African Journal of Biotechnology Vol. 11(54), pp. 11709-11720, 5 July, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.1171
ISSN 1684-5315 ©2012 Academic Journals

Full Length Research Paper

Ethanol production from tropical sugar beet juice

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Accepted 1 June, 2012

Starch and sugar resources have been extensively researched to find a suitable renewable source of energy to supplement the world's ever increasing demand for energy while also abating global warming by stemming the addition of earthbound carbon dioxide into the atmosphere. Sugar beet has been used as a source for sugar production for some time, but its development as a large scale agricultural crop in South Africa has been limited by the large production of sugarcane in tropical areas. Recent trials in the Eastern Cape region have shown some promise for cultivating sugar beets on a large scale. In this study, the influence of process variables such as initial sugar concentration (dilution), pH, yeast concentration and nitrogen source addition were investigated to assess the influence of these variables on the bioethanol production potential of tropical sugar beet. High ethanol yields were obtained without dilution (approximately 0.47 g.g sugar^{-1}) while a pH of 4 and a concentration of 5 g.L^{-1} yeast (*Saccharomyces cerevisiae*) produced the largest amount of ethanol in the shortest fermentation time. The addition of a nitrogen source such as ammonium sulphate significantly increased the ethanol yield. It was concluded from the results of this research that bioethanol can be produced economically from tropical sugar beet cultivars grown in South Africa.

Key words: Tropical sugar beet, fermentation, dilution, pH, bioethanol yield.

INTRODUCTION

Fossil fuels provide 80% of the primary energy needs worldwide and the combustion of fossil fuels account for 73% of worldwide carbon dioxide emissions (Nigam and Singh, 2010; Balat et al., 2008). The progressive depletion of fossil fuel resources, increasing energy demand and concern over the greenhouse gas emissions has increased the research and development of alternative and renewable energy sources (Nigam and Singh, 2010).

Transport plays a major role in the economic activity of South Africa and transport costs constitute approximately 20% of South Africa's gross domestic product (Singh, 2006). Globally transportation accounts for 30% of the energy demand and is responsible for 21% of global greenhouse gas emissions (Markevičius et al., 2010). Currently there are 700 million motor vehicles on the roads worldwide and this is set to increase to 1.3 billion by 2030 and to 2 billion by 2050 with most of the increase coming from developing countries (Balat and Balat, 2009).

The South African economy relies heavily on coal as primary energy source for electricity generation and is reliant on oil imports for transport fuel (Wabiri and Amusa, 2010). The benefits associated with biofuel use are a reduced reliance on foreign oil imports which can lead to long-term energy security, economic growth in rural areas such as job creation and providing an additional income stream for farmers and environmental benefits such as reduced greenhouse gas emissions (Chakauya et al., 2009).

Approximately 60% of the ethanol produced globally comes from sugarcane and 40% from other crops (Balat et al., 2008). The United States and Brazil are the top producers of bioethanol, using maize and sugarcane respectively and account for 70% of the world's

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production while South Africa only produces about 1% of the world's ethanol (Balat et al., 2008).

The biofuel strategy of South Africa proposes a 2% market penetration of biofuels by 2013 and states that bioethanol will be produced from sugarcane and sugar beet, while maize is excluded due to food security concerns (SA, 2007). Sugarcane is a water intensive crop and as South Africa is an already a water-stressed country, the cultivation of sugarcane will be limited to certain areas of the country. Sugar beet however has a high tolerance to a wide range of climatic variations, requires 30-40% less water and fertilizer compared to sugarcane and has sugar content similar to that of sugarcane (Chakauya et al., 2009).

Tropical sugar beet has been successfully introduced in India and currently there are trials being conducted in other tropical countries such as China, Australia, Kenya, South Africa, Brazil and the United States. Tropical sugar beet produces a root of between 0.5 to 2 kg where the majority of the sugar is stored. The root consists mainly of sucrose (15 to 20 wt%), raffinose (0.2 to 0.5 wt%), glucose and fructose (0.05 to 0.1 wt%) and planteose, stachyose and verbascose (Asadi, 2007). The sugar concentration depends on the variety and growth conditions and the average yield of sugar beet is 50-60 tons per hectare (Asadi, 2007).

Sugar beet and the intermediate products produced during the production of sugar can be used as materials for ethanol production. These materials do not require hydrolysis as the sugar content is mainly sucrose which can be easily fermented by *Saccharomyces cerevisiae*. Beet molasses is a commonly used feedstock and is usually diluted to the required sugar concentration and pH for ethanol production (Dodić et al., 2009).

Dodić et al. (2009) investigated the effect of initial sugar concentration on thick sugar beet juice using commercial baker's yeast for 72 h at 30°C. It was seen that if the initial sugar concentration was increased from 20 to 25% ($w.w^{-1}$) the ethanol concentration started to decrease and this showed that the initial sugar concentration does have an effect on the ethanol yield. The study of Dodić et al. (2009) showed that intermediate products of sugar beet processing, such as thick juice can be used for ethanol production and is just as efficient as molasses. Similar results were seen by El-Refai et al. (1992) and Zayed and Foley (1987), who both investigated the influence of fermentation parameters on the ethanol yield from sugar beet molasses. El-Refai et al. (1992) investigated the effect of initial sugar concentration, pH and the addition of nutrients such as urea and magnesium sulphate on ethanol concentration. It was observed that as the initial sugar concentration increased so too did the ethanol yield, but the ethanol yield decreased when the initial sugar concentration was increased further from 200 $g.L^{-1}$ to 250 $g.L^{-1}$ and 350 $g.L^{-1}$. At a high sugar concentration it was reported that the yeast experienced osmotic pressure lead to plasmolysis and a lower ethanol yield.

Zayed and Foley (1987) investigated the use of three different yeast strains in the production of ethanol using sugar beet molasses as well as the effect of initial sugar concentration, fermentation temperature, pH and addition of nutrients. It was found that different yeast strains had a different optimum initial sugar concentration but as seen in Dodić et al. (2009) and El-Refai et al. (1992) the increase in sugar concentration led to an increase in ethanol concentration. Lowering the initial sugar concentration of the broth also dilutes the final ethanol concentration and since experimental errors are not reported in all literature, it is difficult to evaluate whether there is any significant benefit to be gained in terms of ethanol yield when the initial sugar concentration is lowered. The highest ethanol yield reported by Zayed and Foley (1987) was obtained at an initial sugar concentration of 20.8% ($w.v^{-1}$).

The influence of pH on the ethanol production differs in literature, depending on what strain of *S. cerevisiae* or other fermentation organism was used. Zayed and Foley (1987), for example found the optimum pH for ethanol production to be 4.5, while El-Refai et al. (1992) found it to be 5. This is perhaps because two different yeast strains were investigated each with their own optimum.

It has been widely debated whether an additional nitrogen source is necessary for the fermentation of sugar beet juice to ethanol, because it is widely believed that the juice contains enough nitrogen nutrients in its natural state to sustain the fermentation. Zayed and Foley (1987) concluded that the nitrogen naturally occurring in sugar beet juice, although in quantity adequate for the fermentation, is not in a form that is readily accessible to the micro-organism and thus addition of nitrogen sources is necessary. It was shown (Zayed and Foley, 1987) that the addition of nutrients such as urea, phosphorous, sulphur and magnesium substantially increased the ethanol yield.

It is therefore the aim of this study to investigate the potential utilization of South African tropical sugar beet for ethanol production and furthermore to elucidate the effects that some process variables such as initial sugar concentration (dilution), pH, yeast concentration and nitrogen source addition have on ethanol yield.

MATERIALS AND METHODS

Feedstock and chemicals

Tropical sugar beets were received from the Agricultural Research Council of South Africa (ARC) in Rustenburg, South Africa. The tropical sugar beets were washed by hand to remove any soil residue and then chopped into smaller pieces. A juicer was used to extract the juice from the chopped sugar beets. A compositional analysis of sugar beet juice used in this study was done through high performance liquid chromatography (HPLC) and is presented in Table 1.

Table 1. Compositional analysis of tropical sugar beet juice used in this study.

Composition	Weight (%)
Brix index	21.8
Fructose	51.1
Glucose	47.3
Sucrose	1.6

Figure 1. Experimental procedure.

From Table 1 it can be seen that the compositional analysis of the juice used in this study differs significantly from that reported in the literature of Asadi (2007). Literature values reported a high sucrose content while in this study, a high glucose and fructose content was obtained instead of sucrose. Sucrose is a disaccharide consisting of fructose and glucose molecules and is produced by plants during times of stress. The tropical sugar beets used in this study as part of a trial crop that was under irrigation and the assumption can thus be made that these beets did not experience any lack of water or nutrients and thus the plants produced more monomer sugars instead of disaccharides.

Commercial Baker's yeast (*Saccharomyces cerevisiae*) in dried form was obtained from Anchor Yeast. The fermentation broth was used as a growth medium for ten minutes prior to batch fermentation.

Sulphuric acid (98%, Labchem) and Sodium Hydroxide (98%, Fluka) was used to adjust the pH during fermentation. Peptone (Sigma Chemicals), Urea (Fluka), Ammonium Sulphate (Sigma Chemicals) and yeast extract (Fluka) was used as nitrogen sources in the fermentation broth. All chemicals were used without further purification.

Experimental procedure

Tropical sugar beets were washed and chopped whereafter the juice was extracted using a commercial juicer. The juice was fermented at the different experimental conditions without prior sterilization. The effect of the initial sugar concentration on the ethanol concentration and yield was investigated by varying the initial sugar concentration between 43.5 and 218 gL^{-1} and fermentation without any nutrient addition at a pH of 4.5 and a yeast concentration of 1 gL^{-1}. The influence of pH on the ethanol yield was investigated by varying the pH between 4 and 6 and fermentation without nutrient addition with a yeast concentration of 1 gL^{-1} yeast and initial sugar concentration of 109 gL^{-1}. The effect of yeast concentration during fermentation on the ethanol yield was investigated by varying the yeast concentration from 1 to 10 gL^{-1} using a pH of 4 and an initial sugar concentration of 109 gL^{-1}. All fermentations were carried out in an incubator at a constant temperature of 30°C using as shaking speed of 120 rpm. Samples were taken at predetermined intervals and analysed with an Agilent 1200 series high performance liquid chromatograph (HPLC) fitted with a Shodex SP0180 column and a refractive index detector. Sugar and ethanol concentration were quantified using a set of calibration curves. The experimental procedure followed in this study is illustrated in Figure 1.

RESULTS AND DISCUSSION

In this study, the influence of initial sugar concentration, pH, yeast concentration and the addition of nitrogen rich nutrients were investigated.

Influence of initial sugar concentration

The influence of initial sugar concentration on ethanol and glycerol yield (gram per gram sugar) was investigated by diluting the raw, non-sterilised sugar beet juice with distilled water. The experimental error for this set of experiments was calculated to be 6.37% for a 95% confidence level. The sugar uptake curves at different initial sugar concentrations are shown in Figures 2 to 4.

From Figure 2 to 4 it can be seen that the fructose and glucose were completely taken up after 8 h of fermentation while the sucrose was completely utilised after 12 h of fermentation.

Glycerol was detected as the only by-product of fermentation. Ethanol and glycerol production curves are shown in Figures 5 and 6 respectively.

The ethanol concentration in the broth for all initial sugar concentrations used reaches a maximum after

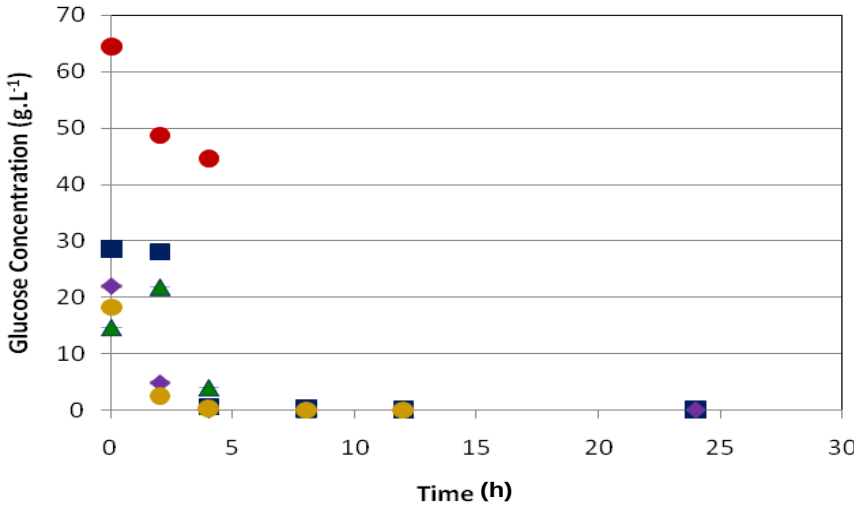

Figure 2. Influence of initial sugar concentration on glucose uptake (• - 218 gL^{-1}, ▲ - 109 gL^{-1}, ■ - 72.7 gL^{-1}, ♦ - 54.5 gL^{-1}, • - 43.5 gL^{-1}).

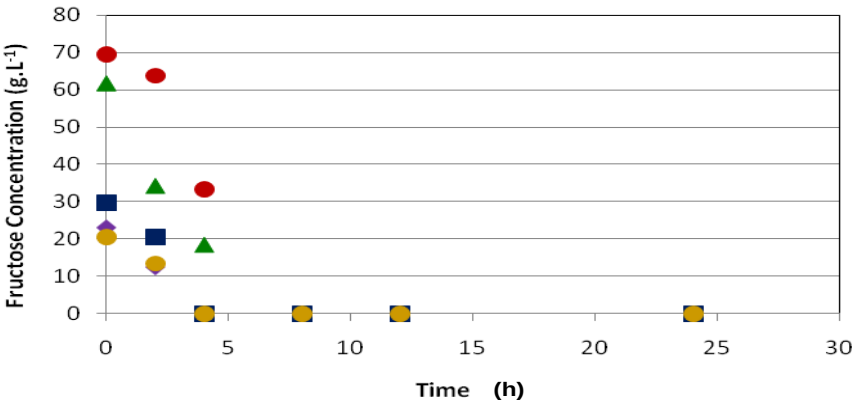

Figure 3. Influence of initial sugar concentration on fructose uptake (• - 218 gL^{-1}, ▲ - 109 gL^{-1}, ■ - 72.7 gL^{-1}, ♦ - 54.5 gL^{-1}, • - 43.5 gL^{-1}).

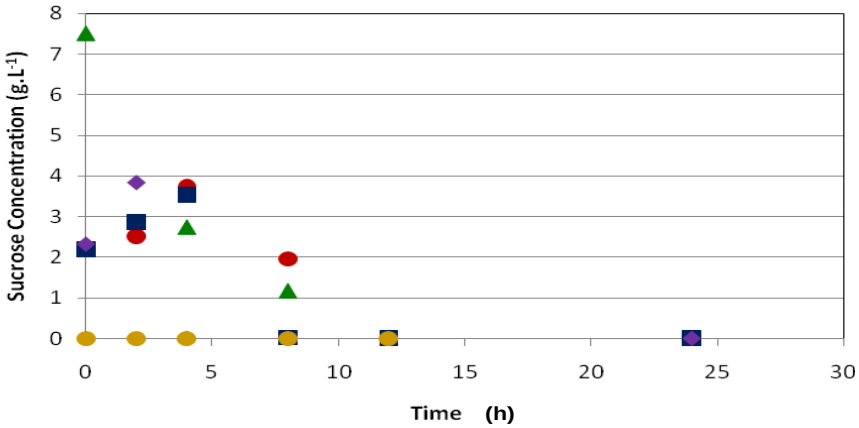

Figure 4. Influence of initial sugar concentration on sucrose uptake (• - 218 gL^{-1}, ▲ - 109 gL^{-1}, ■ - 72.7 gL^{-1}, ♦ - 54.5 gL^{-1}, • - 43.5 gL^{-1}).

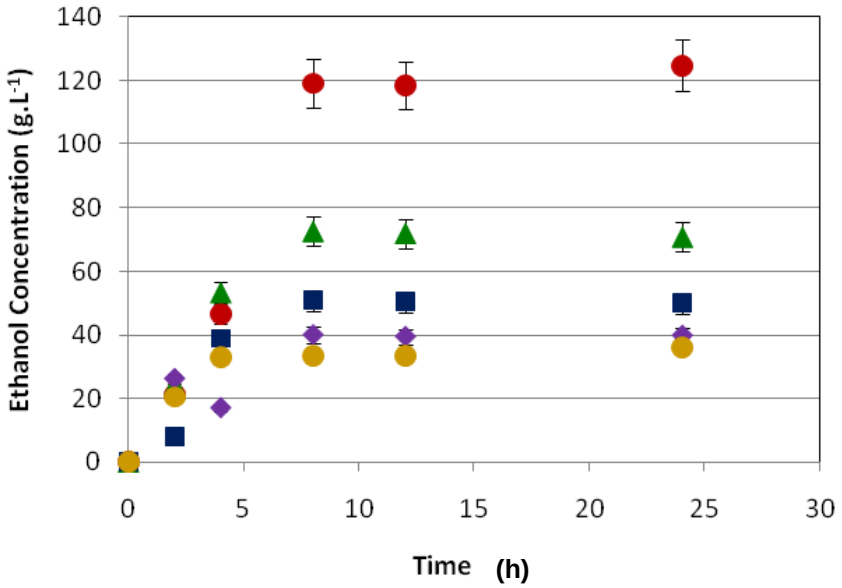

Figure 5. Ethanol production curves at different initial sugar concentrations (● - 218 gL^{-1}, ▲ - 109 gL^{-1}, ■ - 72.7 gL^{-1}, ♦ - 54.5 gL^{-1}, ● - 43.5 gL^{-1}).

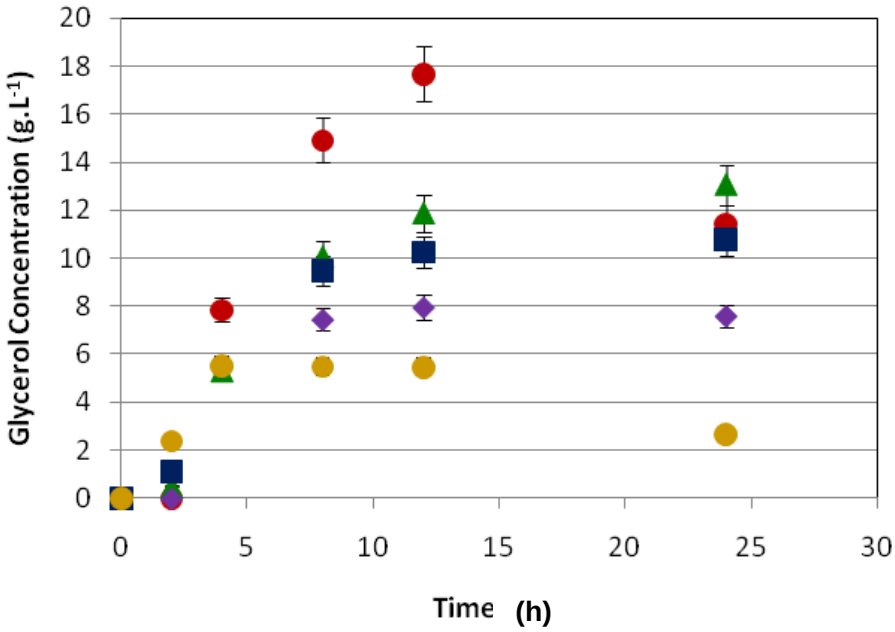

Figure 6. Glycerol production curves at different initial sugar concentrations (● - 218 gL^{-1}, ▲ - 109 gL^{-1}, ■ - 72.7 gL^{-1}, ♦ - 54.5 gL^{-1}, ● - 43.5 gL^{-1}).

approximately 8 h, indicating that no ethanol is produced after the sugar is depleted. The initial sucrose concentration is low enough that the conversion of the sucrose to ethanol after 8 h does not significantly change the ethanol concentration recorded. The glycerol concentration steadily increases with time and is higher for higher initial sugar concentrations. The observation that the glycerol concentration increases after the sugar in the broth has been depleted would suggest that the sugar still being utilised within the cells after 8 h is converted to glycerol instead of ethanol. The amount of glycerol that formed elates to the amount of sucrose

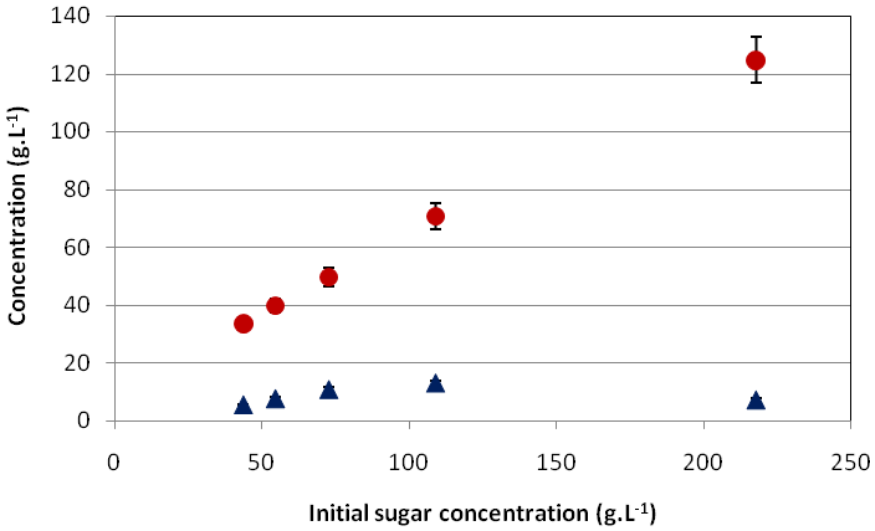

Figure 7. Influence of initial sugar concentration on ethanol (•) and glycerol (▲) concentration after 24 h of fermentation.

depleted between 8 and 12 h for the higher initial sugar concentrations (109 and 218 gL^{-1}) while the amount detected at lower initial sugar concentrations is too low and falls within the experimental error of these experiments. It can thus be deduced that the glucose formed after 8 h for the higher initial sugar concentration is the result of the conversion of residual sucrose to glycerol instead of ethanol. Glucose, fructose and sucrose can theoretically be converted to glycerol through the Embden-Meyerhof-Parnas pathway if the yeast is experiencing osmotic pressure effects, if the uptake rate of sugar exceeds the capacity of the respiratory pathways or if the fermentation is taking place anaerobically. According to literature (Dodić et al., 2009; El-Refai et al., 1992; Zayed and Foley, 1987) *S. cerevisiae* will only experience osmotic pressure effects at initial sugar concentrations above 200 gL^{-1}. Only one initial sugar concentration (218 gL^{-1}) used in this study is higher than the suggested 200 gL^{-1} in literature and therefore it is the only fermentation broth in which there is a chance for osmotic pressure effects on the yeast cells. Dilution of the amount of sugar present should lower the sugar uptake rate (Figures 2 to 4) and thus it is doubtful that the sugar uptake rate exceeds that of the respiratory capacity. The Embden-Meyerhoff-Parnas pathway for pyruvate production and finally ethanol production from glucose is well known. In this pathway, dihydroxyacetone phosphate (DHAP) and glycerol-3-phosphate (G3P) is in equilibrium and as G3P is used up by the sink reaction to ethanol, DHAP is converted to G3P. The presence of glycerol in all fermentation broths in this study would thus suggest that some of he DHAP is converted to glycerol during the fermentation.

The influence of initial sugar concentration on the ethanol and glycerol concentration is shown in Figure 7. The ethanol concentration increases linearly as the initial sugar concentration increases and it is clear from Figure 7 that initial sugar concentration had no influence on the ethanol concentration since the data falls on a straight line that intersects the origin. Decreased ethanol concentration with decreased initial sugar concentration is thus purely the result of dilution of the mixture. Glycerol concentration follows the ethanol production with the only exception being the low glycerol concentration at the highest initial sugar concentration. The change in glycerol concentration falls within the experimental error of this study and thus not much can be deduced from this data. The relationship between ethanol concentration and glycerol concentration can better be seen in Figure 8 where glycerol concentration was plotted as a function of ethanol concentration.

From Figure 8 it can be seen that the glycerol concentration is at maximum when the ethanol concentration is approximately 70 gL^{-1} for an initial sugar concentration of 109 gL^{-1}. This was an unexpected result. The drop in ethanol concentration is explained by the dilution of the same amount of ethanol formed, but it does not explain the increase in glycerol concentration with a decrease in initial sugar concentration. The only conclusion that can be drawn from this data is that initial sugar concentration does not influence the amount of ethanol formed, but does significantly influence the amount of glycerol formed.

Concentration is a volume dependent quantity and to confirm the conclusion drawn from the ethanol and glycerol concentration curves that the amount (mass) of

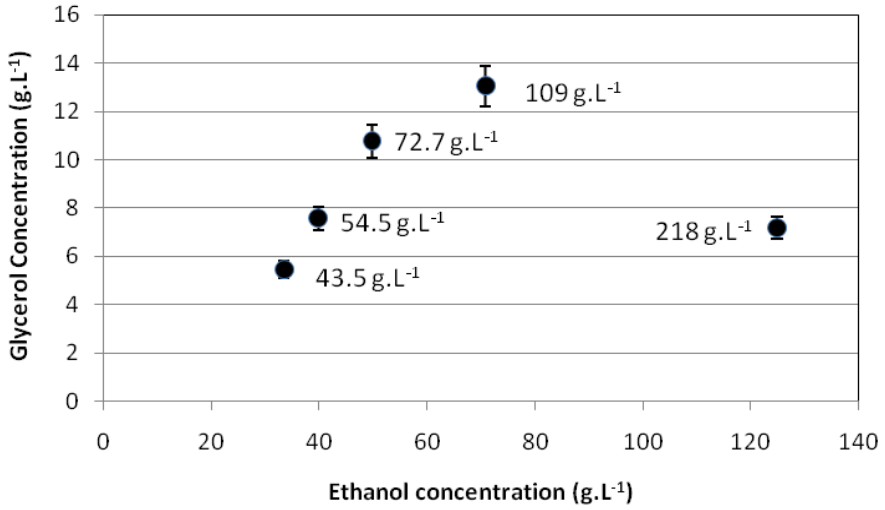

Figure 8. Relationship between ethanol and glycerol concentrations after 24 h of fermentation for different initial sugar concentrations.

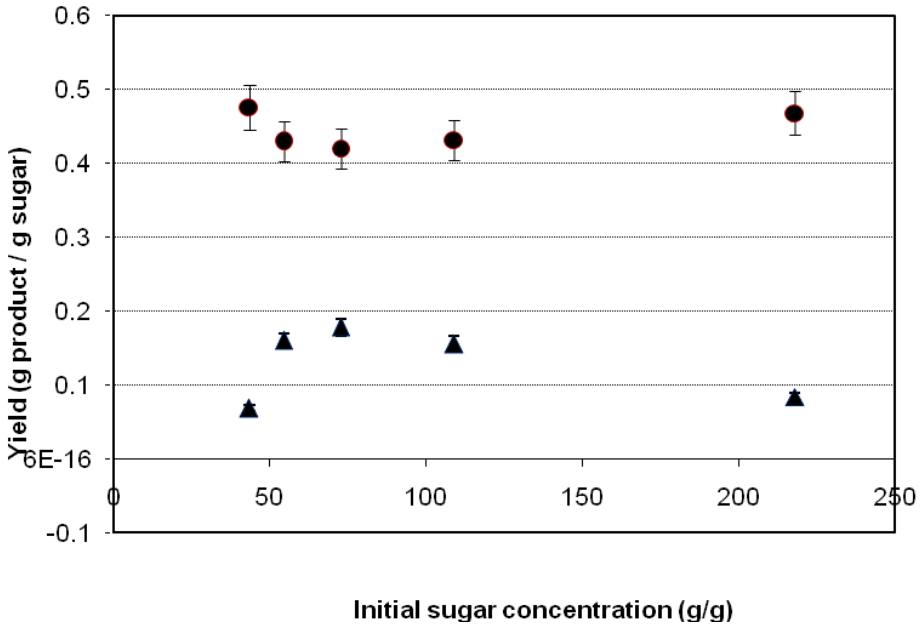

Figure 9. Influence of initial sugar concentration on ethanol (•) and glycerol (▲) yield based on initial mass of fermentable sugars present in broth.

ethanol formed remained approximately the same for all the initial sugar concentrations used, ethanol and glycerol yields based on the initial amount of fermentable sugar present in the broth were calculated. The ethanol and glycerol yields after 24 h of fermentation are shown in Figure 9.

From Figure 9 the results of Figure 7 are confirmed in that there is no significant change in the ethanol yield with a change in initial sugar concentration. The glycerol concentration has a maximum between 70 and 110 gL^{-1} initial sugar concentration. It is clear however that the amount of glycerol formed does significantly change with a change in initial sugar concentration and that it is best to keep the initial sugar concentration as close as possible to 200 gL^{-1}.

Influence of yeast concentration on ethanol yield

The influence of varying yeast concentrations during

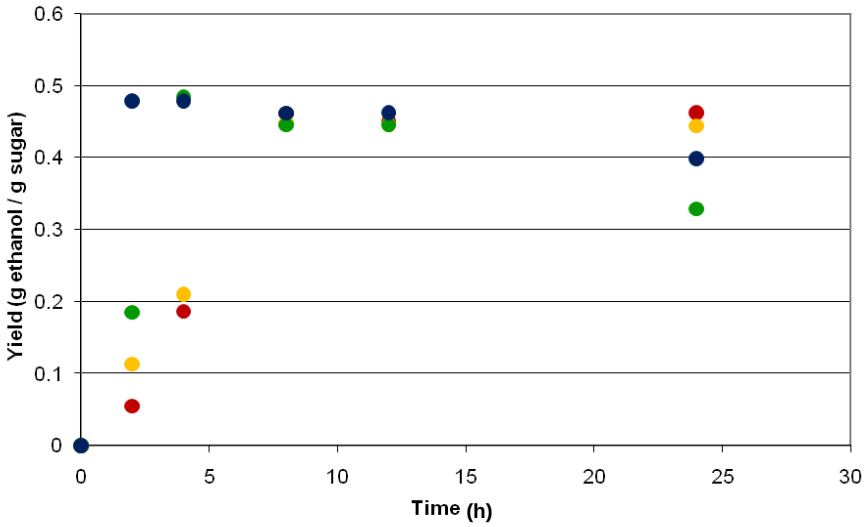

Figure 10. Effect of yeast concentration on the ethanol yield (• - 1 gL^{-1}, • - 3 gL^{-1}, • - 5 gL^{-1}, • - 10 gL^{-1}).

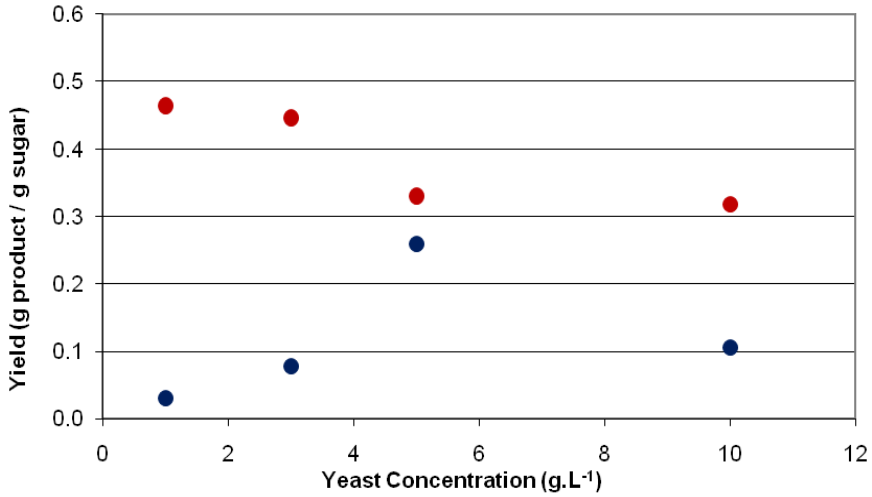

Figure 11. Influence of yeast concentration on ethanol (•) and glycerol (•) yield during fermentation.

fermentation of tropical sugar beet is presented in Figure 10. The experimental error associated with this set of experiments was determined to be 2.57% for a 95% confidence level.

Figure 10 shows that increasing the yeast concentration did have a significant effect on the ethanol yield as well as the fermentation time. A maximum ethanol yield of 0.48 gg^{-1} which corresponds to a conversion efficiency of 95%, was achieved after 2 h of fermentation using a 10 gL^{-1} yeast concentration. The same yield was obtained after 4 h of fermentation using a 5 gL^{-1} yeast concentration. Increasing the yeast concentration increases the amount of yeast cells available to convert the glucose into ethanol and thus the sugar substrate is consumed faster. Arshad et al. (2008) found that increasing the inoculum size from 10 to 30% increased the ethanol yield, but also decreased the formation of by-products such as methanol, fusel alcohols and acetic acid. Only glycerol was observed as a by-product in this study as can be seen in Figure 11.

At higher yeast concentrations, the substrate is consumed faster and if the fermentation is allowed to continue beyond the time of maximum ethanol concentration, the ethanol in the cells will be converted to glycerol and thus if the ethanol and glycerol yields are compared at the same time interval, for example after 24

Table 2. Optimum fermentation times for different yeast concentrations.

Yeast concentration ($g.L^{-1}$)	Optimum time (h)
1	24
3	8
5	4
10	2

h of fermentation, the lower yeast concentrations will yield a higher ethanol concentration (Figure 5). The ethanol yield levelled off at approximately 32 gg^{-1} for 5 and 10 gL^{-1} yeast concentrations, respectively. At these high concentrations, the consumption rate of the sugar substrate and thus the transfer rate across the cell walls are high enough to start to irreparable damage the yeast cells, which explains the lower glycerol concentration at 10 gL^{-1} yeast concentration. Although the cells have been able to produce the ethanol, the cell metabolism is too damaged to further use the ethanol to produce glycerol. This is also evident from the fact that the final fermentation broth still contained a large amount of unconverted fructose while almost all the sugars were converted at yeast concentrations lower than 10 gL^{-1}.

Figure 11 suggests that if higher yeast concentrations are to be used to speed up the fermentation process, the fermentation should be stopped in time to ensure that the ethanol yield is high while the glycerol yield is still low. The optimum time for fermentation for each of the yeast concentrations used in this study is listed in Table 2.

From Table 2 it can be seen that the time for optimum ethanol yield increases exponentially as the yeast concentration is lowered. The fermentation volumes used during these experiments were small (50 ml) and thus heat and mass transfer effects did not play any role in the fermentation times. At higher fermentation volumes, the effect of heat and mass transfer on the diffusion of species to and from the yeast cells can have a significant effect on the time needed to achieve the highest ethanol yield.

Influence of nitrogen supplementation on ethanol yield

The effect of the addition of different nitrogen supplements on the ethanol yield is presented in Figure 12. The different nitrogen supplements were added at a loading of 750 mg NL^{-1}. The experimental error associated with these set of experiments were calculated to be 1.42% for a 95% confidence level.

From Figure 12 it can be seen that all nitrogen supplements investigated had a significant positive effect on the ethanol yield with the yield increasing from 0.44 to 0.47 gg^{-1} when nitrogen was added. Yeast cells can be affected in two ways by the addition of nitrogen: it can increase biomass production and can also increase the sugar utilization rate (Beltran et al., 2005). Yeasts require a constant supply of assimilable nitrogen as it plays a role in the structure and function of the cell (Júnior et al., 2008). It has also been found that yeast might require this extra nitrogen to cope with osmotic pressure (Thomas et al., 1996) and it has been reported that the minimal amount of freely assimilable nitrogen (FAN) required for an adequate fermentation process is 140 mgL^{-1} increasing as the sugar concentration increases (Breisha, 2010). The results from Figure 12 suggest that the tropical sugar beet juice used in this study was slightly deficient in freely assimilable nitrogen (FAN), which can lead to stuck or sluggish fermentation. The addition of the nitrogen supplements thus added the necessary assimilable nitrogen to increase the ethanol yield. It can also be seen from Figure 12 that nitrogen supplementation can be used to prevent the metabolic pathway that will result in the formation of glycerol as by-product and force the reaction towards ethanol production.

Ammonium sulphate was chosen to assess the influence of different concentrations of nitrogen on the ethanol yield. Although the addition of ammonium sulphate produced less ethanol than the other sources used, it is one of the simplest forms of nitrogen (Mendes-Ferreira et al., 2004) and thus also the cheapest to use. The effect of supplementing the fermentation broth with different concentrations of ammonium sulphate is shown in Figure 13.

From Figure 13 it can be seen that the ammonium sulphate concentration did not have a significant effect on the ethanol yield, but did have a significant effect on the glycerol yield with the lowest glycerol yield obtained at a concentration of 750 mg NL^{-1}.

Influence of pH on ethanol yield

The effect of the pH on the ethanol yield is shown in Figure 14. The experimental error associated with this set of experiment was determined to be 4.17% for a 95% confidence level.

From Figure 14 it is seen that changing the pH did have a significant effect on the ethanol yield and that an increase in pH of the broth led to a significant decrease in the ethanol yield. A relatively high yield (0.49 $g.g^{-1}$, corresponding to a 95% conversion efficiency) was obtained using the natural pH of the juice, which is also close to a pH of 4. Ogbonna et al. (2001) found similar results, but since the natural pH of the juice is also dependent on the season and area of cultivation of the tropical sugar beet, pH control might be necessary to maintain high ethanol yields throughout the production season.

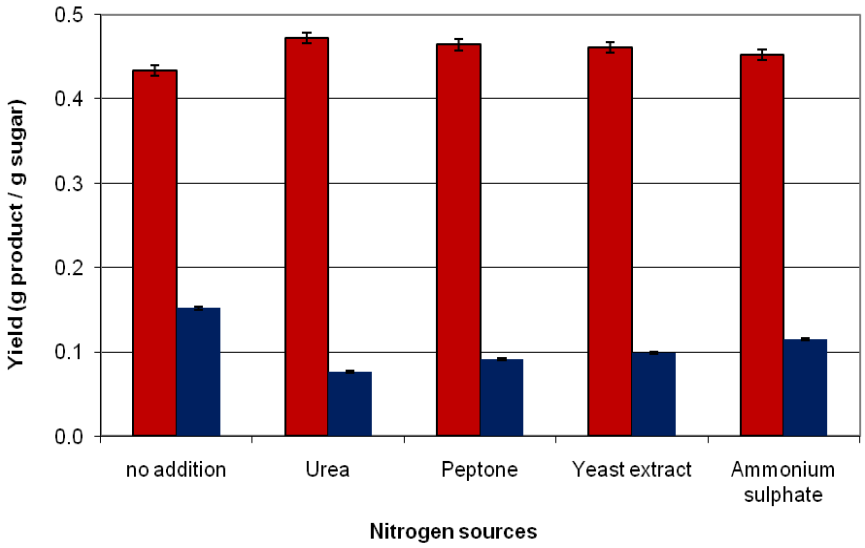

Figure 12. Effect of adding different nitrogen sources to the fermentation broth on the ethanol (•) and glycerol (•) yields after 24 h of fermentation.

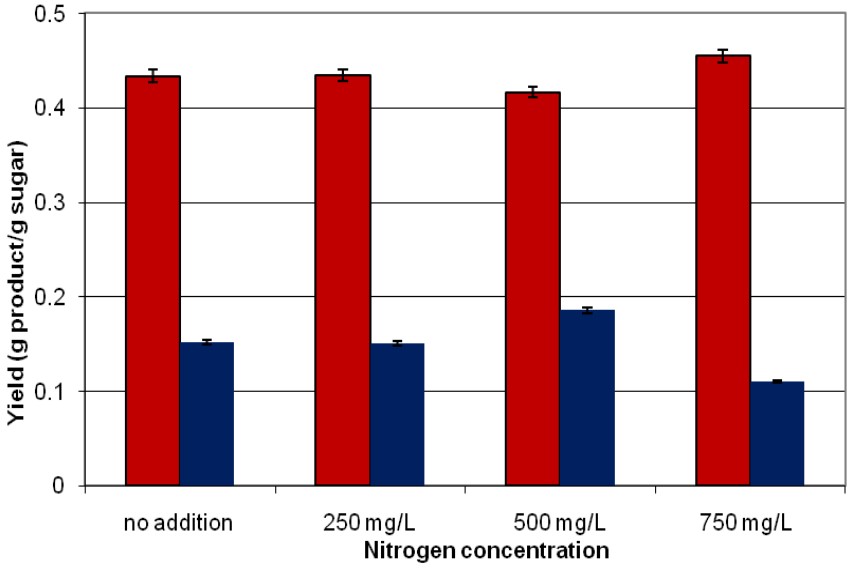

Figure 13. Influence of ammonium sulphate concentration on the ethanol (•) and glycerol (•) yield after 24 h of fermentation.

The ethanol yield as well as the glycerol yield obtained at different pH levels after 24 h of fermentation is presented in Figure 15.

The pH of the fermentation medium is very important as it can influence the ionic state of the mineral components found in the yeasts as well as the surface of the yeast cell, which in turn can influence the activities of the enzymes involved in metabolic activities (Arshad et al., 2008). It has been found that increasing the pH effects some of the enzymes involved in the glycolysis pathway and at a higher pH the activity of the enzyme aldehyde dehydrogenase is increased which converts the metabolic product acetaldehyde to acetic acid instead of ethanol (Munene et al., 2002). The yeast does this in an attempt to lower the pH in the fermentation medium to a more optimal value (20). No acetic acid formation was observed in this study. Zayed and Foley (1987) observed that *S. cerevisiae* will shift its metabolism to produce

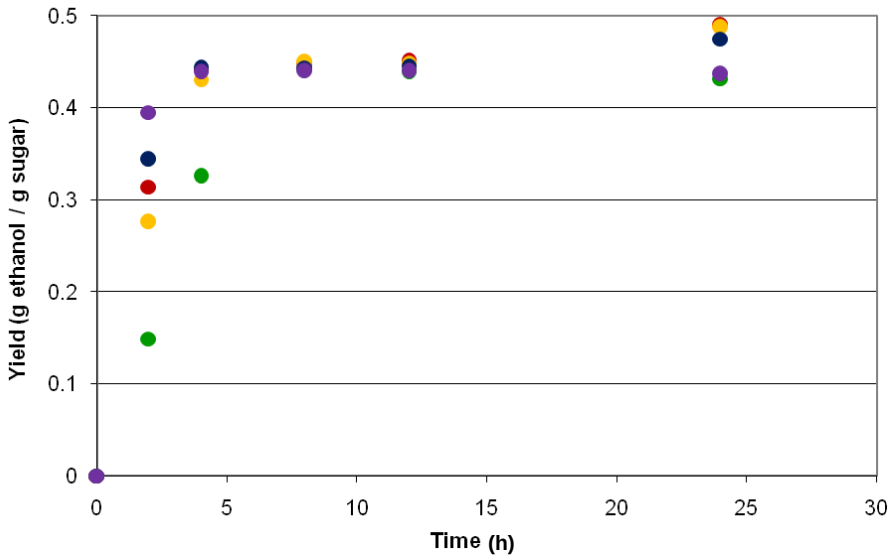

Figure 14. Effect of pH on the ethanol yield (• - No adjustment, • - pH 4, • - pH 4.5, • - pH 5, • - pH 5.5).

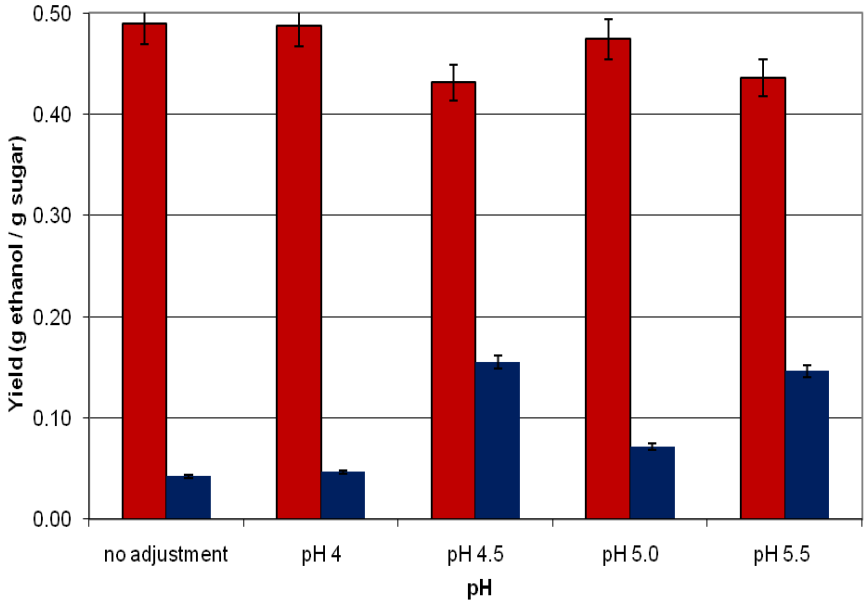

Figure 15. Influence of pH on ethanol (•) and glycerol (•) yield after 24 h of fermentation.

glycerol instead of ethanol at higher pH levels. From Figure 15 it can be seen that in this study, the higher pH levels also led to lower ethanol and higher glycerol yields, confirming the findings of Zayed and Foley (1987).

Conclusion

In this study the process variables that could influence the ethanol yield from tropical sugar beet were investigated. The juice was fermented without prior filtering or sterilisation. It was found that dilution ratio, yeast concentration as well as pH significantly influenced the ethanol yield. Glycerol was primarily formed as by-product. Dilution of the initial sugar concentration significantly decreased the ethanol yield. It could be concluded that both transport of sugars across the cell wall as well as substrate limitation influences the amount of ethanol and glycerol being formed at different dilution

ratios. It was found in this study that the mechanism of ethanol production switched from transport controlling to substrate limited at a dilution ratio of 1:3 (juice:water).

It was further found that higher yeast concentrations produced more ethanol in a shorter amount of time and that there is an optimum fermentation time for each yeast concentration used. Allowing the fermentation to proceed beyond the optimum fermentation time for the yeast concentration used will result in lower final ethanol and higher final glycerol concentrations, because the yeast cells starts using ethanol as an energy source if the substrate is used up and then the glycerol pathway is favoured. It was also seen that at very high yeast concentrations (10 gL^{-1}), the transfer of sugar across the cell walls was high enough to cause permanent damage to the cells, resulting in low ethanol and glycerol yields.

Supplementing the fermentation broth with a nitrogen source had a significantly positive effect on the ethanol yield, while the production of glycerol was suppressed. Urea and peptone supplementation to the fermentation broth resulted in the highest ethanol yields. Ammonium sulphate was used to investigate the influence of different nitrogen concentrations on the ethanol yield and it was found that there was no significant influence on the ethanol yield, although the higher concentrations (750 gL^{-1}) resulted in less glycerol being formed.

Adjustment of pH during fermentation did have a significant effect on the ethanol yield with a lower pH resulting in higher ethanol yields. This was attributed to the fact that the yeast cells prefer a more acidic environment that is better suited to the working of the enzymes during fermentation. The natural pH of the tropical sugar beet juice is close to that of 4 and thus a high ethanol yield is obtained without adjustment of the pH during fermentation.

The highest ethanol yield obtained in this study was 0.49 gg^{-1} which corresponds to a fermentation efficiency of 96%. A kilogram of tropical sugar beet juice will yield approximately 400 ml of juice or 87.2 g of sugar. This translates to an ethanol yield of 110.5 L per kg of tropical sugar beet roots. Tropical sugar beet has a high yield of roots per hectare and the high sugar content relates to an ethanol yield of 8123 L/ha.

It can thus be concluded from this work that tropical sugar beet is a viable crop for ethanol production that requires no additional enzymes and no additional water or pH adjustments to obtain a high yield of ethanol.

ACKNOWLEDGEMENT

The authors would like to acknowledge the South African National Energy Research Institute for funding of this research work.

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