XXI SIMPÓSIO NACIONAL DE BIOPROCESSOS
XII SIMPÓSIO DE HIDRÓLISE ENZIMÁTICA DE BIOMASSA

3 a 6 de setembro de 2017
Aracaju, Sergipe, Brasil

EFFECT OF SOYBEAN PROTEINS ON THE ENZYMATIC HYDROLYSIS OF DIFFERENT PRETREATED SUGARCANE BAGASSE

Camila Florencio[1], Mariana Govoni Brondi [2] and Cristiane Sanchez Farinas [1,2]

[1] Embrapa Instrumentação, Laboratório de Agroenergia

[2] Universidade Federal de São Carlos, Programa de Pós-Graduação em Engenharia Química

E-mail: camila.florencio@gmail.com

ABSTRACT

The enzymatic hydrolysis of lignocellulosic biomass still faces some technical challenges such as the use of high solids loadings, the presence of inhibitors of the biochemical reactions and non-productive adsorption of enzymes onto lignin. The addition of lignin-blocking agents to the hydrolysis medium is a potential strategy and it can contribute to improve the efficiency of the enzymatic hydrolysis process. The aim of this work was evaluate the use of soybean protein as a lignin-blocking additive for the enzymatic hydrolysis of different types of pretreated sugarcane bagasse. For that, steam exploded, liquid hot water and acid pretreated sugarcane bagasse were hydrolyzed using a commercial cellulolytic cocktail in the presence of soybean protein at three concentrations (4, 8 and 12% w/w). The addition of soybean protein increased up to 3.5 times the efficiency of liquid hot water pretreated sugarcane bagasse, relative to the control. Soybean protein has been shown as a potential cost-effective lignin-blocking additive for use in the enzymatic hydrolysis of pretreated sugarcane bagasse.

1. INTRODUCTION

The production of second generation ethanol (2G) from lignocellulosic biomass involves three major steps: physical and/or chemical pretreatment, enzymatic hydrolysis, and fermentation to ethanol production (Jin et al., 2016). The enzymatic hydrolysis (EH) step is one of the main bottlenecks concerning the use of lignocellulosic materials (Méndez Arias et al., 2017). Some major limitations

associated to the EH includes the cost of the cellulolytic enzymes and their efficiencies in converting biomass into monosaccharides.

The use of additives to the hydrolysis medium such as Tween 20 or 80 surfactants, polyethylene glycol (PEG), and bovine serum albumin (BSA) has been shown to increase the yield and rate of enzymatic hydrolysis (Cannella and Jorgensen, 2014, Yang and Wyman, 2006, Jin et al., 2016). This approach has been contribute to improving the efficiency of the enzymatic hydrolysis because reduce the amount of enzyme lost in the process due to unproductive adsorption onto lignin. For this purpose, this work evaluated the addition of soybean protein as a cost-effective lignin-blocking additive in the enzymatic hydrolysis of different types of pretreated sugarcane bagasse.

2. MATERIAL AND METHODS

1.1. Substrates

Six types of pretreated sugarcane bagasse samples were used in the enzymatic hydrolysis reaction: two steam-exploded pretreated sugarcane bagasse (SEB1 and SEB2), three liquid hot water pretreated sugarcane bagasse (LHW1, LHW2 a LHW3) and one acid pretreated sugarcane bagasse (Acid). The SEB1 was provided by Sugarcane Research Center (CTC, Brazil), the steam explosion was conducted at 1667 kPa and 205 °C for 20 min. The SEB2 was donated by a local surgarcane mill (Usina Nardini, Brazil). Liquid hot water pretreatment was performed in a 5 L reactor (Model 4580, Parr Instruments) using a 10% solids loading and three conditions of temperature and time: 195 °C for 10 min, 170 °C for 15 min and 220 °C for 5 min to LHW1, LHW2 and LHW3, respectively. The acid pretreated bagasse was prepared using a solution of dilute sulfuric acid (1.5%, w/w) and a solids loading of 10%, in an autoclave at 121 °C for 30 min. The particle size of all sugarcane bagasse (SB) used was dp ≤ 1mm (particle diameter).

1.2. Enzymatic Hydrolysis

Enzymatic hydrolysis experiments were carried out for 24 h at 50 °C in 5 mL tubes placed in a hybridization incubator operated at an agitation speed of 30 rpm. The enzyme load of commercial enzyme cellulase Cellic Ctec 2 (Novozymes, Araucaria, Brazil) was 5 FPU/g of dry biomass, and it was diluted in 50 mM citrate buffer (pH4.8). The SB materials were used at a concentration of 10% (w/v). Soybean protein was added at 4, 8 and 12% (w/w), and the control experiment was performed in the absence of additive. The glucose released was measured using a D-glucose enzymatic assay kit (Labtest, Brazil). All the hydrolysis experiments were performed in triplicate and the data were calculated as means ± standard deviations.

3. RESULTS AND DISCUSSION

The effect of soybean protein as additive was evaluated on the enzymatic hydrolysis of different pretreated sugarcane bagasse, SEB1, SEB2, LHW1, LHW2, LHW3 and Acid. The most significant positive effect was found for the enzymatic hydrolysis of LHW1 and LHW3 using a concentration of 12% of soybean protein as additive, for which the glucose released achieved were around 2-fold and 3.5-fold higher, respectively, when compared to the control experiment without additive (Figure 1). However, the value released glucose for enzymatic hydrolysis of LHW1 was higher (14±0.22 g/L) than to LHW3 (9.4±0.13 g/L). For the SEB1, SEB2 and acid pretreated SB, the use of soybean protein at 12% w/w increased glucose release up to 1.5 times in relation to the control. The different pretreatment conditions of each sugarcane bagasse directly influenced the results of enzymatic hydrolysis and the action of soybean protein as lignin-blocking additive.

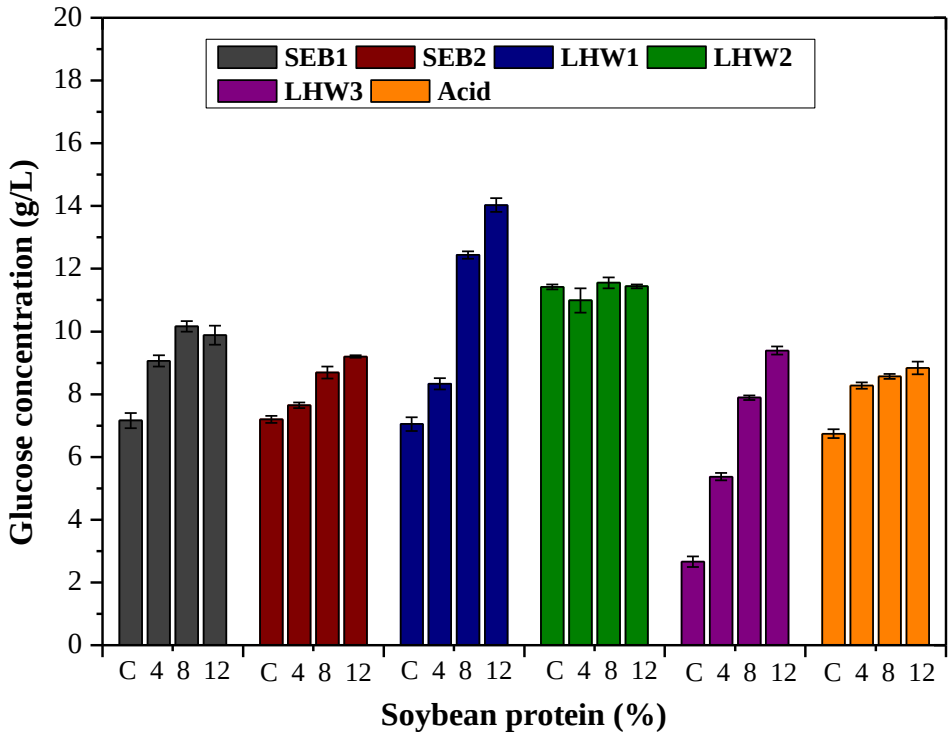

Figure 1. Effect of different concentrations of soybean protein (C: control, 4, 8, 12%) in enzymatic hydrolysis of six type of pretreated sugarcane bagasse.

All pretreatment methods are effective in modify the cell wall structure in lignocellulosic residues and facilitate the enzymatic digestibility of the cellulose. Each pretreatment processes has to be adapted to the different physicochemical characteristics of the lignocellulosic residues. The increase in the severity of LHW pretreatment for example, LHW3 in relation to LHW1, showed lower sugar release despite the positive action of soy protein. The higher temperature in LHW3 pretreatment seemed to have more influence on the pretreatment efficiences. In general, the physicochemical characteristics of each sugarcane bagasse after the pretreatment lead to different results interaction between the cellulose polymer and enzyme cocktail, and consequently the soybean protein action as lignin-blocking additive due to these characteristics and mainly the amount of lignin present in sugarcane bagasse after each pretreatment.

4. CONCLUSION

Addition of soybean protein significantly increased enzymatic hydrolysis of different pretreated sugarcane bagasse. The findings showed that soybean protein is a cost-effective alternative additive for enzymatic hydrolysis, opening up new opportunities for studies of the cellulases-lignin-soybean protein relationship.

5. REFERENCES

CANNELLA, D. & JORGENSEN, H. 2014. Do New Cellulolytic Enzyme Preparations Affect the Industrial Strategies for High Solids Lignocellulosic Ethanol Production? *Biotechnol Bioeng.* 111**,** 59-68.

JIN, W., CHEN, L., HU, M., SUN, D., LI, A., LI, Y., HU, Z., ZHOU, S., TU, Y., XIA, T., WANG, Y., XIE, G., LI, Y., BAI, B. & PENG, L. 2016. Tween-80 is effective for enhancing steam-exploded biomass enzymatic saccharification and ethanol production by specifically lessening cellulase absorption with lignin in common reed. *Appl Energy.* 175**,** 82-90.

MÉNDEZ ARIAS, J., DE OLIVEIRA MORAES, A., MODESTO, L. F. A., DE CASTRO, A. M. & PEREIRA JR, N. 2017. Addition of Surfactants and Non-Hydrolytic Proteins and Their Influence on Enzymatic Hydrolysis of Pretreated Sugarcane Bagasse. *Appl Biochem Biotechnol.* 181**,** 593-603.

YANG, B. & WYMAN, C. E. 2006. BSA treatment to enhance enzymatic hydrolysis of cellulose in lignin containing substrates. *Biotechnol Bioeng.* 94**,** 611-617.