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Production of Anhydrous Ethyl Alcohol from the Hydrolysis and Alcoholic Fermentation of Corn Starch

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Abstract: Ethyl alcohol is an organic substance that contains a functional group, the hydroxyl, attached to the ethyl radical. It is a substance used for sterilization and as an alternative fuel to fossil fuels, especially gasoline. It is obtained by the alcoholic fermentation of biomass containing fermentable sugars, based on the use of yeasts; alternatively, microorganisms in the yeast convert the sugars into ethyl alcohol through aerobic metabolism. In this context, this research aims to produce ethyl alcohol by hydrolysis and alcoholic fermentation of corn starch and to purify the resulting products by distillation. For this, experimental tests were carried out using equipment on a laboratory scale. Alcoholic fermentation tests were carried out with strict control of ºBrix, specific mass and viscosity. After the fermentation, the resulting products with a reduced percentage of alcohol were purified by conventional and extractive distillation, ensuring increased purity and commercial value for the alcohol produced. The results obtained were satisfactory, and the phenomenological analysis of the operations ensured the understanding of the performance of each operation involved, with emphasis on hydrolysis, fermentation and fractional and extractive distillations, which involve strong interaction between the phases in each operation. With this methodology's implementation, it proved possible to produce alcohol with a high degree of purity, known as anhydrous alcohol.

Keywords: hydrolysis, fermentation, ethanol, distillation, extractive distillation

1. Introduction

In developed countries, the ethanol production process has been the object of scientific studies to establish the most relevant parameters used in the microbiological conversion of sugars into alcohol. Understood as a renewable energy source, it is an alternative to fossil fuels. It has been increasingly incorporated into the internal combustion system of engines, and it can be used in several proportions mixing ethanol to gasoline, composing a mixture with fossil fuels, or using pure, without any mixture (Santos, Kugelmeier et al., 2013).

Raw materials rich in sugars or starch are explored to produce ethanol, especially sugarcane and corn. They are the alternatives most adopted in agro-industrial plants. In Angola, maize is one of the promising products in producing fermented beverages. In this context, the Castel Group has shown interest in cultivating corn to produce fermentable beverages, especially beer, to minimize the import of raw materials.

The main carbohydrate in corn is starch, a polysaccharide composed of two macromolecules: amylose and amylopectin. It can be hydrolyzed by acid or enzymatic pathways. Starch is a carbohydrate polymer of glucose. Amylose is formed by glucose units joined by α-1,4 glycosides bonds, which results in the formation of a linear chain. Glucose units form amylopectin joined in α-1,4 and by α-1,6 bonds, thus resulting in a branched structure (Luz et al., 2020; Carvalho et al., 2016).

The analysis of corn's physical and chemical characteristics shows the absence of fermentable sugars in its molecular structure. This condition requires the use of pretreatment that promotes the formation of fermentable sugars. The best pretreatment involves the implementation of hydrolysis when corn starch is converted to glucose.

The decomposition of corn starch is complete when conducted under high temperature and pressure conditions, in the presence of a catalyst, whatever the reaction mechanism. The most important catalysts are alkalis, acids, and enzymes. In this case, enzymatic catalysis has greater selectivity, promotes the formation of products with a higher degree of purity, and requires less energy compared to acid catalysis, which presents more significant difficulties in the production of fermentable sugars (Carvalho et al., 2016; Astolfi, 2019; Luz et al., 2020).

Rocha (2007) evaluated the hydrolysis of corn starch using α-amylase bactericidal and fungal amyloglucosidase. The results suggested that smaller granules are more susceptible to enzymatic action, mainly due to the smaller surface

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area. According to this author, enzymes attack the amorphous and crystalline regions of the starch granules. Piva, Bender and Mibielli (2015) also evaluated the influence of agitation and particle size on the hydrolysis of barley bagasse. Their conclusions were similar to those obtained by Rocha (2007).

Massango (millet, *Pennisetum glaucum L)* is a small, dark-coloured cereal used for human consumption. When processed, it produces flour and animal feed and is used as grain to feed small birds, such as parakeets and canaries. Even with the above description, there are no substantial studies of corn starch hydrolysis using massango as a source of myelitis enzymes. However, Santana (2007) evaluated the hydrolysis process using other sources of myelitis enzymes when producing alcohol using two types of yeasts, *Saccharomyces cerevisiae*, and *Saccharomyces diastaticus*, using cassava starch as raw material. Cereal malt was used as a source of myelitis enzymes in this case. The author evaluated barley, wheat, corn, and rye malt, and the resulting products were analyzed based on the concentration of sugars produced. After hydrolysis, the glucose obtained was subjected to fermentation. The result was the conversion of these sugars into ethyl ethanol by microbial action.

For Santos K. G. et al. (2013), the biological process of fermentation involves reactions of partial oxidation of glucose that result in the growth of yeasts and the partial anaerobic oxidation of hexose to form alcohol and carbon dioxide.

For Folch et al. (2021), the glycolysis is followed by reactions that convert phospho-enol-pyruvate (PEP), pyruvate and/or acetyl-phosphate into the final fermentation products (Figure 1). Product formation has to regenerate the redox cofactors used in glycolysis. The redox reactions are either the reduction of oxo-groups to hydroxy groups (acetaldehyde to ethanol, pyruvate to lactate, acetoacetyl-CoA to 3-hydroxybutyryl-CoA, oxaloacetate to malate), the reduction of 2-oxoacids into amino acids (e.g. pyruvate into alanine) or the reduction of carbon-carbon double bonds (fumarate to succinate, crotonyl-CoA to butyryl-CoA).

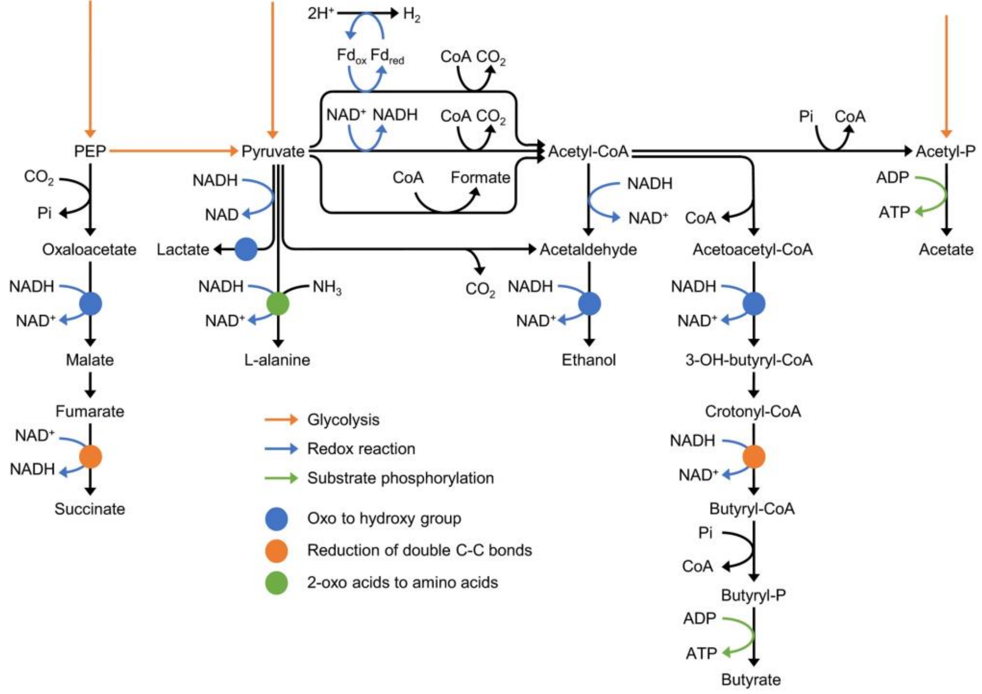

Figure 1. Conversion of PEP, pyruvate and acetyl-phosphate into final products of fermentation processes (Folch et al., 2021)

Fermentation processes are influenced by several factors, mainly the degree Brix, temperature, oxygenation, mineral and organic nutrients, yeast concentration inhibitors, and bacterial contamination. On the other hand, to increase the efficiency of alcoholic fermentation, rigorous monitoring of the referenced parameters is required throughout the process. Generally, fermentation temperatures are between 26 and 35 °C. However, the increase in temperature triggers bacterial contamination rates, increasing the toxic sensitivity of ethanol. Still, from this perspective, fermentation is initially favoured with a low pH, which grows to a range between 3.5 and 4.0.

After alcoholic fermentation, the products are fractionated by conventional and extractive distillation to produce anhydrous ethyl alcohol. In this context, distillation is based on the difference in boiling points of the constituent components of the mixture, especially water (100 °C) and ethyl alcohol (78.4 °C). For Wolf et al. (2001) and Bertoli et al. (2017), the distillation processes are based on the principles of balance between the phases that leave a given stage and make it possible to recover the constituent components of the mixture with a high degree of purity. To evaluate the performance of distillation processes in fractional distillation columns, Noriler et al. (2009) and Siqueira,

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G. A. (2011) evaluated the microscopic behaviour of the flow of liquid and vapour phases in the plates, mainly in terms of interaction between the phases, and related them to the separation transfer efficiency. Soares et al. (2013) related the flow behaviour in liquid stream flow ducts to understand the phenomenological behaviour of mass transfer in distillation columns with a tray. For Zöldy et al. (2007) and Zöldy (2011), ethanol is a renewable resource that could partially contribute to global warming concerns. Ethanol does not contain sulphur, which means it does not emit any sulphur dioxide. The NO_x emission is lower because of the ethanol's higher vapour heat, which cools the combustion temperature.

Based on the literature review, ethanol and ethanol-based chemicals will substantially make mobility more sustainable. The key factor will be the efficiency increase in production. The aim of our research is to develop alcohol production methods for larger yields and better distillate volume. The hypothesis is that a temperature range can be defined for optimal fermentation.

2. Experiment

The study of alcohol production by hydrolysis and fermentation involves 4 phases. The first and second steps of the experiment are preparing the material to be processed, and the definition of the methods must be used to determine the best parameters associated with the hydrolysis and fermentation processes. The third and fourth phases involve conventional and extractive distillation processes to purify and evaluate the alcohol produced.

2.1. Hydrolysis and fermentation procedures

To prepare the material for the experiment, the corn bran mass was weighed and transferred to a beaker, and distilled water was added. The mixture was heated under controlled stirring to 70 °C. After this process, the sugar mass was weighed and dissolved in the water previously weighed. The mixture was then cooled to room temperature, and then hydrolysis was carried out. Data from the operation are described in Table 1:

Table 1. Characteristics of worth samples

Parameters/Sample	A	B	C	D	E
Mass of biomass (g)	65.86	65.568	65.108	65.420	800.00
Mass of water (g)	500.00	501.455	500.90	500.16	6,100.00
Sugar mass (g)	150.05	150.09	50.00	50.314	1,820.00
Temperature (ºC)	40.00	25.00	40.00	40.00	35.00

Source: made by the author, 2021

For samples A and B, the same masses of raw material were used, and the effect of process temperatures was evaluated. The effect of enzymes was investigated for samples C and D with the same temperature. In the case of E, the influence of mass was investigated to see the effect of α-amylase on the yield. The hydrolysis of corn bran was carried out using two different procedures, as follows:

a) The first procedure consisted of using the massango seeds as an enzyme with the raw material and;

b) The second procedure involved using α-amylase enzyme with the raw material.

Sample C was selected for hydrolysis using the massango as an enzyme. For this, 87.178 g of massango was first ground. A solution of this powder was prepared, diluting it in 28.591 g of water, and this solution was added to the prepared must. In the case of enzymatic hydrolysis, 5.4 and 27 g of the enzyme α-amylase were dosed in samples D and E, respectively. After 3 hours of hydrolysis, fermentation was carried out using *Saccharomyces cerevisiae*, i.e. yeast, a material commonly used in the bakery industry.

Yeast preparation involved dissolving this material in distilled water at a ratio of 1:20 (m/m), i.e., 5 g of yeast for every 100.0 g of distilled water, then added to the hydrolyzed mixture. In sample E, as part of the expansion of the amount of biomass, 60.0 g was prepared in 1,200.0 g of distilled water. Fermentation time was measured when yeast was added to the hydrolyzed mixture. Fermentation was carried out with the progressive measurement of the degree Brix for three days.

2.2. Distillation procedures

For this study, fractional distillation was performed using a distillation flask with a capacity of 500 mL and a heating mantle. 250 mL of the fermented must be poured into the flask, and, with heating, the ethanol was progressively recovered at the top of this apparatus, with concentrations higher than those of the fermented must.

During the distillation process, the temperature of the top steam was controlled up to approximately 100 ºC, characteristic of the minimum concentration of ethanol in the mixture. To minimize the thermal dissipation rates by radiation, the flask was insulated with aluminium foil, thus enabling the use of maximum energy for the distillation operation. After the conventional distillation was concluded and considering that the ethanol/water mixture forms an azeotropy point that prevents the production of ethanol with a high degree of purity by conventional distillation, the structure for extractive distillation was implemented. In the extractive distillation processes, Figure 2 was structured, containing a 250.0 mL distillation flask, a heating mantle, and a fractionation column with fillings.

The experimental procedure included inserting 100.0 mL of ethanol from conventional distillation into the volumetric flask. At the top of the distillation column, a funnel with 60.0 ml of glycerol was inserted as solvent.

The fractionation operation started with the activation of the blanket and consequent heating of the mixture contained in the flask. With the beginning of the formation of the ascending ethanol vapour streams, the solvent supply valve was opened, which descends by gravity and interacts with the ascending ethanol vapour.

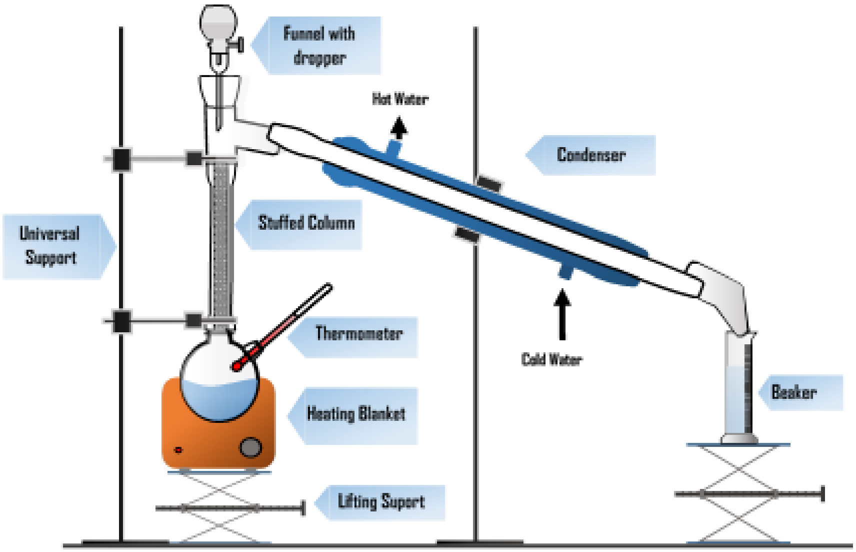

Figure 2. Experimental apparatus used for extractive distillation

The interaction between the phase results in the modification of the thermodynamic properties of the ethanol/water mixture, resulting in the production of anhydrous ethyl alcohol, recovered at the top of the extractive distillation column. This procedure was concluded by maximizing the temperature in the distillation flask, characterizing the minimization of the alcohol content present in the initial mixture.

2.3. Characterization of Alcohol

After the distillation processes were concluded, the ethyl alcohol obtained was characterized by determining the alcohol content (GL and INPM), in compliance with the Brazilian Technical Standards of ABNT 5992:2008. The refractive index, density, and viscosity were also determined by the flow time of the liquid alcohol in an Oswald capillary viscometer.

3. Results and Discussions

3.1. Hydrolysis and Alcoholic Fermentation

Samples C, D, and E were hydrolyzed for 3 hours, and the process was monitored with the evaluation of the ºBrix at the beginning and end of each experiment. In this case, the enzyme used in the process did not significantly influence

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the hydrolysis. It was evaluated during the fermentation of the ºBrix, for all experiments carried out in this study, according to the data in Table 2.

Table 2. Fermentation evolution as a function of ºBrix.

Time (days)	***A***	***B***	***C***	***D***	***E***
0	25.90	26.00	12.20	12.00	25.90
1	13.20	14.10	5.10	5.50	12.20
2	9.30	8.50	5.00	4.80	9.90
3	9.00	8.00	5.00	4.70	9.60

Source: Made by the author, 2021

Qualitatively, for all cases, this parameter decreases with fermentation time, characterized by converting the sugars present in each sample into ethanol, with greater intensity on the first and second days and strong stabilization on the third day. However, the results described in Table 2 demonstrate the need to implement hydrolysis as a strategy for converting non-fermentable sugars into fermentable sugars to increase the conversion rates of sugars into ethanol, thus ensuring a sharp reduction of the ºBrix (%). The data from the experimental tests carried out in this study are shown in Figures 3 and 4. They reveal the influence of the fermentation temperature and enzyme used in the hydrolysis on the evolution of ºBrix measured during the fermentation process.

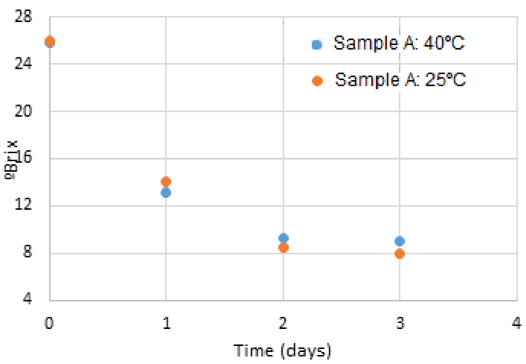

Figure 3. Influence of fermentation temperature.

The temperature evaluated confirms the initial hypothesis that fermentation in the temperature range between 26 and 35 ºC is the most appropriate, with a conversion of 65.25% and 69.23%, for temperatures of 40 ºC and 25 ºC, respectively.

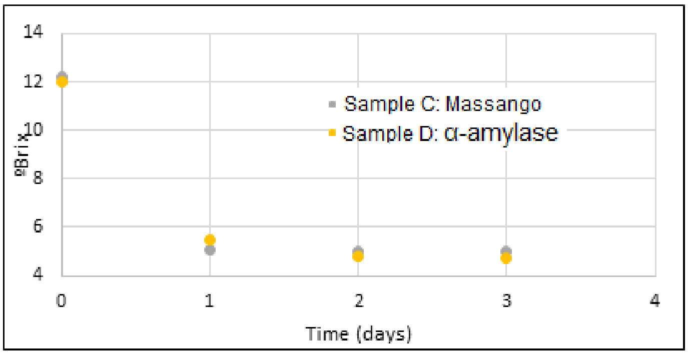

Figure 4. Enzymatic hydrolysis using massango vs α-amylase, both as an enzyme.

Despite the small difference between the two sources of amylolytic enzymes, the use of α-amylase presented a conversion of 60.8%.

3.2. Distillation and characterization of Products

The fermentation products were subjected to conventional and extractive distillation operations to recover and purify the alcohol produced as a recovery strategy, ensuring compliance with international quality standards for use in internal combustion engines, as a reagent, and as an additive to systems, among other utilities. The operating conditions for each experimental test and the results are described in Table 3:

Table 3. Results of different samples obtained from the fractional distillation

Parameters	***A***	***B***	***C***	***D***	***E***
Volume fed (mL)	500.0	500.0	500.0	500.0	8000.0
Volume Distillate (mL)	30.0	78.0	27.0	34.0	1200.0
Process Performance (%)	6.0	15.6	5.4	6.8	15.0
Specific mass (g/cm^3) at 20ºC	0.831	0.832	0.833	0.860	0.865
Refractive index	1.365	1.364	1.3645	1.3645	1.364
Alcohol content (ºGL) (v/v)	90.0	89.0	87.0	82.0	80.0
Alcohol content (ºINPM) (m/m)	85.5	84.4	82.4	75.3	73.0
Viscosity (kg·m^{-1}·s^{-1})	1.819	1.820	1.688	2.183	1.819

(ºGL: Degree Gay-Lussac) **Source:** Made by the author, 2021

From the data contained in Table 3, it is observed that sample C has a lower performance, 5.4%, supported by the reduced rates of alcoholic fermentation observed in Table 3. The highest performance tests are experiments B and E, carried out at temperatures lower than 40 ºC and with similar water and sugar contents. In this case, the tests performed at 40 ºC presented a performance between 5.4% and 6.8%. The limitation is related to the temperature at which the experiments were performed and the smaller masses of sugar added to the system.

The analyses in Table 3 show that the operations carried out involved the production of hydrated alcohol, with concentrations between 80% and 90%, which requires the implementation of extractive or azeotropic distillation to guarantee the production of anhydrous alcohol, a product with higher added value.

This way, experimental tests of extractive distillation were implemented to increase the alcohol content of conventional distillation products based on the thermodynamic modification of the ethanol–water mixture. For these tests, a packed column was used with rings. Rashing and distillation of the distillate from sample E were carried out, with glycerol (G) and ethylene glycol (EG) as solvents. The extractive distillation products were measured, and the results are presented in Table 4.

Table 4. Results obtained from extractive distillation

Number of distillations	***1st***		***2nd***	
Solvents	***EG***	***G***	***EG***	***G***
Volume fed (mL)	100.0	100.0	60.0	52.0
Volume Distillate(mL)	85.0	80.0	35.0	36.0
Specific mass (g/cm^3) at 20ºC	0.801	0.800	0.795	0.797
Refractive index	1.3635	1.3616	1.3615	1.3616
Alcohol content (ºGL) (v/v)	97.5	98.0	100.0	100.0
Alcohol content (ºINPM) (m/m)	96.1	96.7	99.5	100.0
Viscosity (kg·m^{-1}·s^{-1})	1.415	1.310	1.187	1.257

Source: Created by the author, 2021

To increase the contact time between phases (liquid and vapour) and the interaction with the solvent used, the height of the extractive distillation column was increased, and the fillings were introduced. The physicochemical characteristics of the products are shown in Table 4 and show that the solvents used ensured the production of anhydrous alcohol with a high alcohol content that meets international specifications. The alcohol content measurements were based on the determination of the refractive index and specific mass, physical parameters related to the correlations used to determine alcohol concentrations in samples.

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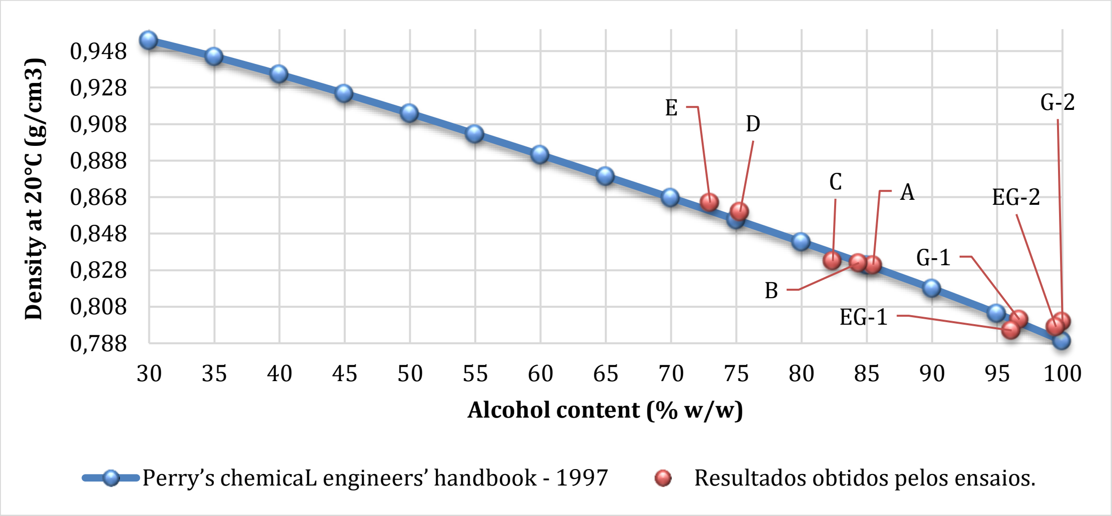

Figure 5. Comparison of density with the literature.

The two solvents used in this study showed efficiency in breaking the azeotropic boiling point, producing anhydrous alcohol with the characteristics of the international standards required for this product. Studies carried out by Barros (1997, 2022) showed that the best procedure to produce anhydrous ethyl alcohol should be associated with extractive distillation, with the use of solvents capable of causing rupture of the azeotropic boiling point, characterized by the deviation of the equilibrium curve from the diagonal. However, studies carried out by Pitt et al. (2019) showed that glycerol, when used as a solvent in extractive distillation processes, has better operational performance compared to ethylene glycol. The use of glycerol in this study is associated with the potential for reusing the by-products of biodiesel production as raw material for extractive distillation processes, guaranteeing an increase in the revenues of industrial units for the production of biofuels.

4. Conclusions

Based on the results contained in this article, it can be concluded that:

a) The methodology used allowed the study of hydrolysis, fermentation, and conventional and extractive distillation procedures capable of producing anhydrous alcohol with international specifications;

b) The implementation of experimental hydrolysis assays, with amylase as an enzyme, presented a better performance in terms of distillate volume;

c) The increase in hydrolysis time should be explored to assess the increase in alcoholic fermentation yield;

d) The structured apparatus to carry out the extractive distillation tests proved viable, as it guaranteed the purification of anhydrous ethanol with physical parameters similar to those in the literature.

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