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Treatments to improve obtention of reducing sugars from agave leaves powder

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ABSTRACT

The leaves of agave plant (*Agave tequilana* Weber) are agricultural crop residues with a high content of carbohydrates represent a potential feedstock for biofuel production. The objective was to study the effect of treatment conditions in agave leaves powder (dried at 100 °C, particle size ≤0.3 mm) to improve the conversion of carbohydrates from lignocellulosic material and fructans to monosaccharides. Lignocellulosic material was pretreated using dilute acid (H_2SO_4 or HCl) at different concentrations (0.5%–2.0% v/v) and enzymatic hydrolysis was performed using four commercial enzymes Viscozyme, Cellic HTec, Celluclast, and Cellic CTec-2 separately. In addition, the enzymatic hydrolysis reaction (pH 5.0, 50 °C) using the commercial cellulases were evaluated at different times (0–36 h) in the lignocellulosic material. Fructans were extracted from agave powder and hydrolysates using a commercial inulinase preparation, Fructozyme L, (pH 5.0, 50 °C, 24 h). The best results of the treatments applied in the lignocellulosic material were H_2SO_4 (0.5% v/v) and enzymatic hydrolysis using Cellic CTec-2 for 18 h. The sugars released from the lignocellulosic material and the fructans represent a saccharification yield of 97%, obtaining 68 g of reducing sugars per 100 g of agave powder. The furfural and hydroxymethylfurfural compounds were not detected in the lignocellulosic material treated with H_2SO_4 (0.5% v/v) and the concentration of phenolics was 239.8 ± 2.1 mg/L. The results show that the treated agave leaves represent a good prospect for the production of biofuels.

1. Introduction

Biofuels research has attracted a lot attention in recent years based on environmental and economic concerns. Among biofuels, second-generation biofuels are developing as an attractive alternative to fossil fuels with the aggregate value that does not compete with the food chain production. This second-generation uses the agricultural and forestry residues composed by lignocellulosic material as biomass source, making it a renewable, economical and readily available, without need for additional land cultivation. Lignocellulosic biomass is a heterogeneous complex of cellulose, hemicellulose and lignin. Cellulose and hemicellulose are potential sources of monosaccharides. But the presence of lignin in the cell walls prevents the hydrolysis of carbohydrates. Therefore, a pretreatment of the material is essential for disrupting the main constituents of the cell wall, allowing the enzymatic activity. The challenge in lignocellulosic materials is the development of physical, physicochemical and biological pretreatments to improve the digestibility of lignocellulose in the ethanol production. (Agbor et al., 2011; Saini et al., 2015; Shi et al., 2016; Sun and Cheng, 2002; Yang and Wyman, 2008).

For ethanol production is primordial to have an appropriate fermentable material, therefore the selection of the biomass type and the pretreatment used are key part of the process. Some pretreatments in lignocellulosic materials have been reported can reduce the yield in the production of biofuels, due to the formation of inhibitory substances, result of certain pretreatment conditions and the characteristics of the biomass. Products formed of the degradation from sugars and lignin during pretreatment, is directly related with a negative influence in the enzymatic activity and alcoholic fermentation process. Phenolic compounds, furfural, and 5-hydroxymethylfurfural are the most studied

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inhibitory substances in hydrolysates. Therefore, is desirable for ethanol production to study and minimize the formation of inhibitors (Klinke et al., 2004; Palmqvist and Hahn-Hägerdal, 2000; Ximenes et al., 2011; Zhai et al., 2016).

The present work proposes the use of residues from the tequila industry, specifically the leaves of agave plant (*Agave tequilana* Weber), as a promising source of carbon for biofuel production and other compounds of industrial interest. Agave leaves constitute around 38% of total mass of the plant (Montañez-Soto et al., 2011), represent a good source of carbohydrates (~70%); nonstructural (direct reducing sugar and reducing sugar polymers such as fructans) and structural (cellulose and hemicellulose). This biomass contains polymers, which can be hydrolyzed to obtain reducing sugars to produce ethanol (Avila-Gaxiola et al., 2017).

Most of the studies in pretreatments applied to the agave plant is realized in the head and bagasse, because it represents the main raw material of the tequila industry (Mancilla-Margalli and Lopez, 2002; Saucedo-Luna et al., 2011; Waleckx et al., 2008). A large number of studies have been presents to improve pretreatment conditions. In the other hand, for the case of the agave leaves only a few works can be mentioned on pretreatment and chemical analysis (Avila-Gaxiola et al., 2017; Corbin et al., 2015; Villegas-Silva et al., 2014), reason why it is considered of great interest to study the effect of pretreatments in the agave leaves, in order to maximize the extraction of sugars and minimize the formation of inhibitory substances that affect the enzymatic reactions and alcoholic fermentations. The objective of this work was to evaluate the effect of pretreatments to release of reducing sugars from fructans and lignocellulosic material presents in agave leaves. Fructans were hydrolyzed by enzymatic reaction, in parallel the lignocellulosic material was pretreated with dilute acid and a subsequent enzymatic saccharification. Phenolic and furans compounds were analyzed in lignocellulosic hydrolysates formed during dilute acid pretreatment, because these compounds are related with an inhibitory effect in enzymatic activity and fermentative process.

2. Materials and methods

2.1. Material

Agave plants with 8 years of ages, were harvested in a same season from a cultivation zone around Culiacán, Sinaloa, México (24°52′50”N, 107°21′20”W). The leaves were randomly selected, cut from the agave plants and transported under ambient conditions (25 °C ± 2 °C; 80% relative humidity) to the laboratory.

2.2. Preparation from the raw material

Agave leaves were washed with chlorinated water (10 mg L^{-1}) to eliminate impurities. The spines were removed with a knife and the leaves were kept under refrigeration (12 °C ± 2 °C; 95% relative humidity) for less than 1 day before obtain a powder. The leaves were cut at 1.0 mm slices with a commercial slicer (Hobart, 1612E, USA), verified the thicknesses employing a precision Vernier (Uchida, M0-1, Japan). The slices were dried according to that reported by (Avila-Gaxiola et al., 2017) using a convective oven at 100 °C for 30.5 min ± 1.0 min, then milled using a blade mill (Molinos Pulvex, Mexico) and sieved (50 mesh) to obtain a particle size ≤ 0.3 mm. The powder produced was stored in polyethylene bags for later analysis. This physical pretreatment facilitates the extraction of sugars and hydrolyze the lignocellulosic material. And besides is a preservation method to store and transport samples, minimize reactions of microbiological contamination and deterioration in the biomaterial.

2.3. Characterization agave leaves powder

2.3.1. Chemical composition

The proximate analysis of the powder of agave leaves quantified according to the official methods of analysis of the AOAC International (AOAC, 2012); moisture (925.09), ash (923.03), lipids (923.05), protein (979.09), crude fiber (962.09). The carbohydrates were determined by taking the difference of the other compounds. Water activity (a_w) was measured for the agave leaves powder with an Aqualab hygrometer (Aqualab CX-2, Decagon, Pullman, USA) previously calibrated with neutral distilled water (a_w = 1.00) and a saturated NaCl solution (a_w = 0.75 at 25 °C). The potential of hydrogen was measured using a digital potentiometer (Hanna,HI 2211, México) according to the official methods of analysis of AOAC International (AOAC, 2012); pH (943.02). Analyses were performed in triplicate.

2.3.2. Aqueous extract (AE) of agave leaves powder

The aqueous extract of agave leaves powder was performed with a solution of powder and distilled water at a solid:liquid ratio of 1:10 and then stirrer (Eppendorf, thermomixer comfort, Hamburg, Germany) at 60 °C, 37 rad s^{-1} for 30 min (Avila-Gaxiola et al., 2017). The obtained suspension was then centrifuged (Eppendorf, 5810R, Hamburg, Germany), at RCF of 3220 × *g* for 30 min at 4 °C and the supernatant liquid was then filtered with a nylon membrane with a 45 × 10^{-8} m pore size (Millipore, SLHN033NK Millex, México) in preparation for the analysis.

2.3.3. Total and reducing sugars quantification

Total sugar content was quantified using the phenol-sulfuric method at 490 nm (Dubois et al., 1956), sugar content was determined comparing the absorbance of sample with respect a standard curve of fructose (Sigma Aldrich, purity ≥99%). The total fructans was determined using a commercial enzyme Fructozyme L (Novozyme, Bagsvaerd, Denmark).

A commercial enzymatic solution was used at 2% (v/v), diluted in acetate buffer solution (1:100) at pH 5.0,equivalent to an enzyme dose of 0.02% (v/v) with respect to the AE from agave leaves powder, then stirrer (Eppendorf, Thermomixer comfort, Hamburg, Germany) at 50 °C, 37 rad s^{-1} for 24 h. Reducing sugars were determined using 3,5-dinitrosalicylic acid (DNS): 0.5 cm^3 of AE was mixed with 0.5 cm^3 of DNS reagent (10 g DNS, 300 g $KNaC_4H_4O_6 4H_2O$, and 16 g NaOH per liter of distilled water), the mix was shaken and then heated for 10 min at 100 °C, the reaction was stopped by immersion in ice for 10 min, and then the reducing sugars were obtained comparing the absorbance of sample at 540 nm with respect a standard curve of fructose. The absorbance was measured in a spectrophotometer (Thermo Spectronic, Genesys 20, USA). The samples were analyzed in triplicate.

2.3.4. High performance liquid chromatography

Sugars were identified and quantified in the extract of agave leaves powder by high performance liquid chromatography (HPLC). Using an Agilent 1220 infinity LC (Agilent Technologies, USA) with a column Aminex HPX-87C (300 mm × 7.8 mm; Biorad, Hercules, CA, USA) at 50 °C, and using a refractive index detector. Elution was performed with 5 × 10^{-3} mol L^{-1} H_2SO_4 (J.T. Baker, NJ, USA, purity ≥95%) at a flow rate of 8 × 10^{-3} cm^3 s^{-1}, the sample volume injected was 20 mm^3. Glucose, fructose, sacarose, and arabinose from Sigma Aldrich (St. Louis, MO, USA) were employed as standards. The samples were diluted in distilled water (100 g L^{-1}) and then filtered with a nylon membrane with a 45 × 10^{-8} m pore size (Millipore, SLHN033NK Millex, México) to inject in the HPLC. The samples were analyzed in triplicate.

Phenolic and furan compounds considered as inhibitors in the fermentation alcoholic and cellulolytic enzymes were identified and quantified in the hydrolysates of agave leaves powder by HPLC. Using equipment Agilent 1220 infinity LC (Agilent Technologies, USA) with a Zorbax Eclipse Plus-C18 column (250 mm x 4.6 mm, 0.005 mm column particle size; Agilent Technologies, USA) at 30 °C, and a UV/VIS