretreatments: alkaline (NaOH 1.5% w/v), acid (H_2SO_4 0.5% v/v) and hydrothermal (water only). Furthermore, the effect of phenols on enzymatic hydrolysis of sugarcane bagasse was examined.

MATERIALS AND METHODS

The sugarcane bagasses were obtained from Jatiboca plant, located in Urucânia – MG. The samples were washed, dried at 70 °C until constant weight and ground in a knife mill to particles smaller than 1 mm (20 mesh). Milled sugarcane bagasse samples, with 50 grams each, was pretreated with diluted acid (H_2SO_4 0.5% (v/v)), alkaline solution (NaOH 1.5% (w/v)) or distilled water, at concentration of 100 $g.L^{-1}$ in an autoclave at 120 °C for 1 hour. The pretreated material (*slurry*) was separated in solid and liquid fractions by vacuum filtration. In this stage, some samples were kept without wash and others were washed three times with distilled water (10:1 water/bagasse weight).

For enzymatic saccharification was used an enzyme blend produced according to Visser *et al*., 2013. This cocktail was obtained by mixing the enzyme extract of fungi *Chrysoporthe cubensis* and *Penicillium pinophilum* in a ratio of 1:1 and showed a great potential to be applied in biomass hydrolysis. This process was conducted in 25 mL flasks with solid loading of 8.0% (w/v) and 5.0 $FPU.g^{-1}$ biomass. The flasks were submitted to stirring at 250 rpm and temperature of 50 ºC. The hydrolysis process (glucose and xylose formation) and the concentration of phenolic compounds were monitored from 0 to 120 hours by HPLC and Prussian Blue method (BUDINI *et al*., 1980), respectively.

RESULTS AND DISCUSSIONS

The presence of phenolic compounds in the saccharification medium indicates degradation and solubilization of lignin after pretreatment. Although the pretreatment reduces the recalcitrance of substrate, lignin and some of its by-products (formed during the removal of lignin from biomass), can inhibit the enzymes that carry out the hydrolysis process, decreasing biofuel yield. Phenols are an obstacle to efficient enzymatic hydrolysis because can act in the deactivation, inhibition and precipitation cellulases (XIMENES *et al.*, 2011).

Figure 1 shows the phenolic compounds concentration in the saccharification medium of the pretreated bagasses.

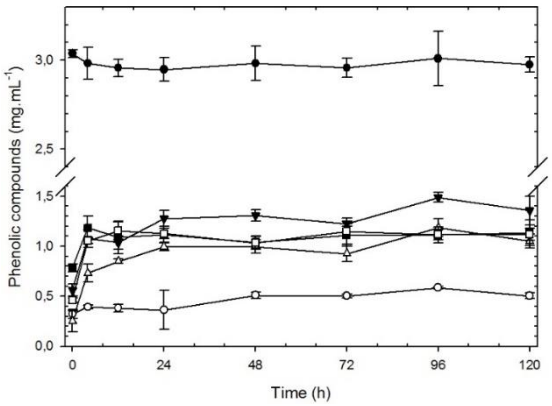

Figure 1. Phenolic compounds in the saccharification medium. (●) Unwashed Alkaline SCB, (○) Washed Alkaline SCB, (▼) Unwashed Hydrothermal SCB, (△) Washed Hydrothermal SCB, (■) Unwashed Acid SCB and (□) Washed Acid SCB.

The alkali pretreated bagasse showed the greatest PC concentration in the saccharification medium (3.0 mg/mL). The wash was efficient to remove phenolics adhered to the biomass (85% reduction) and, possibly, other by-products that may impair enzymatic activity, such as oligo-saccharides. The high release of PC by alkaline pretreatment is a result of chemical bonds present in lignin and between lignin and hemicellulose, which are sensitive to alkalis (saponify) (Martín *et al.*, 2007). Besides promoting lignin degradation, alkaline medium is fundamental for the solubility of phenolics in aqueous phase.

The hydrothermal and acid pretreatments showed similar results in terms of PC concentration. The wash of these bagasses after pretreatment did not provide difference in phenolics concentration. These pretreatments can affect lignin structure, but the acidic condition (pH < 5) not allow the solubilization of phenolics in aqueous medium. In this case, lignin are redistributed on biomass surface as lignin aggregates or "lignin droplets" limiting the hydrolysis (Li *et al.*, 2014). The small release of PC during initial hours of saccharification may be due to temperature, stirring and enzyme action, which can release some lignin droplets more weakly deposited on biomass. Although, washing the bagasses can remove others inhibitors formed, like xylo-oligosaccharides (XOS), organic acids and furfurals.

The hydrolysis of the pretreated bagasses were measured in terms of glucose and xylose formation over 120 hours. Graphs were plotted comparing the same pretreated bagasse, washed and unwashed. Thus, it was possible to analyze the effect of washing on the

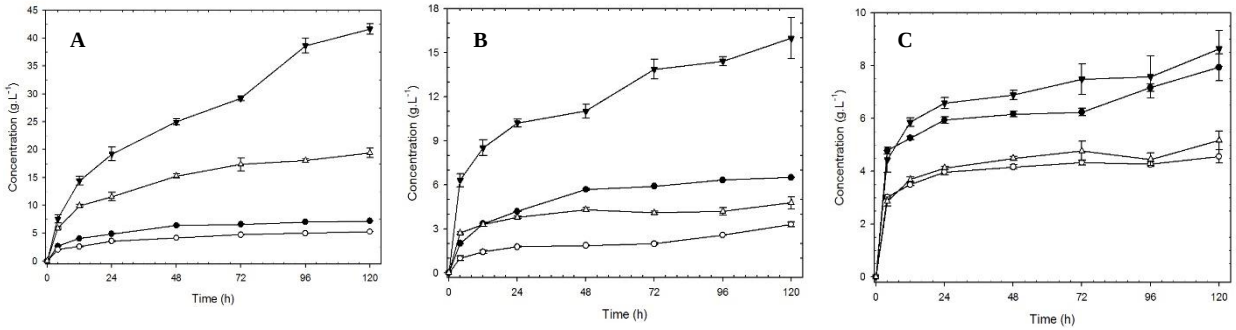

Figure 2. Hydrolysis of the sugarcane bagasses after 120 hours at 50 ºC using enzyme blend *C.cubensis: P.pinophilum* 1:1 (v/v). (**A**) Alkaline SCB, (**B**) Acid SCB, (**C**) Hydrothermal SCB. (●) Glucose (Unwashed SCB), (○) Xylose (Unwashed SCB), (▼) Glucose (Washed SCB), (△) Xylose (Washed SCB).

The greatest difference in the release of glucose and xylose was observed for alkaline pretreated bagasses (540% and 270%, respectively) (Graph A). This is related to the removal of PC in washes, avoiding the inhibition, deactivation and precipitation of enzymes caused by these compounds. Such pretreatment leads to the formation of small PC (readily soluble in water) which acts inhibiting/deactivating enzymes and high-mass lignin fragments that acts precipitating enzymes. Together, these molecules greatly decrease enzymatic activities and thereby, the biomass hydrolysis.

The acid pretreated bagasse also showed increased release of glucose and xylose with washes (140% and 44%, respectively). Nevertheless, this difference was not due to the PC, whereas both acid SCB had similar PC concentration in the saccharification medium. Since the acid pretreatments are described as XOS and xylose formers (acts inhibiting cellulases and hemicellulases, respectively), the washes may have removed many of these compounds, improving the hydrolysis of the washed acid pretreated bagasse (DU *et al*., 2010).

In the case of the hydrothermal pretreated bagasses (washed and unwashed) there was no difference in the glucose and xylose release. Both have the same PC concentration in the saccharification medium (**Figure 1**). Thus, it is not possible to affirm on the effect of these compounds on the enzymatic hydrolysis. The hydrothermal pretreatment used in this study affect slightly the structural polymers of the biomass, whereas not uses chemical catalysts and high temperature. Thereby, there are less formation of by-products and the pH and ionic strength of the medium are more favorable to enzymatic activities, representing a high advantage of the hydrothermal pretreatment compared with others used in this study. Furthermore, the release of glucose and xylose from unwashed hydrothermal bagasse was higher than the acid and alkaline SCB under the same conditions.

CONCLUSIONS

The alkaline pretreatment provides greatest release of PC from biomass, whereas high concentration of these compounds was detected on saccharification medium (~3.0 $mg.mL^{-1}$), negatively affecting this process. Washing the bagasse after this pretreatment is fundamental for a high hydrolysis and therefore, application on an industrial scale becomes environmentally infeasible. The wash of the acid pretreated bagasses not changed the PC concentration, however it had an effect of increasing the hydrolysis. This may be due to removal of other classes of enzyme inhibitors, such the XOS. The hydrothermal pretreated SCB showed the same glucose and xylose release. The unwashed hydrothermal bagasse provides higher glucose and xylose release than other unwashed substrates, suggesting low by-products (inhibitors) formation and representing a great advantage of this pretreatment.

Acknowledgment: We acknowledge the institutions FAPEMIG, CAPES and CNPq for the resources provided to execute this work.

REFERENCES

Budini R, Tonelli D, Girotti S. 1980. Analysis of total phenols using the Prussian blue method. Journal of Agricultural and Food Chemistry 28:1236-1238.

Du B, Sharma LN, Becker C, Chen SF, Mowery RA, Walsum GP, Chambliss CK. 2010. Effect of Varying Feedstock–Pretreatment Chemistry Combinations on the Formation and Accumulation of Potentially Inhibitory Degradation Products in Biomass Hydrolysates. Biotechnology and Bioengineering 107(3):430-440.

Li H, Pu Y, Kumar R, Ragauskas AJ, Wyman CE. 2014. Investigation of Lignin Deposition on Cellulose During Hydrothermal Pretreatment, Its Effect on Cellulose Hydrolysis, and Underlying Mechanisms. Biotechnology and Bioengineering 111(3):485-492.

Martín C, Klinke HB, Marcet M, García L, Hernández E, Thomsen AB. 2007. Study of the phenolic compounds formed during pretreatment of sugarcane bagasse by wet oxidation and steam explosion. Holzforschung 61:xxx-xxx

Visser EM, Falkoski DL, De Almeida MN, Maitan-Alfenas GP, Guimarães VM. 2013. Production and application of an enzyme blend from *Chrysoporthe cubensis* and *Penicillium pinophilum* with potential for hydrolysis of sugarcane bagasse. Bioresource Technology 144:587–594.

Ximenes E, Kim Y, Mosier N, Dien B, Ladisch M. 2011. Deactivation of cellulases by phenols. Enzyme and Microbial Technology 48:54–60.