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Enzymatic hydrolysis of chemically pretreated mango stem bark residues at high solid loading

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ABSTRACT

In the present work, the solid residues of mango stem bark after maceration (MSBAM) were studied as a lignocellulosic material with the potential of serving as a feedstock in the field of bioenergetics. The efficiency of two different chemical pretreatments was evaluated using dilute phosphoric acid and sodium hydroxide. The influence of solid:liquid ratio (w/v), temperature and residence time was assessed on chemical characterization of the pretreated solid fraction of MSBAM. The conversion of glucan to glucose was assessed by enzymatic hydrolysis. Alkaline pretreatment at 120 °C, 15 min and solid: liquid ratio (1:10) was the selected condition for obtaining high glucan content. Also, the enzymatic hydrolysis at high-solids loadings was studied. The results of the enzymatic hydrolysis at 21% showed a glucose production of 71.2 g/L, which corresponds to 56.3% of the enzymatic hydrolysis yield. However, at 15% of solid loading, the glucose concentration obtained was 48.9 g/L and the enzymatic hydrolysis yield was 75.3%. MSBAM has been considered as a raw material, due to its lignocellulosic structure, susceptible to enzymatic attack for obtaining sugars which, in turn, can be converted into ethanol and other products. Using Scanning Electron Microscopy (SEM), visualization of fibers was done for all the solids pretreated.

1. Introduction

One of the major crops in Cuba is the mango trees. *Mangifera indica* L. can be used to produce a crude extract with antioxidant, anti-inflammatory and immunomodulatory properties (Núñez et al., 2007). This crude extract, being used as supplement with pharmaceutical properties, is obtained after maceration of mango stem bark at moderate temperature. The volume of solid residues is 2000 ton/year, generated from 40 production cycles per year. The lignocellulosic characteristic of these residues confers upon them the potential of being used as a renewable energy source. Turning them into fuels (liquids or gases) would decrease the consumption of fossil fuels and their derivatives (Avelino et al., 2014).

A primary and decisive step is the pretreatment of lignocellulose biomass to increase the exposed surface of the lignocellulosic material (cellulose) for subsequent enzymatic hydrolysis. The main objectives of this study were depolymerization and solubilization of the carbohydrates matrix as much as possible, reducing the polymerization degree and crystalline form of the cellulose, and increasing the material's porosity (Kaparaju et al., 2009; Li et al., 2009; Conde et al., 2012; Romaní et al., 2012; Viola et al., 2013). However, pretreatment must achieve other requirements to avoid degradation or loss of carbohydrate and the formation of inhibitory byproducts that can affect subsequent hydrolysis and fermentation stages (Sun and Cheng, 2002; Nantapipat et al., 2013). Different pretreatment strategies have been proposed for a wide variety of biomass materials. Pretreatment processes can be physical, chemical, biological or combinations of these methods. Nevertheless, the cost-effective commercialization of bioethanol production from cellulose at industrial scale still requires further research (Eisentraut, 2010; Wi et al., 2011). Chemical pretreatments include ozonolysis, dilute-acid hydrolysis, alkaline hydrolysis, oxidative delignification and hydrothermal processes, organic solvents and

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ionic liquids (Fernandes et al., 2012; Ruiz et al., 2012; Avci et al., 2013; López-Linares et al., 2013; Ruiz et al., 2013; Behera et al., 2014; Romero-García et al., 2014; Camesasca et al., 2015; Nair et al., 2015).

Typically, by using sulphuric, nitric, hydrochloric or phosphoric acids in soft conditions of temperature and acid concentration allows hemicellulose solubilization, while cellulose is less attacked. This is due to the orientation of the β-1,4 glycosidic bond of cellulose that confers an organized and compact structure, resistant to acid attack (Martin et al., 2006; Avci et al., 2013; De Vasconcelos et al., 2013; Behera et al., 2014).

Phosphoric acid has shown the ability to generate lower concentrations of growth inhibitors of microorganisms, such as furfural or acetic acid. The inhibitor concentrations that have been reported are below the lower limit of the toxic effect from 2 to 4 g/L for acetic acid and 0.5 g/L for furfural (Gámez et al., 2006). Using phosphoric acid at low concentration (1–10% (w/v)) and a moderate temperature (100–180 °C) presents a great capacity of solubilizing the hemicellulose fraction (López-Linares et al., 2013). Hydrolysates can be neutralized with sodium hydroxide (NaOH), forming a sodium phosphate salt, which can be used as nutrient by microorganisms (Nantapipat et al., 2013). Phosphoric acid is less corrosive and less toxic than sulphuric acid, and it minimizes the cost of construction in an industrial plant scale (Nair et al., 2015). Dilute phosphoric acid has been investigated with wheat bran, sugar cane bagasse, rapeseed straw, corn stover, corncobs, switch grass, hybrid poplar, sweet sorghum bagasse, flax shives, triticale straw and poplar wood in order to obtain bioethanol (Hong et al., 2012; Nantapipat et al., 2013; Romero-García et al., 2014; Nair et al., 2015).

Alkaline pretreatment, among the different proposed pretreatment methods for lignocellulosic materials, is one of the most effective processes for remover or modification of lignin of the biomass due to fracturing the ester bonds that form cross-links between xylan and lignin (Mirahmadi et al., 2010; Nieves et al., 2011). Partial delignification combined with swelling of the cellulose takes place, which decreases its polymerization degree, crystallinity and disrupts the lignin structure (Viikari et al., 2012). A higher accessibility of polysaccharides to enzymes improves the enzymatic hydrolysis step (Sánchez et al., 2010; Chen et al., 2013).

According to literature, to obtain a concentrated ethanol solution in the fermentation step, it is important to get a highly concentrated glucose solution from the enzymatic hydrolysis step. To achieve this goal, solids loadings between 15 and 21% (dry basis) in enzymatic hydrolysis are required, but these may result in limited cellulose conversions due to the very high viscosity of the reaction mixture, mass transfer limitation and increased inhibition by intermediates (Cara et al., 2007; Romaní et al., 2012). Increasing the amount of glucose obtained per liquid volume is an important parameter of enzymatic hydrolysis which makes the process more economical (Kristensen et al., 2009). The objective of this study was to evaluate: (1) two chemical pretreatments, phosphoric acid and alkaline on mango stem bark after maceration (MSBAM) as raw material and to assess the influence of the main operational variables, residence time, temperature, and solid: liquid (S:L) ratio, on the cellulose content in the solid fraction; (2) the enzymatic hydrolysis at high loadings of pretreated solids.

2. Experimental

2.1. Raw material

MSBAM were air dried, milled to a particle size of 0.5–2 mm using a vibratory ball mill and stored in plastic bags at room temperature. This milling process was carried out in laboratory scale using high frequency vibrations (from 250 to 1800 rpm) up to 50 g loading; although, this condition is industrially unviable to be applied on biomass pretreatment (Silva et al., 2012). The selection of particle size was based on the reports by Sun and Cheng (2002) and Silva et al. (2012). The representation of the methodology followed in this study is as shown in Fig. 1.

Table 1
Variables studied in the alkaline and dilute phosphoric acid pretreatments.

Pretreatment	Alkaline pretreatment	Dilute phosphoric acid
Temperature (°C)	80, 100, 120	80,100,120
Solid: liquid (S:L) ratio (w/v)	1:10, 1:15, 1:20	1:10, 1:15, 1:20
Residence time (min)	15, 22.5, 30	30, 45, 60

2.2. Chemical characterization of the raw material

The moisture content of the raw material was determined using a moisture analyzer (OHAUS, MB23). The extractives content was measured according to Sluiter et al. (2005) employing extraction with 95% ethanol for 6 h.

The carbohydrates and lignin content in solid fraction of MSBAM were determined using a two-step acid hydrolysis procedure, according to the standard method of the National Renewable Energy Laboratory (NREL) using high performance liquid chromatography (HPLC) (Sluiter et al., 2008). Glucose, xylose, arabinose, galactose and mannose were separated on an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) using 4 mM H_2SO_4 as mobile phase at a flow rate of 0.8 mL/min at 60 °C and detected with a refractive index (RI) detector (Shimadzu Corp.). The concentrations of the monosaccharides were determined from standard curves obtained with high purity reagents (99%, SIGMA). Subsequently, the concentration of polymeric sugars (glucan, xylan, arabinan, galactan and mannan) was calculated from the concentration of the corresponding monomeric sugars, using anhydro correction of 0.88 (or 132/150) for xylose and arabinose, and a correction of 0.90 (or 162/180) for glucose, galactose and mannose (Sluiter et al., 2008). The acid insoluble lignin was measured using gravimetric analysis while the acid soluble lignin was measured using UV–vis spectroscopy (Sluiter et al., 2008).

2.3. Pretreatment

A three-factor central composite design was selected for each pretreatment: diluted phosphoric acid at 3% (w/w) and alkaline with NaOH at 3% (w/w) (Table 1). Test runs were developed with varying pretreatment conditions, temperature (80, 100, and 120 °C), S:L ratio (1:10, 1:15, and 1:20 (w/v)), and residence time (15, 22.5, and 30 min) for alkaline pretreatment. The pretreatment conditions were selected according to previous experiences with other lignocellulosic materials (Michelena et al., 2009; Nieves et al., 2011). For dilute phosphoric acid, the assay was performed with varying temperature and S:L ratio variables at the same values like alkaline pretreatment and residence time at 30, 45, and 60 min.

After pretreatment, the solid fraction was separated by vacuum filtration, washed with distilled water and stored at 4 °C until further analysis. The recovered pretreated solids of each pretreatment condition of design were chemically characterized using HPLC, as previously described for raw material (see chemical characterization of raw material section).

2.4. Structural characterization of untreated and pretreated solids

Morphological analyses of untreated and pretreated solids were performed using Scanning Electronic Microscopy (SEM). Random air-dried particles of solids were coated with gold-palladium in