



Comparison of chemical composition and calculated ethanol yields of sugarcane varieties harvested for two growing seasons



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ABSTRACT

In this study the agronomic properties, chemical composition, sugar yield, and calculated combined ethanol yield from different varieties of sugarcane were compared for two different harvest seasons. Results showed wide variations in agronomic parameters (cane yield, juice sugar and fiber content), chemical composition and sugar released after pretreatment-hydrolysis of bagasse, and calculated combined ethanol yield. Calculated combined ethanol yields ranged from 1695 to 15,975 L/ha. Classical breeding varieties showed improved biomass yield per hectare. Precision breeding varieties for improving sucrose content largely failed during growth season with sub-optimal rain fall. Prolonged drought also affected the performance of all varieties represented by lower and intermediate lignin content for cane yield compared to that had highest lignin content. Therefore, an attempt to reduce lignin content in the bagasse to reduce processing costs for ethanol production should also target improved crop tolerance toward drought.

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1. Introduction

Sugarcane (*Saccharum* spp. hybrids) is a major bio-energy crop planted in tropical and subtropical countries. Its main distinguishing features include high biomass yield, high sucrose content (Somerville et al., 2010), high efficiency in assimilating solar energy (Tammisola, 2010), and high water requirements in terms of liters per kilogram of aerial biomass comparable to many other annual grain or sugar crops (Tammisola, 2010). Sugarcane has been used for ethanol production, with Brazil leading production (Goldemberg, 2008). During the harvest of sugarcane, leaves, tops and trash are left in cane field, while the sugarcane stalks are transported to the mills, crushed to extract juice (sugar juice) for sugar and ethanol production (Gullett et al., 2006). Along with the fibrous residue (bagasse) following juice extraction, the left over harvest residue (leaves, tops and trash) have potential to be converted to ethanol in the “whole plant” conversion strategy. However, bagasse and harvest residues differ in physical nature and process requirements (Ferreira-Leitão et al., 2010; Krishnan et al., 2010). These differences in the process requirements, suggest that separation of

the two residues is important during processing for optimal ethanol production. Therefore, this study was limited to bagasse.

Presently in most of the sugar industries only sugar syrup is fermented to ethanol. Converting bagasse to ethanol requires specialized enzymatic hydrolysis and fermentation, but pretreatment is required to further increase ethanol production from sugarcane per unit per unit land (Wyman, 2007). However, prior to enzymatic hydrolysis of the residues, a costly pretreatment process is required to make its structural carbohydrates accessible to enzymes (Sun and Cheng, 2002). The chemical composition of bagasse determines in part the quality of the feedstock (Masarin et al., 2011). Enrichment for structural carbohydrates content (cellulose and hemicellulose) and low lignin content is favorable for ethanol production, while high lignin content impedes ethanol fermentation because lignin can reduce ethanol yield (Isci et al., 2008).

Available moisture, soil, fertilizers, supplementary irrigation, diseases, and temperature are the factors that can affect the agronomic properties of sugarcane (cane yield, sucrose content and content of fibrous components). These factors have direct impact on the combined ethanol yield from sugar juice and bagasse (Basnayake et al., 2012; Patel, 1985; Singels et al., 2011; Tammisola, 2010; Waclawovsky et al., 2010).

Moreover, chemical compositions of the bagasse from different sugarcane varieties may vary depending on genotype (Masarin et al., 2011). Studies on other types of energy feedstocks such as

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sweet sorghum (Zhao et al., 2009), switchgrass (Kim et al., 2011) and winter triticale (Kučerová, 2007) have shown that the chemical composition of the biomass can vary depending on genotype, location, year, age of crop, harvests, environmental and cultivation parameters. However, limited information is available on the variability in agronomic properties combined with chemical composition of bagasse and combined ethanol yield of sugarcane varieties from different harvests. The variation in properties between harvests should be considered in feedstock (genotype) selection for combined ethanol production from both sugar juice and bagasse.

To identify novel varieties of sugarcane with improved properties for combined ethanol production from sugar juice and bagasse, 115 varieties from the breeding program at South Africa Sugarcane Research Institute (SASRI) were screened. Of these samples 100 varieties originated from classical breeding and 15 were from precision breeding (genetic engineering). These varieties were screened in terms of potential ethanol yield per hectare from both, sugar juice and bagasse, and bagasse conversion, as reported previously (Benjamin et al., 2013), including optimization of pretreatment conditions to maximize fermentable sugar yields from bagasse (Benjamin et al., 2014a). In the present study, preferred varieties were compared using sugarcane materials from different harvest years, to assess the impact of seasonal variations on the combined ethanol yield, from sugar juice and bagasse. Ethanol yields from sugar juice were estimated from soluble sugar content in the juice based on literature, while ethanol yields from bagasse were determined by pretreatment-hydrolysis-fermentation experiments. Such multi-harvest assessment of raw materials is expected to identify the most promising variety for ethanol production from the sugarcane.

2. Materials and methods

2.1. Raw material and samples preparation

The sugarcane varieties used in the present study were developed by South African Sugarcane Research Institute (SASRI) through classical and precision breeding. The precision breeding varieties aimed for increasing sucrose, were developed by down-regulating the expression of an endogenous enzyme UDP glucose dehydrogenase as described elsewhere (Bekker, 2007). The classical breeding varieties were developed through traditional method of cross breeding for increasing biomass yielding. The experimental field trial was conducted at the SASRI, Mount Edgecombe, KwaZulu Natal (latitude: 29.7000° S; longitude: 31.0333° E) site. The varieties were of South Africa origin and were first planted in field in 2006. This means that the varieties evaluated in this study were from 3rd and 5th ratoon crops. Clones were planted in 35 m² plots with 3 replications, with a row spacing of 1 m and plants to plant spacing of 0.5 m. The crop was grown under rainfed conditions throughout the experiment. In total, six varieties were included in the study, where varieties 99F2004 (55), 00F0884 (70) and 01G1662 (74) were derived from classical breeding and 05TG004 (101), 05TG008 (104) and 05TG018 (114) from precision breeding. The numbers in parenthesis 55, 70, 74, 101, 104 and 114 will be used to describe and discuss the varieties further in the manuscript.

Twenty stalks per variety per plot were cut from the experimental field at the time of harvest and used to determine cane yield, juice sugar content (sucrose, glucose and fructose), fiber contents and others important measurements as described by standard mill-room analysis (Anonymous, 2009).

For chemical characterization and pretreatment-hydrolysis study of the bagasse, 20–30 stalks were used. The stalks were shredded using a mechanical shredder/disintegrator (locally

manufactured) and then blended with water (1.5 kg of sample and 3 L of water) for 20 min using a mixer (locally manufactured). 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 remaining fiber was pressed to reduce water content and dried at 40 °C for four days. The average moisture content of the materials after drying was about 6%. Prior to its use, the milled (shredded + blended) sugarcane bagasse was sieved to obtain a representative particle size suitable for raw material composition analysis and 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 with a relative humidity of 65% up until processed.

2.2. Dilute sulphuric acid pretreatment of bagasse

Dilute H₂SO₄ pretreatment of bagasse samples was performed in a tubular reactor (22.8 mL-volume, and constructed out of Hastelloy C276 tubes, capped by Swagelok fittings). Heat was provided through the immersion in two 4 kW fluidized sand bath (Model SBL-2D, Techne Co., Minneapolis, MN), each coupled with a temperature controller (Model TC-8D, Techne, Minneapolis, MN).

The reactor was loaded with pre-soaked material containing 1.5 g (dry weight) of bagasse and 5 mL acid solution (0.45 and 0.65% (w/w)). The wet material was compressed by a metal rod to ensure uniform heat and mass transfer. The compression also assured that approximately two third of the reactor volume was unfilled with material. The reactor was submerged into a heating-up fluidized sand bath set at 30 °C above the target temperature, of which were 170, 180 and 190 °C. Once the target temperature was reached (approximately within 120 s), the reactor was transferred into the second fluidized sand bath set at the target reaction temperature. The pretreatment times were 5, 10 and 15 min. The reaction was stopped by submerging the reactor into cold water bath.

After pretreatment, the solid material was separated by centrifugation and 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). 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 solid (WIS) fraction. The wet WIS was packed in plastic and stored in a freezer until was used for enzymatic hydrolysis.

2.3. Enzymatic hydrolysis of pretreated bagasse

The WIS fractions of pretreated bagasse samples were subjected to enzymatic hydrolysis to evaluate the effect of pretreatment on enzyme accessibility. These experiments were conducted in 24 mL glass tubes. The tubes were loaded with 200 mg (dry weight) of wet WIS and 0.05 M citrate buffer (pH 4.8) with the enzyme solution was added to attain the final solid loading of 2% (w/v). 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 cellulase activity of 65 FPU/mL and Novozym 188 (Novozymes A/S, Denmark) with β-glucosidase activity of 995 IU/mL. Enzyme activities of both undiluted enzymes were determined according to García-Aparicio et al. (2010). Cellulase loading of 15 FPU/g WIS of Spezyme CP supplemented with β-glucosidase of 15 IU/g WIS was applied in all the experiments. Tubes loaded with the mixtures were placed in water bath shaker maintained at 50 °C and shaken at 90 rpm. Samples were withdrawn after 72 h, prepared as described below and

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