

Comparative analysis of 2G sugars production from sugarcane bagasse after pre-treatment with nitric acid and sodium hydroxide

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Introduction

The use of lignocellulosic biomass is the key to establish an integrated sustainable bio-based economy that offers an important advantage as a feedstock for biofuels production and other value-added bioproducts. In this study, sugarcane bagasse (SCB) was selected as biomass due to the presence of lignin, hemicellulose and cellulose representing almost 50% in its chemical composition, which are key to the obtaining of fermentable sugars (Canilha *et al.* 2012).

A pre-treatment process is required to disrupt the complex structure of the SCB that allows reducing the crystallinity of cellulose and remove the lignin content. Some of the most common pre-treatment technologies are milling, pyrolysis, microwave, steam explosion or hydrothermal, ammonia fiber explosion (AFEX), CO₂ explosion, hot water, acid pre-treatment, alkaline pre-treatment, oxidative delignification, ozonolysis, organosolv, wet oxidation and biological pre-treatment (Canilha *et al.* 2012).

The use of nitric acid as a catalytic agent during pretreatment has shown the improvement in hydrolysis of corn stover at low concentrations (Zhang *et al.* 2011). On the other hand, alkaline pre-treatment with sodium hydroxide is a delignification process wherein a representative amount of hemicellulose is solubilized but compared to acid pre-treatments where hemicellulose are almost completely removed, alkaline pre-treatments generate less sugar degradation (Canilha *et al.* 2012).

Material and methods

Raw material

Sugarcane bagasse was kindly provided by Ipiranga Agroindustrial (Descalvado/SP, Brazil). Raw sample was milled by rotatory knife (Marconi MA-680, São Paulo, Brazil) and sieved through 20 mesh, with 7% moisture percentage (w/w).

Acid pre-treatment

All experiments were performed in triplicates in 250 mL Erlenmeyer flasks with HNO₃ solution concentration (1% w/v) and 1:10 solid/liquid ratio. All flasks were properly closed with aluminum foils. The experimental conditions for autoclaving were: 121 °C, 1 atm pressure for 30 minutes. Cellulignin samples were separated of hemicellulose by filtration thoroughly washed for pH neutralization with water and sun dried followed by storing for further chemical characterization and enzymatic hydrolysis reactions.

Alkaline pre-treatment

Alkaline hydrolysis of SCB was performed in triplicates in 250 mL Erlenmeyer flasks with 1% (w/v) NaOH solution and 1:10 of solid to liquid ratio. All flasks were properly closed with aluminum foils and autoclaved at 121 °C, 1atm for 30 min. After the reaction, the solubilized lignin was separated by filtration and the recovered a solid fraction (cellulose + hemicellulose) washed with distilled water for pH neutralization, sun dried and then stored for further analysis.

Chemical characterization of natural and pre-treated sugarcane bagasse

Natural SCB, SCB after nitric acid pretreated (cellulignin) and SCB after alkaline pretreated were chemically characterized in triplicates in regard to cellulose, hemicellulose, lignin and ash (Gouveia *et al.* 2009).

Enzymatic saccharification

The samples of extracted bagasse, cellulignin and delignified bagasse were submitted to enzymatic hydrolysis. The enzymatic digestibility tests were performed in 125 mL Erlenmeyer flasks containing 4 g of biomass (dry weight), 40 mL of sodium citrate buffer (50 mM, pH 5.2) were digested by commercial cellulase enzyme from Novozyme Inc, Curitiba, Brazil with the loading of 15 FPU g⁻¹. The experimental conditions were as follows: 50 ± 0.5 °C, 200 rpm in an incubator shaker New Brunswick Scientific Innova 4000. Liquid samples were collected at 0, 12, 24, 48 and 72 h of incubation and stored for analysis. Glucose and xylose present in the hydrolysates were quantified by HPLC (Chandel *et al.* 2012).

Results and discussion

Chemical characterization of natural and pre-treated sugarcane bagasse

The chemical characterization of the natural SCB and the cellulignin and delignified bagasse were examined. Table 1 shows cellulose, hemicellulose, lignin and ash contents for all biomass samples. Natural SCB showed average amounts of cellulose (39.24 ± 1.74%), hemicellulose (29.83 ± 2.36%), lignin (23.63 ± 0.98%) and ash (3.28 ± 0.03%). Cellulignin pre-treated with diluted nitric acid showed an increase cellulose content of 65.61% because solubilizes the hemicellulose

decreasing its content by up to 44.55%. Delignification of SCB by dilute sodium hydroxide pre-treatment led to the average removal of 37.7 % of lignin. This pre-treatment increases the cellulose content by up to 78.22%. Amount of hemicellulose and structural ash remain basically the same.

Table 1. Chemical composition (% w/w, dry basis) of natural SB, acid and alkaline bagasse pre-treated.

Biomass	Cellulose	Hemicellulose	Lignin	Ash	Mass balance (%)
Natural SCB	39.24 (\pm 1.74)	29.83 (\pm 2.36)	23.63 (\pm 0.98)	3.28 (\pm 0.03)	95.98 (\pm 3.21)
Nitric acid pre-treated (Cellulignin)	59.80 (\pm 0.75)	13.29 (\pm 0.03)	25.96 (\pm 0.71)	3.30 (\pm 0.01)	102.34 (\pm 1.43)
Sodium hydroxide pre-treated (Delignified bagasse)	50.16 (\pm 1.07)	32.22 (\pm 0.53)	8.91 (\pm 0.72)	3.32 (\pm 0.01)	96.59 (\pm 1.95)

Enzymatic saccharification

Biomass characterization of natural and pre-treated bagasse samples (Nitric acid and sodium hydroxide pre-treated) are presented in Table 1. It is clear from the results that nitric acid removed hemicellulose and sodium hydroxide mediated pre-treatment removed lignin from bagasse. Both the samples were enzymatically hydrolysed. Nitric acid pre-treated (cellulignin) samples showed less sugars recovery than sodium hydroxide pre-treated (delignified bagasse). Figure 1 summarise the enzymatic hydrolysis profile of both pre-treated bagasse. Nitric acid pre-treated bagasse showed maximum sugars (glucose + xylose) recovery of 23.12 g/l (yield, 31%) after 48 h of hydrolysis. On the other hand, alkaline pre-treated bagasse showed maximum sugars (glucose + xylose) recovery of 56.91g/l (yield, 67.7%) after 72 h of enzymatic hydrolysis.

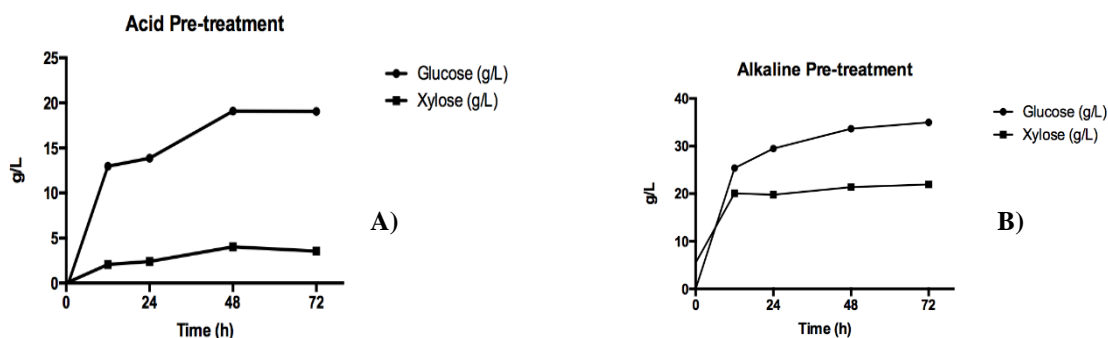


Figure 1: Enzymatic hydrolysis profile of nitric acid and sodium hydroxide pretreated sugarcane bagasse

Conclusions

This study shows a comparative analysis of two pre-treatment (nitric acid and sodium hydroxide) performed on sugarcane bagasse. Nitric acid efficiently removed hemicellulose while sodium hydroxide removed lignin. Delignified bagasse showed more sugars recovery (56.91g/l, yield, 67.7%) than nitric acid bagasse (23.12 g/l, yield, 31%) after 48 h and 72 h, respectively. Thus, it is clear from study that sodium hydroxide is better catalytic agent than nitric acid warranted higher sugars production after enzymatic hydrolysis.

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