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Production of fermentable sugars from sugarcane bagasse by enzymatic hydrolysis after autohydrolysis and mechanical refining

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HIGHLIGHTS

- Autohydrolysis conditions affect the chemical composition of pretreated bagasse.
- The pretreatment severity affects the enzymatic hydrolysis efficiency.
- Sugar yield of sugarcane bagasse was improved after mechanical refining.
- 87% of carbohydrate in the raw material could be converted into fermentable sugars.

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ABSTRACT

The autohydrolysis process has been considered a simple, low-cost and environmental friendly technology for generation of sugars from biomass. In order to improve accessibility of enzymes during enzymatic hydrolysis as well as to allow the recovery of hemicellulose in the filtrate, the sugarcane bagasse was pretreated using autohydrolysis followed by a mechanical refining process. The autohydrolysis was carried out in three different conditions. Autohydrolysis at 190 °C for 10 min provided the highest overall sugar (19.2/100 g raw bagasse) in prehydrolyzate. The enzymatic hydrolysis step was performed for all the post-treated solids with and without refining at enzyme loadings of 5 and 10 FPU/g for 96 h. A total of 84.4% of sugar can be recovered from sugarcane bagasse at 180 °C for 20 min with 5 FPU/g enzyme charge. The economic analysis for the proposed method showed that the bioethanol production can have a financial return larger than 12%.

1. Introduction

The negative impact of fossil fuels on the environment as well as the increased concern for the security of the oil supply has stimulated the search for renewable fuel alternatives. At present, the most common renewable fuel is ethanol produced from sugar or grain (starch); however, this raw material base by itself may not be sustainable to meet the worldwide needs for ethanol in the future. Consequently, future large-scale use of ethanol will greatly be based on production of ethanol from lignocellulosic materials. In addition, the bioethanol produced from lignocellulosic biomass is an attractive alternative since lignocellulosic raw materials do not compete with food crops and they are less expensive than conventional agricultural feedstocks (Rabelo et al., 2011).

Lignocellulosic biomass, mainly composed of cellulose, hemicellulose, and lignin, is the most abundant renewable resource available for the industrial production of fuel ethanol. Generally, the production of bioethanol from lignocellulosic biomass via biological process involves the following steps: (1) pretreatment either to remove lignin or hemicellulose to make the cellulose more accessible to enzymatic attack, (2) depolymerization of carbohydrate polymers to produce free sugars by cellulase mediated action, (3) fermentation of hexose and/or pentose sugars to produce ethanol, and (4) distillation of ethanol (Canilha et al., 2012).

The biomass pretreatment is the most important processing challenge in the production of biofuel. Pretreatment is required for removing the lignin and/or hemicelluloses, reducing cellulose crystallinity, and increasing the porosity of the material (Sarkar

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et al., 2012) to enhance the enzymatic susceptibility of the carbohydrates (Mosier et al., 2005). An effective pretreatment must preserve the utility of the hemicelluloses and avoid the formation of inhibitors (Margeot et al., 2009). An economical pretreatment should use inexpensive chemicals and require simple equipment and procedures (Margeot et al., 2009). In general, pretreatment technologies can be divided into different categories: physical (milling and grinding), physicochemical (steam pretreatment/autohydrolysis, hydrothermolysis, and wet oxidation), chemical (alkali, dilute acid, oxidizing agents, and organic solvents), biological, electrical, or a combination of these. The following pretreatment technologies have promise for cost-effective pretreatment of lignocellulosic biomass for biological conversion to fuels and chemicals (Kumar et al., 2009).

Autohydrolysis is one method often used for pretreatment of lignocellulosic materials. This method has been considered a simple, low-cost, and environmentally friendly pretreatment technology for generation of sugars from lignocellulosic materials (Garrote et al., 1999). The autohydrolysis process uses water and lignocellulosic feedstocks as the only reagents (Ertas et al., 2014) and occurs over a wide range of temperatures (130–230 °C) and pretreatment times (from a few seconds to several hours) (Saska and Ozer, 1995). The autohydrolysis pretreatment promotes hemicellulose depolymerization (mainly converted into soluble oligomers as a major reaction product) and lignin transformation due to the high temperature (Lee et al., 2009), which enhances the accessibility of enzymes to solid substrates during the subsequent enzymatic hydrolysis to mono-sugars (Mosier et al., 2005). In spite of the simplicity of autohydrolysis, it has not received much attention due to the low sugar recoveries at economical enzyme dosages.

Mechanical refining has been commonly used in the pulp and paper industry. The main effects of refining in fibers are internal and external fibrillation, fines formation, fiber shortening or cutting, and fiber curling or straightening (Gil et al., 2009). Those effects improve the enzyme accessibility of cellulose through the increase of surface area and reduction of particle size. It has been reported that the refining can significantly improve the enzymatic conversion of pretreated ligonocellulosics at low enzyme dosages (Koo et al., 2011; Han et al., 2014; Ertas et al., 2014). In this study autohydrolysis was combined with refining to improve the overall pretreatment step.

Lignocellulosic materials from different crop residues have been used for conversion to ethanol; among them, sugarcane bagasse is the most abundant lignocellulosic material in tropical countries (Rabelo et al., 2011). Brazil is the biggest producer of sugarcane in the world. It has been estimated that production will be around 652 million tons for sugarcane for 2014/2015. The sugarcane basically consists of stem and straw. The residual fraction from the sugarcane stem after juice extraction is named bagasse (Canilha et al., 2012). In general, 1 ton of sugarcane generates 280 kg of bagasse (Rabelo et al., 2011). Normally, sugarcane bagasse is used as the main source of the energy required in sugar mills and ethanol distilleries and also for generating electricity to be sold to the grids. Nevertheless, a significant portion of the produced bagasse is underexplored. It has been reported that upon the technological improvements made to the boilers it is possible to satisfy the energy requirements of the plants with only half of the produced bagasse. Due to the large capacity of this biomass as an industrial waste, there is a growing interest in developing biorefinery concepts and methods for the production of fuels and chemicals that offer economic, environmental, and strategic advantages (Rabelo et al., 2011).

The present paper deals with pretreatment and biological transformation of sugarcane bagasse into an added value product, emphasizing on fuel ethanol production. A simple process that consists of autohydrolysis followed by refining was proposed. The aim of this study was to assess the efficiency of autohydrolysis and refining pretreatments in the production of fermentable sugars from sugarcane bagasse.

2. Methods

2.1. Raw material

Sugarcane bagasse chips were provided by a sugarcane manufacturer located in the Brazilian Southeastern region. A fraction of the sugarcane bagasse chips were converted into sawdust, classified according to TAPPI T257-cm85 standard procedure, dried to 20% moisture, and stored in glass jars for compositional analysis.

2.2. Compositional analysis

The total extractives and ash content of original and pretreated raw materials were measured according to TAPPI T264 cm-97 and TAPPI T211 om-93. The moisture, ash, Klason lignin (acid–insoluble lignin), acid–soluble lignin, and acetyl group contents of original and pretreated raw materials were determined by National Renewable Energy Laboratory's (NREL) Laboratory Analytical Procedures (Ehrman, 1994, 1996; Templeton and Ehrman, 1994). For sugar analysis, 0.3 g samples were hydrolyzed with 3.0 mL of 72% (w/w) H_2SO_4 for 2 h at room temperature. Hydrolysates were diluted to 4% (w/w) H_2SO_4 with 84 mL deionized water (DI) and autoclaved for 1 h at 121 °C. Mono-sugars were analyzed by a HPLC system (Agilent 1200, Agilent, Santa Clara, CA), including a Shodex SP0810 column (8 × 300 mm, Showa Denko, Tokyo, Japan). All samples were eluted at 80 °C with Mili-Q water at a flow rate of 0.5 mL/min with peak detection using a refractive index detector, set at 35 °C. Before analysis, all the samples were filtered through a 0.20 μm nylon syringe filter (Millipore, Billerica, MA). Sugar contents were quantitatively determined by comparison with standard sugars. The 4-O-methylglucuronic acid was measured according to Scott, 1979. All experiments were duplicated.

2.3. Autohydrolysis pretreatment

Three different autohydrolysis conditions were studied: 180 °C for 20 min, 180 °C for 40 min, and 190 °C for 10 min. The pretreatments were carried out in a 1.0 L alloy C-276 reactor (Parr Instruments Company, Moline, IL). For each batch cook, 50 g of oven dry sugarcane bagasse samples were placed in the reactor and supplemented with the proper amount of deionized water in order to set a water to solid ratio of 10:1. The autohydrolysis process was quantify by severity factor. This factor was calculated by equation below (Overend and Chornet, 1987):

$$\text{severity factor} = \log_{10}[t_1 \times \exp(T_1 - 100/14.75)]$$

where t_1 and T_1 are the pretreatment time (min) and temperature (°C), respectively. The value of 14.75 is an empirical parameter related to temperature and activation energy. The values of severity factor are shown in Table 2. This express the influence of temperature and time on autohydrolysis process.

After the autohydrolysis stage was completed, the reactor was cooled to room temperature with running tap water, and pretreated samples were filtered through cheese cloth. After the filtration, filtrate was collected in a plastic vial and stored in a refrigerator at 4 °C for pH, sugar, and byproduct analyses. Monosugars of the separated filtrate were analyzed by the HPLC system after acid hydrolysis of samples by using 4% (w/w) H_2SO_4 for 1 h at 121 °C. The filtrates were filtered through a 0.20 μm nylon syringe filter prior to analysis. All autohydrolysis pretreatments were performed in duplicate. The remaining solid residues were washed, approximately, to neutral pH. After that, the solid residues were centrifuged to achieve relatively uniform moisture content. The