

## Suitability of some Sweet Sorghum Varieties for Bioethanol Production

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### ABSTRACT

Bio-ethanol or microbial ethanol is a renewable eco-friendly energy source can be produced from bio-mass (hemicelluloses). Sweet sorghum, with sugar-rich stalks and water-use efficiency, has a very good potential as an alternative feedstock for ethanol production and also non-competing with human food on land. This study evaluates the exploitation of juice and bagasse of four varieties of sweet sorghum for microbial ethanol production which can further improve the energy yield of the sweet sorghum crops. The sweet sorghum varieties, Ramada, GK-coba, SS-301 and Mn-4508 were tested for their productivity, also, its sugar and fiber contents were determined. The four sweet sorghum varieties significantly differed in juice, bagasse and stripped stalk yield. The fiber-rich bagasse, resulting from squeezing the striped stalks and the sugar-rich juice, were used for microbial ethanol production by two microorganisms; *Saccharomyces cerevisiae* and *Zymomonas mobilis*. Varieties GK-coba, Mn-4508 and SS-301 were utilized for microbial ethanol production directly from juice where their stalks contained high sugar contents. While, stalks of varieties Ramada and SS-301 had higher content of fibers, so their bagasse were used for microbial ethanol production. Bagasse of sweet sorghum was pretreated and hydrolyzed thermo-chemically for bioethanol production. *Zymomonas mobilis* and *Saccharomyces cerevisiae* or mixed-culture of both organisms at a ratio (1:1) were used to ferment sweet sorghum sugars or hydrolyzed neutralized sweet sorghum bagasse to produce microbial ethanol. Results indicated that juice and bagasse of sweet sorghum variety SS-301, by the mixed-culture treatment, gave the highest microbial ethanol production. From the juice, microbial ethanol concentration was 51.36ml/l, whereas from sweet sorghum bagasse, microbial ethanol concentration was 10.5ml/l. 210ml of microbial ethanol can be produced from 1kg of sweet sorghum bagasse for variety SS-301. **keywords:** *Zymomonas mobilis*, *Saccharomyces cerevisiae*; Bioethanol, Sweet sorghum; Bagasse, Juice

### INTRODUCTION

The annual production of sweet sorghum crop in 2009 was 56000000 tons of grain, this value make sweet sorghum crop ranking as the fifth crop in the world, beyond maize, wheat, rice and barley. The greater region which cultivate sweet sorghum crop is India and sub Saharan Africa, sweet sorghum crop is an essential crop for food and feed, also, as a fuel source (Kassam *et al.*, 2012 and Serna-Saldivar *et al.*, 2012). Ahmed *et al.* (2010) in Egypt reported that, sweet sorghum crop is quite cultivated specially in Upper Egypt, this area in year 2002 was 384000 Feddan. On the other side, Reddy *et al.* (2005) published that, there are new strains of sweet sorghum crop developed specially for bio-energy, it is evaluated to be 760 L/ha of grain, 1400 L/ha of stalk juice and 1000 L/ha of the agricultural wastes. Annual global crude oil production is decreasing. The prediction value of crude oil will be 5000 million by 2050. Therefore, it is useful to find non-oil alternative sources of clean energy, renewable and not incompatible with feed and food (Bajpai, 2013). Bioethanol is considered as a favorable biofuel, displays many benefit, like high heat evaporation, elevate octane number, mover it is decreasing the dangerous gases. The production of microbial ethanol was by microbial fermentation of sugars which extracted from crops which a high contents of starch. or from biomass. The use of crops which human sued it for feed and food (corn or sugar beet and cane) to produce microbial ethanol from can create warfare in land and water used (Pimentel *et al.*, 2008). So that, the production microbial ethanol should be from imperfect crops or from substrates which contains low sugar to be economically competitive (Farako, 2013). Microbial production from sweet sorghum is suitable because it has a high carbohydrate production. The crops was cut for releasing the sweet juice with a high sugars level about 12 - 20%, sugars such as fructose and glucose is considered a good substances for microbial ethanol (Serna-Saldivar *et al.*, 2012). For each 10000kg of mashed sweet sorghum, 5000 to 6000kg of moisten bagasse can be

obtained, so that, many authors used bagasse to produce microbial ethanol, also they use it for producing methane and hydrogen, or a fuel or for feeding the animals. (Negro *et al.*, 1999; Zaldivar *et al.*, 2001; Antonopoulou *et al.*, 2008; Bennett and Anex, 2009, Wu *et al.*, 2010 and Venkata *et al.*, 2012). These are many reasons which make the sweet sorghum crop to be a suitable crop for microbial production of ethanol production. The water which required for sweet sorghum crop is less than that required for by sugarcane being about 1:3. Also, sweet sorghum crop is resistant crop for dryness and sweet sorghum crop can acclimatize for growing in tropical regions or subtropical regions. Furthermore sweet sorghum crop cultivation requires low levels of fertilizers and stay in the ground for short time (30 - 150 days) (Almodares and Hadi, 2009; Woods, 2000 and Reddy *et al.*, 2005). Kerem *et al.* (1992) produce microbial ethanol from lignocellulose. The lignocellulosic biomass was dried and cut into small particles for easy hydrolysis. Delignification was done by breaking down the lignin layer of the lignocellulosic biomass to expose cellulose, this process was done using steam explosion or high heat combined with acids or alkaline which called thermochemically process, or by fungi such as *Pleurotus ostreatus* or *Phanerochaete chrysosporium* which called biologically process. El-Tayebet *et al.* (2012) hydrolysis sugarcane bagasse, sugar beet waste, corn stalks and rice straw with H<sub>2</sub>SO<sub>4</sub>, HCl or H<sub>3</sub>PO<sub>4</sub> and he establish that the increasing of acids levels from 1% to 5% decreased the transformation of biomass. Faraco (2013) fermented cellulose into sugars by *Zymomonas mobilis* and *Saccharomyces cerevisiae* to ethanol.

Thus, the propose of this study is determined the bioethanol production from sweet sorghum juice and sweet sorghum bagasse using *Saccharomyces cerevisiae* and *Zymomonas mobilis*, single or mixed culture. Also, sugar and bagasse were determined.

## MATERIALS AND METHODS

### Sweet sorghum crop varieties and its cultivation

This experiment was conducted at Agricultural Research Station, Giza governorate, Egypt. Four varieties of sweet sorghum crops (*Sorghum bicolor*), namely, Mn4508 Ramada, SS-301 and GK-cobawere obtained from Sugar Crops Research Institute (SCRI), Agricultural Research Centre (ARC), Giza, Egypt. During summer season of the year 2016, sweet sorghum crops were cultivated and cut for extracting their stalk juice. The cultivation process started at the twelfth of June and the sweet sorghum crops were harvested after 5 months, this stage (dough) which considered suitable for giving high juice quality.

### The productivity

Ten samples from each variety were randomly taken of sweet sorghum stalks, removed the crust and cleaned. Sweet sorghum was stripped stalks, passed through three roller mill, for stalk juice extraction. Layers of cheesecloth was used to remove the large pieces of raw juice (A.O.A.C., 2005).

Extracted juice of sweet sorghum crops and gross yields/feddan of stripped stalks were calculated. Also, sweet sorghum bagasse yield per feddan was calculated (A.O.A.C., 2005): Yield of wet sweet sorghum bagasse (ton perfeddan) = yield of stripped stalks (ton perfeddan) - yield of juice (ton perfeddan).

### Juice quantitative analysis

The method described by Plews (1970)- Brix hydrometer standardized - was used for total soluble solids (TSS%) in the sweet sorghum juice crops at 20°C. Also, the method of Dolciotti *et al.* (1998) and Long *et al.* (2006) was used for determining the juice sugars of sweet sorghum.

### Quantitative analysis

Determination of moisture content was by weighting five grams of fresh sweet sorghum bagasse and an oven was used to dry it at 105 °C for constant weight. The cooling was done using a desiccators. Sweet sorghum bagasse crude fiber was conducted according to A.O.A.C. (2005). The method of Georging and Van Soest (1975) was used for sweet sorghum bagasse fiber fractions using dried sweet sorghum bagasse, fiber fractions of lignin, hemicelluloses and cellulose was calculated as following:

**NDF(neutral detergent fiber)**

=lignin+hemicellulose+Cellulose.

**ADF(acid detergent fiber) =lignin+Cellulose**

**Hemicelluloses=NDF-ADF.**

**Lignin was determined as natural detergent fiber**

**(NDF) – (hemicelluloses+cellulose).**

Cellulose was determined as weight loss of ADF upon extraction with 72% H<sub>2</sub>SO<sub>4</sub>.

### The source of microbial strains and propagation

*Saccharomyces cerevisiae* ATCC 7754 was obtained from Department of Microbiology, Soil, Water and Environment Res. Institute, Agriculture Research Center (ARC), Giza, Egypt, and *Zymomonas mobilis* ATCC 29191 were obtained from Department of Microbiology, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. Two broth medium were used for propagation of *Saccharomyces cerevisiae* and

*Zymomonas mobilis* the first was ATCC 948 broth medium (Swings and Deley, 1977) and the second was YM (Wickerham, 1946). The first medium (ATCC 948) was composed of: 20g glucose; 5g yeast extract; 1000 ml distiller water and pH was 6.5 ± 0.2. The second medium (YM) was composed of: 10g glucose; 5g peptone; 3g malt extract; 3g yeast extract; 1000 ml distiller water and pH was 6 ± 0.2. 15 g of agar agar was added for solid medium. These media were sterilized in autoclave at 121°C for 20 min. then inoculated with microbes and incubated at 30 °C for 48 h.

### Te pretreatment of sweet sorghum bagasse for producing bioethanol

Two stages were used, the first stage was the sweet sorghum bagasse pretreatment and the second stage was the fermentation process to produce microbial ethanol. The method of Abdelhafez *et al.* (2014) which was used for acid hydrolyzing of sweet sorghum bagasse by adding 95ml of 2% (v/v) of H<sub>2</sub>SO<sub>4</sub>(98%) or 95ml of tap water to 5 g of sweet sorghum bagasse into 250 ml Erlenmeyer flask and pH was 6.7 ± 0.2 (the control treatment) and then pH was adjusted to 5.5 ± 0.2. The method of Pattana *et al.* (2010) which hydrolyzed sweet sorghum bagasse thermochemically at 120°C for 60 minutes was used. For obtaining the sugar-rich liquid, the hydrolyzed sweet sorghum bagasse was left to cold and filtered to remove the bag solid fractions. The pH of hydrolyzate sweet sorghum bagasse was adjusted to 5.8 using two stages, the first stage using NaOH pellets until pH reach to 3 and second stage using NH<sub>3</sub> solution (33%) until pH reach to 5.5. Ethanol production was done using the neutralized sweet sorghum bagasse and inoculated with *Saccharomyces cerevisiae* and *Zymomonas mobilis*.

### Total sugars determination in sweet sorghum bagasse hydrolyzate

The method of Dubois *et al.* (1956) and Pak and Simon (2004) was used for determination total sugars. In a test tube, five ml of hydrolyzate sweet sorghum bagasse was mixed with one ml of phenol solution (2% w/v), 2.5ml of H<sub>2</sub>SO<sub>4</sub>(98%) was addition. Tubes were left into the darkness for 10 minutes. Tubes were cooled at 25°C for 30 minutes. Spectrophotometer (Shimadzu UV-1601) was used to measured sugars at 490 nm with a blank of distilled water. Glucose was used for preparing a standard curve under similar conditions. Total sugars were determined as glucose according to this equation:

$$X = (Y - 0.032) / 0.064$$

Where X = concentration of glucose (µg/ml), Y = optical density.

### Microbial ethanol production from sweet sorghum bagasse hydrolyzate

The ethanol production medium of *Saccharomyces cerevisiae* was composed of each 100 ml of sweet sorghum stalk juice was supplemented with 0.3g yeast extract; 0.35g peptone; 0.2g KH<sub>2</sub>PO<sub>4</sub>; 0.1g MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.1g (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, (Arapoglou *et al.*, 2010). Also, the medium of *Zymomonas mobilis* composed of each 100 ml of sweet sorghum stalk juice was supplemented with: 0.2g KH<sub>2</sub>PO<sub>4</sub>; 0.1g MgSO<sub>4</sub>.7H<sub>2</sub>O; 0.1g (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>(Davis *et al.*, 2006). All media were autoclaved at 121°C for 20 minutes. The inoculation volume was 5 ml of standard inoculum (standard inoculum after incubation period 2 days, it will be described below) of *Saccharomyces cerevisiae* or

*Zymomonas mobilis* individually or mixed cultures at ratio1:1. Incubation was done in anaerobic incubator (Labconco Manufacturing Corp., USA) at 30°C for four days. A rotary evaporator was used for extracting bioethanol for about 10 to 20 minutes at 78.5°C, all experiments were done in triplicate. Also, bioethanol was produced from neutralized hydrolyzate sweet sorghum crop bagasse, which supplemented, autoclaved, inoculated, incubated, and bioethanol extracted as the pervious method. For preparing standard inoculum five ml of broth media (YM for *Saccharomyces cerevisiae* and ATCC 948 for *Zymomonas mobilis*) was inoculated with a full loop of cultures and incubated at 30°C for 2 days.

**Determination of ethanol produced from tested yeasts**

Potassium dichromate method which described by Crowell and Ough (1979) was used for determining ethanol calorimetrically. Potassium dichromate reagent was prepared by dissolving potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) (34g) in distilled water (400ml) and sulfuric acid (325ml), the volume was completed to be one liter with distilled water. Two ml of distilled sample was added to 10ml of Potassium dichromate reagent in a test tube and mixed well. In a water bath, tubes were incubated at 60°C for 20 minutes, tubes cooled to room temperature. Spectrophotometer (Shimadzu UV-1601) was used for determining the reaction mixture absorption at 600nm. The blank experiment was done using 2ml of distilled water and 10 ml of the previous reagent (potassium dichromate). The standard curve was done using ethanol diluted with distilled water to made a standard solutions. The following equation was used for determining ethanol,

$$X = (Y - 0.1196) / 0.0988$$

Where X = concentration of ethanol (µl/ml). Y = optical density.

**Statistical analysis of the experiments**

The mean of data were expressed in 3 replicates. The data statistically analyzed using (ANOVA). Duncan’s Multiple Range Test (Snedecor and Cochran, 1980) was used to compare the differences between means by with *p* >0.05.

**RESULTS AND DISCUSSION**

**Total soluble solids (TSS %)of sweet sorghum varieties juice and Productivity of crops.**

Data in Table 1 showed the yield productivity of sweet sorghum and its TSS percentage of juice. All varieties differed in the TSS%, bagasse, stalk, yield of stripped and juice significantly. The highest value in stalk yield, juice content, wet bagasse yield and juice TSS% was 29.51ton/feddan,10%, 20% and 20%,respectively in variety SS-301.

**Table 1. Total soluble solids (TSS%) of juice and yield productivity of four sweet sorghum varieties**

Sweet sorghum crops varieties	Yield productivity			Juice TSS %
	Strip stalk yield (Ton/feddan)	Juice yield (Ton/feddan)	Wet baggas yield (Ton/feddan)	
Ramada	26.42 <sup>b</sup>	7.10 <sup>b</sup>	20.27 <sup>a</sup>	13.90 <sup>c</sup>
GK-coba	25.03 <sup>b</sup>	9.18 <sup>a</sup>	14.90 <sup>b</sup>	16.50 <sup>b</sup>
Mn-4508	29.40 <sup>a</sup>	10.30 <sup>a</sup>	18.20 <sup>b</sup>	17.10 <sup>b</sup>
SS-301	29.51 <sup>a</sup>	10.10 <sup>a</sup>	19.73 <sup>a</sup>	19.90 <sup>a</sup>

Means values are not significant at (p< 0.05).

These results was suitable for producing bioethanol, based on sugar content which is the major factor responsible of the process. Sweet sorghum varieties Mn-4508 and SS-301 were the highest in juice yield being 10.30 and 10.10 ton/feddan, respectively. Similar results were obtained by El-Geddawy *et al.* (2014) who reported that Sweet sorghum varieties SS-301 had 21.4% TSS and high bagasse yield (20.27 ton/feddan). Also, similar results with Negro *et al.* (1999) who published that, 50–60% wet bagasse was produced from crushed sweet sorghum stalk.

**Quantitative analysis of sweet sorghum varieties juice:**

Total sugars and sugars composition of sweet sorghum varieties were presented in Table 2.Total sugars and their composition were significant deferent among the sweet sorghum varieties. The highest values of total sugars was in the case of variety SS-301 which contained 18.72% and sucrose was 17.13%. The concentration of glucose and fructose in the juices of varieties Ramada and SS-301 was insignificantly different and also between GK-coba and Mn-4508.Abo-El-Wafa and Abo-El-Hamid (2001) and El-Geddawy *et al.* (2014) were obtained similar results where they determined the sugars in juice of sweet sorghum variety SS-301 and they recorded total sugars and sucrose concentrations of 19.3% and 13.95%, respectively.

**Table 2. Juice sugar composition content of four sweet sorghum varieties**

Sweet sorghum crops varieties	Total sugars%	Composition of sugars (%)		
		Sucrose	Glucose	Fructose
Ramada	10.87 <sup>c</sup>	9.33 <sup>c</sup>	0.95 <sup>b</sup>	0.37 <sup>b</sup>
GK-coba	16.37 <sup>b</sup>	14.22 <sup>b</sup>	1.23 <sup>a</sup>	0.90 <sup>a</sup>
Mn-4508	16.83 <sup>b</sup>	15.09 <sup>b</sup>	1.11 <sup>a</sup>	0.81 <sup>a</sup>
SS-301	18.72 <sup>a</sup>	17.13 <sup>a</sup>	1.01 <sup>b</sup>	0.55 <sup>b</sup>

Means values are not significant at (p< 0.05).

In the same direction, Almodares *et al.* (2007)published that, total sugar and sucrose had a positive correlation, but the negative correlation was done between glucose, maltose, sucrose and fructose. Moreover, sucrose content of sweet sorghum ranging of 6–16% (Almodares and Hadi, 2009). Finally, the varieties which were high content of sucrose tended to lower reducing sugars and to be high total soluble solids content (Abazied, 2013). According to the high level of sugar content in the juice yield, only three varieties were selected to produce bioethanol which were SS-301, Mn-4508 and GK-coba.

**Fractionation of bagasse fibers of sweet sorghum varieties:**

Data in Table 3 indicated that crude fiber (%) of the tested sweet sorghum bagasse ranged between 20.15% and 37.54%. The highest values of crud fiber (%) were obtained by varieties Mn-4508 and GK-coba, which represented 37.54 and 37.43 %, respectively. In a similar way, sweet sorghum bagasse of 10 sweet sorghum varieties contained about 20.90–38.98% crude fiber (Bhoyar and Thakare, 2009).

Composition of fibers showed that Mn-4508 contains the highest concentrations of hemicellulose (14.39%), while SS-301 containing low ratio of lignin% (4.69%) and hemicellulose (10.73%) compared to the other varieties. Fortunately, these low values of lignin was suitable for the production of microbial ethanol from lignocellulosic biomass. There was difference between SS-301 and Ramada in lignin concentration, also between

Ramada and GK-coba in cellulose concentration, these differences were insignificant. Because of the low concentrations of lignin and of variety SS-301, the hydrolysis of it was easier. Moreover, this variety (SS-301) had the highest concentration of Total soluble solids and juice yield. These results were similar to the founding of Dolciotti *et al.* (1998), who reported that the sweet sorghum crops significantly differed in lignin, cellulose and hemicellulose. So that, bagasse of SS-301 and Ramada were chosen to produce microbial ethanol, because of it had a high level of cellulose ratio and bagasse and it had also, low level of lignin.

**Table 3. Composition of bagasse fibers (%) of four sweet sorghum crops varieties**

Sweet sorghum varieties	Crude fibers (%)	Composition of fibers (%)			Moisture content (%)
		Cellulose	Hemicellulose	Lignin	
Ramada	23.03b	24.11a	12.02b	5.04c	16.6
GK-coba	37.43a	23.74a	11.60b	11.00a	18.5
Mn-4508	37.54a	19.48c	14.39a	6.81b	17.1
SS-301	20.15c	21.53b	10.73c	4.69c	18.8

Means values are not significant at ( $p < 0.05$ ).

**Table 4. Production of microbial ethanol by *Saccharomyces cerevisiae* and *Zymomonas mobilis* from the juice of sweet sorghum crops**

Sweet sorghum variety crops	Microbes	Ethanol values		Sugars consumed (g/l)	Conversion coefficient of sugars (w/w%)	Total yield of ethanol /feddan	Efficiency of sugar utilization (w/w%)
		(ml/l)	(g/l)				
Mn-4508 (containing 138 g/l sugar)	<i>Z. mobilis</i>	41.77 <sup>ef</sup>	32.2 <sup>ef</sup>	71.6	45 <sup>a</sup>	492.88	51 <sup>BC</sup>
	<i>S. cerevisiae</i>	45.10 <sup>d</sup>	34.9 <sup>d</sup>	76.0	46 <sup>a</sup>	532.18	55 <sup>AB</sup>
	<i>Z. mobilis</i> + <i>S. cerevisiae</i>	47.92 <sup>bc</sup>	37.1 <sup>bc</sup>	76.5	48 <sup>a</sup>	565.46	56 <sup>AB</sup>
GK-coba (containing 135 g/l sugar)	<i>Z. mobilis</i>	40.29 <sup>f</sup>	31.2 <sup>f</sup>	69.5	45 <sup>a</sup>	402.18	51 <sup>BC</sup>
	<i>S. cerevisiae</i>	42.67 <sup>e</sup>	33.2 <sup>e</sup>	71.0	46 <sup>a</sup>	425.85	52 <sup>ABC</sup>
	<i>Z. mobilis</i> + <i>S. cerevisiae</i>	46.26 <sup>cd</sup>	35.8 <sup>cd</sup>	76.5	47 <sup>a</sup>	461.67	57 <sup>A</sup>
SS-301 (containing 146 g/l sugar)	<i>Z. mobilis</i>	42.28 <sup>ef</sup>	33.0 <sup>ef</sup>	70.3	46 <sup>a</sup>	435.48	48 <sup>C</sup>
	<i>S. cerevisiae</i>	49.28 <sup>b</sup>	38.1 <sup>b</sup>	80.9	47 <sup>a</sup>	507.58	55 <sup>AB</sup>
	<i>Z. mobilis</i> + <i>S. cerevisiae</i>	51.36 <sup>a</sup>	39.9 <sup>a</sup>	83.5	48 <sup>a</sup>	529.00	57 <sup>A</sup>

#### Bioethanol production from acid hydrolyzed bagasse of sweet sorghum varieties:

In this experiment, the bagasse of two sweet sorghum varieties were hydrolyzated by 2% (v/v) of H<sub>2</sub>SO<sub>4</sub> at 120°C for 60 minutes and supplemented with nutrients and used for producing ethanol from *Zymomonas mobilis* and *Saccharomyces cerevisiae* in single or mixed cultures at 30°C for four days. Table 5 showed that, microbial production of ethanol from the bagasse (hydrolyzated and supplemented with nutrients) of Ramada and SS-301 sweet sorghum crops from *Saccharomyces cerevisiae* and *Zymomonas mobilis* either individual or in mixed culture by a ration (1:1). Initial sugars varied according to sweet sorghum varieties. The concentrations initial sugars were 430 and 376mg/g of hydrolyzated bagasse from SS-301 and Ramada, respectively. Ethanol produced mixed culture was the highest concentration, these values were 8.3 and 7.2g/l obtained from SS-301 and Ramada, respectively. Similar results were obtained from Abdelhafez *et al.* (2014) where they fermented sugarcane bagasse which treated with H<sub>2</sub>SO<sub>4</sub> at a same condition

#### Bioethanol production of from juice of three selected sweet sorghum varieties:

Because of varieties Mn-4508, SS-301 and GK-coba had the highest levels in total sugar, juice yield Total soluble solids (Tables 1 and 2), the juice of these varieties were chosen to produce bioethanol. Table 4 should that, the highest values of juice containing sugars was 146g/l and microbial ethanol value was 39.9 g/l equal to 51.36 ml/l in the case of variety SS-301 using *Zymomonas mobilis* and *Saccharomyces cerevisiae* together. The concentration of consumed sugars in this case was 57% and the converted to ethanol was 48%. Generally, the mixed culture was the best treatment for microbial ethanol values, bioethanol total yield, conversion efficiency of sugars.

Table 4 indicated that *Zymomonas mobilis* for all tested varieties gave the lowest values of all parameters while mixed culture resulted the highest values. From this Table, using mixed culture of microorganisms, although the SS-301 variety gave the highest concentration of bioethanol (51.36ml/l), Variety Mn-4508 gave the highest bioethanol total yield, 565.46L/fed, where it produce the maximum yield of raw juice per feddan (11800 l/feddan).

(120°C for 60 minutes), they produced 474 mg/g of total sugars from hydrolyzed sugarcane bagasse. Also, they fermented these sugars using *Saccharomyces cerevisiae* for four days at 30°C and they produced 10.3 g/l ethanol.

Table 5 also, showed that, mixed culture of *Zymomonas mobilis* and *Saccharomyces cerevisiae* which used for fermenting hydrolyzed sweet sorghum bagasse of all varieties had a high levels from microbial ethanol, total yield microbial ethanol and sugar conversion coefficient. Similar results were obtained from Oyeleke *et al.* (2012) who used cassava peels and sweet potato peels to produce microbial ethanol yield being 26% and 12%, respectively using *Zymomonas mobilis* and *Saccharomyces cerevisiae*. The study of Qian *et al.* (2006) which also used mixed culture of *Escherichia coli* (recombinant with *pdC* and *adh B* genes which obtained from *Zymomonas mobilis*) and *Saccharomyces cerevisiae* to ferment softwood (acid hydrolyzate) to microbial ethanol and they gain a high yield of ethanol being 0.49g/g consumed sugars after only one day.

**Table 5. Production of microbial ethanol by *Saccharomyces cerevisiae* and *Zymomonas mobilis* from hydrolyzed bagasse of sweet sorghum crops.**

Sweet sorghum variety crops	Microbes	Ethanol values		Sugars consumed (g/l)	Conversion coefficient of sugars (w/w%)	Total yield of ethanol /feddan	Efficiency of sugar utilization (w/w%)
		(ml/l)	(g/l)				
Ramada (containing 376 mg/g sugar)	<i>Z. mobilis</i>	5.2 <sup>e</sup>	4.1 <sup>e</sup>	9.0	45 <sup>a</sup>	106	48 <sup>e</sup>
	<i>S. cerevisiae</i>	8.2 <sup>c</sup>	6.5 <sup>c</sup>	14.0	46 <sup>a</sup>	161	74 <sup>b</sup>
	<i>Z. mobilis</i> + <i>S. cerevisiae</i>	9.2 <sup>b</sup>	7.2 <sup>b</sup>	15.3	47 <sup>a</sup>	180	81 <sup>A</sup>
SS-301 Ramada (containing 430 mg/g sugar)	<i>Z. mobilis</i>	7.3 <sup>d</sup>	5.7 <sup>d</sup>	12.2	46 <sup>a</sup>	146	57 <sup>d</sup>
	<i>S. cerevisiae</i>	9.8 <sup>b</sup>	7.7 <sup>b</sup>	16.3	47 <sup>a</sup>	190	76 <sup>b</sup>
	<i>Z. mobilis</i> + <i>S. cerevisiae</i>	10.5 <sup>a</sup>	8.3 <sup>a</sup>	17.2	48 <sup>a</sup>	210	80 <sup>c</sup>

### CONCLUSION

This work is very significant because in the future the world's need much renewable energy to maintain the world's energy crisis and to protect the environment. Among four varieties of sweet sorghum crops (*Sorghum bicolor* L.), Ramada, GK-coba, SS-301 and Mn-4508, variety SS-301 showed to be the best sweet sorghum for its high gross yield/feddan of bagasse, juice and stalks, being 19.73, 10.1, 29.51, respectively. Moreover, the juice of this sweet sorghum varieties contained high Total soluble solids (20%). The stalks of the sweet sorghum variety (SS-301) contain about 34% and 66% juice and bagasse, respectively. Bagasse used for microbial ethanol production by *Saccharomyces cerevisiae* and *Zymomonas mobilis* of sweet sorghum crops produced about 210ml ethanol/kg striped stalks. Authors calculate the follows:

**Yield of ethanol in the case of sweet sorghum crop SS-301 from juice/feddan=266ml/1X10300=2740 L/feddan.**

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## ملاءمة بعض أصناف الذرة الرفيعة لإنتاج الإيثانول الحيوي

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يعتبر الإيثانول الحيوي مصدر طاقة قابل للتجديد و صديق للبيئة ويمكن إنتاجه من الهيميسيليلوزات. تتمتع الذرة الرفيعة ، التي تحتوي على سيقان غنية بالسكر وذات كفاءة في استخدام المياه ، بإمكانيات جيدة للغاية باعتبارها مادة وسيطة بديلة لإنتاج الإيثانول ولتستخدم كغذاء للإنسان. وتقوم هذه الدراسة بتقييم استخدام العصير والمصاصة لأربع أصناف من الذرة الرفيعة لإنتاج الإيثانول الحيوي والتي يمكن أن تزيد من تحسين إنتاجية الطاقه من المحصول. تم تحليل أصناف الذرة الرفيعة ، GK-coba ، Ramada ، Mn-4508 و SS-301 ، لتقدير إنتاجيتها ومحتوياتها من السكر والألياف. واختلفت الأصناف الأربعة اختلافاً كبيراً في المحصول من السيقان والعصائر والمصاصة. تم استخدام مصاصة الذرة الغنية بالألياف الناتجة عن عصر السيقان والعصير الغني بالسكر ، في إنتاج الإيثانول الحيوي بواسطة اثنين من الكائنات الحية الدقيقة هما: سكاروميسيس سيرفيسيا وزيموموناس موبليس . وقد تم استخدام الأصناف GK-coba و Mn-4508 و SS-301 لإنتاج الإيثانول الحيوي مباشرة من العصير حيث تحتوي سيقانهم على محتويات عالية من السكر. في حين أن السيقان من الأصناف رامادا و SS-301 كانت محتوية على نسبة عالية من الألياف ، لذلك تم استخدام مصاصة الذرة في إنتاج البيوايثانول. تم استخدام المصاصة - المعالجة حرارياً و كيميائياً - لإنتاج الإيثانول الحيوي بواسطة سكاروميسيس سيرفيسيا وزيموموناس موبليس أو المزارع المختلطة لكلا الميكروبات بنسبة 1: 1. أثبتت النتائج أن العصير و المصاصة من صنف SS-301 ، من خلال المعاملة الخليطة بالميكروبات أعطى أعلى إنتاج للإيثانول الحيوي. وكان تركيز البيوايثانول 51.36 مل/لتر من العصير ، في حين كان تركيز البيوايثانول 10.5 مل / لتر من المصاصة. أخيراً ، يمكن القول أنه يمكن إنتاج 210 مل من البيوايثانول من كل 1 كجم من الصنف SS-301 ، عند استخدام المصاصة.