Industrial Crops and Products 51 (2013) 7–18

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Evaluation of bagasse from different varieties of sugarcane by dilute acid pretreatment and enzymatic hydrolysis

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ARTICLE INFO

Article history:
Received 19 February 2013
Received in revised form 19 August 2013
Accepted 20 August 2013

Keywords:
Sugarcane bagasse
Classical breeding
Precision breeding
Pretreatment
Enzymatic hydrolysis

ABSTRACT

Lignocellulosic ethanol is a promising alternative to gasoline that can be produced by fermentation of sugars present in lignocellulosic biomass. Improved properties of energy crops and reduction of lignocellulose recalcitrance to biological conversion have the potential to reduce production costs. This study evaluated bagasse from 115 varieties of sugarcane for fermentable sugar yield. The purpose was to select the preferred varieties with fiber of high processability without compromising juice ethanol and cane yield. Dilute acid pretreatment was employed to improve the sugars yield from the bagasse. The results showed wide variations in structural carbohydrates (as monosaccharide) content (66.6–77.6% dry matter (DM)) and lignin content (14.4–23.1% DM) between varieties. Combined sugar yield obtained after pretreatment and enzymatic hydrolysis also varied significantly (27.3–55.2 g/100 g DM). Further, it was demonstrated that some of the varieties had combined characteristics of high cane productivity and combined sugar yield after pretreatment-hydrolysis of the bagasse. These results suggest the incorporation of selection of varieties, given its contribution for developing a cost-efficient pretreatment and saccharification process.

1. Introduction

Sugarcane is one of the preferred crops for ethanol production due to high biomass yields and high fermentable sugar content (Somerville et al., 2010). However, the fibrous residue (bagasse) generated after sucrose extraction for ethanol production may also be used to increase the ethanol yield per ton of harvested cane. The positive utilization of this abundant residue will bring a breakthrough to a complete utilization of the whole crop for ethanol production.

Like other agricultural residues, sugarcane bagasse (SB) is mainly composed of cellulose, hemicellulose and lignin (Cardona et al., 2010). Cellulose is a homopolymer composed of glucose molecules with the major part bounded by hydrogen bonds and forms a crystalline microfibril structure (Klemm et al., 2005). Hemicellulose is an amorphous polymer, mainly composed of xylose and arabinose monomers (Lavarack et al., 2002). Hemicellulose is linked to cellulose and lignin by covalent bonds and fewer hydrogen bonds. Lignin acts like a glue and bind cellulose and hemicellulose, which in turn makes structure more moisture resistant and recalcitrant to biological degradation. Due to this matrix structure, it is difficult for the enzymes to access cellulose if the material is in a native form (Cardona et al., 2010; Sun and Cheng, 2002). Employing a pretreatment process is an efficient way of reducing natural recalcitrance of the lignocellulose cell wall. In this process, the matrix structure is altered to increase the accessibility prior to enzymatic hydrolysis (Alvira et al., 2010; Wyman, 2007). Several pretreatment options have been actively researched (Alvira et al., 2010; Cardona et al., 2010; Wyman, 2007). Among these methods, dilute acid has been studied extensively since it satisfies most of the requirements of the pretreatment process (Sun and Cheng, 2002). The fundamental concept of the dilute acid pretreatment is based on the solubilization of the hemicellulose, thereby increasing the cellulose accessibility by enzymes (Taherzadeh and Karimi, 2007).

Although pretreatment can significantly improve the cellulose accessibility it still remains a limiting factor in industrial application, due to the cost of processing However, the cost of pretreatment could be reduced by breeding or selection of lignocellulose feedstocks that are more easily hydrolysable (possibly low lignin content) and with high structural carbohydrates content, without compromising other important agronomic characteristics such as high biomass yields and high sucrose/grain yields.

Previous studies have reported on reduction of the lignin content by breeding new varieties, as a way of diminishing the recalcitrance of the lignocellulose feedstock (Dien et al., 2009; Li et al., 2011). A study on grain yield and stover quality for cellulose ethanol of test crosses of 223 × Mo17 inbred of maize (*Zea mays L.*) has proven that the corn breeding programs are able to improve the stover digestibility without adversely affecting for the grain yield and agronomic traits (Lewis et al., 2010). Working on 79

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http://dx.doi.org/10.1016/j.indcrop.2013.08.067

wheat straw samples, Lindedam et al. (2012) found that ethanol yield estimated from sugar released after the pretreatment and enzymatic hydrolysis varied from 161 to 203 l per ton dry straw between cultivars. The sugar yield showed a strong negative correlation with lignin as well as ash contents, and was positively correlated with structural carbohydrates. These findings are also relevant during breeding and selection of sugarcane for biorefinery. Masarin et al. (2011) showed a strong negative correlation between enzymatic digestibility of sugarcane bagasse from and lignin content, thus favoring sugarcane clones with reduced lignin content. However, what has not been known is the selection criteria used during screening to reach to eleven clones and also the correlation between sugar yields and other chemical components such as structural carbohydrates and ash.

For many years, sugarcane breeding program at South Africa Sugarcane Research Institute (SASRI) has been focusing on only how to increase the sucrose content per unit biomass (Bekker, 2007). With the recent knowledge of producing ethanol from lignocellulose materials, it is also equally important to increase both total fermentable sugars and fiber yields per hectare, to maximize energy production per land used. Following recent developments, various research initiatives have been developed to find a way of enhancing ethanol production from SB. One of these initiatives is the use classical and precision breeding (genetic engineering) technologies to produce sugarcane with preferred fiber characteristics, such as higher biomass yields per hectare and physico-chemical properties that are more amenable to hydrolysis, which will significantly reduce the lignocellulosic ethanol production costs.

In this study, bagasse of one hundred and fifteen varieties of sugarcane developed by classical and precision breeding technologies were evaluated in terms of fiber compositions and fermentable sugar yields from dilute acid pretreatment and enzymatic hydrolysis. The influence of chemical composition and variety type in pretreatment and enzymatic hydrolysis were also evaluated.

2. Materials and methods

2.1. Raw material and samples preparation

One hundred and fifteen samples of bagasse from different sugarcane varieties were provided by SASRI. The feed stocks were sampled from mature sugarcane (12 months old) in an experimental field located at Mount Edgecombe (29.7000° S and 31.0333° E), KwaZulu-Natal. Fifty six varieties had a South African origin and the rest (59) were imported from USA, Barbados, Australia, India and Reunion. The local and imported varieties are shown in Table 1. Numbers enclosed in parenthesis were used for varieties identification from the two breeding technologies, labels 1–100 represented classical breeding varieties and the remaining varieties (labeled 101–115) were from precision breeding, as reported previously (Bekker, 2007). The international and local genotypes were first planted in field trials in 2002 and 2006 respectively. This means that the bagasse evaluated in this study were from 3rd ratoon for the local crops and 7th ratoon for the international crops. The plants were rain fed and no fertilizer was applied.

Twenty to thirty of cane stalks (not less than 6 kg) from each variety were randomly cut from the experimental field. The stacks were shredded and then blended with water (1.5 kg of sample and 3 l of water) for 20 min. Thereafter, the finely crushed shredded canes from the blending jar were washed with water three times and each wash was collected and measured for residue sucrose and other soluble sugars. The remained fiber was pressed to reduce water content and finally it was dried at 40 °C for four days until dry. The average moisture content of the materials after drying was about 6%. Prior to its use, the milled SB was sieved to obtain a representative particle size suitable for the raw material composition analysis and for the pretreatment studies. The particles retained between 425 and 825 μm were packed in zipped plastic bags. The prepared samples were stored in a temperature and moisture controlled room set at 20 °C and relative humidity of 65% until needed. The total storage time of the samples was 12 months.

2.2. Dilute sulphuric acid pretreatment

Dilute sulphuric acid pretreatment was carried out in a small tubular reactor (18 cm long and 1.27 cm internal diameter), according to Yang and Wyman (2009). 1.5 g Dry Material (DM) was soaked in 30 ml of dilute sulphuric acid solution or water for 12 h. Soaked samples were concentrated through filtering to a solid loading of 30% (w/v). The obtained wet biomass was loaded into the reactor and compressed by a metal rod to ensure uniform heat and mass transfer. The reactor was first submerged into a heating-up fluidized sand bath set at 30 °C above the target temperature. The reactor was heated until the target temperature was reached (approximately within 120 s), after which it was transferred into the second fluidized sand bath set at the target reaction temperature. After the reaction time was completed, the reactor was quenched by submerging into cold water bath. After cooling, the whole slurry was mixed with 100 ml of distilled water and vacuum-filtered into a solid and a liquid fraction. The solid fraction was further washed in three washes (each wash with 100 ml) to raise the pH up to 5 prior to enzymatic hydrolysis, and is subsequently referred to as Water Insoluble Solids (WIS). One part of filtrate was analyzed for monomeric sugars content and the other part was used to determine the total sugars in the pretreated liquor as monomers and oligomers by post-hydrolysis as described elsewhere (Jacobsen and Wyman, 2002). All pretreatments were performed on duplicate and average results are shown.

The pretreatment study was done in two phases. In the first phase, all 115 SB varieties were pretreated at 180 °C, 0.5% (w/w) acid for 15 min. The temperature and residence time were selected from Diedericks (2013). In the second phase, 34 varieties were selected and pretreated at four different pretreatment conditions based on the preliminary study performed on one variety: (150 °C, 0.96%, w/w acid for 15 min); (160 °C, 0.96%, w/w acid for 15 min); (190 °C, 0.07%, w/w acid for 15 min); (200 °C, no-acid for 10 min). The pretreatment severities used in this study were those reported by others (Canilha et al., 2011; Jacobsen and Wyman, 2002; Neureiter et al., 2002; Um and Bae, 2011). The condition used in the first phase (180 °C, 0.5%, w/w acid for 15 min) was repeated to check the effect of storage time on the sugar yield.

2.3. Enzymatic hydrolysis

The WIS fraction was subjected to enzymatic hydrolysis to evaluate the effect of pretreatment on the enzyme accessibility for each of SB. These experiments were conducted in 24 ml glass tubes. The tubes were loaded with 200 mg (dry weight) of WIS and 10 ml of 0.05 M citrate buffer (pH 4.8) with the enzyme solution. Sodium azide was added at a concentration of 0.02% (w/v) to prevent microbial contamination. Two commercial enzymes preparations were used: Spezyme CP (Genencor-Danisco, Denmark) with protein concentration of 140 mg/ml (cellulase activity of 65 FPU/ml) and Novozym 188 (Novozymes A/S, Denmark) with protein concentration of 95 mg/ml (β-glucosidase activity of 700 IU/ml). Protein concentration and activities of undiluted enzymes (Spezyme and Novozym 188) were determined by applying analysis protocol