

Ethanol production from sweet sorghum

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Abstract

The use of fossil fuels contributes to global warming and thus there is a need to resort to clean and renewable fuels. The major concerns with using agricultural crops for the production of energy are food and water security. Crops that do not threaten food security can be fermented with a relatively low amount of water and produce high yields of fermentable sugars is thus needed. Sweet sorghum is a fast growing crop that can be harvested twice a year and can produce both food (grain) and energy (sugar juice from stems).

The aim of this study was to determine the sugar content of different sweet sorghum cultivars at different harvest times and also determine the cultivar that will produce the highest ethanol yield at optimized fermentation conditions. Four sweet sorghum cultivars USA 1, USA 2, Honey green and Sugar graze were harvested at 3 and 6 months and the juice was extracted from the stems. The juice was used for ethanol production and the effect of pH, yeast concentration (*Saccharomyces cerevisiae*), dilution factor and the addition of a nitrogen source on the ethanol yield was investigated.

The results showed that the USA 1 cultivar contained the highest sugar content at 3 months. A maximum ethanol yield ($0.48\text{g}\cdot\text{g}^{-1}$) was observed at a pH of 4.5, a yeast concentration of 3 wt%, a dilution rate of 1:1 and when ammonium sulphate was added to the fermentation broth as nitrogen source. Glycerol yield formed as a by-product during fermentation and at a maximum ethanol yield was $0.05\text{g}\cdot\text{g}^{-1}$.

Keywords: Sweet sorghum, Fermentation, *Saccharomyces cerevisiae*, Ethanol, Sugar content

1. Introduction

High contents of carbon dioxide in the atmosphere that are contributing towards global warming is said to be caused by the use of fossil fuels around the world. (Archer, 2005). India, Europe, Brazil and the United States have resorted to the production of biofuels from plants in an attempt to reduce the carbon dioxide levels in the atmosphere (Roseiro *et al.*, 1991, Krishna and Chowdary, 2000, Kim and Dale, 2004).

Bio-ethanol produced from agricultural crops or plants is a clean burning renewable fuel that is being increasingly used as a substitute fuel for road transport applications (Sanchez, 2007). In 2009 worldwide ethanol fuel production reached about 19.5 billion gallons (RFA, 2010). The market for bio ethanol road fuel is growing so rapidly that demand is starting to exceed supply (Kennedy and Turner, 2004). There is an urgent need for alternative plant species that can produce large volumes of biomass for conversion into cost effective bio ethanol on a large industrial scale. Plants used for ethanol production include maize, sugar beet, sugarcane, sweet sorghum and cassava (Adelekan, 2010). Food security is a very serious issue in terms of using food crops for energy production. Crops that are chosen for future energy production should thus be able to produce both food and energy.

Sweet sorghum (also called Sorgho) is an indigenous African plant belonging to the family *Sorghum bicolor* (L) Moench (Bryan, 1990). Sweet sorghum produces grain, which is harvested for human consumption and contains sweet juice in the stalk. After harvest, the stalks are squeezed for the sweet juice, which can be turned into sugar or fermented to ethanol. The stalk material remaining after the sugar juice has been squeezed out is called the bagasse and can be used as animal feed or pre-treated, hydrolyzed and fermented to ethanol. Sweet sorghum is considered as one of the most promising crops for the production of ethanol at low cost (Barbanti *et al.*, 2006; Yun-long *et al.*, 2006; Wang and Liu, 2009).

There are approximately four thousand varieties of sweet sorghum throughout the world (Grassi *et al.*, 2002). The area planted to commercially grown sorghum in South Africa in the years 1995/6-2004/5 was approximately 120 000 ha with an average yield of 2.9 tonnes per hectare (NDA, 2006). Ripe sweet sorghum typically consists of approximately 75% cane, 10% leaves, 5% seeds and 10% roots by weight (Grassi *et al.*, 2002). Using both the sweet juice and the bagasse for bio ethanol production increases the bio ethanol yield that can be obtained per hectare of sweet sorghum (Dolciotti *et al.*, 1998).

Fermentation of sweet sorghum using yeast has an advantage of rapid fermentation (Liu and Shen, 2008). Sufficient nutrients like carbon and nitrogen are required for the yeast to grow and reproduce but inorganic salts present in sweet sorghum juice is not enough to meet the need of fermentation (Mei *et al.*, 2009). Bafrcova *et al.* (1999); Sipos *et al.* (2008) and Jones *et al.* (2004) have shown that yeast extract, ammonium, urea, calcium and magnesium have effects on both growth and fermentation, thus stimulating fermentation rate and ethanol production.

Asli (2010) studied the fermentation of sweet sorghum juice at different pH levels using different nitrogen sources and dilution rates and found that the highest ethanol yield was obtained at a pH of 4.5 using ammonium sulphate as a nitrogen source and an initial sugar concentration of 100 g.L⁻¹. Kundiyana *et al.* (2010) studied the fermentation of sweet sorghum juice at different pH levels, using urea as nitrogen source. The authors concluded that the highest ethanol yield could be obtained at a pH of 4.3 without the addition of urea and without prior sterilisation of the juice.

D'amore *et al.* (1989) studied the optimization of yeast concentration for ethanol production using a *Saccharomyces* strain. The best results for high ethanol yield were observed at a yeast concentration of between 2 and 3.5% (w/v).

Liu *et al.* (2008) studied the effect of adding different inorganic salts on the ethanol yield. Ammonium sulphate produced the highest bio ethanol yield. Ammonium sulphate adjusts and stabilizes the pH of fermentation juice, while the sulphur in the sulphate group is utilized by yeast cells as nutrient source.

Dilution is simply the addition of water to adjust the amount of sugar in the juice. It is necessary because the yeast used for the fermentation process, can be killed by too great a concentration of alcohol produced during fermentation (Mathewson, 1980). It is believed that a sugar concentration of approximately 16 to 18 % causes osmotic pressure effects on the yeast during fermentation and the added stress in the cells yields lower ethanol concentrations (Roukas, 1996). Zanette *et al.* (2007) observed that at low dilution rates the system to be fermented has enough time to transform the sugars into ethanol, but at the same time the hydraulic retention time is very high and a high production of by-products is observed. In this study the fermentation of sweet sorghum juice harvested from different cultivars were investigated with regard to pH, yeast concentration, nitrogen source and dilution rate.

2 Experimental

2.1 Materials and chemicals

Four different sweet sorghum cultivars, USA 1, USA 2, Honey green and Sugar Graze, were harvested at 3 and 6 months from the Agricol Research Company in Potchefstroom. The leaves were stripped off the stalks by hand after harvest and the juice was extracted using a hydraulic three-roller press. The extracted juice was used without any further sterilisation or purification.

Baker's yeast (*Saccharomyces cerevisiae*) was obtained from a local grocery store. The dried yeast was revived at 32°C for 5 minutes using some of the sweet sorghum juice as growth medium. The pH was adjusted during experiments using 50 % (v/v) sulphuric acid (98% Assay) from Labchem and 50% (v/v) sodium hydroxide (98% Assay) from Fluka Chemika.

2.2 Experimental procedure
The experimental steps followed in fermenting sweet sorghum juice is shown in Figure 1.

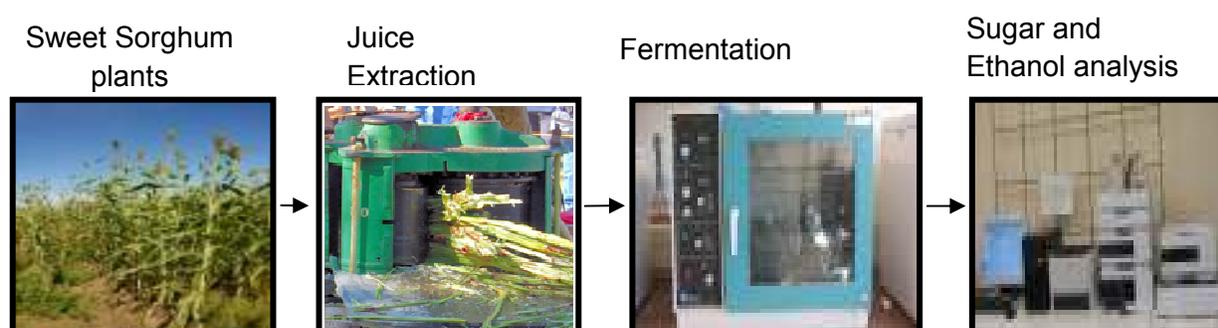


Figure 1: Ethanol production from sweet sorghum

The sweet sorghum juice of cultivar USA 1 was used to investigate the influence of different fermentation variables on the ethanol yield in this study. The optimum fermentation parameters found for the USA 1 cultivar were employed in the fermentation of the other three cultivars for the production of ethanol to compare the yield for the different cultivars.

Fermentation of sweet sorghum juice was carried out in 250ml conical flasks. The dilution rate was varied between 1:1 to 1:3 juice to water ratio while the pH was kept constant at 4.5. *Saccharomyces cerevisiae* was added (3 wt%) and the fermentation was carried out in an incubator at 30°C for 24 hours at a shaking speed of 120 rpm. Samples were collected and analysed for sugars and ethanol at set time intervals.

The effect of pH on the ethanol yield was investigated by varying the pH of the fermentation broth between 4.0 and 6.0 using no dilution. *Saccharomyces cerevisiae* was added (3 wt%) and the fermentation was carried out in an incubator at 30°C for 24 hours at a shaking speed of 120 rpm. Samples were collected and analysed for sugars and ethanol at set time intervals.

The effect of yeast concentration on the ethanol yield was determined by varying the yeast concentration between 1 and 5 wt% at a constant pH of 4.5 and no dilution. The fermentation was carried out in an incubator at 30°C for 12 hours at a shaking speed of 120 rpm. Samples were collected and analysed for sugars and ethanol at set time intervals.

The effect of adding different nitrogen sources to the fermentation broth on the ethanol yield was investigated by adding urea, yeast extract, peptone or ammonium sulphate at a fixed dosage of 1 wt% to the fermentation broth. All other variables were kept constant (pH 4.5, 3wt% yeast concentration, no dilution).

2.3 Analysis

All sugar analyses were done using high performance liquid chromatography (HPLC). An Agilent series 1200 HPLC fitted with a Shodex SP0810 column and using water as mobile phase was used to analyse samples for sucrose, fructose, glucose, ethanol and glycerol. The different sugars, ethanol and glycerol were quantified using a set of standard calibration curves. Samples were filtered through a set of micro filters before analyses to remove all yeast cells and other solids from the samples before analysis.

3 Results

3.1 Effect of cultivar on total sugar content

After juice extraction, samples were taken from different sweet sorghum cultivars and analysed for sugar content. Sweet sorghum juice is known to contain different amount of sugars depending on the type of cultivar (Prasad *et al.*, 2007). The total sugar content of the juices extracted from the different cultivars used in this study at different harvesting times is presented in Table 1.

Table1: The total sugar content of sweet sorghum juice of different cultivars at different harvesting times

Cultivar	Sugar content (g.L ⁻¹)	
	3 months	6 months
USA1	401.22	119.02
USA2	161.36	135.95
Honey Green	128.10	212.63
Sugar graze	275.32	197.00

The highest sugar content for all cultivars and harvest times were obtained at 3 months for the USA1 cultivar. The second highest was at 3 months for the Sugar graze cultivar. USA 1 and Sugar graze are early flowering plants and USA 2 and Honey green are late flowering plant. As soon as the sweet sorghum plant flowers, the sugar content increases. USA 1 and Sugar graze flowered at 3 months which is why the sugar content is high. After flowering, the sugar in the stalks is used to produce the starch rich grains, thus a decrease in sugar content is observed at 6 months. The USA 2 cultivar flowered at 5 months and Honey green at 6 months, which explains why the Honey green sugar content is high compared to USA 2 at 6 months. . The USA 1 cultivar harvested at 3 months was used in all subsequent experiments.

3.2 The effect of dilution rate

Water was added to the sweet sorghum juice in different ratio to assess the influence of dilution rate and thus initial sugar concentration on the ethanol yield. The experimental error associated with these experiments was 9%, calculated for a 95% confidence limit. The effect of varying dilution rate on the ethanol yield is shown in Figure 2.

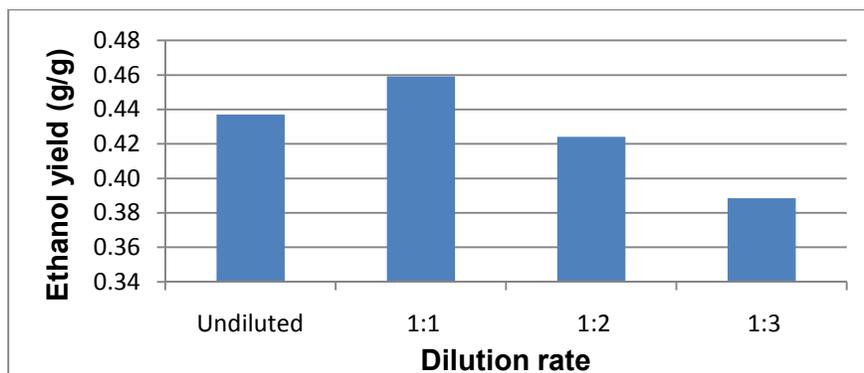


Figure 2: Effect of sugar dilution on ethanol yield after 24 hours

Figure 2 shows the results obtained at the end of fermentation at 24 hour. The highest ethanol yield of 0.45 g.g⁻¹ was observed at a dilution of 1:1. The lowest ethanol yield was observed at 1:3 dilution. Gaur (2006) reported that high sugar concentrations above 20% cause an increase in osmotic pressure and the increase of osmotic pressure has been related with the increase in glycerol yield. Yeast cells adapt to osmotic stress environments by producing glycerol. Figure 2

shows that at high sugar concentration (undiluted) the ethanol yield was low compared to 1:1 dilution rate with reduced sugar concentration.

3.3 The effect of pH

The effect of pH on the ethanol yield is shown in Figure 3. The experimental error associated with these experiments was 7%, calculated for a 95% confidence limit.

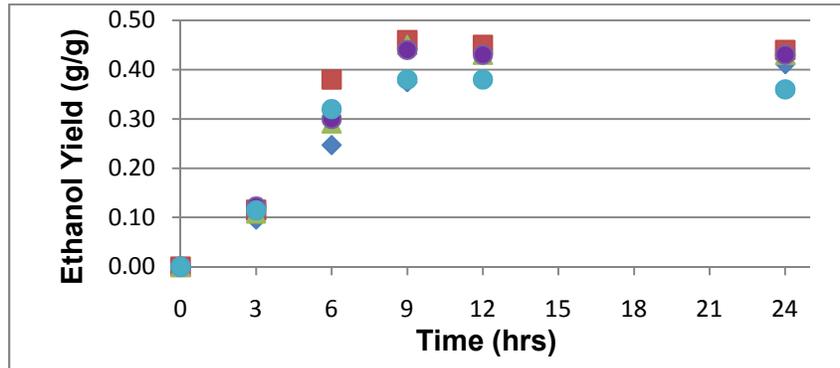


Figure 3: The effect of pH on ethanol yield

(◆-pH 4.0 ■-pH 4.5 ▲-pH 5.0 ●-pH 4.5 ●-pH 6.0)

Saccharomyces cerevisiae contains invertase enzymes that are affected by changes in pH. Bio-ethanol yield was the highest at a pH of 4.5 and the lowest at pH of 6.0. The change in ethanol yield with pH is probably due to the activity of the enzymes expressed by the yeast cells at different pH levels.

3.4 The effect of wt% yeast concentration

The effect of yeast concentration on ethanol yield at is presented in Figure 4. The experimental error associated with these experiments was 7% calculated for a 95% confidence limit.

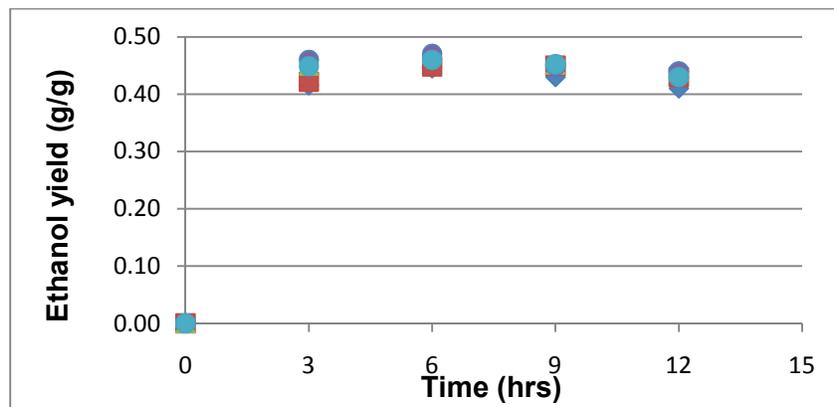


Figure 4: Effect of yeast concentration (wt %) on ethanol yield

(◆1% ■2% ▲3% ●4% ●5%)

Figure 4 shows that the highest ethanol yield was achieved at a yeast concentration of 3 % and 4% at 6 hours and then decreased at 5%. It was observed that as the ethanol yield decrease, the glycerol yield increase. At yeast concentration of 5% ethanol yield decreased and the glycerol yield increased. High glycerol yield is associated with high sugar concentrations of more than 20%. The results obtained suggest that the ethanol yield increases with an increase in yeast concentration up to a certain concentration and starts to decrease. The yeast concentration of 3 wt% was efficient for high bio-ethanol yield at short fermentation time. Table 2 below shows the ethanol and glycerol yield at 24 hours

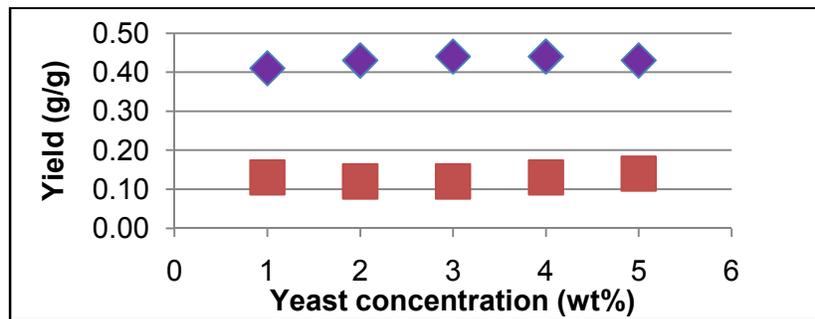


Figure 5: Effect of yeast concentration on ethanol yield (◆ - Ethanol yield ■ - Glycerol yield)

Table 3 shows that at the end of fermentation glycerol yield was high at a yeast concentration of 4% and 5%. The optimum ethanol yield was reached at 6 hours (Figure 4), and started to decrease. $5g.L^{-1}$ has been found as the optimum yeast concentration for high ethanol yield therefore increasing the yeast concentration is associated with activation of glycerol biosynthesis pathways in the yeast (Brumm and Hebeda, 1988).

3.5 The effect of different nitrogen sources

All nitrogen sources were added at a concentration of 1%. Addition of nutrients such as ammonium sulphate, urea, yeast extract and peptone has been reported to play a very important role in increasing the ethanol yield and the rate of fermentation (Fundora *et al.*, 2000). The effect the different nitrogen sources had on the ethanol yield in this study is presented in Figure 5. The experimental error associated with these experiments was 2%, calculated for a 95% confidence limit.

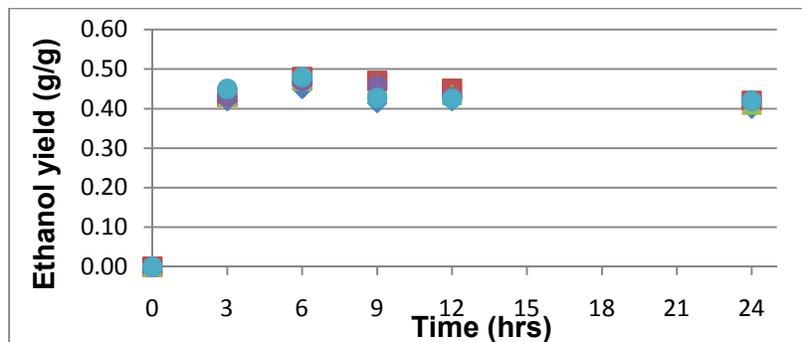


Figure.5: Effect of different nitrogen sources on ethanol yield.

(◆ - No nitrogen ■ -Urea ▲ - Peptone ● -Yeast Extract ● - Ammonium Sulfate)

All the nitrogen sources used had a positive effect on the ethanol yield and sugar utilization compared to the control. The most efficient nitrogen sources during fermentation were ammonium sulphate and urea. Ammonium sulphate is a simpler molecule compared to urea and therefore also cheaper. Jones *et al.* (1994) and Bafrcova *et al.* (1999) observed that nitrogen sources including yeast extract, urea and ammonium sulphate, stimulates the rate of fermentation and ethanol production. The fermentation conversion of sugars to ethanol using the nitrogen source increased by 3 % compared to the control.

4. Conclusions and recommendations

The highest sugar concentration was found at 3 months for cultivar USA 1 and Sugar graze. The Honey green cultivar showed the highest sugar concentration at 6 months. The maximum ethanol yield was achieved at pH 4.5, 3% yeast concentration, 1:1 dilution rate and with ammonium sulphate and urea nitrogen sources. The maximum ethanol yield was 0.48 g.g⁻¹ of sugar representing 94% fermentation conversion efficiency.

5. References

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