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Enzymatic hydrolysis of sugarcane bagasse pre-treated by alkaline solution in fluidized bed reactor

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ABSTRACT

Alkaline pretreatment is an efficient method that solubilize lignin of biomass, becoming a more accessible material to the action of enzymes that could breakdown remaining polymeric carbohydrates of its content in fermentable sugars, for use, e.g., as carbon source in bioprocess. Moreover, in Brazil, sugarcane bagasse is one of the most generated lignocellulosic material, due to the high production of alcohol and sugar industries that use sugarcane juice as carbon source. Within this context, this work presents enzymatic hydrolysis of sugarcane bagasse pre-treated by alkaline solution in fluidized bed reactor. Biomass was pretreated in bioreactor by fluidization conducted by recirculation of sodium hydroxide solution. After hydrolysis, solid portion was washed, dried and submitted to enzymatic saccharification. Results showed that enzymes could act significantly better in the pre-treated material, compared to a non pre-treated raw material, presenting hydrolysate of 9,51 g/L of xylose and 21,4g/L of glucose .

Keywords: *sugarcane bagasse, enzymatic hydrolysis, alkaline pretreatment, fluidized bed reactor.*

INTRODUCTION

In Brazil, sugarcane bagasse (SB) is one of the most generated lignocellulosic material, due to the high production of alcohol and sugar industries, that use sugarcane juice as raw material. In last years, Brazil has produced around 680 million tons of SB annually (Conab 2015). Once this material is composed mainly by cellulose, hemicellulose and lignin, it can be used as carbon source in fermentative process. However, recalcitrance of biomass is one of the challenges to be studied in order to release fermentable sugars of its composition. Cellulose is a polymer composed by glucose, linked by β (1→4)-glycosidic bonds, while hemicellulose is a heteropolysaccharide composed by different compounds such as hexoses and pentoses sugars such as xylose, L-arabinose, besides some organics acids. Lignin, the third most abundant fraction is a complex aromatic compound, formed mainly by three phenyl-propane alcohols, such as p-coumarilic, coniferilic, and synapilic (Canilha et al., 2012). Different pre-treatment of biomass, such as physical, chemicals and biologicals

methods can be carried out to separated these three main contents of biomass. For example, alkaline pre-treatment of lignocellulosic material is a method that removes high portion of lignin from biomass, providing a liquid fraction of lignin, and a remaining solid composed by cellulose and hemicellulose, called holocellulose (Alvira et al., 2010). However, fermentable sugars such as glucose and xylose are already in polymeric fraction of holocellulose. Thus, one strategy is the submission of this material to an enzymatic hydrolysis, where enzymes could cleave these polymers in monomeric sugars (KUMAR et al., 2009). We highlight that the importance of preliminary stage of delignification that promotes better accessibility of enzymes into biomass, enhancing enzymatic digestibility (Chandel et al., 2014). However, novel and differentiated strategies to enhance process yields must be investigated in more details. For example, process conducted in column reactors usually presents high efficiency (Sarrouh and Silva, 2013). Colum reactors, operated in fluidized bed configuration promotes high homogenization of its content just by recirculation of fluids, without requirement of mechanical agitators or impellers, that need high energy demand, increasing process costs. Within this context, in this work, SB was conducted to alkaline pre-treatment mediated sodium hydroxide solution in fluidized bed reactor. After hydrolysis, solid portion was separated and submitted to enzymatic hydrolysis by using a complex of cellulase enzymes.

MATERIAL AND METHODS

Alkaline pre-treatment of sugarcane bagasse (kindly provided by Usina Vale Onda Verde, located in Onda Verde-SP) was carried out by using fluidized bed reactor of 2.0L (PID Fermenter AWS - Bioengineering AG, Wald, Switzerland). Bed of reactor was filled with 30 g of milled sugarcane bagasse (14-20 Mesh) and solution of 0,1M of sodium hydroxide was recirculated in flow of 1600 mL/min by 90 min at 90 °C. After hydrolysis, solid portion was separated by solubilized lignin by filtration. Remained solid portion was washed and dried at 60°C.

Enzymatic hydrolysis was performed in 125 mL Erlenmeyer flasks, containing 3 g of crude raw material or pre-treated sugarcane bagasse, 50 mL of citrate buffer (50 mM, pH 4.8), 0.10 g of surfactant Tween 20 and 20 FPU of cellulase complex (Cellulase CP CONC) from the Dyadic. Experiments were conducted by 72h in at 50°C at 150 rpm in an incubator shaker (Innova 4000; New Brunswick Scientific, Enfield, CT, USA).

The content of glucose and xylose in the enzymatic hydrolysate were verified by high performance liquid chromatography (HPLC) (chromatograph Schimadzu LC-10 AD (Kyoto, Japan) with column equipped with BIO-RAD Aminex HPX-87H (300 × 7.8 mm) coupled to refractive index detector (RID-6A), with 0.01 N sulfuric acid as an eluent at a flow rate of 0.6 mL/min, column temperature of 45∘C). For these analysis, samples were previously filtered through Sep Pak C18 filter.

RESULTS AND DISCUSSION

Sugarcane bagasse pre-treated by alkaline solution in fluidized bed reactor and crude raw material were submitted to enzymatic hydrolysis. The content of both enzymatic hydrolysate after 72h of saccharification are presented in figure 1.

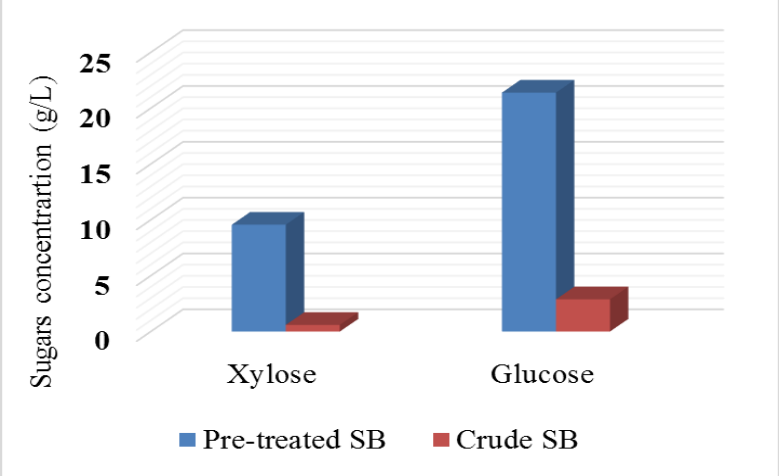

Figure 1. Concentration of xylose and glucose of enzymatic hydrolysis of sugarcane bagasse pre-treated by alkaline solution in fluidized bed reactor and crude raw material

Results showed that hydrolysate of alkaline pre-treatment material in fluidized bed reactor presented 21,4 g/L and 9,51 g/L of glucose and xylose, respectively, while crude raw material showed just 2,8 g/L and 0,6 g/L for these same sugars. Pre-treatment enhanced enzymatic digestibility, where it was achieved around 7.5 times more glucose releasing in pre-treated SB than crude raw material. Moreover, xylose, a C5 sugar, was also released around 16 times more, by the same comparing. Releasing of C5 sugars was probably due to hemicelullase content in the cellulase complex used. The requirement of a pre-treatment for enhance enzymatic digestibility is reported by different authors. For example, by working in Erlenmeyer flasks, Chandel et al. (2014) reported less sugars recovery of cellulignin from sugarcane bagasse when compared to the same sample mediated by alkaline solution (NaOH (1% m/v) at 121°C for 1 hour). In addition, Chandel et al., (2013) also presented 28.43 g/L of reducing sugars from saccharification of sugarcane bagasse pre-treated after soaking in concentrated aqueous ammonia (20% v/v ammonia solution, at 70°C; for 24 h after enzymatic hydrolysis). Moreover, Teran-Hilares et al., (2016), by using also column reactor, but in packed bed flow configuration, conducted alkaline pretreatment for sugarcane bagasse delignification. In that work, authors reported lignin removal and hydrolysis of about 50% of cellulosic and 57% of hemicellulosic fractions of pretreated SB. Although authors have reported the efficiency of the process, new strategies and developments, such as use of column reactors in fluidized bed configuration must continue to be investigated in details, aiming to maximize process feasibility and increasing of enzymatic digestibility of biomass.

CONCLUSIONS

Enzymatic hydrolysate of pre-treated sugarcane bagasse showed considerable sugars concentration than crude raw material without pre-treatment. This present work indicates the potential of use column reactor operated in fluidized bed configuration for enhancing of sugarcane bagasse digestibility for action of enzymes and optimization of glucose and other sugars releasing in enzymatic hydrolysate.

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