



## Comparison of two pretreatments methods to produce second-generation bioethanol resulting from sugarcane bagasse



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### ABSTRACT

Non-centrifugal cane sugar (NCS) is a high carbohydrate-content food obtained by boil evaporation of the sugarcane juice. In the manufacturing process, the by-product of NCS called bagasse is used as fuel for the boilers, underestimating its potential as a lignocellulosic source for second-generation bioethanol. In order to assess the bioethanol potential of the by-product resulting from the NCS production, pretreatments were applied to breakdown the lignocellulosic structure for the study in hand. Therefore, this study carried out a comparison of two pretreatment methods steam explosion (SE) and acid hydrolysis (AH) applied to the sugarcane bagasse to produce second-generation bioethanol. It was evidenced that both pretreatments redistribute lignin and hemicelluloses and enhances the accessible surface area of the biomass which further benefitted the subsequent fermentation process as the reducing sugars were available enough to foster the production of bioethanol. The study found that the SE pretreatment was likely to obtain more bioethanol as the reducing sugars (glucose) production outperformed other pretreatments under different residence times and concentrations. Considering the necessity to develop second-generation biofuel production, this study presented the sugarcane bagasse as a promising and abundance lignocellulosic substrate to be investigated more in depth in the Colombian context.

### 1. Introduction

Nowadays the growing demand of energy for transportation and industrial processes have increased substantially. Large quantities of fuel, especially petroleum-based liquid fuel, are constantly required to meet this demand. Nevertheless, this is a highly pollutant non-renewable fuel extracted from fossils. Hence, it is crucial to foster the quest for fuels obtained from renewable sources that do not contaminate the environment (Saini et al., 2015). Under those circumstances, biofuels such as bioethanol have emerged as an ideal renewable fuel that meets these requirements in a sustainable fashion. Essentially bioethanol has higher oxygen content than petroleum, which can boost combustion and reduce hydrocarbon, carbon monoxide and particulate emissions and can be obtained from different low-priced substrates (Zabed et al., 2016).

The substrates used to produce biofuel are numerous which has led to a considerably increasing in the worldwide production from 31 billion barrels in 2001 up to 50 billion barrels in 2015 (Sarkar et al.,

2012). The U.S.A and Brazil are regarded the largest producers of the 'first generation' bioethanol in the world with a 62% of market share. So far, U.S has supported corn-based bioethanol production and Brazil has focused on sugar-based bioethanol (Jonker et al., 2016). Although corn-based and sugar based-ethanol are promising substitutes to petroleum-based fuel production, they are not enough to substitute a considerable portion of the one trillion gallons of fossil fuels consumed worldwide each year (Cai et al., 2017).

With regards to the Colombian market, sugarcane-based ethanol production was of 366.75 million liters in 2017 harvested in 238,304 ha, which has permitted to comply with the mandatory 10% blend of ethanol in gasoline (E10) in cities with populations larger than 500,000 inhabitants (Fedebiocombustibles, 2015). However, it is expected to observe a high-demand for bioethanol due to a mandatory blend of 30% of ethanol in gasoline projected by the year 2030. The production of solely "first-generation bioethanol" from sugarcane stance uncertainties on the sustainable production of this biofuel as the harvest of this crop might cause competition over land-use (Larsen et al., 2012).

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Despite the concerns on land-use, the biofuel production, so far, have had a positive impact to National policies on environmental sustainability initiatives such as the National Biofuels Program, the Low Carbon Development Strategy, and the implementation of a Bill on renewable energy and energy efficiency in which biomass is included. As a result of this environmental package backed by the Ministry of Environment and Sustainable Development, the bioethanol is also contributing to achieve Colombia's climate change commitment of 20% reduction of Greenhouse Gases (GHG) emissions under the Paris Climate Agreement (MINAMBIENTE et al., 2015). This evidence was observed in a study carried out by the Inter-American Development bank (IDB) and Ministry of Mines and Energy in which the bioethanol produced in Colombia could reduce the GHG emissions in about 74% compared to the emissions released by the burning of fossil fuels (IDB et al., 2012). Therefore, it is essential to obtain biofuels from other sources that does not compete for the land-use and in the same fashion reduce GHG emissions. (Larsen et al., 2012)

Thus, the sort of biofuel that might contribute to meet those requirements is the second-generation bioethanol. It is currently the most advanced, environmentally friendly biofuel made from non-edible crops and it can substitute fossil fuels as transport fuel (Zucaro et al., 2016). Among several raw materials, the lignocellulosic biomass appears as a suitable option that can be used in the second-generation bioethanol industry (Akgul et al., 2012; Matías et al., 2015). Additionally, lignocellulosic biomass can be supplied on a large-scale basis from different sources as the lignocellulosic materials are no longer used in the value chain. So, their availability and low-cost make them appropriate for use as a “second generation biofuel” (Singh et al., 2016). Currently the most favorable cellulosic feedstocks derived from plant residues in the U.S., South America, Asia and Europe are from corn stover, sugarcane bagasse, rice and wheat straws, respectively (Aditiya et al., 2016).

Despite the benefits of using this sort of feedstock, the lignocellulosic biomass contains a high degree of complexity inherent to the processing of this material due to its physicochemical matrix and composition. This means that the biomass requires an intensive labor to break down the cellulose and hemicellulose into fermentable sugars (Quintero et al., 2013). So, in order to obtain the second-generation bioethanol, it is necessary to apply previous processes that reorganize the structural composition of lignin, hemicellulose and cellulose contained in it. This previous step is called pretreatment and is significant to reduce operational costs and to increase the efficiency gains in further fermentation process (Chen et al., 2014).

The pretreatments are a crucial step that influences the effectiveness of the lignocellulosic materials to be converted into second generation biofuel (Haghighi Mood et al., 2013). The pretreatment, as the beginning stage of bioethanol production, therefore, must be carried out carefully to optimize the result. For this reason, it is necessary to assess different pretreatments applied to agricultural by-products to evaluate the concentration of fermentable sugars to produce bioethanol (Aditiya et al., 2016).

This study aimed to determine the technical viability of sugarcane-bagasse bioethanol through the comparison of the most effective pretreatment among a physical pretreatment (SE) and a chemical pretreatment (AH) applied to sugarcane bagasse. Afterwards, the effects of pretreatments on sugarcane bagasse were assessed together with the determination of the inhibitory compounds. Later, the pretreatment that delivered a high yield of fermentable sugars was then submitted in a subsequent enzymatic saccharification and a further fermentation process. A non-linear regression analysis was carried out to model the enzymatic saccharification. Finally, conclusions are presented. Since there is no a fit-for-all pretreatment solution to any sort of lignocellulosic material, it is expected that this study builds up a state-of-art regarding sugarcane bagasse as a raw material to produce second-generation bioethanol in Colombia to help to reduce the current 8% GHG emitted by the transportation sector in Colombia (United Nations,

2015).

## 2. Materials and methods

### 2.1. Raw material

Sugarcane bagasse was obtained from a commercial farm located in the municipality of Alvarado (Tolima, Colombia), located at 439 masl, 26 °C mean annual temperature, and 70% mean annual relative humidity. The raw material was dried in a forced convection dryer (Thermolab TH 53, DIES, Colombia) at 70 °C for 48 h until constant weight was obtained. The dried sugarcane bagasse, with a moisture content of  $10 \pm 0.2\%$  (dry basis), were then ground in a hammer mill (Model 4 Thomas-Wiley Laboratory Mill, Thomas Scientific, USA) to reduce particle size to pass a 2 mm screen. The resulting material was stored at 4 °C for 3 days prior to use.

### 2.2. Pretreatments

#### 2.2.1. Steam Explosion (SE)

The SE pretreatment was based on the method proposed by Viola et al. (2013). Samples of sugarcane bagasse were placed in a laboratory bioreactor (ITEMSA, Colombia) with saturated steam supplied from a fire-tube boiler of 2 BHP and flow of 69 lb/h (Equipos y Calderas Industriales EU, Colombia). SE pretreatments were performed at 100 psi over different residence times (15, 30 and 60 min). Afterwards, the pretreated material was filtered and washed out with distilled water to remove inhibitory substances along with the water-soluble hemicellulose. The resulting liquid fraction was stored at 4 °C for 1 h to be used for determination of total carbohydrates (TC) and reducing sugars (RS). The solid residue remaining after filtration was dried (at 70 °C for 18 h) to be used in the enzymatic hydrolysis.

#### 2.2.2. Acid hydrolysis (AH)

The AH was carried out based on the method described by Jackson de Moraes Rocha et al. (2011). Samples of sugarcane bagasse were mixed with different sulphuric acid solutions (2.5%, 5% and 10%) in a sample/solution ratio of 1:30 (w/v) and submitted to AH at 100 °C for 60 min. As in the SE pretreatment, each hydrolysate was filtered and washed. Liquid fractions were used for determination of total carbohydrates and reducing sugars, and solid fractions were dried and used in the enzymatic hydrolysis.

### 2.3. Effects of pretreatments on lignocellulosic biomass (sugarcane bagasse)

#### 2.3.1. Total carbohydrate determination

The total carbohydrates of the lignocellulosic material were quantified before and after the pretreatments were completed (SE and AH). The contents of TC were measured spectrophotometrically at 620 nm (Helios gamma spectrophotometer, Thermo Fisher Scientific, Waltham, USA) according to the anthrone-sulfuric acid methodology (Hackmann et al., 2013; Leyva et al., 2008).

#### 2.3.2. Determination of reducing sugars

The liquid fractions resulting from enzymatic hydrolysis and fermentation (0–48 h) was quantified spectrophotometrically at a wavelength of 540 nm through the DNS (glucose) method with a Helios gamma spectrophotometer (Thermo Fisher Scientific, Waltham, USA) (Ghose, 1987; Miller, 1959; Saqib and Whitney, 2011).

#### 2.3.3. Characterization of Lignocellulosic biomass

The biomass was submitted to an acid detergent fiber (ADF), neutral detergent fiber (NDF) and acid detergent lignin (ADL) tests to determine the contents of cellulose, hemicellulose and lignin using a modified method described by Van Soest et al. (1991).

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