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## **Ethanol Fermentation from Sweet Sorghum Juice**

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**Abstract.** *In recent years bio-ethanol, considered the cleanest liquid fuel alternative to fossil fuels derived from agricultural staples or waste has been of great interest as the ethanol consumption is expected to reach 11.2 billion gallons by 2012. Sweet sorghum containing 18-20% fermentable sugar is an ideal feedstock grown in the Southeast and Midwest states for its easy ethanol fermentation by yeast. The objective is to optimize the fermentation efficiency and ethanol production by varying strategies to process the juice before fermentation, and perform kinetic study to determine the factors that may affect the rate of sugar consumption and ethanol production during fermentation of two different varieties of juice. Even though variety 2 has a higher ethanol yield (35 g/L) than variety 1 (25 g/L), yet variety 1 has a faster consumption and production rate due to its' lower glucose and sucrose proportion in the juice than in variety 2. Applying the Michaelis-Menten model, the consumption rate for variety 1 juice is 5.8 g/L.hr while variety 2 is 3.8 g/L.hr. Microbe concentration may need to be increased for a higher rate for variety 2. Fermentation efficiency is above 90% for frozen and autoclaved juice, and 25% sugar content juice except 30% sugar content juice had the lowest fermentation efficiency of 79%. All these results help us understand the different processing conditions of sweet sorghum juice during fermentation.*

**Keywords.** . Ethanol, sweet sorghum, fermentation, sugar and ethanol profile, kinetics.

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## Introduction

Bioethanol, a form of renewable energy can be produced from agricultural feedstocks such as sugar cane, sorghum, potato, manioc, and maize. However, there has been considerable debate about how useful bioethanol will be in replacing gasoline. Concerns on ethanol production and its' use relate to the large amount of arable land required for crops (UNEP, 2009). Conversely, the reduced energy usage and pollution due to ethanol as an eco-friendly alternative fuel usage are of importance. Added in small amounts, 10% of ethanol to gasoline that fuels our cars, it reduces greenhouse emissions like carbon monoxide and nitrogen oxides (UNEP, 2009; F.O Licht et al., 2008). Many aspects of ethanol production from sweet sorghum have been conducted during the past two decades. Agricultural practices on sweet sorghum performance to improve soil and water conservation (Buxton et al., 1999); different harvest approaches (Worley et al 1991) and juice processing techniques (Reidenbach et al., 1985) on juice recovery and ethanol yield; various yeast strain performance on ethanol production (Wu et al., 2008) 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 climate and soil condition makes sweet sorghum attractive for bio-ethanol production (Phowchinda et al., 2009). This plant is composed of sugars; saccharose, glucose, and fructose and can readily produce fermentable sugars in its' juice, starch and lignocelluloses that can be used in both starch-based and cellulosic ethanol plants. Increasing the juice yield or making use of the remaining sugar in the juice is crucial for the high ethanol yield of sweet sorghum and is of economical value. Possible ethanol yield can be 600-650 gal/a if all the fermentable sugars in sweet sorghum are converted to ethanol (Wu et al., 2008).

This work evaluates the use of two different varieties of sweet sorghum juice; Variety1 (V-1) and Variety2 (V-2) as fermentation substrate to study the kinetics of the sugar consumption in the juice and ethanol production during fermentation using a 3 L fermenter. It also compares pre-fermentation processes; autoclaved juice, non-autoclaved juice directly from the refrigerator, juice containing 25% and 30% sugar behavior during fermentation to determine the optimum condition.

## Materials and Methods

***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. These were stored in the refrigerator and activated right before fermentation. Activation of dry yeast was conducted by adding 0.5 g of dry yeast (ethanol red) in to 10 ml of preculture broth. 10 ml of the pre-culture broth contains: 0.2 g glucose, 0.05 g peptone, 0.03 g yeast extracts, 0.01 g  $\text{KH}_2\text{PO}_4$ , 0.005 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ . The pre-culture broth was shaken at 200 rpm in an incubator shaker at 38 °C for 25-30 min. The concentration of the inoculated cells was  $1 \times 10^6$  cells/ml.

**Substrate:** Two varieties; V1 and V2 of sweet sorghum were obtained from Sorghum Breeding, Soil and Crop Sciences Department, Texas A&M University, College Station, TX. These plants were pressed to obtain the juice. About 2.5 kg of plants gave about 1 kg of the juice.

**Fermentation Process:** The sorghum juice as it was obtained after pressing the sorghum was first filtered using 25mm filter paper. Samples of 1 L sorghum juice, one straight from the refrigerator and the other autoclaved for 30 min at 60 °C were taken for fermentation experiment. 1 L of the juice was supplemented with 3 g of yeast extract in a 1.5 L Erlenmeyer flask. The pH of the juice was adjusted with extract to about 4.2 to 4.3 with 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 hours at 32 °C and 750 rpm for ethanol production. All experiments are run in triplicates to determine the ethanol production from variable pre-fermentation conditions and kinetic study.

**Analytical Methods:** Microbial cell culture were serially diluted using peptone saline diluents (1g/L peptone and 8.5 g/L NaCl) and counted on a Plate Count Agar (PCA) that consisted of glucose (1g/L), yeast extract (2.5 g/L), tryptone (5 g/L) and agar (15 g/L). Sugar and ethanol concentration was 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 °C. Each sample was run for 25 minutes at a flow rate of 0.7 ml/min using water as the eluent.

**Kinetic Model:** Fermentation data are fit in to Michaelis-Menten model to determine the 2 kinetic constants; maximum reaction rate,  $V_m$  and dissociation constant,  $K_m$ . High  $V_m$  shows faster consumption of the sugar in the juice or faster production of the product ethanol. While, bigger  $K_m$  shows lower affinity of the yeast for the sugar in the juice. The equation is shown below:

$$v = V_m [S] / K_m + [S] \quad (1)$$

Where,

v- Rate of reaction (g/l.hr)

$V_m$  – Maximum rate of reaction (g/l.hr)

S – Substrate/Product concentration (g/l)

$K_d$  - dissociation constant (g/l)

## Results and Discussion

### ***Influence of substrate composition on kinetics***

Kinetics of ethanol production was studied using the 3-L fermenter reactor. Total sugar consumption in sorghum juice, and ethanol production were measured during continuous fermentation process. The kinetics of glucose consumption and ethanol production from 2 different varieties of sorghum juice; V1 and V2 are shown in Figure 1.

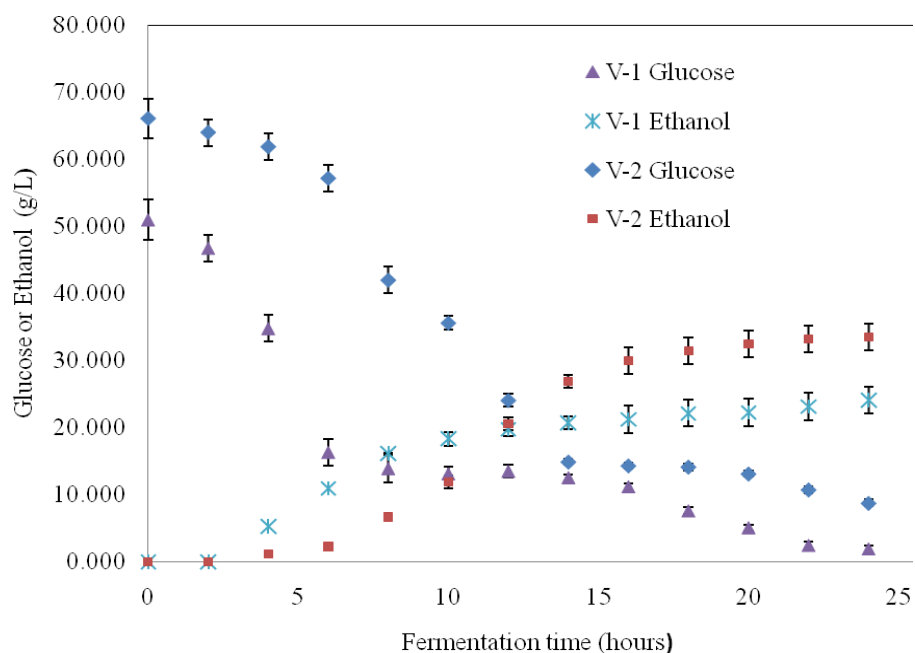


Figure 1. Kinetics of ethanol fermentation from 2 different varieties (V-1 and V-2) of sorghum juice containing different sugar concentration by *Saccharomyces cerevisiae* in a 3-L Fermenter

From Figure 1, the kinetic study divides the fermentation into 3 stages. Variety 1 sorghum juice has a faster initial sugar reduction and ethanol production than variety 2. For variety 1, the initial decrease takes place after the 2<sup>th</sup> hour while for variety 2 the initial decrease takes place after the 6<sup>th</sup> hour. This means that it is easier for the inoculated yeast cells in variety 1 to go through the adjustment to the environment of the fermentation than in variety 2. Sugar consumption and ethanol production is seen to be very low for the first 6 hours for variety 2 sorghum juice. This may be explained by the difference in the proportions of the different sugars in the 2 different varieties of juice. Therefore, studying the influence of the substrate composition on the kinetics of fermentation is important to increase yield 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 enzyme compared to a lower concentrated juice of mixed sugars.

Most glucose consumption and ethanol production takes place during the second stage from Figure 1. For V-1 juice after the 2<sup>nd</sup> hour and for V-2 after the 6<sup>th</sup> hour high glucose consumption is seen together with high ethanol production. Both cases, it goes up to about 20<sup>th</sup> hour almost linearly. Even though most glucose seems to be absorbed during this time, ethanol concentration continues to increase slightly in both cases. This may be due to other fermentable sugars such as maltose, maltotriose, fructose, and dextrans were hydrolyzed into glucose and resulted in ethanol generation after the original glucose was consumed. At the final stage, ethanol concentration increased very slowly by fermentation due to the release of glucose from any residual dextrans. When this experiment was run for 72 hours, there was not much change after the 48<sup>th</sup> hour in ethanol production.

Further, Michaelis-Menten kinetics is applied to determine the maximum rate ( $V_m$ ) and dissociation constant ( $K_m$ ) for both the varieties of sorghum juices and the results are presented in Table 1.

Table 1. Kinetic constants based on Michaelis-Menten model

Variety	Maximum Rate, $V_m$ (g/L.hr)	Dissociation Constant, $K_m$ (g/L)
V-1	$5.8 \pm 0.5$	$22 \pm 1.5$
V-2	$3.8 \pm 1.0$	$24 \pm 3.0$

Table 1 shows higher reaction rate for variety 1 juice than variety 2 juice. V-1 juice has a rate of 5.8 g/L.hr which means the rate of consumption of glucose or production of ethanol is 5.8 g/L per hour during the first 18 hours of fermentation as there is a linear increase during this period. While for variety 2 this linear increase is until almost 22 hours with a maximum consumption rate of 3.8 g/L.hr. This explains the faster glucose consumption and ethanol production for variety 1 juice compared to variety 2. This may be due to lower sugar concentration in V-1 that allows the yeast to easily use up the juice compared to the V-2 juice that has higher concentration of sugars.

The higher the dissociation constant, the lower the affinity of the yeast cells to the juice or the growing condition. The dissociation constant,  $K_m$  in this case is not an accurate representation of the affinity to the sorghum juice. This is because yeast initially takes a longer time to get used to the variety 2 juice environment. Then the growth trend is similar to V-1 but slightly slower rate of growth and the production of the product, ethanol continue to increase slightly for a longer time. This may be due to the left over residual sugars in V-2 that hydrolyzed later into glucose to further continue to produce ethanol.

### ***Bacterial counts and pH changes during fermentation***

For the 2 different varieties of juices discussed above growth of the yeast cells were studied and the results are presented in Figure 2.

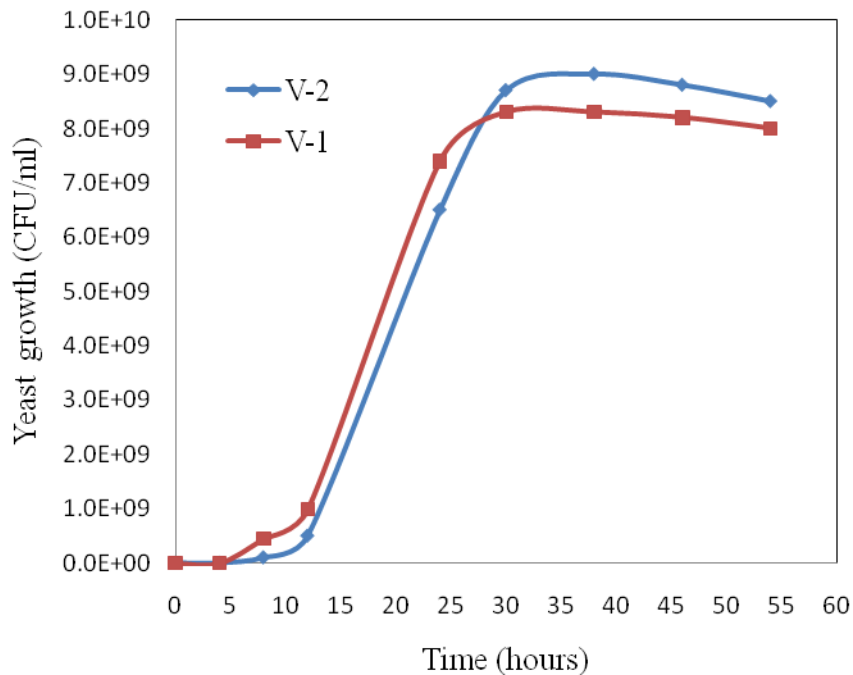


Figure 2. Average *Saccharomyces cerevisiae* cell counts in 2 different varieties of sorghum juice for fermentation

The yeast from the 2 different juices was cultivated. Figure 2 shows the yeast cells growth having four stages: lag phase; exponential phase; stationary phase; death phase. Yeast cells had a shorter lag phase for V-1 than V-2. The initial sugar concentration (51 g/L for V-1 versus 61 g/L) was optimum for the initial phase for V-1 therefore from the 8<sup>th</sup> - 24<sup>th</sup> hour yeast in V-1 has a higher growth than in V-2. During the 2<sup>nd</sup> growth phase (exponential phase) V-2 and V-1 shows similar growth. Later during the 3<sup>rd</sup> and 4<sup>th</sup> phase, yeast in V-2 seems to have a shorter stationary phase and dies off 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 45<sup>th</sup> hour (25 g/L for V-1 versus 36 g/L for V-2).

The pH of the mixture remained constant at 4.3 to 4.4 during the first few hours and then decreased to about 3.9 after about 18 hours of fermentation. This shows ethanol production to be stable.

### ***Fermentation Efficiency for various pre-fermentation processes for juice***

When fermentation was performed on autoclaved juice, frozen juice straight from refrigerator, and various concentrated juices, efficiency of fermentation was different. The results are presented in Figure 3.

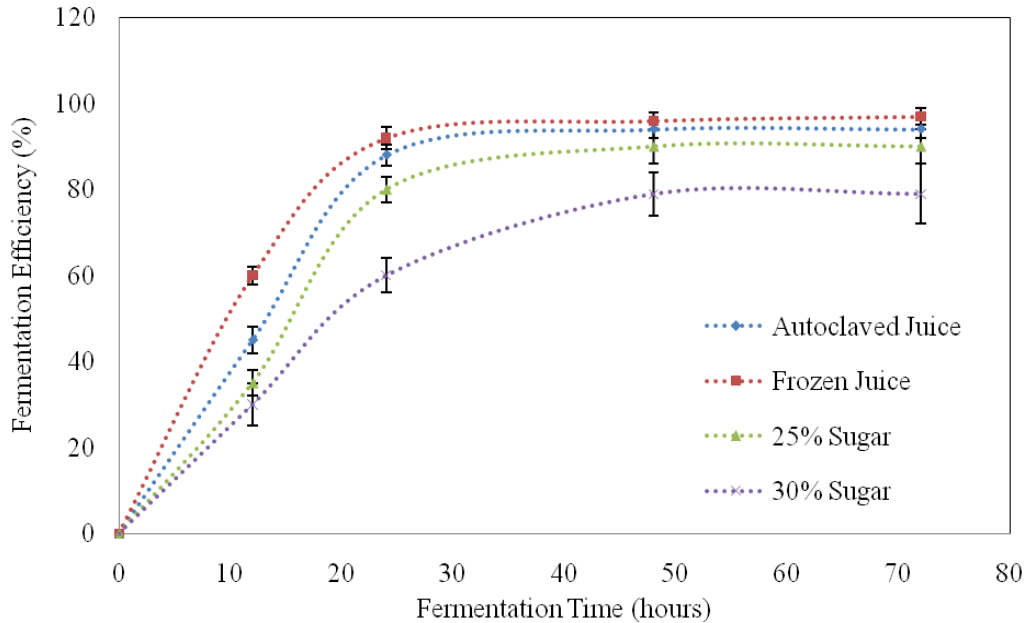


Figure 3. Comparison of ethanol fermentation efficiency between the different juice processing

Fermentation efficiencies of frozen juices were higher than those autoclaved juices or highly concentrated juices. This can be explained by low bacterial contamination due to low pH and (5) and low temperature. Also, adjusting the pH of the juice to about 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 generate inhibitors in the juice that might have decreased the fermentation efficiencies of the autoclaved juices. Concentrated juices had the lowest fermentation efficiencies compared to the rest. This might have been due to the inhibiting effects of high ethanol concentration, aconitric acid or the combination of both on yeast.

From Figure 3 it can be said that the sorghum juice do not need to be autoclaved for better fermentation efficiencies. It is best to keep the concentration at about 20% for higher efficiency. Further, highly concentrated juice (above 20%) had left over residual sugars; about 15% fermentable sugars and others (fructose) in the final alcohol product after the 72 hours period of fermentation compared to the lower concentration frozen or autoclaved juice. Other contents like glycerol, and lactose was also found in the highly concentrated juice compared to the low concentrated juice which might have also contributed to the lower fermentation efficiencies of the concentrated juices. The corresponding ethanol yield to the efficiencies presented in Figure 3 are 10, 13, 15-16 and 17-18% for the autoclaved, frozen, 25% and 30% juice respectively. All has fermentation efficiencies greater than 90% except for the 30% juice at the end of fermentation.

## Conclusion

Ethanol production and fermentation efficiency vary depending on the sweet sorghum crop and the amount and proportion of sugar in them. Rate of glucose consumption, ethanol production, and cell growth is higher for an optimum concentration of sugar with a combination of yeast specific to the substrate. This should always be determined for optimizing any fermentation

process. In this study, both variety 1 and 2 juice worked efficiently with 0.5 g of yeast/ L juice. Yet rate of consumption and production was higher for V-1 due to its' lower concentration of sugar that's makes it easy for the yeast to use it up and different proportions that was verified by the Michaelis-Menten rate constant,  $V_m$  of 5.8 g/L.hr for V-1 versus 3.8 g/L.hr for V-2. Fermentation efficiencies for frozen, autoclaved, and containing 25% sugar were greater than 90% except for the one containing 30% sugar as fermentation did not go to completion.

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