Fermentation kinetics and ethanol production from different sweet sorghum varieties

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Abstract: In recent years bioethanol fuels derived from agricultural biomass resources or waste have been considered the cleanest liquid fuel alternative to fossil fuels. Ethanol consumption is expected to reach 11.2 billion gallons by 2012. Sweet sorghum, grown in the Southeast and Midwest states in US, contains 14%-20% fermentable sugar and is an ideal feedstock, owing to its ease of fermentation by yeast. The objectives of this research were to perform kinetic studies to determine the factors that may affect the rates of sugar consumption and ethanol production during fermentation of two varieties of sweet sorghum juice, Variety 1 (V-1) and Variety 2 (V-2), and to optimize the fermentation efficiency and ethanol production by varying strategies to process the juice before fermentation. Kinetic parameters of fermentation provide total sugar consumption rate of 3.4 g/(L·h) for V-1 juice and 2.2 g/(L·h) for V-2 juice, with ethanol production rates of 1.8 g/(L·h) for V-1 juice and 1.6 g/(L·h) for V-2 juice. Maximum ethanol production (V/V) was 8.5% for V-1 and 9.2% for V-2; ethanol yield was 0.065 g of ethanol/g of V-1 juice and 0.072 g ethanol/g of V-2 juice. Even though V-2 has a higher ethanol yield than V-1, V-1 has faster consumption and production rates due to its lower initial glucose and sucrose proportions in the juice, relative to V-2. Fermentation efficiency is greater than 90% for frozen, autoclaved juice and 25% sugar content juice. The lowest fermentation efficiency (79%) was for 30% sugar-content juice. These results can be used to optimize processing conditions of sweet sorghum juice during fermentation.

Keywords: ethanol, sweet sorghum, fermentation, kinetics, sugar and ethanol profile

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1 Introduction

Bioethanol is a form of renewable fuel that can be produced from agricultural feedstocks such as sugar cane[1,2], sorghum[3,4], potato[5,6], manioc[7,8], and maize[9,10]. However, there has been considerable debate about how useful bioethanol will be in replacing gasoline. Concerns about ethanol production and its use relate to the large amount of arable land required for crops[11,12]. Conversely, the reduced energy usage and pollution due to ethanol as an eco-friendly alternative fuel usage are important[13]. Small amounts (10%) of ethanol added to the gasoline that fuels cars can reduce greenhouse emissions like carbon monoxide and nitrogen oxides[14,15]. Many aspects of ethanol production from sweet sorghum have been studied during the past two decades. Effects of agricultural practices on sweet sorghum performance to improve soil and water conservation[16]; different harvest approaches[17]; effects of juice processing techniques[18] on juice recovery and ethanol yield; and performance of different yeast strains on ethanol production[18,19] are all significant to this research.

High fermentable sugars and yield of green biomass, low requirement for fertilizer, high efficiency in water usage, short growth period and its adaptability to diverse

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climate and soil conditions make sweet sorghum attractive for bioethanol production[20,21]. Sweet sorghum juices are composed of sugars: saccharose; glucose; and fructose. Also, the sorghum plant contains cellulose and lignocellulose that can be used to produce ethanol[22]. To improve economic value and ethanol yield, increasing the juice yield from the sorghum plants and making use of remaining sugars in the juice are both crucial. Ethanol yield may be as great as 5 612-6 080 L/ha, if all the fermentable sugars in sweet sorghum are converted to ethanol[21,23,24].

In this research, the use of two varieties of sweet sorghum juice; Umbrella, Variety 1 (V-1) and M-81E, Variety 2 (V-2) that contained 14% to 16% sugars as fermentation substrates were evaluated to study the kinetics of the sugar consumption in juice and ethanol production during fermentation, using a 3 L fermenter. To determine the optimum condition behavior during fermentation, we also compared pre-fermentation processes: autoclaved juice; non-autoclaved juice direct from the refrigerator; and room temperature juices containing 25% and 30% sugar.

2 Materials and methods

2.1 Micro-organisms and culture media

The dry alcohol yeast, *Saccharomyces cerevisiae* (Ethanol Red) provided by Fermentis (Lesaffre Yeast Corp., Milwaukee, WI) in vacuum-packed bags was used for ethanol fermentation. The yeast was stored in a refrigerator and activated immediately before fermentation. Dry yeast was activated by adding 0.5 g of dry yeast (Ethanol Red) to 10 mL of preculture broth. The 10 mL of pre-culture broth contains: 0.2 g glucose; 0.05 g peptone; 0.03 g yeast extracts; 0.01 g KH_2PO_4; and 0.005 g $MgSO_4.7H_2O$. The pre-culture broth was shaken at 200 r/min in an incubator shaker at 38℃ for 25-30 min. The concentration of the inoculated cells was 1×10^6 cells/mL determined by the Plate Count Agar (PCA) method (see 2.4).

2.2 Substrate

Two varieties; V-1 and V-2 of sweet sorghum were obtained from Sorghum Breeding, Soil and Crop Sciences Department, Texas A&M University, College Station, TX. These Texas grown plants were pressed to obtain the juices, which were refrigerated immediately. The juice yield from pressing the plants was 40%-50%[21, 25]. V-1 contains 64% sucrose, 22% glucose, and 14% fructose, whereas V-2 contains 56% sucrose, 30% glucose, and 14% fructose as determined by the High Performance Liquid Chromatography (HPLC) method (see 2.4). Table 1 below presents the content of each variety of juices.

Table 1 Sucrose, glucose and fructose content in V-1 and V-2 sweet sorghum juice

Sugar composition	V-1 Concentration /g · L^{-1}	Composition of V-1/%	V-2 Concentration /g · L^{-1}	Composition of V-2/%
Sucrose	(89±2)	64	(83±5)	56
Glucose	(31±3)	22	(44±1)	30
Fructose	(20±1)	14	(21±2)	14
Total sugars	(140±6)	-	(148±8)	-

2.3 Fermentation process

The sorghum juice obtained from pressing the sorghum was first filtered by using 25-mm Whatman 1005325 Grade 5 qualitative filter paper**.** Fermentation efficiency was tested for autoclaved (30 min at 60℃), non-autoclaved (frozen), 25% and 30% concentrated juice. The concentration of juice was increased by freezing the juice, allowing the water to rise and removing the ice from top. Then, the sugar content was measured on HPLC (refer to 2.4) and diluted with deionized water if needed to maintain the required (25% and 30%) concentration under study. One liter of juice was supplemented with 3 g of yeast extract in a 1.5 L Erlenmeyer flask. The pH value of the juice was adjusted with extract to approximately 4.2 to 4.3 by using 2N hydrochloric acid. The juice was then inoculated with 10 mL of freshly activated dry yeast (Ethanol Red) and run in the 3-L fermenter for a period of 72 h at 32℃ and 750 r/min for ethanol production. All experiments were run in triplicate to determine the ethanol production.

2.4 Analytical methods

Microbial cell cultures were serially diluted using peptone saline diluents (1 g/L peptone and 8.5 g/L NaCl) and were counted on a Plate Count Agar (PCA) that consisted of glucose (1 g/L), yeast extract (2.5 g/L), tryptone (5 g/L) and agar (15 g/L). Sugar and ethanol concentrations were determined on High Performance

Liquid Chromatography (Consta Metric 3200 solvent delivery system from LCD Analytical) equipped with auto sampler, Shodex SP 810 packed column and a Refractive Index (RI) detector. Column temperature was maintained at 60℃. Each sample was run for 25 min at a flow rate of 0.7 mL/min by using water as the eluent.

2.5 Parameter calculations

Fermentation efficiency was calculated from the ratio between the average produced ethanol and the theoretical ethanol production of 51.1 g of ethanol generated per 100 g of glucose[22] in the biochemical conversion of the sugar consumption. The maximum rates of sugar consumption, S_m (g/(L·h)) and ethanol production, P_m (g/(L·h)) were obtained from the slopes (a plot between sugar/ethanol (g/L) and time (h) of fermentation) during the initial fermentation period of 4 h to 18 h [26,27]. Ethanol concentration, P (% V/V) was the product concentration produced in the fermentation broth as determined by HPLC (refer to 2.4). Ethanol yield, $Y_{p/s}$ (%), was calculated as the ethanol (g) produced per g of the different varieties of juice[28].

2.6 Statistical model

Fermentation data: glucose consumption; ethanol production; and microbial growth were statistically tested for significant differences with the t-test. Software used for the t-test was Design Expert.

3 Results and discussion

3.1 Influence of substrate composition on kinetics

The kinetics of ethanol production was studied by using the 3-L fermenter reactor. Total sugar consumption in sorghum juice and ethanol production was measured during continuous fermentation. The kinetics of total sugar consumption and ethanol production from V-1 and V-2 varieties of sorghum juice are shown in Figure 1.

The ethanol concentrations of V-1 and V-2 juices are (7.8%±1%) and (8.5%±1%) at the end of the 24 h period (Figure 1). The kinetic study divides the fermentation into three stages (Figure 1). V-1 sorghum juice has a faster initial total sugar reduction and ethanol production than V-2. For V-1, the initial decrease takes place after the 2nd hour, whereas for V-2 the initial decrease takes place after the 6th hour. Therefore, it is easier for the inoculated yeast cells in V-1 to go through the adjustment to fermentation than for V-2. This is explained by the differences in the proportions of the glucose and sucrose in the two varieties of juice (Table 1). Sugar consumption and ethanol production are low for the first 6 h for V-2 sorghum juice. Therefore, studying the influence of the substrate composition on the kinetics of fermentation is important to increasing yields of ethanol. For the initial stage of fermentation, it also shows that starting with a higher concentration of juice that has mixed sugars is less efficient in utilizing the substrate by the yeast compared to a lower concentrated juice of mixed sugars.

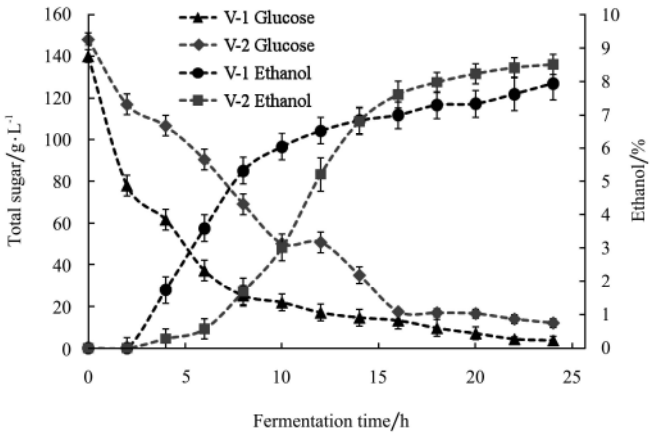

Figure 1 Fermentation kinetics of V-1 and V-2 sorghum juice to ethanol by *Saccharomyces cerevisiae* in a 3-L fermenter, truncated at 24 h

Most rapid glucose consumption and ethanol production occur at the time between 2 h and 10 h for V-1 and 6 h and 16 h for V-2 sweet sorghum juice (Figure 1). Glucose consumption decreases and ethanol production increases nearly linearly between the stated hours for V-1 and V-2. Even though most glucose seems to be absorbed by the 20th hour for V-1 and 24th hour for V-2, ethanol concentration continues to increase slightly in both cases. This is due to remaining fermentable sugars; fructose and sucrose that were hydrolyzed to glucose and resulted in ethanol generation after the initial glucose was consumed. At the final stage, ethanol concentration increased very slowly by fermentation due to the release of glucose from residual sucrose. When this experiment was run for 72 h, there was little change after the 48th

hour of ethanol production.

Further, fermentation kinetic parameters were determined; maximum sugar consumption rate (S_m), maximum ethanol production rate (P_m), maximum ethanol concentration, P, at the end of the fermentation period and ethanol yield, $Y_{p/s}$, for both varieties of sorghum juices (Table 2). There were higher sugar consumption and ethanol production rates for V-1 juice than for V-2 juice (Table 2). V-1 juice had a sugar consumption rate of 3.3 g/(L·h), which means the rate of the consumption of total sugar was 3.3 g/(L·h) during the first 18 h of fermentation, as there was a linear decrease during this period. For V-2 this linear decrease lasted nearly 22 h with a maximum consumption rate of 2.2 g/(L·h). This explains the faster total sugar consumption and ethanol production for V-1 juice compared to V-2. This may be due to lower sugar concentration (Table 1), in V-1 that allowed the yeast to easily use up the juice, compared to the V-2 juice, which has slightly higher concentration of sugars.

Table 2 Fermentation kinetic parameters of ethanol production

Variety	Max total sugar consumption rate, S_m/g·(L·h)$^{-1}$ (± Std deviation)	Max ethanol production rate, P_m/g·(L·h)$^{-1}$ (± Std deviation)	Maximum ethanol concentration, P/%(V/V) (± Std deviation)	Ethanol yield, $Y_{p/s}$ (w.t. ethanol/wt juice) (± Std deviation)
V-1	(3.3± 0.3)	(1.8± 0.2)	(8.3±0.5)	(0.065±0.003)
V-2	(2.2± 0.2)	(1.6± 0.5)	(9.2±0.4)	(0.072±0.001)

Note: *Kinetic parameters calculated for fermentation between 4 and 18 h when sugar consumption and ethanol production rapidly changed (Figure 1).

The maximum ethanol concentration, P in the fermentation broth was slightly higher for V-2 juice (9.2%) than for V-1 juice (8.3%), due to slightly greater amount of initial sugar in V-2 than V-1 (Table 1). These results are comparable to those of Laopaiboon and Belloch.[28,29] who reported most yeast strains being able to ferment juices up to 20%, producing ethanol yields of 10% to 12% V/V with high fermentation efficiency. Also, the ethanol yield, $Y_{p/s}$ was greater for V-2 juice than V-1 juice (Table 2). The yield of 0.065 w.t.% for V-1 juice implied that, for every 100 g of V-1 juice, 6.5 g of ethanol was produced whereas for every 100 g of V-2 juice, 7.2 g of ethanol was produced. This yield comparison between the different varieties of juice is important for ethanol production, since sweet sorghum juice is being used as a substrate for ethanol production in many parts of the world.

3.2 Bacterial counts and pH changes during fermentation

For the two varieties of juices, growth of the yeast cells was analyzed (Figure 2). The yeast from the two juices was cultivated. The yeast cells show four growth stages: lag phase; exponential phase; stationary phase; and death phase (Figure 2). Yeast cells had a shorter lag phase for V-1 than for V-2. The initial sugar concentration (140 g/L for V-1 versus 148 g/L for V-2) was optimal for the initial phase for V-1 and V-2. Therefore, from the 8th - 24th hour, yeast had a higher growth in V-1 than in V-2. During the 2nd growth phase (exponential phase), V-2 and V-1 show similar growth. Later, during the 3rd and 4th phase, yeast in V-2 seems to have a shorter stationary phase and dies off more quickly than the yeast in V-1. This may be due to the higher alcohol content that V-2 provides, compared to that of V-1 towards the end of fermentation, after the 55th hour (9.2% for V-2 vs. 8.3% for V-1).

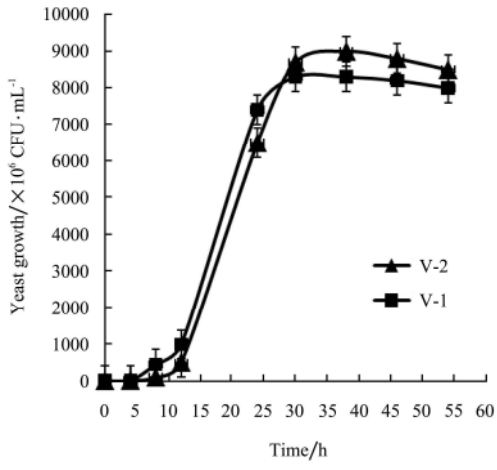

Figure 2 Average *Saccharomyces cerevisiae* cell counts in the two varieties of sorghum juice during fermentation

The pH value of the mixture remained constant at 4.3 to 4.4 during the first few hours and then decreased to

about 3.9 after approximately 18 h of fermentation. This result shows ethanol production to be stable.

3.3 Fermentation efficiency for various pre-fermentation juice processes

When fermentation was performed on autoclaved juice, frozen juice straight from refrigerator, and various concentrated juices, fermentation efficiency differed (Figure 3). Fermentation efficiencies of frozen juices were higher than those autoclaved juices or highly concentrated juices (Figure 3). This can be explained by low bacterial contamination due to low pH (5) and low temperature. Also, adjusting the pH value of the juice to 4.2 to 4.4 before yeast inoculation prevented contaminated bacteria from competing with the inoculated yeast. Autoclaved juices on the other hand may have lost some heat-sensitive nutrients and generated inhibitors in the juice that might have decreased the fermentation efficiencies of the autoclaved juices[30]. Concentrated juices had the lowest fermentation efficiencies. This may have been due to the inhibiting effects of high ethanol concentration, aconitric acid or the combination of both on yeast[21,31].

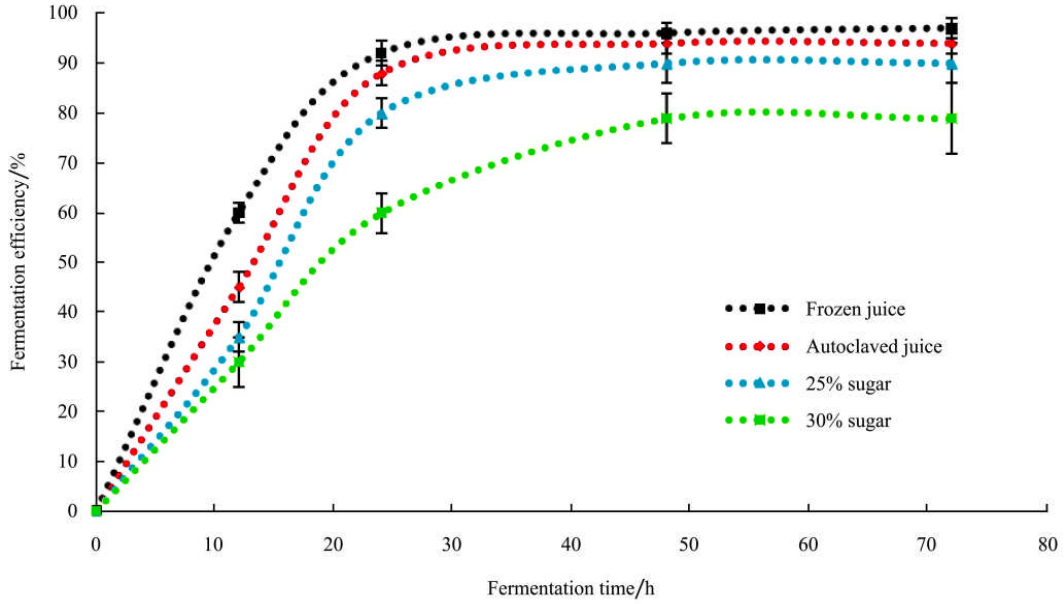

Figure 3 Comparison of ethanol fermentation efficiency among the different juices processed

From Figure 3, we conclude that the sorghum juices do not need to be autoclaved for better fermentation efficiencies. It is best to keep the concentration below 25% for higher efficiency (Figure 3). Further, highly concentrated juices of 25% and 30% had residual sugars of (3%±2%) and (10%±5%), respectively (Table 3), containing mostly fructose and little sucrose in the final alcohol product after the 72 h period of fermentation, when compared to the lower concentration frozen or autoclaved juice that had negligible remaining sugars.

Table 3 Total residual sugars contents in the final product from concentrated juices

Concentrated juice	Residual sugars/%
25% sugar	(3±2)
30% sugar	(10±5)

Previous report that various other ingredients, such as glycerol and lactose, are more abundant in the highly concentrated juice than in the low-concentrated juice, which may also have contributed to the lower fermentation efficiencies of the concentrated juices[21,30]. The corresponding ethanol yields for the four pre-fermentation conditions presented in Figure 3 are 12%-14%, 11%-13%, 11%-12%, and 9%-10% for the non-autoclaved frozen juice, autoclaved juice, 25% juice and 30% juice, respectively. At the end of fermentation, all juices had fermentation efficiencies greater than 90%, except for the 30% juice. In comparison, for M81E varieties (V-2) of sweet sorghum juice from the Riley and Doniphan counties of Kansas, Wu et al.[21] reported fermentation efficiencies with different pre-fermentation processes, where frozen juice had the highest

fermentation efficiency (94%). They also found similar ranges of results for other conditions as observed in this study. In contrast, Rein et al.[30] reported fermentation efficiencies of 41% for unheated raw juice and greater than 90% for autoclaved juice.

Table 4 summarizes the ethanol production per acre of land based on the two varieties of sorghum juice. V-2 juice has approximately 10% higher ethanol yield per acre of land, as compared to V-1. These results are similar to previously reported values of yield for sweet sorghum. Wu et al.[21] reported ethanol yield of 2 134-2 470 L/ha for M81-E (V-2) from sweet sorghum grown in Kansas. In comparison, Texas grown V-2 sweet sorghum (this study) produced 1 704-2 273 L/ha (Table 4). Similarly, sweet sorghum (variety unknown) grown in India yielded 2 816-4 052 L ethanol/ha[31]. V-1 ethanol yield data are not readily available in literature but this variety has a more rapid rate of ethanol production compared to V-2, even though the yield is lower.

Table 4 Approximate ethanol production per acre of land

Variety	*Ethanol/Acre/(L • ha^{-1})
1	1 537-2 050
2	1 704-2 273

Note: *Assuming average growth of 15-20 dry tons of sorghum / acre.

The results in Table 4 may be used to compare efficiencies of ethanol production of sweet sorghum varieties to one another. Also, it may be used to assess the efficiencies of sweet sorghum varieties relative to other agricultural media, such as maize, sugarcane and many others, for generating ethanol from biomass. Because of the ease of plant growth, V-1 may be more profitable than V-2 for ethanol production. V-1 is day-length insensitive, and its maturing time is shorter, in general, than that of V-2. On the other hand, V-2 may be a better sweet sorghum option during the fall season, as these plants are day-length sensitive.

Fermentation data for mean ethanol production were significantly different when statistically tested on Design Expert. P-value was smaller than 0.05 (0.0059), showing a significant difference of (65±3) g/L or 8.3% (V/V) for V-1 versus (72 ±1) g/L or 9.2% (V/V) for V-2. Fermentation data for mean cell growth were significantly different when statistically tested. P-value was smaller than 0.05 (0.0036) showing significant difference of ($8.0\times10^9 \pm 5.8\times10^7$) CFU/mL for V-1 versus ($9.0\times10^9 \pm 5.2\times10^7$) CFU/mL for V-2.

4 Conclusions

Ethanol production and fermentation efficiency vary depending on sorghum variety or crop and the amount and proportion of sugar in the sweet sorghum. The rates of glucose consumption, ethanol production, and cell growth are higher for an optimum concentration of sugar with a combination of yeast specific to the substrate. This should always be determined to optimize any fermentation process. In this study, V-1 had a smaller ethanol yield compared to V-2. However, rates of sugar consumption and ethanol production were higher for V-1 due to its initial lower concentration of sugar. This was verified by the fermentation kinetic parameters; maximum sugar consumption rate was 3.3 g/(L·h) for V-1 juice and 2.2 g/(L·h) for V-2 juice, maximum ethanol production rate of 1.8 g/(L·h) for V-1 juice and 1.6 g/(L·h) for V-2 juice. Maximum ethanol concentration in the final fermentation broth was 8.3% for V-1 juice and 9.2% for V-2 juice. In terms of energy efficiency, V-1 may be a better crop in the long run, because of its higher rate of ethanol production and shorter maturation. In terms of ethanol yield, V-2 may be a better choice. Ethanol fermentation efficiency varied among the four pre-fermentation preparations. Fermentation efficiencies for frozen, autoclaved, and juice containing 25% sugar were greater than 90%. Juice containing 30% sugar had lower efficiency (79%) because fermentation did not go to completion.

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