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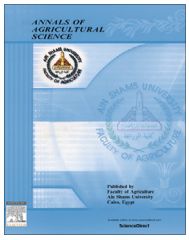

Evaluation of bioethanol production from juice and bagasse of some sweet sorghum varieties

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Abstract Sweet sorghum, with sugar-rich stalks and water-use efficiency, has a very good potential as an alternative feedstock for ethanol and also non-competing with human feed on land. The present study evaluates the exploitation of juice and bagasse of five varieties of sweet sorghum for bioethanol production which can further improve the energy yield of the crop. The sweet sorghum varieties, GK-coba, Mn-1054, Ramada, Mn-4508 and SS-301, were analyzed for their productivity, and sugar and fiber contents. All varieties significantly differed in yield of stripped stalk, juice and bagasse. The sugar-rich juice and the fiber-rich bagasse, resulting from squeezing the striped stalks, were used for bioethanol production by two microorganisms; *Saccharomyces cerevisiae* ATCC 7754 and *Zymomonas mobilis* ATCC 29191. Stalks of varieties GK-coba, Mn-4508 and SS-301 contained high sugar contents and thus were utilized for bioethanol production directly from juice. Stalks of varieties Mn-1054, Ramada and SS-301 had higher content of fibers, so their bagasses were used for bioethanol production. Bagasse was pretreated and hydrolyzed thermo-chemically with 2% (v/v) sulfuric acid (98%) at 120 °C for 60 min and filtered and the sugar-rich filtrate was neutralized and supplemented with nutrients for bioethanol production. Fermentation of sweet sorghum sugars or acid-hydrolyzed neutralized bagasse into bioethanol was conducted by *Sacch. cerevisiae*, *Z. mobilis* or mixed-culture of both organisms at 1:1 ratio. The highest bioethanol production was obtained from juice and bagasse of variety SS-301, by the mixed-culture treatment. From the juice, bioethanol concentration was 50.26 mL L^{-1}, whereas from bagasse, bioethanol concentration was 10.5 mL L^{-1}. Finally, it could be estimated that 160 mL of bioethanol can be produced out of each 1 kg of variety SS-301, when using both juice and bagasse.

Introduction

As the world energy consumption is rapidly increasing, annual world production of crude oil is declining, predicted to reach 5 billion barrels in 2050 (Bajpai, 2013). Thus, it is a fateful interest to find non-petroleum-based alternative sources of energy

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that are clean, renewable and do not conflict with human feeding, and these criteria apply to biofuel.

As a promising type of biofuel, bioethanol exhibits several advantages, such as high octane number, high heat of vaporization and most importantly, reduction of greenhouse gas emissions. Bioethanol is made by microbial fermentation of sugars extracted from sugar-rich and starch-rich crops, or from non-food lignocellulosic biomass (Faraco, 2013).

In addition to the fact the average available cropland per capita worldwide has now diminished to less than 0.22 ha (IWMI, 2007), the use of edible crops, such as corn and sugarcane, for bioethanol production would create conflicts in the use of land, water, energy resources for either food or biofuel production (Pimentel et al., 2008). Thus, to be economic and competitive alternative fuel, bioethanol has to be produced either from non-edible crops or from low-cost biomass, viz lignocellulosic materials, as sustainable substrate, to eliminate the conflicts (Faraco, 2013).

As a promising crop for bioethanol production, sweet sorghum (*Sorghum bicolor* L. Moench) is characterized by high biomass yield and rich in carbohydrates. The stalk of sweet sorghum is squeezed, similar to sugarcane, releasing sweet juice with high levels of sugar (12–20%) composed mainly of sucrose, glucose, and fructose, good substrates for fermentation to ethanol, and leaves behind lignocellulosic biomass, the bagasse (Serna-Saldívar et al., 2012).

The bagasse has several potential uses; for bioethanol production (Zaldivar et al., 2001), for hydrogen and methane production (Antonopoulou et al., 2008), as fuel source for processing plant (Bennett and Anex, 2009) or as animal feed, having higher biological value for animals than sugarcane bagasse (Wu et al., 2010; Venkata et al., 2012). For each 10 tons of crushed sweet sorghum, 5–6 tons of wet bagasse can be obtained (Negro et al., 1999).

Cultivation of sweet sorghum requires relatively low nutrient inputs and last for short period of 3–5 months, allowing to fit into many double-crop management rotations and to be planted on fallow sugarcane land (at most 5% of total sugarcane area) for harvesting and processing before the start of the sugarcane planting season (Woods, 2000). More interestingly, sweet sorghum requires one-third, or less, of the water required by sugarcane (Almodares and Hadi, 2009). It is also drought resistant crop due to its capacity to remain dormant during the driest periods, and well adapted to grow in a wide variety of climates including tropical, subtropical, and arid regions (Reddy et al., 2005). These properties entitle sweet sorghum to be a promising and competitive crop for bioethanol production and industry.

Worldwide production of sorghum in 2009 reached 56 million tons of grain, ranking it as the fifth most widely grown cereal crop in the world, behind maize, wheat, rice and barley (Serna-Saldívar et al., 2012) The largest region cultivated with sorghum is in sub-Saharan Africa and India, where it is a staple crop, providing food, feed grain and forage, and is even used in industry as a fuel source (Kassam et al., 2012). In Egypt, sorghum is widely cultivated in Upper Egypt with area reaching to 384 thousand Feddan[1] in 2002 (Ahmed et al., 2010).

New sweet sorghum varieties are being developed for bioenergy, where the current bioethanol production is estimated to be 760 L/ha from grain, 1400 L/ha from stalk juice and 1000 L/ha from the residues (Reddy et al., 2005). Some hybrids are especially suited tropical regions where drought and crop rotation restrictions limit sugarcane cultivation. Research in India has tested and used these hybrids for bioethanol production (Zhao et al., 2009).

Conversion of lignocellulosic biomass to bioethanol involves consequent steps. The biomass is dried and ground to fine size particles for better hydrolysis. Following step is delignification; breaking lignin layer of the ground biomass to expose cellulose, which is done either thermo-chemically, using high heat, or steam explosion, combined with alkali or dilute acid, or biologically, using fungi such as *Pleurotus ostreatus* and *Phanerochaete chrysosporium* (Kerem et al., 1992). The released cellulose is hydrolyzed to sugars (saccharification) which are fermented by certain microorganisms, such as *Sacch. cerevisiae* and *Zymomonas mobilis*, to bioethanol (Faraco, 2013).

El-Tayeb et al. (2012) treated rice straw, corn stalks, sugar beet waste and sugarcane bagasse with H_3PO_4, HCl or H_2SO_4 at 1.0–5.0% (v/v) for 15 to 120 min at 120 °C and found that increasing acid concentration from 1% to 5% decreased the conversion % of the above tested biomass. The fungus *Trichoderma viride* was also found capable of lignin removal of the same above-mentioned biomass, but required longer retention times than thermo-chemical method (El-Tayeb et al., 2014). Treatment with gamma irradiation, combined with dilute acid hydrolysis, was also used for sugarcane bagasse and potato peels, giving higher concentrations of fermentable sugars than using dilute acid alone (Abdelhafez et al., 2015).

Therefore, the current study aimed to evaluate the efficiency of bioethanol production from five varieties of sweet sorghum juice and bagasse. The five varieties were compared on the basis of their sugar and bagasse yields per feddan, and sugar and fiber contents. The effect of using *Saccharomyces cerevisiae* ATCC 7754 and *Z. mobilis* ATCC 29191 microorganisms, either individually or in mixed culture on bioethanol yield was also studied.

Material and methods

Sorghum varieties and cultivation

Five varieties of sweet sorghum (*Sorghum bicolor*, L., Moench), namely GK-Coba, Mn 1054, Ramada, Mn4508 and SS-301 were obtained from Sugar Crops Research Institute (SCRI), Agricultural Research Centre (ARC), Giza, Egypt. These varieties were planted and harvested and their stalk juice was extracted at Agricultural Research Station, Giza governorate, Egypt, during the summer season of 2013. Sowing started at the 1st week of June and the crop was harvested 120 days later, the dough stage, which is considered suitable stage to give high juice quality.

Productivity of sweet sorghum varieties

Samples of twenty stalks were taken at random from each variety, stripped and cleaned. For stalk juice extraction, stripped stalks of sweet sorghum were passed through three roller mill.

[1] Feddan (fed) is a unit of land area used in Egypt, Sudan and Oman, 1 fed = 4200 m^2 = 0.42 ha = 1.038 acres.

The raw juice was screened though layers of clean cheesecloth to remove the large pieces of suspended matter (A.O.A.C., 2005).

Gross yields per feddan for stripped stalks and extracted juice were calculated. Bagasse gross yield/fed was calculated by the following equation (A.O.A.C., 2005):

Wet bagasse yield ton/fed = Stripped stalks yield ton/fed – juice yield ton/fed

Quantitative analysis of sweet sorghum juice

Total soluble solids (TSS%) in the sorghum juice were determined by Brix hydrometer standardized at 20 °C, as described by Plews (1970).

Juice sugars were determined according to Dolciotti et al. (1998) and Long et al. (2006) using HPLC (Knauer, Germany) equipped with two pumps, RI detector, UV detector, column oven and operated by Clarity-Chrom Software as described in the following steps: 5 g sample was dissolved in 12 mL methanol (HPLC grade), quantitatively transferred to 50 mL measuring flask, filled up to the mark with HPLC grade water, sonicated for 20 min, filtered through PTFE filter (0.2 mm) and stored at 0 °C until analysis. The flow rate was adjusted at 2 mL/min, and the column was Luna NH_2 column for carbohydrates analysis. The column oven temperature was kept constant at 40 °C, the RI detector operated at room temperature and the mobile phase was Acetonitrile: HPLC grade (80/20, v/v).

Quantitative analysis of sweet sorghum bagasse

For moisture content determination of bagasse, 5 g of fresh bagasse was dried in oven at 105 °C until a constant weight is reached and left to cooling in a desiccator and moisture content was calculated.

Determination of bagasse crude fiber was conducted according to A.O.A.C. (2005) as follows: two grams of ground sample was mixed with 200 mL sulfuric acid (1.25%, w/v) and the mixture was boiled under reflux condenser for 30 min, filtered through a gooch crucible provided with asbestos mat and thoroughly washed with hot distilled water. The residue and the asbestos were boiled with aqueous sodium hydroxide solution (200 mL, 1.25% w/v) for 30 min, then filtered through a gooch crucible as the previous step. The residue was washed with distilled water followed by ethyl alcohol and acetone, then dried at 100 °C to a constant weight. The ash content was determined and subtracted from the dry weight to calculate the fiber content.

Determination of bagasse fiber fractions (cellulose, hemicelluloses and lignin) in dried sweet sorghum bagasse was conducted according to Georging and Van Soest (1975) where samples were analyzed to acid-detergent fiber fraction (ADF), neutral detergent fiber fraction (NDF) and acid-detergent lignin (ADL). Calculations were done as follows:

Cellulose was determined as weight loss of ADF upon extraction with 72% H_2SO_4.

NDF (neutral detergent fiber) = Cellulose + hemicellulose + lignin.

ADF (acid detergent fiber) = Cellulose + lignin

Hemicelluloses = NDF – ADF.

Bioethanol-producing microorganisms and their media

Saccharomyces cerevisiae ATCC 7754 was obtained from the Department of Microbiology, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, and *Zymomonas mobilis* ATCC 29191 was obtained from Microbiological Resources Center (Cairo MIRCEN), Cairo, Egypt.

YM broth medium (Wickerham, 1946) was used for propagation of *Sacch. cerevisiae* and ATCC 948 broth medium (Swings and Deley, 1977) for *Z. mobilis*. YM medium consists of the following ingredients (g L^{-1}): glucose 10, peptone 5, malt extract 3, and yeast extract 3. Medium pH was adjusted to 6 ± 0.2. ATCC 948 medium consists of the following ingredients (g L^{-1}): glucose 20, yeast extract 5 and the pH was adjusted to 6.5 ± 0.2. For solid medium, 15 g of agar was added to each liter of the medium.

Bagasse pretreatment for bioethanol production

The aim of this experiment is to further improve the energy yield of sweet sorghum by producing bioethanol from the sorghum bagasse; the lignocellulosic residues remain after sugar extraction from sorghum stalks. Bioethanol production from these residues consisted of two main stages: (1) pretreatment of bagasse and (2) bioethanol production (fermentation). Bagasse pretreatment, according to Abdelhafez et al. (2014), was carried out by dilute acid hydrolysis and then adjusted at pH 5.5 ± 0.2. Dilute acid hydrolysis was performed by adding 5 g of sweet sorghum bagasse to 250 mL Erlenmeyer flask containing 95 mL of 2% (v/v) of sulfuric acid (98%) or 95 mL of tap water, and pH was 6.7 ± 0.2 (the control treatment). Hydrolysis was run at 120 °C for 60 min (Pattana et al., 2010). The pretreated bagasse was left to cool then filtered to remove the solid fraction and the sugar-rich liquid filtrate was neutralized, as follows: the pH of the separated hydrolyzate was adjusted to 5.8 in two steps, first by NaOH pellets to pH of 3 and second by NH_3 solution (33%) to pH of 5.5. Bioethanol production was performed by inoculating the neutralized pretreated bagasse with *Sacch. cerevisiae* and *Z. mobilis* to ferment released sugars into ethanol.

Determination of total sugars in bagasse hydrolyzate

In a test tube, 0.5 mL of hydrolyzate bagasse was mixed with 1 mL of phenol solution (2% w/v) followed by addition of 2.5 mL sulfuric acid (98%). Tubes were left in dark for 10 min and then cooled to 25 °C for 30 min. Absorbance was measured at 490 nm using spectrophotometer (Shimadzu UV-1601). Distilled water was used as a blank. A standard curve was prepared under similar set of conditions using standard solutions of glucose (Dubois et al., 1956; Pak and Simon, 2004).

Bioethanol production

For bioethanol production from stalks juice, 100 mL of juice was supplemented with the following nutrients (g L^{-1}): KH_2PO_4, 2; $MgSO_4.7H_2O$, 1; and $(NH_4)_2SO_4$, 1 for *Z. mobilis* (Davis et al., 2006) or yeast extract, 3; peptone, 3.5; KH_2PO_4, 2; $MgSO_4.7H_2O$, 1; and $(NH_4)_2SO_4$, 1 for *Sacch. cerevisiae* (Arapoglou et al., 2010), then autoclaved at 121 °C for 20 min. Flasks containing 95 mL of nutrients-supplemented

juice were inoculated with 5 mL of 48 h old liquid seed cultures of *Sacch. cerevisiae*, *Z. mobilis* or mixed-cultures of both organisms (at 1:1 ratio) and flasks were incubated in anaerobic incubator (Labconco Manufacturing Corp., USA) at 30 °C for 4 days. After incubation, bioethanol was extracted by transferring the grown culture to a rotary evaporator (R206D 2L–SENCO) and the apparatus was run for 10–20 min at 78.5 °C. The distillate was used to determine bioethanol concentration as described later. All tests were performed in triplicate.

For bioethanol production from sweet sorghum bagasse, neutralized hydrolyzates were supplemented as was done with the juice, then autoclaved at 121 °C for 20 min. Flasks containing 95 mL of nutrients-supplemented sterilized acid-hydrolyzates were inoculated and incubated, and bioethanol was extracted as described above.

Standard inoculum was prepared by inoculating test tubes containing 5 mL broth media of YM (for *Sacch. cerevisiae* cultivation) or ATCC 948 (for *Z. mobilis* cultivation) with a full loop of tested culture and incubated at 30 °C for 48 h. Flasks were incubated in anaerobic incubator (Labconco Manufacturing Corp., USA) at 30 °C for 4 days.

Bioethanol determination

Distillate obtained from rotary evaporator was used to determine bioethanol concentration colorimetrically using potassium dichromate method (Crowell and Ough, 1979) as follows: in a test tube containing 10 mL of acidic potassium dichromate reagent, 2 mL of distillated sample was added and mixed well. Tubes were caped and kept in a water bath at 60 °C for 20 min then cooled to room temperature. The absorption of the reaction mixture was measured at 600 nm by spectrophotometer (Shimadzu UV-1601). Blank consisted of 2 mL of distilled water mixed with 10 mL of potassium dichromate acidic reagent. This reagent was prepared by dissolving 34 g of potassium dichromate ($K_2Cr_2O_7$) in 400 mL of distilled water with 325 mL of sulfuric acid and by making up the volume to 1 L. A standard curve was prepared under similar conditions using standard solutions of ethanol in distilled water.

Statistical analysis

Represented data were expressed as mean of three replicates and statistically analyzed using one way analysis of variance (ANOVA). Differences between means were compared by Duncan's Multiple Range Test with $p > 0.05$ (Snedecor and Cochran, 1980).

Result and discussion

Productivity and quantitative analysis of sweet sorghum varieties

Data presented in Table 1 indicated that all varieties significantly differed in the yield of stripped stalk, juice, and bagasse and TSS%. Results showed that variety **Mn-1054** was the highest in stalk yield with 33.63 ton/fed, and its juice content and juice TSS% were 24% and 14%, respectively. These values are suitable to bioethanol production, since sugar content is a major factor to ensure the feasibility of the process. The highest juice yield is recorded in varieties **Mn-4508** and **SS-301**, being 11.80 and 10.30 ton/fed (28.10 and 24.52 ton/ha), respectively, given that SS-301 was the highest in TSS% with 20.2% value. Differences between cultivars in values of the investigated traits may be attributed largely to the genetic makeup of them. El-Geddawy et al. (2014) evaluated variety SS-301, in addition to other five sweet sorghum varieties, and reported that SS-301 has 21.4% TSS. In terms of bagasse yield, variety Mn-1054 has the highest bagasse yield (24.35 ton/fed) followed by Ramada and SS-301 with 21.27 and 20.73 ton/fed, respectively. High bagasse yield is favored when biomass is the primary target. This finding is in agreement with that of Negro et al. (1999) who stated that each ton of crushed sweet sorghum stalk produces 50–60% wet bagasse, depending on the genotype.

Table 2 illustrates total sugar and sugar fractionation for the five varieties of sweet sorghum. Data showed significant differences exist among the five sweet sorghum varieties in total sugar and their fraction of sugar content. Sweet sorghum variety SS-301 contained the highest values of total sugars (19.12%), and sucrose (17.63%) compared to the other four varieties. The percentage of glucose and fructose of varieties Mn-4508 and GK-coba juices are insignificantly different and also between SS-301 and Ramada. Moreover, the highest values of glucose and fructose% were recorded in GK-coba variety. Similar findings were reported by Abo-El-Wafa and Abo-El-Hamid (2001) and El-Geddawy et al. (2014) where the extracted juice of sweet sorghum variety SS-301 recorded total sugars and sucrose values of 19.3% and 13.95%, respec-

Table 1 Yield productivity and TSS juice percentages of five sweet sorghum varieties.

Sweet sorghum varieties	Yield productivity						Juice TSS %
	Strip stalk yield		Juice yield		Wet bagasse yield		
	ton/fed	ton/ha	ton/fed	ton/ha	ton/fed	ton/ha	
GK-coba	26.03^{c}	61.98	9.98^{a}	23.76	15.90^{c}	37.86	16.9^{b}
Mn-1054	33.63^{a}	80.07	8.15^{b}	19.40	24.35^{a}	57.98	14.2^{c}
Ramada	27.42^{c}	65.29	6.90^{c}	16.43	21.27^{b}	50.64	14.7^{c}
Mn-4508	30.40^{b}	72.38	11.80^{a}	28.10	18.6^{c}	44.29	18.0^{b}
SS-301	30.51^{b}	72.64	10.30^{a}	24.52	20.73^{b}	49.36	20.2^{a}

Means with the same superscripts at the same column are not significant at ($p < 0.05$).

Table 2 Fractionation of juice sugar content of five sweet sorghum varieties.

Sweet sorghum variety	Total sugar %	Fractionation of sugar%		
		Sucrose	Glucose	Fructose
GK-coba	16.87^{b}	14.68^{b}	1.66^{a}	1.00^{a}
Mn-1054	11.26^{c}	10.27^{c}	0.66^{c}	0.33^{c}
Ramada	11.47^{c}	9.66^{c}	1.16^{b}	0.65^{b}
Mn-4508	17.43^{b}	15.49^{b}	1.31^{a}	0.83^{a}
SS-301	19.12^{a}	17.63^{a}	1.16^{b}	0.65^{b}

Means with the same superscripts at the same column are not significant at ($p < 0.05$).

Table 3 Fractionation of bagasse fibers and moisture % of five sweet sorghum varieties.

Sweet sorghum variety	Crude Fibers%	Fractionation of fibers%			Moisture %
		Hemi-cellulose	Cellulose	Lignin	
GK-coba	38.43[a]	12.60[c]	24.14[a]	11.30[a]	19.3
Mn-1054	24.09[b]	17.20[a]	26.14[a]	5.62[c]	16.2
Ramada	23.43[b]	12.72[c]	24.31[a]	5.34[c]	16.6
Mn-4508	38.14[a]	15.19[b]	20.18[c]	7.21[b]	18.1
SS-301	21.15[c]	11.73[d]	22.13[b]	5.19[c]	19.8

Means with the same superscripts at the same column are not significant at ($p < 0.05$).

tively, as average of two seasons. In a review, Almodares and Hadi (2009) listed 19 cultivars of sweet sorghum with sucrose content range of 6–16%. The variation among the tested sweet sorghum varieties might be due to their genotypes. In addition, Abazied and Sakina (2013) showed that the variety with high sucrose content tended to have high TSS% and lower reducing sugars content. Moreover, Almodares et al., (2007) reported that at hard dough stage, sucrose and total sugar exhibited a positive correlation, while a negative correlation was found between sucrose and glucose, fructose and maltose. Based on high juice yield and sugar content, varieties GK-coba, Mn-4508 and SS-301 were selected for bioethanol production from their juice.

Data presented in Table 3 indicated that crude fiber% of sweet sorghum bagasse ranged between 21.15% and 38.43%. Similarly, Bhoyar and Thakare (2009) found that sweet sorghum bagasse of 10 sweet sorghum varieties contained about 20.90–38.98% crude fiber.

Fractionation of fibers showed that **Mn-1054** contains the highest concentrations of hemi-cellulose%, cellulose%, while containing low ratio of lignin% compared to the other two the varieties of (**Gk-coba** and **Mn-4508**). Fortunately, these values are favorable for bioethanol production from lignocellulosic biomass, since high lignin content would require more energy and chemicals for hydrolysis step before conducting fermentation by the yeast. Moreover data showed insignificant difference among varieties Mn-1054, Ramada and SS-301 in lignin content and also between Mn-1054 and Ramada in cellulose content. Variety SS-301 has the lowest value of crude fiber% and lignin, making it easier for hydrolysis, in addition to having the highest values of both juice yield and TSS% (Table 1). Similar results were achieved by Dolciotti et al. (1998), who indicated that sweet sorghum hybrids significantly differed in their insoluble dietary fibers (hemi-cellulose, cellulose and lignin).

Based on high yield of wet bagasse and cellulose ratio and low lignin content, the three varieties of Mn-1054, Ramada and SS-301 were selected for bioethanol production from their bagasse.

Production of bioethanol from juice of three selected varieties of sweet sorghum

As previously shown, varieties **GK-coba**, **Mn-4508** and **SS-301** were the highest in juice yield, TSS and thus total sugar (Tables 1 and 2). Therefore these varieties were selected for bioethanol production directly from their juice. These parameters were reflected in the data presented in Table 4. Variety SS-301 had the highest values of juice initial sugars (143 g L^{-1}, see Table 4 footnote) as well as bioethanol concentration, regardless of the fermenting organisms. The highest bioethanol concentration (39.2 g L^{-1} equal to 50.26 mL L^{-1}) was obtained from the juice of **SS-301** by the mixed-culture of *Sacch. cerevisiae* and *Z. mobilis*, which consumed 58% of the available sugars, of which 48% were converted to ethanol. From all the tested varieties, the mixed-culture treatment gave the best values for bioethanol concentration, sugar conversion efficiency and bioethanol total yield.

Table 4 Production of bioethanol by *Z. mobilis, Sacch. cerevisiae* or mixed-culture of both (1:1) from the juice of three selected varieties of sweet sorghum.

Sweet sorghum variety (Initial sugar g L^{-1})	Microorganism	Bioethanol concentration		Consumed sugar	Sugar conversion coefficient	Bioethanol total yield per cultivated area		Sugar utilization efficiency
		mL L^{-1}	g L^{-1}	g L^{-1}	w/w%	L/fed	L/ha	w/w%
GK-coba (132 g L^{-1})	*Z. mobilis*	39.49[F]	30.8[F]	68.5	45[A]	394.11	938.36	52[BC]
	Sacch. cerevisiae	41.67[E]	32.5[E]	70	46[A]	415.87	990.16	53[ABC]
	Mixed-culture (1:1)	45.26[CD]	35.3[CD]	75.5	47[A]	451.69	1075.46	58[A]
Mn-4508 (136 g L^{-1})	*Z. mobilis*	40.77[EF]	31.8[EF]	70.6	45[A]	481.09	1145.44	52[BC]
	Sacch. cerevisiae	44.10[D]	34.4[D]	75	46[A]	520.38	1239.00	55[AB]
	Mixed-culture (1:1)	46.92[BC]	36.6[BC]	75.5	48[A]	553.66	1318.23	56[AB]
SS-301 (143 g L^{-1})	*Z. mobilis*	41.28[EF]	32.2[EF]	69.3	46[A]	425.18	1012.34	48[C]
	Sacch. cerevisiae	48.08[B]	37.5[B]	79.9	47[A]	495.22	1179.10	56[AB]
	Mixed-culture (1:1)	**50.26[A]**	**39.2[A]**	**82.5**	**48[A]**	**517.68**	1232.57	**58[A]**

Bioethanol concentration: ethanol in mL or ethanol in g (density: 0.789/ml) per L of fermenting juice (20%).
Sugar conversion coefficient (w/w%) = [Bioethanol concentration (g L^{-1}) ÷ consumed sugars (g L^{-1})] × 100,
Bioethanol total yield: estimated amount of bioethanol L to be produced from yield of sorghum raw juice per fed; juice yield of **GK-coba:** 9980**, Mn-4508:** 1180**, and SS-301:** 10300 L/fed.
Sugar utilization efficiency (w/w%) = consumed sugars (g L^{-1}) ÷ initial sugars (g L^{-1}) (Ramadan et al., 1985).
Initial sugars concentrations of sweet sorghum juice (10%) were 132 g L^{-1} **(GK-coba),** 136 g L^{-1} **(Mn-4508)** and 143 g L^{-1} **(SS-301).**
The values are mean of three replicates. Standard deviation was within 10%. Means with the same letter are not significantly different.
Values shown in bold are the highest in their corresponding measurement.

Table 5 Production of bioethanol by *Z. mobilis, Sacch. cerevisiae* or mixed-culture of both (1:1) from bagasse of three varieties of sweet sorghum hydrolyzed by 2% H_2SO_4 (v/v) at 120 °C for 60 min.

Sweet sorghum variety (initial sugar mg g^{-1})	Microorganism	Bioethanol concentration		Consumed sugars		Sugar Conversion coefficient	Bioethanol total yield	Sugar utilization efficiency
		mL L^{-1}	g L^{-1}	g L^{-1}	mg g^{-1} (%)	w/w%	mL kg^{-1}	w/w%
Mn-1054 (306 mg g^{-1})	*Z. mobilis*	4.6^{G}	3.6^{G}	8.1	162	44^{A}	92	53^{F}
	Sacch. cerevisiae	7.5^{D}	5.9^{D}	12.9	259	46^{A}	150	85^{B}
	Mixed-culture (1:1)	8.2^{C}	6.5^{C}	13.9	299	47^{A}	164	**98^{A}**
Ramada (376 mg g^{-1})	*Z. mobilis*	5.3^{F}	4.2^{F}	9.1	185	46^{A}	106	49^{F}
	Sacch. cerevisiae	8.1^{CD}	6.4^{CD}	13.9	277	46^{A}	162	74^{C}
	Mixed-culture (1:1)	9.0^{B}	7.1^{B}	**15.2**	**354**	47^{A}	180	94^{A}
SS-301 (430 mg g^{-1})	*Z. mobilis*	7.4^{DE}	5.8^{DE}	12.3	250	47^{A}	148	58^{E}
	Sacch. cerevisiae	9.6^{B}	7.6^{B}	16.2	320	47^{A}	192	74^{C}
	Mixed-culture (1:1)	**10.5^{A}**	**8.3^{A}**	17.2	270	**48^{A}**	**210**	63^{D}

Bioethanol concentration: mL ethanol per L of fermenting solution, or g of ethanol (density: 0.789/ml) per L of fermenting solution.
Sugar conversion coefficient (w/w%) = [Bioethanol concentration (g L^{-1}) ÷ consumed sugars (g L^{-1})] × 100,
Bioethanol total yield (mL kg^{-1}): amount of bioethanol (mL) per 1 kg of bagasse, yield of sorghum bagasse **(ton/fed)** for **Mn-1054:** 24.35**, Ramada:** 21.27**, and SS-301:** 20.73**.**
Sugar utilizing efficiency (w/w%) = consumed sugars (g L^{-1}) ÷ initial sugars (g L^{-1}) (Ramadan et al., 1985).
Initial sugars concentrations of sweet sorghum bagasse hydrolyzed by 2% H_2SO_4 (v/v) at 120 °C for 60 min were 15.3 g L^{-1} (306 mg g^{-1}) for **Mn-1054,** 18.8 g L^{-1} (376 mg g^{-1}) for **Ramada** and 21.5 g L^{-1} (430 mg g^{-1}) for **SS-301.**
The values are mean of three replicates. Standard deviation was within 10%. Means with the same letter are not significantly different.
Values shown in bold are the highest in their corresponding measurement.

Production of bioethanol from acid hydrolyzed bagasse of sweet sorghum

Acid hydrolysis of bagasse of three sweet sorghum varieties was performed using 2% (v/v) of sulfuric acid (H_2SO_4) at 120 °C for 60 min of retention time. The neutralized nutrient-supplemented acid hydrolyzates of sweet sorghum bagasse were used for bioethanol production by *Sacch. cerevisiae, Z. mobilis* or mixed-culture of both (at 1:1 ratio) at 30 °C for 4 days.

Data in Table 5 show initial sugar liberated from acid-hydrolysis varied with sorghum varieties, where concentrations were 306, 376 and 430 mg g^{-1} of bagasse from varieties **Mn-1054**, **Ramada** and **SS-301**, respectively. Consequently, fermenting these sugar solutions with either *Sacch. cerevisiae, Z. mobilis* or mixed-culture of both produced variable bioethanol concentrations depending on sorghum variety and fermenting organism. Mixed culture produced the highest bioethanol concentration from all varieties, where 6.5, 7.1 and 8.3 g L^{-1} of ethanol were obtained from Mn-1054, Ramada and SS-301, respectively.

In a similar study, sugarcane bagasse was treated with 2% (v/v) of sulfuric acid at 120 °C for 60 min, liberating total sugars of 474 mg g^{-1} of bagasse. When fermenting these sugars for 4 days at 30 °C using gamma-radiated *Sacch. cerevisiae* ATCC 7754, 10.3 g L^{-1} of ethanol was obtained, which is equal to 146 g of ethanol per kg bagasse (Abdelhafez et al., 2014).

The highest bioethanol concentration was obtained from fermenting the treated bagasse of **SS-301**, regardless of the microorganism employed in fermentation. The utmost bioethanol concentration (10.5 mL L^{-1}, or 8.3 g L^{-1}) was produced by mixed-culture with high sugar conversion coefficient of 48% (w/w) and sugar utilization efficiency of 63% (w/w), Table 5. Comparable study, done by Abdelhafez et al. (2015), produced 8.2 g L^{-1} of ethanol by mixed-culture of *Sacch. cerevisiae* and *Z. mobilis* from sugarcane bagasse hydrolyzed by combined treatment of acid hydrolysis and gamma irradiation.

Results also showed that using mixed-culture of *Sacch. cerevisiae* and *Z. mobilis* for fermentation of acid-hydrolyzed bagasse of all sweet sorghum varieties had the highest values in bioethanol concentrations, sugar conversion coefficient and bioethanol total yield compared to using any of the two organisms solely. In this context, *Sacch. cerevisiae* possessed better values of bioethanol concentration, sugar conversion coefficient and bioethanol total yield than those values recorded by *Z. mobilis*. Overall performance of mixed-culture in producing bioethanol from bagasse sweet sorghum varieties was significantly higher than that of one-organism culture. Similar study produced bioethanol yield of 26% from cassava peels and 12% from sweet potato peels using a mixed culture of *Sacch. cerevisiae* and *Z. mobilis* and these results were attributed to the combined activity of the two organisms to produce bioethanol (Oyeleke et al., 2012). Another study also used co-culture of *Sacch. cerevisiae* and *recombinant Escherichia coli* (carrying both *pdc* and *adhB* genes derived from *Z. mobilis*) to ferment acid hydrolyzate of softwood to bioethanol and achieved a high ethanol yield of 0.49 g ethanol/g sugars after 24 h, corresponding to 96.1% of the maximum theoretical bioethanol yield (Qian et al., 2006)**.**

Conclusions

In this study, among five varieties of sweet sorghum (*Sorghum bicolor* L.), variety **SS-301** showed to be the best for its high gross yield/fed of stalks, juice and bagasse, being 72.62, 24.5 and 49.35 ton/ha, respectively. Moreover, the juice of this variety contained the highest TSS content of 20%. Thus this variety is profitable if used for both first and second-generation

bioethanol production. Consequently, given that the stalks of the **SS-301** variety contain 34% juice and 66% bagasse, using both components for bioethanol production would produce approximately 160 mL of ethanol from each kg of striped stalks of this variety. In this context, a formula can be created to calculate the total yield of ethanol produced from the juice per feddan as follows:

Yield of ethanol (L) from juice per feddan = L ethanol/L juice × L juice/feddan

In the case of sweet sorghum variety SS-301 the yield of total produced ethanol will be = 251 mL L^{-1} × 10300 = 2585 L/fed.

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