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Short Communication

Production of ethanol from sweet sorghum bagasse pretreated with different chemical and physical processes and saccharified with fiber degrading enzymes

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HIGHLIGHTS

- Enzymatic hydrolysis achieved 50% of total sugars in untreated sorghum bagasse.
- The extrusion treatment did not generate inhibitors.
- The enzymatic hydrolysis improved 30% the sugar yields in acid hydrolysates.
- The *Saccharomyces cerevisiae* and *Issatchenkia orientalis* had good ethanol yields after 24 h.
- The *S. cerevisiae* and *I. orientalis* reduced inhibitors during fermentation.

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ABSTRACT

The C5 and C6 sugars generated from sweet sorghum bagasse pretreated with five different chemical or physical schemes and then further hydrolyzed with a fibrolytic cocktail were determined. Hydrolysates were fermented with three yeast strains in order to determine which combination generated the highest amount of bioethanol. The bagasse only treated with the enzyme complex generated 50% of the total C5 and C6 sugars available. The pressure-cooked and extruded pretreatments further hydrolyzed with the enzymes generated 17% more sugars compared to the enzyme alone treatment. The enzyme increased the total sugar content in approximately 40% in the three acid pretreated hydrolysates. Among the different pretreatments, only the extrusion process did not generate inhibitors acetic acid, furfural and 5-hydroxymethylfurfural. At 24 h fermentation, the strains *Saccharomyces cerevisiae* and *Issatchenkia orientalis* produced, respectively 183.9 and 209.2 mg ethanol/g dry bagasse previously treated with HCl and enzymes.

1. Introduction

The sweet sorghum is one of the most attractive biomass resources for bioethanol due to its adaptability to adverse conditions (Yu et al., 2011). The juice and spent bagasse of the sweet sorghum crop can be effectively bioconverted into ethanol using first and second generation processes, respectively. The lignocellulosic residues need three key steps for this transformation: (1) a pretreatment to modify the lignocellulose structure; (2) enzymatic hydrolysis to obtain C5 and C6 sugars; and (3) fermentation (Zang et al., 2007). The pretreatments have the target of modifying, removing or releasing the three main fiber components (cellulose, hemicellulose and lignin). Many pretreatments generate undesirable compounds such as acetic acid, furfural and 5-hydroxymethylfurfural that are known to inhibit fermenting microorganisms and therefore they are commonly removed beforehand. However, the expensive and environmental unfriendly detoxification step could be skipped with the use of resistant or ethanologenic microorganisms capable of enduring the remaining inhibitors (Tomás-Pejó et al., 2010). In this way, most of the current research focuses in the use of the same reactor for pretreatment, detoxification, enzymatic saccharification and fermentation to reduce energy expenditure and production cost (McIntosh and Vancov, 2010). In a previous investigation Heredia-Olea et al. (2012) documented the application of different acid hydrolyses schemes in preparation for fermentation and bioethanol production of sweet sorghum bagasse. The aim of this research was to evaluate the improvement in yields of C5 and C6 sugars after fibrous degrading enzymes of sweet sorghum bagasse previously pretreated with different chem-

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ical or physical pretreatments. In addition, the dual treated and detoxified hydrolysates with the highest C5 and C6 sugar concentrations were fermented with three different sorts of yeast to evaluate ethanol performance.

2. Methods

2.1. Materials

Sweet sorghum (*Sorghum bicolor* (L.) Moench) bagasse was procured from the research plots of Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) C.E., located in Celaya, Guanajuato at 1760 m above sea level (coordinates 20°34′47″N, 100°49′13″W). The sweet sorghum bagasse was dried at 50–60 °C for 24 h and then ground as previously described by Heredia-Olea et al. (2012). The dehydrated sweet sorghum bagasse used in the pretreatments contained an initial moisture content of 1.6% and a chemical composition of 42.4% glucans, 12.4% xylans, and 10.5% arabinans (Heredia-Olea et al., 2012).

2.2. Pretreatments

Before the enzymatic saccharification, the sweet sorghum bagasse was hydrolyzed with five pretreatments consisting of three diluted acid schemes, one hydrolysis with water and one mechanically extruded. All pretreatments were autoclaved at 15 psi (121 °C). The conditions of acid hydrolysates were: (1) 1.48% w/w of H_2SO_4 and 101 min hydrolysis, (2) 1.28% w/w of HCl and 53 min hydrolysis, (3) 0.74% w/w of H_2SO_4 and 0.64% of HCl, and 53 min hydrolysis. These conditions were previously optimized (Heredia-Olea et al., 2012). All acid and the water hydrolysis treatments were carried using a bagasse (db):liquid ratio of 6% w/v. Then, the hydrolysates were detoxified by liming to increase the pH to 5.0 and heating to 60 °C for 60 min. The water hydrolysis was carried out with the same bagasse:liquid ratio and 75 min of hydrolysis. The last pretreatment tested was the thermo-mechanical extrusion. A twin-screw corotating extruder (BTSK-20/40, Bühler AG, Uzwil, Switzerland) was used. The dry sweet sorghum ground bagasse feed rate and conditioning water were 5.7 kg/h and 1.5 l/h, respectively. The temperature of the last chamber of the barrel and the speed of the screws were set at 154.1 °C and 99 rpm, respectively. The extruded feedstock was dried overnight to approximately 2.9% moisture at 60 °C and reground as described by Heredia-Olea et al. (2012).

2.3. Enzymatic saccharification

The solubles and insoluble of the different pretreated sorghum bagasses were further hydrolyzed with a fiber degrading enzyme complex. The modified method of McIntosh and Vancov (2010) was used for biocatalysis. The assays were made with a total volume of 250 ml in 500 ml flasks at 5% bagasse (w/v) using a citrate buffer 50 mM pH 5.0 with 10 mM of sodium azide. The reaction was performed in an orbital shaking incubator (VWR Model 1575) at 50 °C and 150 rpm for 3 days. The fibrolytic cocktail of Novozymes® consisted of cellobiase (NS22084), β-glucosidase (NS50010), hemicelluloses (NS50012), endo-xilanase (NS22036), β-glucanase and xilanase (NS22002), and glucoamylase (NS22035). The declared activity for each enzyme was 1000 endo-glucanase unit (EGU)/g, 250 cellobiase unit (CBU)/g, 100 fungal beta-glucanase unit (FBG)/g (13,700 polygalacturonase unit (PGU)/g), 1000 fungal xylanase unit (FXU-S)/g, 45 FBG/g, and 750 PGU. The dosages used were 5.00%, 0.25%, 0.60%, 0.40%, 2.00%, 0.06% w/w total solids, respectively.

2.4. Microorganisms

The strains *Saccharomyces cerevisiae* ATCC® 20252™, *Pichia stipitis* ATCC® 66278™, and *Issatchenkia orientalis* ATCC® 20381™ were used to ferment the hydrolysates. The three yeasts stocks were maintained in YM Difco™ media (Franklin Lakes, NJ, USA) with 20% glycerol at −80 °C. The seed stocks were inoculated with 250 ml of YM media for 12 h, 100 rpm at 28 °C for *S. cerevisiae* and *I. orientalis*, and 24 °C for *P. stipitis*.

2.5. Fermentation process

The fermentations were performed by triplicate in 500 ml Erlenmeyer flasks with 200 ml of the hydrolysates. The strains *S. cerevisiae* and *I. orientalis* were incubated in an orbital shaking incubator (VWR Model 1575) at 28 °C and 100 rpm. The strain of *P. stipitis* was incubated similarly but at 24 °C. Yeasts were inoculated at a rate of 1×10^6 cells/ml/Brix in each prepared flask. Aliquots were taken at 0, 24, 48 and 72 h fermentation to determine ethanol, residual sugars and inhibitors. All fermentations were done without any yeast nutrient supplementation.

2.6. HPLC quantification of sugars and inhibitors

After the different hydrolyses schemes, samples were placed in ice to stop the reaction and centrifuged at 8000g and 4 °C for 5 min. Fermenting aliquots were centrifuged at 4500 rpm. Then, the supernatant was filtered through a 0.22 μm pore size filter into a HPLC vial. The sugar and inhibitor compounds were assayed with a high performance liquid chromatography as previously described by Heredia-Olea et al. (2012).

2.7. Statistical analysis

The amounts of sugars, inhibitors, and ethanol were analyzed with ANOVA procedures using the software Minitab 14 and a statistical significance (α) of 0.05 to evaluate the significant differences among mean treatments.

3. Results and discussion

3.1. Enzymatic saccharification

Most of previous research works with sweet sorghum bagasse only employed cellulase and β-glucosidase to hydrolyze the cellulose (Salvi et al., 2010; Cao et al., 2012). Furthermore, the addition or supplementation of different hemicellulases (xylanases, arabinases, etc.) enhances cellulose digestion due to the heterogeneous structure of this type of fiber. In this research a cell wall degrading complex consisting of eight different enzymes was used to hydrolyze both cellulose and hemicellulose into C6 and C5 fermentable sugars. This enzyme cocktail could be used alone or to improve the amount of fermenting sugars associated to hydrolysates pretreated with chemical or physical processes. Table 1 depicts sugars and inhibitors generated after biocatalysis with the fiber degrading enzymes of hydrolysates previously chemically or physically treated. The amounts of cellobiose were low for the six pretreatments. Previous studies have failed to report the concentration of cellobiose which is a good indicator of exoglucanase activity and a non-competitive inhibitor of cellulases (Gruno et al., 2004). Therefore, it is important to reduce cellobiose concentration in the hydrolysates with the use of higher concentrations of β-glucosidases. In the case of glucose, the untreated sorghum had the lowest concentration, followed by the extruded and H_2O hydrolysates which generated approximately 320 mg glucose/g bagasse. The exoglucanase

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