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ORIGINAL ARTICLE

Suitability of *Sorghum bicolor* L. stalks and grains for bioproduction of ethanol

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Abstract The goal of this proposed research is to utilize each of stalks juice, the acid hydrolysate of both lignocellulosic components of the juice extracted stalks and grains of non food sweet sorghum crop as a carbon source during bioproduction of ethanol using *Saccharomyces cerevisiae* and simultaneous saccharification fermentation (SSF) process. The obtained results revealed variation in proximate composition of carbohydrate compounds and minerals content between the sweet sorghum crop parts used in this study. The acid hydrolysis of the lignocellulosic components of stalks led to hydrolyze 11.18% of cellulose, 76.91% of hemicelluloses and 24.27% of lignin. It was also converted 67% of the starch in the grains into reducing sugars. After 72 h of fermentation, the medium containing starch grains hydrolysate gave the highest ethanol production (23.93 g/l) with yield of 0.50 g alcohol per g sugar and fermentation efficiency of 97.39% comparing with other two mediums having stalks juice and stalks hydrolysate (SH) as carbon sources. Also the yeasts in this medium consumed highest amount of sugars and gave the highest biomass yield followed by that containing stalks hydrolysate. Waste water which was remained after recovering of ethanol and removing the biomass from the fermentation medium had considerable levels of potassium, sodium, Mg, Ca, Fe, Cu and Mn and can be recommended for use in plant irrigation.

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Introduction

Production of bio-ethanol as an alternative energy source should be produced from non food crops, an agro-industrial wastes and lignocellulosic feedstock rich in carbohydrates (Oliveira et al., 2006; Balat, 2011).The available carbohydrates in such sources can be converted into ethanol either by simultaneous saccharification then fermentation (SSF) or by separate enzymatic hydrolysis and fermentation (SHF) processes (Endo et al., 2008). SSF process is more favored due to it's low potential costs (Hamelink et al., 2005).

Saccharomyces cerevisiae can be used for fermentation. It has been used in an industrial large-scale in fermentation of sugar- and starch-based materials into ethanol (Hahn-Hägerdal et al., 2007; Tian et al., 2009).

Sweet sorghum (*Sorghum bicolor* L. Moench) is a non food crop. It gives high green biomass yield (20–30 dry tons/ha), needs low water (1/3 of sugarcane and 1/2 of corn), and fertilizer requirements, short period (3–5 months) for growth, grows at diverse climate and soil conditions. It contains fermentable sugars (sucrose, glucose and fructose) in its stalks juice, starch in its grain, and lignocelluloses feed stock in its juice extracted stalks (Kresovich and Henderlong, 1984; Prasad et al., 2007; Ronghou et al., 2008; She et al., 2010). Therefore this plant considers one of the promising non food crop for fuel ethanol production (Wu et al., 2010).

The main objective of this study was to evaluate the suitability of using stalks juice, lignocellulosic components of the juice extracted stalks and grains of sweet sorghum crop in bioproduction of ethanol using *S. cerevisiae* and SSF process.

Materials and methods

Materials

The stalks free from leaves and husks as well as the grains of sweet sorghum (*S. bicolor* L. Moench) crop were obtained from Sabahia Agric. Research Station, Agric. Research Center, Alexandria, Egypt. The crop was planted in May, 2010 and harvested in late October of the same year. The following substrates were prepared to use as a carbon source in the broth used for the bioproduction of ethanol by *S. cerevisiae*.

Stalks juice (SJ)

The obtained stalks were washed with tap water then squeezed using sugar cane roller mill at Technological Lab. in Sugar Crops Res. Inst. of Sabahia, Agric. Res. Station. The collected extracted juice was filtered through fine plastic screen to remove suspended matters, packed in polyethylene pouches and stored at −18 °C in deep freezer until used.

Lignocellulosic hydrolysate of the juice extracted stalks (HS)

After juice extraction, the extracted stalks were collected, washed several times with hot water to remove their content of any residual sugars, then dried in an electric oven (E. Schulg & Co. Inh. Franz. KG), at 60 °C ± 2 °C for 5 h, ground and sifted before subjecting to acid hydrolysis using H_2SO_4 at ratio of 1 g sample: 10 mL of 3% acid solution 121 °C for 30 min in a labtech. autoclave (Labtech, USA). After cooling, the resulted hydrolysate was filtered under vacuum, neutralized with 2% NaOH, packed in glass jars and stored at −20 °C until used.

Starch hydrolysate of grains (HG)

The grains were dried at 50 °C ± 2 °C for 5 h using the above mentioned electric oven, then ground, screened through 30 mesh sieve and subjected for acid hydrolysis, filtration, neutralization packing and storage as mentioned above.

Methods

Bio-ethanol production

Preparation of yeast inoculums

Dry *S. cerevisiae* was activated by adding 10 g of dry yeast to 50 mL of pre-culture broth containing 1 g glucose, 0.4 g peptone, 0.15 g yeast extracts, 0.05 g KH_2PO_4, and 0.025 g $MgSO_4{\cdot}7H_2O$, and incubated in an a rotary incubator shaker, at 38 °C and 200 rpm for 60 min. before using it as an inoculum for ethanol production.

Fermentation method

One hundred milliliter of each of SJ, acid hydrolysate of HS and/or HG was added as a carbon source to flask containing 5 g of yeast extract, 5 g of peptone, 1.2 g $(NH_4)_2SO_4$, 1 g KH_2PO_4, and 0.5 g $MgSO_4{\cdot}7H_2O$, to prepare one liter of the required fermentation medium for ethanol production .The pH value of the medium was adjusted to 6 ± 0.3 before autoclaving at 121 °C for 20 min .The sterilized fermentation medium was incubated with 10 mL (~10% inoculums size) of activated yeast, then, incubated in a shaking (rotatory incubator Innova 4230, Edison, NJ., USA) at 30 °C and 200 rpm for 72 h (Wu et al., 2006). Through this period both ethanol production and sugars consumption in medium were periodically determined. At the end of the fermentation period, the temperature of broth was raised to above the boiling point of ethanol to recover alcohol by distillation. The biomass in the fermentation broth, was separated by centrifugation, at 5000 rpm for 20 min, dried at 80 °C and weighed (Norris and Ribbons, 1970). Meanwhile some minerals (Mg, Na, Fe, K Mn, Cu and Ca) were estimated in the waste water of the fermentation broth.

Analytical methods

Total solid, ash, crude, protein, crude fat and crude fiber of the used sweet sorghum raw materials were analyzed by AOAC approved methods (1998). Total carbohydrate (%) was calculated by difference. Total sugars were determined by phenol–sulfuric method (Dubois et al., 1956). The concentration of reducing sugars was determined by dinitrosalicylic colorimetric assay using glucose as sugar standard (Miller, 1959). The hemicellulose, cellulose and lignin were analyzed using the method of Goering and Van Soest (1970). Minerals (Na, K, Mg, Ca, Fe, Mn and Cu) were estimated using Perklin Elmer atomic absorption spectrophotometer (Model 2380), England, as described in the AOAC (1998). The produced ethanol was measured by gas chromatography (Shimadzu, GC-17A, Japan) using a RTX-1 column (20 m by 0.25 mm) packed with 100% dimethyl polysiloxane and a flame ionization detector (Palo Alto, CA), N_2 as carrier gas at a flow rate of 30 mL/min, and maintaining the injector and detector temperature at 150 °C (Laopaiboon et al., 2007). All the experiments were carried out in triplicate.

Calculation

Ethanol concentration (P, g L^{-1}), ethanol yield (Y), volumetric productivity of ethanol; fermentation efficiency (FE) were

Table 1 Proximate composition and minerals content of stalk juice (SJ), free sugar stalks (SS) and grain (SG) of sweet sorghum.*

Component	Stalk juice (SJ)	Free sugar stalk (SS)	Grain (SG)
Total solids (%)	17.52 ± 0.44	94.2 ± 1.55	92.13 ± 1.86
Protein (%)	2.14 ± 0.63	4.55 ± 0.18	12.06 ± 0.24
Crude fat (%)	1.21 ± 0.05	5.93 ± 0.75	5.93 ± 0.11
Crude fiber (%)	2.85 ± 0.16	49.47 ± 1.38	4.02 ± 0.95
Total carbohydrate (%)	91.74 ± 1.22	32.81 ± 3.04	74.97 ± 1.79
Ash (%)	2.06 ± 0.38	7.24 ± 0.73	3.02 ± 0.49
Mineral content (ppm) Mg	1340.33 ± 1.14	3048.21 ± 0.55	1899.59 ± 0.26
Na	2710.55 ± 0.87	3734.28 ± 0.66	627.09 ± 0.07
Fe	85.1 ± 0.02	267.98 ± 0.07	128.24 ± 0.05
K	3028.8 ± 0.16	26854.24 ± 0.08	65041.58 ± 0.011
Mn	37.94 ± 0.03	57.23 ± 0.03	18.45 ± 0.06
Cu	18.05 ± 0.02	55.39 ± 0.05	20.84 ± 0.02
Ca	66.5 ± 0.06	3710.34 ± 0.06	735.51 ± 0.08

* On dry weight basis.

calculated using the following equation as described by Laopaiboon et al. (2009):

1. Ethanol concentration (P, g L^{-1}) = Ethanol produced (g) per liter of fermentation broth.
2. Sugar utilization (%) = (Grams of original sugar − Grams of residual sugar/Grams of original sugar) × 100.
3. Ethanol yield (Y, g g^{-1}) = Produced ethanol/Total utilized sugar.
4. The volumetric productivity (g L^{-1} h^{-1}) = Produced ethanol (g/L)/Fermentation time (hr).
5. Fermentation Efficiency (FE)% = (Actual yield/Theoretical yield) × 100.

Whereas: Actual yield = the produced ethanol from 100 g consumed sugars,Theoretical yield = the calculated amount of ethanol from 100 g sugar.

Results and discussion

Proximate composition and minerals content of stalks juice (SJ), juiced extracted stalks (SS) and grains (SG) of sweet sorghum

Data in Table 1 showed large variations in the proximate composition and minerals content of the sweet sorghum raw materials used for ethanol production. Except carbohydrates, the SJ contained the lower levels of other's proximate composition than SS and SG. SS had nearly similar crude fat, lower protein and total carbohydrates contents, higher crude fiber and ash comparing with SG. According to Ratnavathi et al. (2010) protein, crude fiber, ash and fat contents ranged from 1.04% to 2.16%, 5.68% to 7.48%, 2.04% to 2.52% and 0.89% to 1.21% respectively in five genotypes of sweet sorghum stalk juice.

Results of the minerals analysis in Table 1 indicated that Mg, Na, Fe, Mn, Cu and calcium were found in higher levels in SS than both SG and SJ. While SG contained the highest level of K followed by SS and SJ respectively. The ash of SJ had only higher level of Na and Mn than that of SG.

Generally these minerals may play a role as coenzymes, activators and inhibitors to the yeast enzymes required to achieve fermentation process and produce ethanol. Results of Monti et al. (2008) showed that the ash of sweet sorghum stem contained 3446, 112, 12.991, 2079, 195.804, 681 mg/kg of Ca, Fe, K, Mg, Na and P, respectively. Generally type of soil, genetic properties of plants, chemical composition of fertilizers, climatic and environmental conditions affect the proximate composition and minerals contents of plant products (Ratnavathi et al., 2010).

Carbohydrate composition of stalk juice (SJ), raw and acid hydrolysate of sugar stalks (SS) and grain (SG)

Results in Table 2 indicated that total sugars represented 83.36% of the total carbohydrates in SJ. These sugars consisted of 35.09% reducing sugars and 64.91% non reducing sugars. This means that nearly all SJ carbohydrates are simple

Table 2 Carbohydrates composition of stalks juice, raw and acid hydrolysate of juiced extracted stalks and grains.

Component (%)	Stalk juice (SJ)	Juice extracted stalks		Grain	
		Unhydrolysate (SS)	Acid hydrolysate (HS)	Unhydrolysate (SG)	Acid hydrolysate (HG)
Total sugar	76.47	9.62	26.01	13.95	48.52
Reducing sugar	26.84	7.53	26.01	8.45	48.52
Sucrose	47.15	0.87	0.0	2.06	0.0
Starch	6.33	ND	ND	54.27	17.91
Celluose	ND	29.61	26.3	ND	ND
Hemiocelluose	ND	16.28	3.76	ND	ND
Lignin	ND	3.09	2.34	ND	ND

ND: Not determined.

Table 3 Effect of stalk juice (SJ), lignocellulosic hydrolysate of the juice extracted stalks (HS), and starch hydrolysate of grains (HG) on the ethanol bioproduction.

Parameter	Ethanol fermentation medium containing		
	SJ	HS	HG
Initial total reducing sugar (g L^{-1})	76.47	26.01	48.52
Initial reducing sugar (g L^{-1})	26.84	26.01	48.52
Total sugars utilization (%)	70.56	98.76	99.11
Ethanol content (g L^{-1})	10.7	12.4	23.93
Ethanol yield (g/g of sugar)	0.20	0.48	0.50
Maximum volumetric productivity of ethanol (g $L^{-1} h^{-1}$)	0.15	0.17	0.33
Biomass (g L^{-1})	3.13	5.56	6.98
Fermentation Efficiency (%)	38.81	94.45	97.39

fermentable sugars. According to Liu et al. (2008) the total sugars and sucrose varied from 11–13% and 6–9%, respectively in SJ. Wu et al. (2010) stated that the fermentable sugars of SJ were sucrose, glucose and fructose.

On the other side only 29.32% and 18.8% of the total carbohydrates of raw SS and SG, respectively were found as total sugars. Reducing sugars represented 76.39% and 60.56% of the total sugars in both of SS and SG, respectively. The main carbohydrate of raw SG was starch 54.27%. Meanwhile the lignocellulosic compounds (cellulose, hemicelluloses and lignin) were the major carbohydrates in raw SS. Therefore the acid hydrolysis of such complicated carbohydrates, starch and lignocellulosic compounds, in both SG and SS is required to increase their contents of available fermentable sugars before using in fermentation .Total sugars increased from 9.2% to 26.01% in HS and from 13.95% to 48.52% in HG after acid hydrolysis. These sugars were free from non reducing sugars. The acid hydrolysis conditions used in this study led to hydrolyse 11.18% of cellulose, 76.91% of hemicelluloses, 24.27% of lignin in raw SS and 67% of the starch in raw SG. The hydrolysate products of the previous carbohydrates compounds consisted mainly of reducing sugars. Ioannis et al. (2009) and Zhao et al. (2009) found that the level of cellulose, lignin and hemicellulose ranged from 20.5% to 31.7%, 4.7% to 7.1% and 18.7% to 29.9%, respectively in sweet sorghum stalks. Richie and McBee (1991) stated that starch content varied from 38.8% to 48.2% in sweet sorghum grains. Balat (2011) mentioned the following advantages of the acid hydrolysis of lignocellulosic compounds (1) produce syrup of monomeric sugars, (2) remove hemicelluloses, part of the lignin, and heavy metals. Such removal helps in the enzyme digestion of unhydrolyzed cellulose during fermentation process, (3) effective and inexpensive treatment when the sulfuric acid concentration was less than 4% (Kumar et al., 2009).

Effect of using stalk juice (SJ), lignocellulosic hydrolysate of the juice extracted stalks (HS) and starch hydrolysate of grains (HG) as a carbon source on ethanol production

As shown from Table 3 the ethanol content, yield, productivity and efficiency in addition to biomass yield and consumed sugars were differed according to type and level of sugars in the fermentation medium. After 72 h of fermentation, SJ medium, containing high levels of total sugars and sucrose, gave lower content, yield, productivity and fermentation efficiency ratio of ethanol as well as biomass yield, and consumed sugars than those having HS and HG hydrolysates. HG medium containing nearly 63.45% of the total sugars of SJ medium and free from sucrose gave the highest yield, productivity and efficiency ratio of ethanol in addition to biomass yield and consumed sugars than that containing HS hydrolysate. The later medium had nearly 34% and 53% of total sugars of each of SJ and HG mediums respectively as well as free from sucrose. These results indicated that *S. cerevisiae* preferred reducing sugars than non reducing ones. The lower fermentation efficiency of SJ could be due to the inhibiting effects of it's high contents of sugar, aconitic acid, or the combination of both on yeast. Berthels et al. (2004) mentioned that glucose and other monosaccharides were utilized first during ethanol production by yeast. Katahira et al. (2006) identified different monosaccharides in lignocellulosic hydrolysate beside glucose such as galactose, arabinose, xylose, mannose, and some oligosaccharides. Hahn-Hägerdal et al. (2007) stated that microorganisms are able to ferment mono and oligosaccharides into ethanol.

Table 4 Macro and micro-elements content in waste water of fermentation medium.

Mineral content (ppm)	Stalk juice (SJ)	Lignocellulosic hydrolysate of the juice extracted stalks (HS)	Starch hydrolysate of grains (HG)
Mg	916.0 ± 0.08	264.72 ± 0.06	68.68 ± 0.03
Na	2735.15 ± 0.43	1280.76 ± 1.03	33262.30 ± 0.91
Fe	33.12 ± 0.04	15.49 ± 0.01	7.137 ± 0.003
K	4755.54 ± 0.55	3389.76 ± 0.22	1356.28 ± 0.35
Mn	1.31 ± 0.02	1.78 ± 0.003	0.229 ± 0.04
Cu	9.78 ± 0.001	14.07 ± 0.02	8.61 ± 0.01
Ca	35.27 ± 0.03	155.291 ± 0.05	54.14 ± 0.02

Yu et al. (2009) increased the rate of ethanol production by adding 0.77 g phosphorus and 2.15 g nitrogen to 1 L of SJ medium.

Data in Table 3 showed that to obtain high ethanol yield the level of reducing sugars in fermentation medium should not less than 26 g L^{-1}. According to Laopaiboon et al. (2009) SJ containing 28% total sugars gave maximum ethanol production, yield and fermentation efficiency.

Minerals content (ppm) in the waste water of ethanol fermentation medium

Data in Table 4 showed macro and micro-elements content in waste water of ethanol fermentation medium after recovering of ethanol and removing biomass. Potassium and sodium were the dominant minerals in this water followed by Mg, Ca, Fe, Cu and Mn. The levels of these minerals differed according to the sweet sorghum part used as a carbon source in fermentation medium. Waste water of SJ medium had the higher levels of Mg, K and Fe and lower value of Ca comparing with those of HS and HG fermentation media. Meanwhile Na was the only mineral found in higher level in. waste water of HG than HS fermentation medium. Generally these minerals presence in soluble form and can easily absorb by plant roots. Therefore such waste waters can recommend for plant irrigation.

Conclusion

This study proved that the bio-ethanol can be produced from both stalks juice, acid hydrolysate of the juice extracted stalks lignocellulosic and grains starch of non food sweet sorghum crop at the same time reduced environmental pollution.

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