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Article

Approach before alcoholic fermentation of mixtures with syrup in a Cuban distillery

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ABSTRACT

Because of the competition with final molasses in different productions, it is necessary to look for other sources of sugar substrates to obtain ethanol. Streams derived from sugar production, such as final molasses, syrup, or filter juice, contain fermentable sugars, representing an opportunity for ethanol production. This work conducted a preliminary study in the alcoholic fermentation stage using a mixture of filter juice, molasses, and syrup. It also analyzed the feasibility of using syrup as a raw ferment material obtained from low-quality sugarcane. The experimental study was carried out using a 2^{k-1} experimental design, considering as variables: substrate (molasses or syrup), dilution agent (water and filter juice), and type of acid (H_2SO_4 and H_3PO_4), and the response variable was the alcoholic percentage obtained. Syrup, diluted with water using H_3PO_4, is a viable option when low-quality sugarcane is present, allowing the alcoholic degrees between 5.45 and 5.47%. With filter juice, alcoholic degrees between 5.22 and 5.30% were obtained, which are lower than in other studies with filter juices from sugarcane of adequate quality. The most influential variables were the dilution and acidifying agents in the statistical model obtained using Statgraphics Centurion XV 15.1.0.2 software.

Keywords: experiment design; mixture; fermentation; syrup; substrate.

INTRODUCTION

Bioethanol production is a promising solution to the challenge of carbon emissions from global fuel consumption. However, several obstacles must be overcome to fully exploit its potential, including low product yield, the food versus fuel factor, and feedstock logistics[1].
According to data from Ethanol Market Report[2], the liquid ethanol market is forecast to increase by USD 63.07 billion from 2022 to 2032. Ethanol is widely produced through biochemical (fermentation) and thermochemical (gasification) routes for its applications in diversified areas, e.g., cosmetics, food and beverages, pharmaceutical, and transportation sectors as a promising biofuel[3]. The leading bioethanol producers are the

United States of America and Brazil, which use food crops, corn, and sugarcane juice as feedstock through biochemical routes[4].

Most ethyl alcohol produced worldwide is fermented from sugar sources such as *Saccharum officinarum* sugarcane[5]. This contains readily fermentable sugars, which allow for an easy and economical ethanol production process. In countries such as Cuba, the molasses from the sugar production process is used as a carbon source, and the yeast *Saccharomyces cerevisiae* is used as the alcohol-producing microorganism[6,7]. With these two, combined with ammonium salts as sources of nitrogen and phosphorus and a pH adjusted with sulfuric acid in the equipment suitable for the process, it is possible to obtain satisfactory alcohol production [8,9, 10].

During alcoholic fermentation, contaminating bacteria compete with yeast for sugar and nutrients, causing a significant decrease in ethanol production; therefore, the lower the pH, the more the fermentation medium is protected against possible bacterial attack. Sulfuric acid (H_2SO_4) is the most commonly used to adjust the pH of the fermentation medium, although there are studies investigating the feasibility of using phosphoric acid (H_3PO_4) and nitric acid (HNO_3)[11].

Worldwide trends in ethanol production are leading to the use of substrates that are alternative to the traditional sugar cane and sugar beet molasses. Among them are the lower-quality juices that are processed with sugar. Research by [9, 12-17] addresses the use of other substrates for alcoholic fermentation, such as juices from the sugar process, bagasse hydrolysate, and stillage from alcohol distillation, offering great significance for the use of intermediate products from the sugar industry, which at the same time contribute to improving the sugar process [18, 19].

According to Martínez Y [20], the economic efficiency of ethanol production is strongly influenced by the availability, market prices, and destinations of the best use of raw materials. Therefore, several studies have been conducted using combined substrates such as juice from mud filters, diluted juices, clarified juices, B molasses, and final molasses in the fermentation stage[19, 21, 22-23]. Fabelo[24] made significant contributions with his study on the modeling and optimization of the fermentation stage, using stillage and filter juice mixed with final molasses in different proportions. Mixtures of final molasses (0.299), filter juices (0.342), and hydrolyzed bagasse liquor with molasses (0.358) at 5.26% alcohol have also been used as a source of carbohydrates[25, 26]. These studies have shown that it is possible to reduce production costs by using mixtures of different substrates. For the mixture, as mentioned earlier, Morales[25] demonstrates that it is possible to save 67 % of the molasses to be purchased in the non-harvest period and 22.73% of the water.

By redirecting 30 % of the juices from the filters, the sugar produced is reduced by 5 %; at the same time, the incorporation into the process of material with a high content of non-sugars, colloids, and microorganisms, which ultimately damages the quality of the sugar, is avoided[27].

The extraction of these secondary juice streams for ethanol production allows for a reduction of harmful substances in the raw sugar production process, such as insoluble solids, polysaccharides, ash, and other impurities that hinder the evaporation, concentration, and crystallization stages of sugar, as well as contributing to water savings in the fermentation process since part of it is replaced by these juices. In addition, it leads to greater efficiency in the clarification stage of the sugar process, obtaining a higher quality sugar, a decrease in steam consumption, and an increase in the availability of excess bagasse[19, 28-30].

An essential aspect that frequently occurs in Cuban sugar factories is that sometimes the sugar cane that arrives at the sugar mills does not have the appropriate or standard quality for the production of raw sugar (low sucrose content (Pol), high content of soluble solids other than sucrose (average is 10-16 %) and high levels of cane fiber (average is 11-16 %)), which can be caused by various reasons that are not the purpose of this work. In view of this situation, an analysis is needed on what would be the most favorable scenario, whether to produce raw sugar or to produce only syrup and use it as a substrate to obtain ethanol.

The goal of this work is to carry out a study of alcoholic fermentation using a mixture of filter juices, molasses, and syrup from low-quality sugarcane.

MATERIAL AND METHODS

Raw materials

For the study of mixtures in the fermentation stage, final molasses (MF), filter juice (JF), and syrup (M) are used as substrates, all of them coming from the process of obtaining raw sugar in the Cuban sugar industry. Initially, the substrates were characterized to know their conditions before they were used in the subsequent experiments.

Final Molasses

The final molasses is the liquid separated from the sucrose crystals by centrifugation. It is a dense and viscous syrup, separated from the final cooked mass itself and from which it is not possible to crystallize more sugar by conventional methods, given the inverted sugar and high viscosity. It contains approximately 45-55% sucrose, 20-30% glucose and fructose, 10-20% water[3], and also has small amounts of mannose in stored molasses[31, 7]. In the present study, 600 ml were taken at 26 ºC from the final molasses used as raw material in the fermentation stage, which was stored in the sugar factory.

Filter juice

Filter juice is the intermediate stream obtained during the separation operations of the mill mud extracted from the clarified juice in the raw sugar manufacturing process [32, 33]. Clarified filter juice has almost no unfermentable sugars, so all sugars are considered fermentable[31]. In this case, 800 ml of the juice obtained at the sugar factory's rotary vacuum filter outlet was taken at approximately 40 ºC and allowed to decant at room temperature for subsequent studies.

Syrup

Syrup is a liquid product resulting from the evaporation of clarified juices, by evaporation, of excess water in the evaporators without removing the sugar. This syrup is close to saturation point and has 55-65% dissolved solids. It contains high sugars (33% to 75%) in sucrose, glucose, and fructose[34]. For the study, the syrup was taken at the outlet of the third evaporator, approximately 50 ºC. The sample taken was 600 ml and was allowed to cool to room temperature for subsequent studies[10].

It appears relatively similar to molasses, but its color is darker and almost black. It has a sweet taste, similar to licorice, but with a bitter touch. In turn, it has a high nutritional content of carbohydrates, B vitamins, minerals, and a low water content[16].

Raw materials characterization

The substrates used are characterized based on the main components that develop the fermentation. In each case, they are analyzed in the distillery laboratory in question using standardized methods.

Fermentation conditions

Saccharomyces cerevisiae yeast is used in fermentation, the inoculum is prepared similarly to the conventional factory, and the final molasses is used as substrate[24, 35]. In the pre-fermentation process, the molasses is diluted with water in the dissolver until it reaches the desired brix to feed the preferments and the fermenters, this mixture being known as the cushion. Subsequently, the yeast culture is carried out in the preferments in three phases. In the first phase, 20% of the total volume of the mattress pre-fermenters is

added from the dissolver, phosphoric acid is added to regulate the pH from 4 to 5, and the necessary amount of urea, phosphate, and yeast is also added. In the second phase, the cushion is added up to half of the total capacity, adding urea, phosphoric acid, and phosphate in the same proportions. Finally, the pre-fermenters are completely filled, finishing the brix in a range of 10-11, repeating the same dosage of the previously added compounds. Samples are taken every two hours, and when the brix measurement is half of the initial value, it is ready to be transferred to a fermenter, leaving 20% of the volume for seed in said pre-fermenters.
Fermentation is carried out in an anaerobic beaker of 1 L capacity at ambient temperature (28 ºC) for 10 hours because the brix had already dropped and remained constant. For process control, the Brix degree was determined during propagation and pre-fermentation every one hour and during fermentation every two hours. Brix was determined using an optical refractometer with ATC-Precisso, according to the approved Cuban standards[36-38]. The concentration of reducing sugars was determined by the Eynon-Lane method[24], while the alcoholic strength was determined by pycnometry at 20 ºC. A Reischauer glass pycnometer is used to determine the density of the alcohol by weighing differences using a balance with a precision of 0.1 mg according to standard [39].

Experimental design

A 2^{k-1} type design of experiments with replication is carried out according to the distribution of Table 1. The following are considered as independent variables: primary substrate [final molasses (1) or syrup (-1)], dilution agent [water (1) and filter juice (-1)], and acidifying agent [sulfuric acid H_2SO_4 (1) and phosphoric acid H_3PO_4 (-1)]. The experiments take into account up to 100% of the total reducing sugars (TRS) of the substrates, and the alcoholic percentage is considered a response variable. Table 2 shows the quantities of each stream used in the experiments.

Experiments	Variables		
	Main substrate	Dilution agent	Acidifying agent
1	1	1	1
2	1	-1	-1
3	-1	-1	1
4	-1	1	-1
5	1	1	1
6	1	-1	-1
7	-1	-1	1
8	-1	1	-1

Table 1. Experiment planning

Experiments	Molasses (mL)	Syrup (mL)	Water (mL)	Filter Juices (mL)	H_2SO_4 (mL)	H_3PO_4 (mL)
1	188.72	-	811.28	-	0.83	
2	124.41	-	-	875.59	-	0.4
3	-	128.98	-	871.02	0.83	
4	-	143.05	856.95	-	-	0.4
5	188.72	-	811.28	-	0.83	
6	124.41	-	-	875.59	-	0.4
7	-	128.98	-	871.02	0.83	
8	-	143.05	856.95	-	-	0.4

Table 2. Values of the streams in each experiment

RESULTS AND DISCUSSIONS

Substrates characterization

Table 3 shows the main results obtained from characterizing the substrates used in the fermentation process.

Parameters	Substrate		
	Filter juice when syrup is produced	Final molasses	Syrup
Bx	14.15	77.60	75.35
pH	5.80	6.000	6.200
Density (Kg/L)	1.0406	1.396	1.381
ART (g/L)	10.45	14.20	12.55
RL(%)	-	14.150	14.563
RT(%)	-	57.904	58.441
Sucrose	-	41.566	41.684
AzT(%)	-	55.716	56.247
AzF(%)		53.013	53.480

Where RL: Light reductants, RT: Total reductants, AzT: Total sugars and AzF: Fermentable sugars.

Table 3. Main characteristics of the substrates used in fermentation

Fermentation results

The procedure applied in fermentation is similar to that developed in the distillery. First, a small bed of molasses is added, adding the volume of pre-fermenters needed to inoculate the pre-fermenters. The main characteristics of the pre-fermenters are shown in Table 4.

Parameter	Value
ºBrix	7,45
ART (g/L)	26
Alcoholic degree	4,1 ºGL
Cell count (10^6)	281 cells/mL

Table 4. Characteristics of the preferment used

Once the preferment is added to the diluted molasses bed, it is left to ferment under anaerobic conditions to ensure the transformation of sugars into alcohol and not to seek yeast propagation. The first step is carried out to gradually adapt the microorganism to the medium. The fermenter is filled when activity begins to be observed in the medium. The filling is carried out by refreshment, as in the industrial scale, considering the decrease of the concentration of total solids in the medium to a value equal to half plus one.

In the experimental fermentation study, the behavior of the Brix degrees was measured for each experiment (Figure 1). Samples were taken for analysis every hour until a constant value was reached (most of them after 6 hours), after which time cell death was expected to occur to determine the alcohol content (Figure 2). The time analyzed was 10 hours[10].

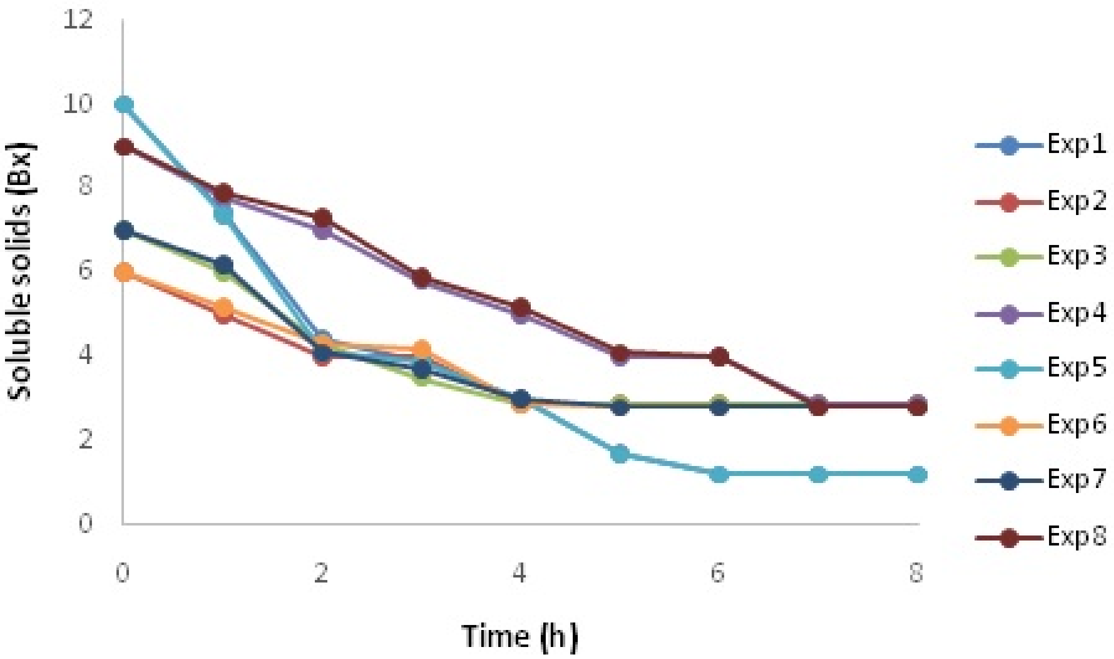


Figure 1. Brix behavior during fermentation

In all the variants, it was observed that there is consumption of substrates by the yeast, and no considerable alterations were perceived in indicators such as Brix; which indicates that the substrates used separately and in combination do not have inhibitory action due to the presence of salts, metabolites and other compounds. The variation of soluble solids in the fermented musts behaved similarly to the results achieved industrially and to those reported in studies carried out by authors such as [24, 16]. In the first five hours of fermentation, a more than 44% decrease in soluble solids was observed in all the experiments, demonstrating substrate consumption at this stage.

The best results are achieved when only the final molasses is used (experiment 1), which supports the procedure applied in the factory when fermenting with this stream coming from the plant. While the experiments where the juice from the filters is used (2, 3 and their replies 6, 7), lower values (5.22-5.3 %) are obtained than those obtained by García[15] (5.72), and Cortés[40] (5.56). These results may be influenced by the quality of the cane from which the juice was extracted for fermentation. Figure 2 shows the alcoholic percentage and pH at the end of fermentation.

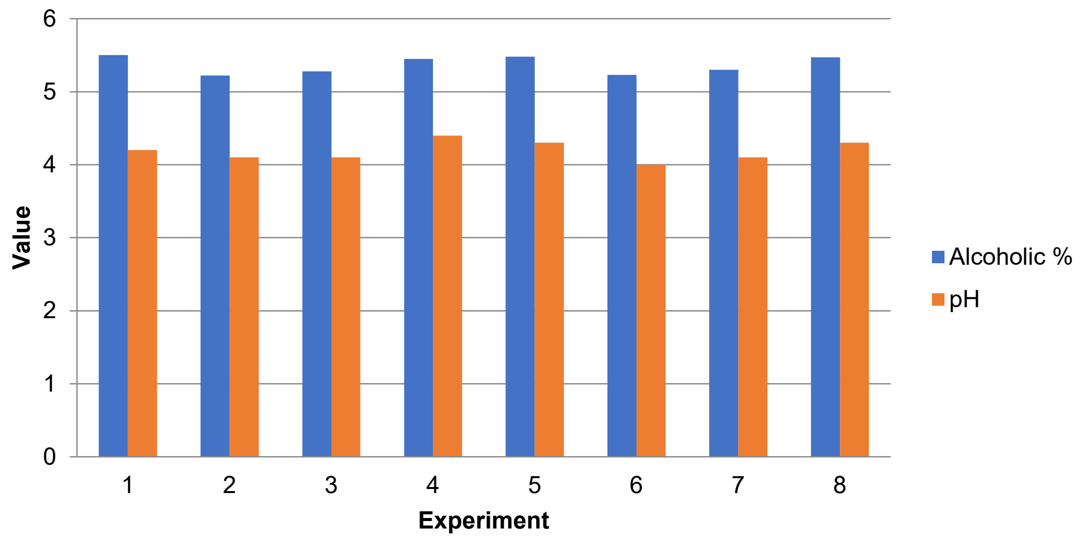


Figure 2. Alcoholic percentage and pH at the end of fermentation

The fermented musts have an alcoholic percentage between 5.22 and 5.50, these values are between the ranges obtained by Rivero[19] [4.81 to 5.90 %], García[15] [5.02 to 5.85 %] and Díaz[16] [5.53 to 6.70 %], when they used filter juice, secondary juices and final molasses as substrates. When using final molasses in the fermentation diluted with water and H_2SO_4 as pH controller[11], the range reached is 5.48 to 5.5 %, higher than that obtained by García[15] (5.15 %) and equal to that of Cortés[40] (5.5 %). This supports the traditional Cuban industrial process.

The best results were obtained in the experiments using the syrup diluted with water using H_3PO_4 (experiments 4 and 8). Alcoholic percentages between 5.45 and 5.47 % were obtained. The syrup is an attraction of the present work for low quality canes, confirming the viability of H_3PO_4 as an acidifying agent[11].

The worst results, were obtained where the juice of the filters (2, 3 and their replicates 6, 7) was used when obtained in this study from low quality canes, ranges between 5.22 and 5.3 % are obtained, lower than those obtained by García[15] (5.72 %) and Cortés[40] (5.56 %). This was caused because these juices were extracted from low quality canes, this was discusses at the conference[41].

The lowest pH value at the end of fermentation was reached when using the juice from the filters and the final molasses (experiment 2 and 6), which indicates that under these conditions there was a higher production of weak organic acids such as succinic and acetic acids, which can lower the pH of the culture medium, as explained by[42]. The pH obtained at the end of fermentation was similar to the values reported by[43, 16], authors who likewise worked with molasses and sugarcane juice fermentations.

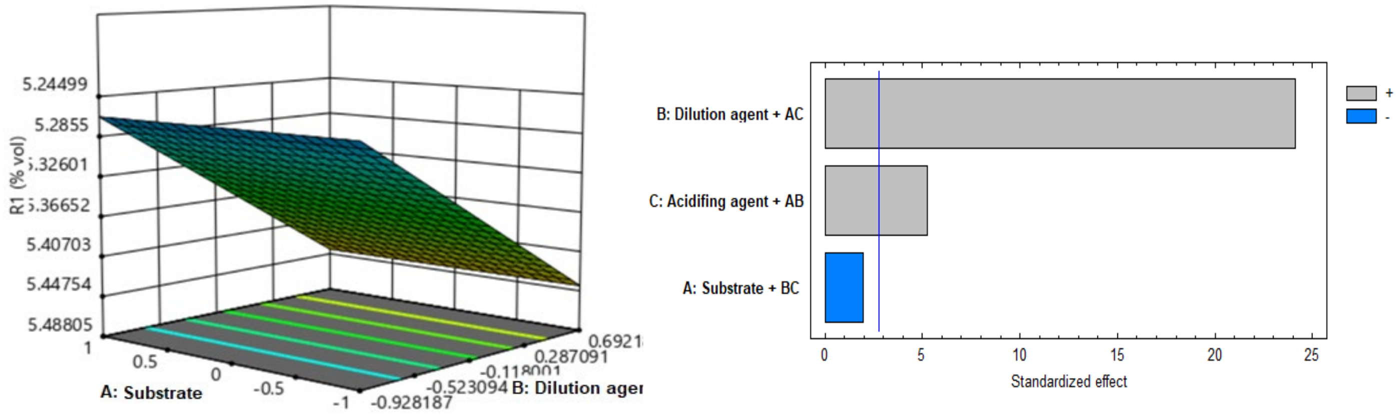
Statistical analysis of the experimental design

The statistical results were obtained by processing the experimental design in *Statgraphics Centurion* XV 15.1.0.2 software. By means of the regression study for the alcoholic percentage, the statistical model that adjusts to each of its coefficients was obtained, which is reported by equation (1). The R^2 value reached indicates that the model thus adjusted explains 99.3525% of the variability in the alcoholic strength, there being a statistically significant relationship between the variables with a confidence level of 95.0%.

$$\text{Alcoholic Degree (\%)} = 5.366 - 0.0087 \cdot \text{Substrate} + 0.1087 \cdot \text{Dilution agent} + 0.0237 \cdot \text{Acid}$$

(1)

The graphical representation of the above can be seen through the response surface graph and the Pareto Diagram in Figure 3(a) and 3(b), respectively.



(a) **(b)**

Figure 3. Response surface plot (a) and Pareto plot (b) for the results of the experimental design

It is observed that the most significant influence is exerted by the dilution agent, followed by the type of acid used to regulate the pH. In this case, the substrate does not show a significant influence from the qualitative point of view since it is used as a base to establish, at a constant value, the starting point of the soluble solids at the beginning of the fermentation.

In summary, the results of this study suggest that syrup, diluted with water and using H3PO4 as a pH control agent, is a viable option for ethanol production when using low-quality sugarcane. Alcohol degrees between 5.45 and 5.47% were obtained, indicating that this method may be an efficient alternative for utilizing sugarcane that is not optimal for sugar production. However, using filter juice in alcoholic fermentation is not recommended when the cane is of low quality, as lower alcohol degrees are obtained (between 5.22 and 5.30%) compared to other studies using filter juice from adequate-quality cane. The statistical analysis of the experimental design confirmed that the dilution and acidifying agents are the variables that most influence the alcoholic fermentation process.

CONCLUSIONS

This preliminary study on alcoholic fermentation using filter juice, molasses, and syrup as substrates has provided valuable information on the feasibility of using different sugar sources for ethanol production, especially in the Cuban sugar industry.

The results show that syrup, obtained from low-quality sugarcane, can be a viable alternative to final molasses in ethanol production, provided it is diluted with water, and H3PO4 is used to control pH. This finding is relevant to the Cuban sugar industry, as it allows sugarcane that does not meet the quality standards for sugar production.

On the other hand, filter juice is not recommended as a substrate for alcoholic fermentation when the sugarcane is of low quality since the alcohol degrees obtained are lower than those reported in previous studies with filter juice from good-quality sugarcane.

Statistical analysis of the 2k-1 experimental design confirmed the influence of the dilution agent and the type of acid used to regulate the pH on the final alcohol content.

This study offers an overview of the possibilities of diversifying sugar sources for ethanol production in the Cuban sugar industry, contributing to the search for sustainable and efficient resource-use alternatives.

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