A Comparative Analysis of Sugar Cane and Sugar Beet Fermentation

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Abstract

With a view to curb global warming, it is required that countries pursue a net zero emission of carbon dioxide. CO_2 comes mainly from the burning of fossil fuel. To this effect, alternative energy sources such as bioethanol must be sought. Two feedstocks are sugar cane and beet fruit. The latter is not a sustainable feedstock, whereas sugar cane is. These were fermented to produce bioethanol using the biological catalyst *Saccharomyces cervisae*. The sugar cane demonstrated a high initial average Brix content of 18.97, compared to the average Brix content of 7.87 for beet fruit. The % reduction of the Brix content seems to be the same in both instances (72%). The ethanolic content of the distillates were analysed using Gas Chromatography, GC. The highest average % yield of bio ethanol (10.18%) was noted for the sugar cane juice, whereas a value of 4.1 % was observed for sugar beet. The Gas Chromatograph for both substrates also indicated the presence of other minor components such as acetone, methylacetate, propanol, ethylacetate, butan-2-ol, isobutanol, butanol, acetal, pentanol and furfuraldehyde. The sugar beet GC was much cleaner with side products: propanol, ethylacetate and isobutanol. This demonstrated that sugar cane is a far superior source for bioethanol, compared with sugar beet.

Keywords: *Bioethanol production, Sugar cane, Beet, Fermentation, Environmental impact*

Introduction

With a view to decrease dependence on fossil fuel, as a result of depletion, increasing global fuel price, increasing population and increasing global warming, there has been increased interest in the use of renewable energy sources of which bioethanol is one **[1,2,3]**. Bioethanol (b.p: 78.5°C) can be used for a variety of purposes, of which blending with gasoline to produce gas alcohol to power automobiles is of current utilisation **[1,2,3, 24-25]**. In addition, ethanol is a clean burning renewable energy source[4]. Ethanol is also an important component of alcoholic beverages such as wine, beer, cider, vodka, gin. whisky, brandy etc. It is also an important starting materials for aldehydes, ketones, carboxylic acid, carboxylic acid derivatives and the hydroxyl group is a component of many pharmaceutical drugs [5]. Ethanol can be used in the perfume, disinfectant, tincture, biological and biofuel industries. Ethanol production through Fermentation has been one of the world most significant approaches to aid in the Advancement of Commercial Industry.

Ethanol doesn't have significant environmental impact as fossil fuel combustion **[3]**. It has low air polluting effect and low atmospheric photochemical reactivity, further reducing impact on the ozone layer **[6]**. It contributes little net CO_2 accumulation to the atmosphere and thus should curb global warming[6-9].

Ethanol can be used in three primary ways as biofuel, namely, E10 which is a blend of 10% ethanol and 90% unleaded gasoline, a component of reformulated gasoline both directly

and or as ethyl tertiary butyl ether (ETBE) and as E85 which is 85% ethanol and 15% unleaded gasoline. When mixed with unleaded gasoline, ethanol increases octane levels, decreases exhaust emissions and extends the supply of gasoline. Bio-ethanol is made by fermenting almost any material that contain starch or sugar. Grains such as corn and sorghum are good sources, but fruits that are high in sugar concentration are excellent sources as well, since they contain ready to ferment sugars [10].

To solve the above problem, emanating from fossil fuel, one alternative is to produce bioethanol from fruits, other grown organic matter or waste [3,4,6-8,9]. Bioethanol can be obtained via the fermentation of glucose, fructose or sucrose under the influence of *Saccharomyces cerevisiae* at room temperature, [4,6-35]. This is shown in Figure 1. Also, acid hydrolysis of lignocellulose material followed by subsequent fermentation

[7-21]. Sugar rich sources include ripe fruits [8-18] etc. Other sources include biodegradable fraction of products, waste and residues from agriculture like vegetables, fruit wastes and animal origin [11-12, 17, 20-21] etc. The percentage yield of ethanol, ranging from 4.0 -10.0 v/v) have been reported [3-12]. Fruits that are high in sugar concentration are favourable to the fermentation process, since they can produce high percentage volume of ethanol

Fermentation is the process of energy production in a cell in an anerobic environment with the lack of an external electron acceptor [22]. Sugars are the common substrate of fermentation and the products include ethanol, lactic acid and hydrogen. In some instances, compounds such as butyric acid and acetone are produced.
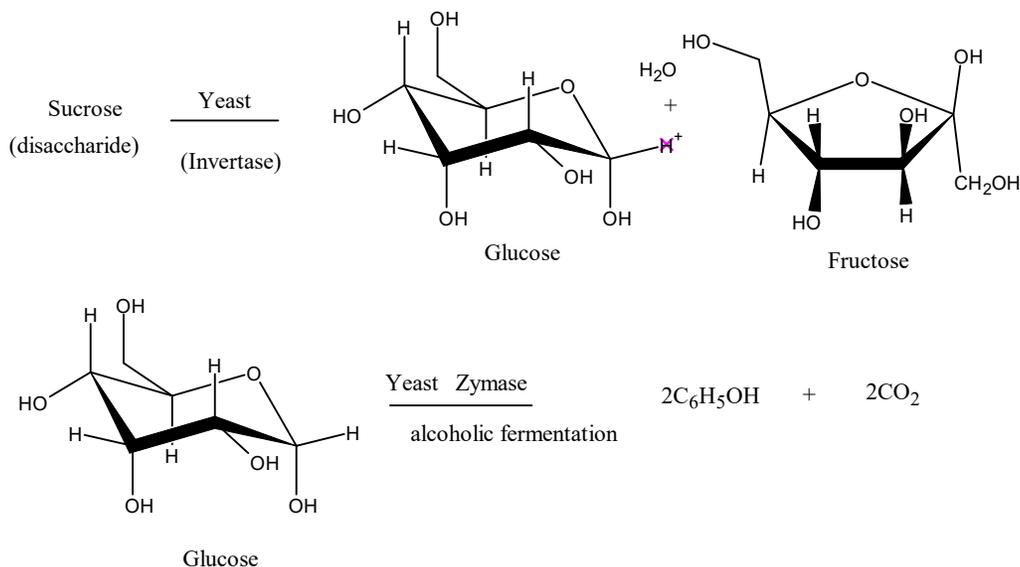

Figure 1: Fermentation route of glucose and sucrose

The fermentation process begins with the yeast breaking down the different forms of sugar in any fermenting matrix. *Saccharomyces cerevisiae* contains two enzymes that is very important for the yeast enzyme activity in the fermentation process. These two enzymes are called Invertase and Zymase and they functions are similar but somewhat prerequisite to each other. Invertase aids in converting any sucrose sugar that is present in any biomass that is used in fermentation to glucose and fructose while zymase aids in the conversion of glucose to ethanol [22]., Figure 1.

During Fermentation, starch is first hydrolysed to maltose by the action of the enzyme diastase. This enzyme is obtained from germinating barley seeds or malt. Maltose is converted to glucose by the enzyme maltase. Glucose is then fermented to ethanol via the enzyme zymase [23], Figure 2. Once the sugars are broken down into monosaccharides, the yeast can now use them. *Saccharomyces cerevisiae* is able to perform both aerobic and anaerobic respiration.
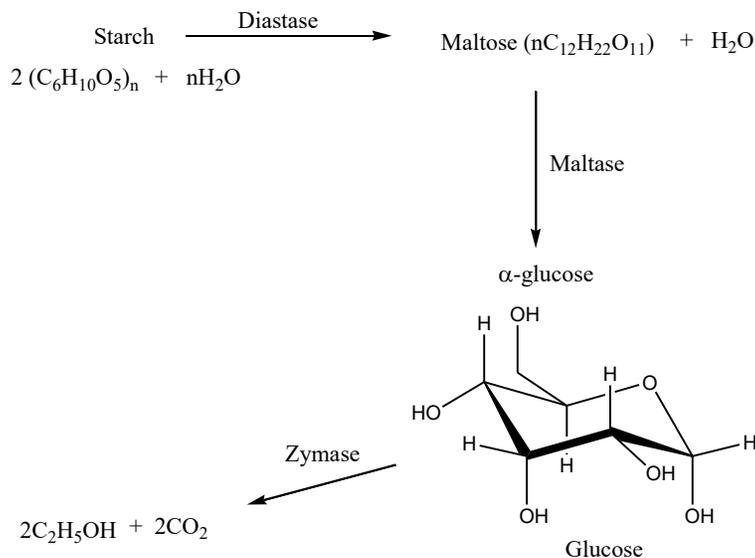
$$2\ (C_6H_{10}O_5)_n\ +\ nH_2O \xrightarrow{\text{Diastase}} \text{Maltose}\ (nC_{12}H_{22}O_{11})\ +\ H_2O$$

Maltase

α-glucose

Zymase

$$2C_2H_5OH\ +\ 2CO_2$$

Glucose

Fig. 2.0

Twenty research articles were reviewed, with the view to identify research gaps and several points can be noted **[24-63]**. Several feedstocks were reported and these are beet molasses, sugar cane, sugar beet molasses, sugar beet syrup, sugar beet pulp, beet root wine, beet juice, sugar beet. To the best of knowledge, only one article reported the fermentation of sugar cane vs beet sugar in the production of sparkling wines. Sixteen of these articles reported the use of *Saccharomyces cervisae*. One article reported the use of *L. acidophilus*, *L. plantarum*, and *scheffersomyces stipites*, *Aspergillus niger* was reported as biological agent to convert sugar rich feedstock above to bioethanol. Varying % yield for bioethanol were obtained. No experimental design was mentioned for twelve of these articles. For ten of these articles, no statistical analyses was used. ANOVA was the dominant statistical tool to test for significance difference. For one article, the Duncan T-test was employed. For another article, Linear Regression and T-tests were used to analyse results. One of the experimental design employed a 2 x 2 Factorial design with two factors: sugar type and time points.

The objectives of this research were to compare the fermentation yield of sugar cane versus sugar beet. The rationale for using these two substrates is that sugar cane is an abundant sustainable feedstock in the Caribbean. In addition, sugar beet can be grown in certain parts of Guyana. Sugar beet is also an alternative sweetener in the Europe and its fermentation profile must be evaluated. This has made it necessary to compare the ethanolic fermentation yield of both sugar rich substrates. These information will dictate the further expansion of the sugar cane industry in Guyana and the rest of the Caribbean.

Methodology

Experimental Design:

The experimental design used was the Completely Randomised Block Design (CRD). The three treatments were replicated three times. There were a total of 6 experimental units

Sugar Cane Method:

The process began by obtaining mature sugar cane stalks from three different sources. Care was taken to ensure the sugar cane was healthy and free from diseases or pests. Following harvesting, the juice was extracted from the sugar cane stalks using a mechanical mill. The sugar cane was randomly selected. A refractometer was used to measure the initial sugar content (°Brix) of the sugar cane juice.

Experiments were conducted in triplicates. The jars were collected and labelled Jar 1, Jar 2, and Jar 3. 500 ml of sugar cane juice was added to each of the jars. The initial pH was measured and citric acid was added to each medium to lower the pH. This is because the organisms, specifically yeast, which produce the enzymes needed for glucose fermentation, have adapted to thrive in acidic environments (Yalcin & Ozbas, 2008).

For the fermentation process, the jars were closed, and tubes were connected to the jars leading to a test tube with paraffin. During fermentation, microorganisms consumed the sugars in the sugar cane juice and produced carbon dioxide (CO_2) as a byproduct (Pepin & Marzzacco, 2021). The gas needed to escape from the jar to prevent pressure buildup. The tube allowed the CO_2 to bubble out into the paraffin solution.

After 72 hours, the mixture was filtered. The filtrate was obtained and collected in 500 ml water bottles. The Brix of each filtrate sample was measured. The filtrate was distilled using simple distillation at a temperature range of 80°C to 95°C. For each jar, six fractions were collected and added to six test tubes. The filtrate's composition was tested for the percentage of alcohol using a pictometer. The ethanol concentration and other components were analysed using gas chromatography. The ethanol standard used had a 40% ethanol concentration and 3.775 g/HL for the 14 analytes.

Sugar Beet Method:

The beet was washed with distilled water, dried, weighed, and cut into smaller pieces. Experiments were conducted in triplicates. Drying the beet removed excess water, providing a more accurate measurement of the actual biomass. Blending the beet into smaller pieces increased the surface area available for enzymatic and microbial activity during fermentation, enhancing the efficiency of the process. 200 g of beetroot was weighed. The determination of the volume of beet was conducted using a graduated measuring cylinder. The beet was introduced into a cylinder filled with 40 ml of water. The volume difference observed before and after the complete immersion of the feedstock represented its volume (Vf). The specific gravity of the feedstock was computed as the ratio of the feedstock's mass (Mf) to its volume (Vf). Specific gravity holds significance in fermentation studies, aiding in the assessment of feedstock density, providing insights into its composition, and predicting fermentation efficiency.

The jars were collected and labelled Jar 1, Jar 2, and Jar 3. 605 g of beetroot was blended with 500 ml of water and added to each of the jars. For the fermentation process, a solution of inoculated yeast, *Saccharomyces cerevisiae*, was added to the medium. After 72 hours, the mixture was filtered. The filtrate was obtained and collected in 500 ml water bottles. The Brix of each filtrate sample was measured for Jar 1, Jar 2, and Jar 3. The filtrate was distilled using simple distillation at a temperature range of 90°C to 98°C. For each jar, six fractions were collected and added to six test tubes. The filtrate's composition was tested for the percentage of alcohol using a pictometer. The ethanol concentration and further analysis were done on the sample using gas chromatography. The ethanol standard used had a 40% ethanol concentration and 3.775 g/HL for the 14 analytes.

Preparation of 12% Yeast Solution:

To prepare the 12% yeast solution, 44 ml of deionized water was added to a 100 ml beaker, and then 6 g of dry yeast (*Saccharomyces cerevisiae*) were introduced. Two brands of yeast were used at equal mass, Fermipan and Pakmaya. The mixture was allowed to incubate for 90 minutes at 27°C to attain a yeast solution with a concentration of 12%.

GC Analyses:

The ethanol concentration and Gas chromatography analysis were done at the Central Laboratory at the Quality Assurance Department at Demerara Distillers Ltd.

Results

Table 1. Fermentation of Sugar cane juice

Cane Juice	Initial º Brix	Final º Brix	Initial pH	Final pH	Weight of Residue	Final Specific Gravity	Average Ethanol Content %
Jar 1	19.00	5.20	6.17	2.91	1.737	1.00	9.86
Jar 2	19.10	5.40	5.35	3.29	4.683	1.08	10.65
Jar 3	18.80	4.90	4.96	4.42	3.221	1.02	10.03
Average	18.967	5.167	5.493	3.540	3.214	1.033	10.181

Table 2. Fermentation of Sugar beet

Sugar beet	Initial º Brix	Final º Brix	Initial pH	Final pH	Weight of Residue	Final Specific Gravity	Average Ethanol Content %
Jar 1	7.90	2.00	5.91	3.17	529.44	1.00	3.89
Jar 2	7.80	2.50	5.86	2.74	516.84	1.08	4.935
Jar 3	7.90	2.10	5.89	3.19	493.84	1.02	3.47
Average	7.867	2.200	5.887	3.033	513.37	1.033	4.103

Table 3: Showing the ethanol content % for Sugar cane and sugar beet in the various fractions

	Fractions	Ethanol Content %			
		Jar 1	Jar 2	Jar 3	Average
Sugar cane	1	35.01	58.17	32.65	41.94
	2	3.58	0.18	18.48	7.41
	3	1.56	1.46	8.2	3.74
	4	11.35	0	0.11	3.82
	5	7.48	4.11	0.73	12.32
	6	0.18	0	0	0.06
Sugar beet	1	11.72	12.44	11.29	11.82
	2	10.86	12	8.81	10.56
	3	0.23	4.49	0.2	1.64
	4	0.26	0.68	0.29	0.41
	5	0.13	0	0.26	0.13
	6	0.13	0	0.07	0.07

Table 4: Fermentation Profile for Sugar Cane vs. Sugar Beet

Components	Sugar Cane (G/HL)	Sugar Beet (G/HL)
Acetaldehyde	(+)	(+)
Methanol	(+)	(+)
Acetone	(+)	(-)
1 Methylacetate	(+)	(-)
2 Propanol	(+)	(+)
1 Ethylacetate	(+)	(+)
2 Butan-2 ol	(+)	(-)
2 Isobutanol	(+)	(+)
2 Butan-1 ol	(+)	(-)
Acetal	(+)	(-)
2 Isoamyl Alcohol		(-)
2 Activeamyl Alcohol		(-)
2 1 pentanol	-(+)	(-)
Furfuraldehyde	(+)	(-)

Figure 3: Showing the results for GC analysis for the ethanol standard

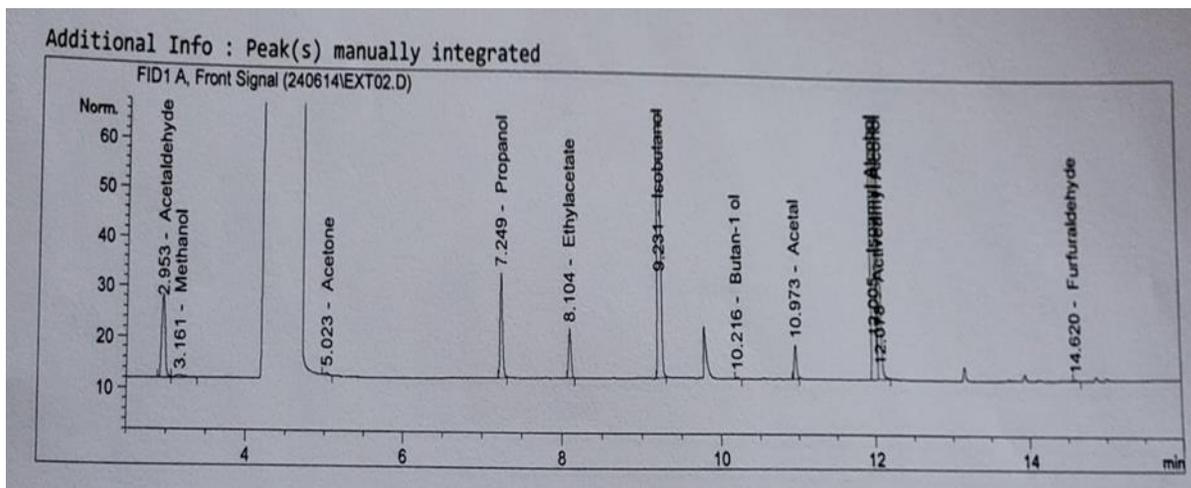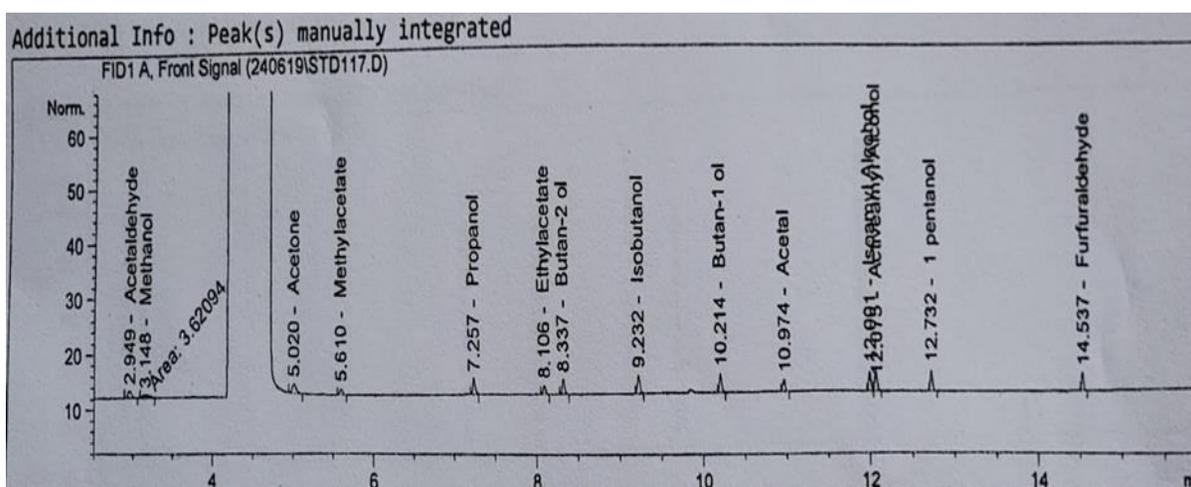**Figure 4:** Showing the results for GC analysis for sugar cane sample

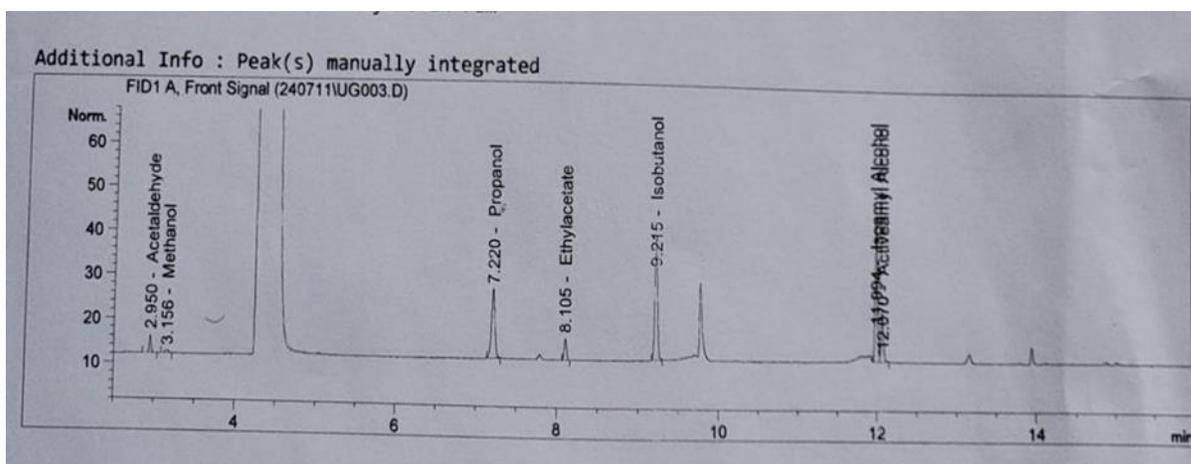**Figure 5.** Showing the results for GC analysis for the sugar beet sample

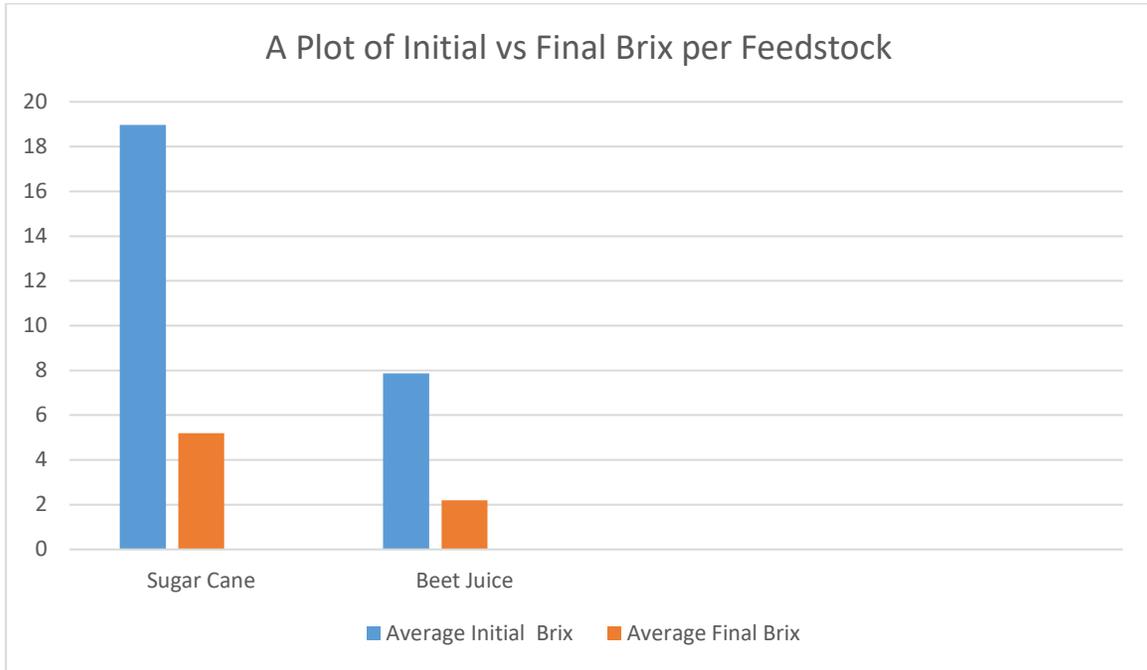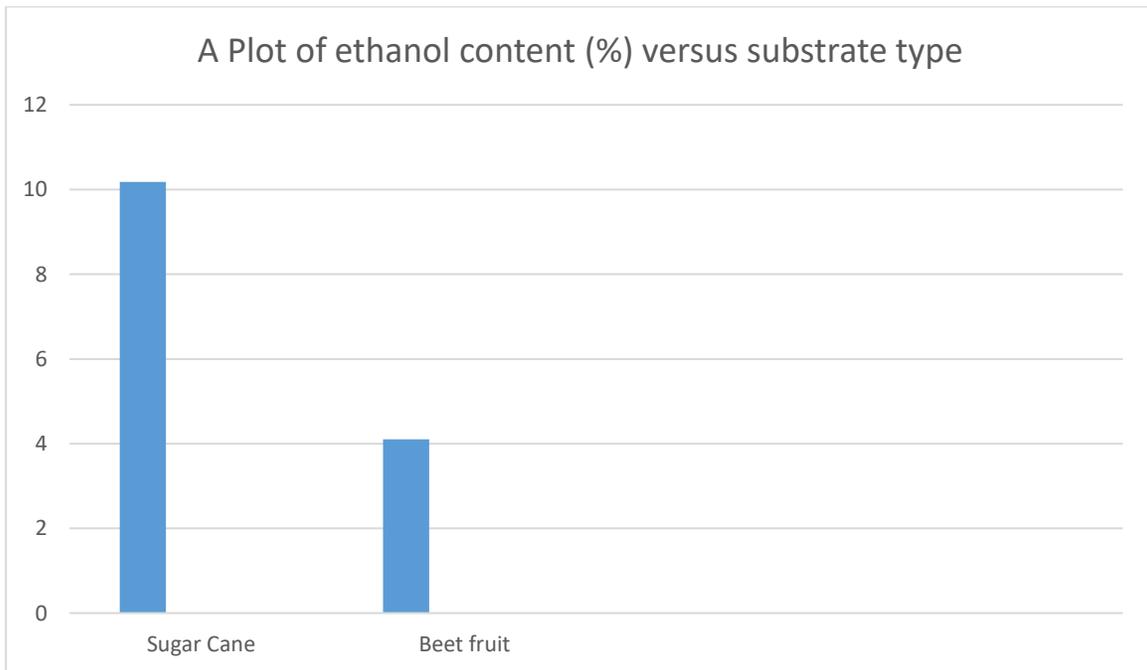**Figure 6:** A Plot of Initial Brix vs Final Brix per Feedstock

Figure 7: A Plot of ethanol content versus feedstock type

Analysis of Results

Statistical analysis will be performed using Microsoft Excel 365, employing Analysis of Variance (ANOVA) at a 95% confidence interval to analyse performance data.

Table 5: Anova: Two-Factor with Replication

SUMMARY	Jar 1	Jar 2	Jar 3	Total		
Sugar cane						
Count	6	6	6	18		
Sum	59.16	63.92	60.17	183.25		
Average	9.86	10.65333	10.02833	10.18056		
Variance	168.4876	544.3688	174.3826	261.076		
Sugar beet						
Count	6	6	6	18		
Sum	23.33	29.61	20.92	73.86		
Average	3.888333	4.935	3.486667	4.103333		
Variance	32.9475	34.65015	26.46715	28.06076		
Total						
Count	12	12	12			
Sum	82.49	93.53	81.09			
Average	6.874167	7.794167	6.7575			
Variance	101.2871	272.1084	102.9663			
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	332.3937	1	332.3937	2.03236	0.164304	4.170877
Columns	7.738756	2	3.869378	0.023659	0.976637	3.31583
Interaction	1.066956	2	0.533478	0.003262	0.996744	3.31583
Within	4906.519	30	163.5506			
Total	5247.718	35				

Table 6: Anova: Two Factor without Replication, a comparison of the average % mean of ethanol amongst the different fractions (1-6)

Anova: Two-Factor Without Replication						
SUMMARY	*Count*	*Sum*	*Average*	*Variance*		
Row 1	2	53.76	26.88	453.6072		
Row 2	2	17.97	8.985	4.96125		
Row 3	2	5.38	2.69	2.205		
Row 4	2	4.23	2.115	5.81405		
Row 5	2	12.45	6.225	74.29805		

Row 6	2	0.13	0.065	5E-05		
Column 1	6	69.29	11.54833	238.8107		
Column 2	6	24.63	4.105	30.59987		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	972.3771	5	194.4754	2.595248	0.15932	5.050329
Columns	166.2096	1	166.2096	2.218045	0.196584	6.607891
Error	374.676	5	74.93519			
Total	1513.263	11				

A comparison of the average % mean of ethanol amongst the major fractions (1-2)

Table 7: Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*		
Row 1	2	53.76	26.88	453.6072		
Row 2	2	17.97	8.985	4.96125		
Column 1	2	49.35	24.675	596.1605		
Column 2	2	22.38	11.19	0.7938		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	320.231025	1	320.231	1.157225	0.47678	161.4476
Columns	181.845225	1	181.8452	0.657138	0.566338	161.4476
Error	276.723225	1	276.7232			
Total	778.799475	3				

Discussion

Table 1.0 shows the fermentation data for the sugar cane juice. Of significance, the average inititial Brix decreases from 18.967 to 5.1667 (i.e 72.75% reduction) at the 72 hours stipulated fermentation period. This probably indicated that more yeast were needed for the fermentation process. Brix is a measure of the sugar content in a solution*. This probably indicated that more yeast were needed for the fermentation process. The average ethanol content was found to be 10.18%. There was also a decrease in pH from 5.493 to 3.54. This decrease in pH was probably due to acetic acid formation via oxidation of ethanol. Table 2.0 shows the fermentation profile for sugar beet. It can be seen that the average Brix decrease from 7.867 to 2.20 i.e 72.03% reduction. The pH also decreased from 5.887 to 3.03. This is probably due again to acetic acid formation. The average ethanol content for sugar beet was 4.103 which is lower than that for sugar cane (10.18) = 40.36%. This shows that the sugar cane juice is more fermentable than sugar beet. Table 1.0 and Table 2.0 show that sugar cane juice has a higher average initial Brix content (18.97) than sugar beet (7.87), indicating that it's a far better feedstock for bioethanol production, compared with sugar beet. However, the sugar substrate conversion efficiency to bioethanol seems to be the same for both, 73 %. .

The initial ethanol concentrations fractions are significantly higher in the sugarcane samples (41.9 %) compared to the sugar beet samples (11.82%). This suggests that sugarcane has a higher initial ethanol concentration. For sugarcane, the ethanol concentration decreases sharply after the first fraction, with some variability, but generally follows a trend of diminishing ethanol content across subsequent fractions. However, its noticeable for fraction 5, the % yield was 12.32. For sugar beet, the ethanol concentration decreases more gradually across the fractions, from 11.82% to 0.07%. Sugar beet samples, while starting with lower ethanol concentrations, show a more gradual decrease in the % yield of ethanol.

Table 3.0 shows the average ethanol content per fractions of distillate collected. As can be seen, the average ethanol content was higher for sugar cane than sugar beet. Six fractions were collected from distillate of both samples and these were progressively lower in ethanol content as the more volatile ethanol content were distilled off. Figure 4 and Figure 5 show the GC profile for the sugar cane and sugar beet samples and these can be compared with the GC profile for the Standard, Figure 3. In addition to ethanol, as the main component, other components were observed from the GCs. These are shown in the Table 4.0. The prominent ethanol peak within the region, 4 to 4.4 is noticeable.

Based on the results, 530g of sugar cane juice will produce 10.18ml of bioethanol. Thus, in terms of scalability, 1 tonne $(1.0 \times 10^6 g)$ of sugar cane juice will produce 19.21 litres of ethanol. On a parallel trend, 605g of beet will yield 4.103 ml of bioethanol. Thus, 1 tonne $(1.0 \times 10^6 g)$ of beet will produce 6.781 litres of bioethanol. Thus, the sugar cane which is a sustainable feedstock in Guyana, can be grown for bioethanol production in addition to its processing to make sugar. Also, for the sugar cane and sugar beet, the Brix conversion ratio is 0.191 Brix per hour and 0.079 Brix per hour respectively.

Based on the ANOVA results, Table 5, there are no statistically significant differences in ethanol concentration between the sugarcane and sugar beet samples, across different fractions, or in the interaction between the type of sample and the fraction. The F-value for the sample effect is 2.03236, which is less than the critical F-value of 4.17. The P-value of 0.164304 is greater than the significance level of 0.05. The ANOVA analyses amongst the different fractions (1-6) for both substrates also revealed no statistically significant different. A P-value of 0.159 was noted, Table 6. When the ANOVA analyses were also applied to the two major fractions (1-2) for both substrates, a P-value of 0.47 was noted. This is shown in Table 6. Therefore, the hypothesis (H_1) that either sugarcane or sugar beet is significantly better for ethanol production is not supported by these ANOVA analyses.

Limitations to research: The inability to conduct the GC analyses on the distillates at the University of Guyana. The student had to seek analyses at the Quality Assurance Department at Demerara Distillers Limited. This imposes a time and transportation constraints.

Conclusions

This study demonstrated that both sugarcane and sugar beet are viable feedstocks for bioethanol production, with each exhibiting unique fermentation profiles. Sugarcane showed a higher % ethanol concentrations of 10.18% and by-products compared to sugar beet (4.1%), indicating its potential for higher ethanol yields. In addition, sugar cane demonstrated a higher initial average Brix content of 18.97, compared to the average Brix content of 7.87 for beet fruit. This demonstrated that sugar cane has the potential to produce more bioethanol, compared with sugar beet. Guyana has one bioethanol plant to date, with a production capacity of 1000 litres per day. However, the abundance of sugar cane should allow for the establishment of more bioethanol plant around the country.

Acknowledgements

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