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Application of acid hydrolysis and fermentation to barley hulls for the production of bioethanol

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Abstract*– In the Peruvian brewing industry, approximately 60% of the barley grain is used for beer production, while the other 40% is lost in the husk. In order to make the most of the lignocellulosic residue contained in the barley husk in a different way, the main objective of this research is to obtain bioethanol through acid hydrolysis and fermentation using yeast as a microorganism capable of decomposing the substrate. The result was obtained after making changes in temperature, acid and yeast concentration that the highest concentration of ethanol was 33.58%, the color of the solution was light brown and that the boiling temperature is 84.43°C. It is concluded that temperature and acid concentration have a positive effect on the hydrolysis of the compound but there is a point where additional increases may not be as beneficial. To optimize the process, the cost-benefit of using higher acid concentrations and temperatures must be evaluated.

Keywords--* Barley husks, bioethanol; acid hydrolysis; fermentation.

I. Introduction

Oil accounts for 80% of the world's energy production today, but it is a non-renewable energy source. In contrast, one of the seventeen sustainable development goals of most countries is to obtain new, clean, and affordable energy sources [1][2]. Biodiesel is a viable alternative due to its biodegradability, renewability, and minimal toxicity [3]. Obtained from raw materials with high oil content and, due to its variety of biomasses, soybeans, and sunflowers are the first-generation option. However, sources that do not compete with food crops were chosen, giving rise to the second generation [4].

This type of biofuel is characterized by the reuse of biomass through biological processes that allow obtaining more sustainable energy, such as biogas, bioalcohols, bioethanol, and biodiesel [5]. Cellulosic ethanol production was expected to support bioethanol supply as it is obtained from crop residues such as stems, leaves, and husks, as well as non-food crops, forest residues, and lignocellulosic industrial waste [6].

Sugarcane bagasse, corn stover, rice straw, eucalyptus wood chips, and palm waste are agricultural wastes with high potential, currently under investigation for their conversion into a range of value-added products [7]. In the Peruvian brewing industry, 40% of the barley grain for beer production is lost in the husk containing cellulose [8]. For this reason, in this research, barley husks will be used, which will undergo a pretreatment and will be subjected to the acid hydrolysis process, subsequently to alcoholic fermentation using the yeast Saccharomyces cerevisiae to decompose the substrate and be able to produce second-generation bioethanol.

II. Theoretical Bases

A. *Fermentation Process*

Fermentation is the intentional cultivation of microorganisms such as bacteria, yeasts and fungi to manufacture useful products for humans (biomass, enzymes, primary and secondary metabolites, recombinant products and biotransformation products [9] that have application within the energy production industries, materials, pharmaceutical, chemical and food [10].

Therefore, the activity of microorganisms plays an important role in the fermentation of foods by showing changes in the chemical and physical properties of foods [11].

1) Types of fermentation.

There are two types of fermentation: natural and artificial. Natural fermentation occurs under environmental conditions that allow spontaneous interaction between microorganisms and susceptible organic substrates, without direct human intervention. In contrast, artificial fermentation is a controlled process in which humans intervene by favoring the conditions and the contact involved. Depending on the type of substrate used, fermentation can be classified into different categories, such as alcoholic, lactic, acetic, and butyric, each with specific applications in the food, energy, and chemical industries [12].

- Alcoholic Fermentation.

Alcoholic fermentation is a process carried out by yeasts of the genus *Saccharomyces cerevisiae* under anaerobic conditions; which acts as an enzyme complex for the degradation of sugars such as sucrose and maltose [13].

- Lactic Fermentation.

It is a metabolic process carried out by bacteria and fungi that act on the sugars present in foods (see Fig. 1), from which they transform the sugars into carbon dioxide and lactic acid, the latter is produced by the oxidation of glucose to two molecules of pyruvic acid, generating NADH [14].

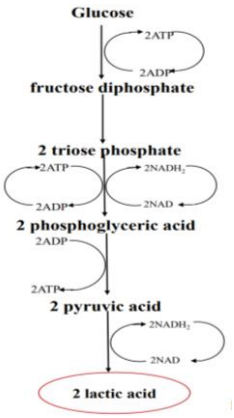

Fig. 1 Lactic fermentation

- Acetic fermentation.

Acetic fermentation is defined as the conversion of ethanol produced in anaerobic fermentation, carried out in the presence of oxygen.

$$CH_3CH_2OH + O_2 + Acetobacter\ aceti \rightarrow CH_3CO_2H + H_2O \quad (1)$$

Ethyl alcohol Oxygen Acetic Bacterium *Acetic Acid Water*

Generally the fermentation stops when there is the presence of a minimum, although finite residue of ethanol to avoid over-oxidation to CO_2 and water [15].

- Butyric fermentation.

It is a process that consists of the conversion of carbohydrates into butyric acid and the production of gases such as carbon dioxide, methane, volatile fatty acids, and hydrogen. Fermentation occurs from the degradation of lactose by the action of strictly anaerobic bacteria such as the *Clostridium butyricum* species [16] [17].

2) Natural substrates for bioethanol production.

Obtaining ethanol from lignocellulosic hydrolysates is an alternative widely studied today in the world, with a view to reducing the cost of fuel ethanol. Among the alternative substrates due to their biomass composition and the possibility of producing bioethanol are [18]:

- Barley
- Wheat
- Corn
- Beetroot
- Sugar cane

Fig. 2 shows each of them with their different yields (L/ha).

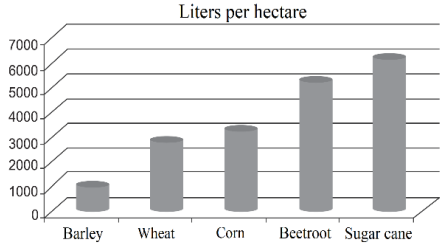

Fig. 2 Liters of bioethanol produced per hectare of different crops. [19]

3) *Main nutrients required by microorganisms in the fermentation process.*

For cell duplication or growth to occur, various anabolic and catabolic reactions must take place, which require specific microbial nutrition. Yeasts can utilize various carbon and energy sources, with Saccharomyces cerevisiae and *Candida utilis* being the predominant species in the microbial community. These yeasts require ammonia, urea, or ammonium salts, as well as a mixture of amino acids, as a source of nitrogen [20].

Other nutrients required by these microorganisms are phosphorus, which can be administered in the form of phosphoric acid, magnesium (magnesium sulfate), calcium, iron, copper, zinc and B complex vitamins. As part of their metabolism fermentative produce ethanol [21].

4) Factors influencing fermentation for ethanol production.

In fermentation, the factors that influence the ethanol yield depend on the inoculum, the composition of the culture medium, the environmental conditions, and the microbial growth [22]. The latter will depend on the morphological complexity of the microorganism, since the lower the complexity, the higher the specific growth rate (μ_max) and will decrease as the complexity increases [23]. The μmax is related to the time it takes for a microorganism to duplicate (t_D) as can be seen in Table 1.

TABLE I
μ_{max} AND t_D FOR DIFFERENT TYPES OF MICROORGANISMS AND CELLS

Microorganisms/cells	μ_{max} (h^{-1})	t_D (h)
Bacteria	0.6 – 1.4	0.5 – 1.15
Yeast and filamentous fungi	0.2 – 0.6	1.15 - 3
Animal cells	0.01 – 0.04	17 - 70
Plant cells	0.007 – 0.03	23 - 100

5) Microorganisms in ethanol production.

Alcoholic fermentation in sugary musts originates from the anaerobic metabolism of Saccharomyces cerevisiae (yeast). Yeast is a fungus whose enzymes help break down glucose into ethanol and carbon dioxide anaerobically. This reaction takes place in the absence of oxygen [24]. This fermentation can be represented by the Gay-Lussac stoichiometric equation.

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \quad (2)$$

glucose ethanol

6) Metabolic pathway for Bioethanol production.

The sugar fermentation pathway for Saccharomyces is shown in Fig. 3. Glycolysis is used for the fermentation and/or production of bioethanol. Under anaerobic conditions pyruvate is reduced to ethanol with the release of CO_2 (see Fig. 3).

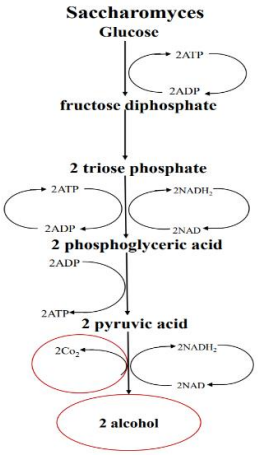

Fig. 3 Sugar metabolism by Saccharomyces as representative of yeasts [11]

B. *Acid Hydrolysis*

The acid hydrolysis of lignocellulosics has been the most widely used technology for obtaining reducing (fermentable) sugars, as can be seen in Fig. 4, which are subsequently converted to bioethanol.

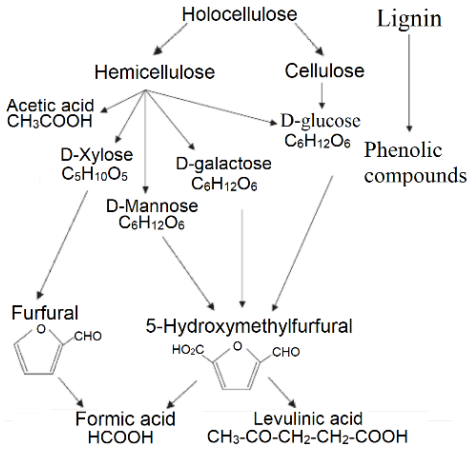

Fig. 4 Products and by-products formed during the acid hydrolysis of lignocellulosic biomass [25]

The degree of bagasse degradation depends on the acid concentration and the hydrolysis time [26]. Concentrated acid hydrolysis employs concentrated H_2SO_4 in the range of 10-30%, this process provides a complete and rapid conversion of cellulose and hemicellulose. The reaction times are generally longer than those of the dilute acid hydrolyzation [27].

C. *Barley Husk*

They are the most abundant compounds in nature and are formed by three different types of polymers: cellulose, hemicellulose, and lignin. These are intertwined in a complex structure that protects against microbial attack and, at the same time, allows the depolymerization of cellulose and hemicellulose to fermentable sugars through the action of lignin. For their use, pretreatments are required to facilitate their release, followed by hydrolysis and, finally, fermentation of cellulose and hemicellulose [28]. Table 2 shows the average polymer composition of different substrates.

TABLE II
AVERAGE COMPOSITION IN POLYMERS [8]

Lignocellulosic Material	% (w/w) BS Cellulose	% (w/w) BS Hemicellulose	% (w/w) BS Lignin
Barley hulls	25.89-35.5	18.1-21.35	18.20-24.6
Cane bagasse	48.81	24.42	25.82
Banana by-products	13.2	14.8	14.00

D. *Bioethanol*

Considered an alternative fuel for diesel engines, biomethane is obtained through fermentation mediated by the interaction of various organisms. It is defined as a mixture of monoalkyl esters of fatty acids, derived from plant matter, animal fats, or vegetable oils [29]. There are 4 generations or options to obtain bioethanol; the first generation is produced by fermentation of sugary products such as beets, sugar cane, and cereal grains, but these compete in the food market; the second generation is obtained from lignocellulosic waste such as barley husk [30]; the third generation is organisms capable of feeding on light and CO_2 such as algae and microalgae due to their high concentration of lipids, proteins, and carbohydrates; finally the fourth generation are genetically modified organisms allowing a greater production of sugars or oils, as can be seen in Fig. 5.

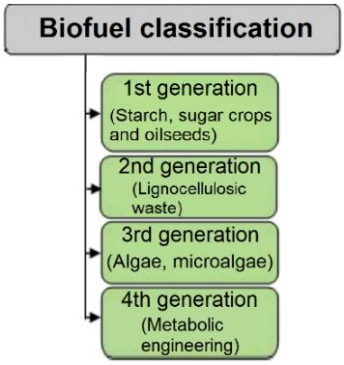

Fig. 5 Scheme of the classification of biofuels [24].

III. METHODS AND MATERIALS

A. *Procedure to determine optimal parameters of acid hydrolysis*

1) *Pretreatment*

Pretreatment of the sample to obtain alcohol from the acid hydrolysis of the barley husk (see Fig. 6).

- Barley grinding in an industrial mill.
- Sieving of the barley sample.
- Barley husk drying.

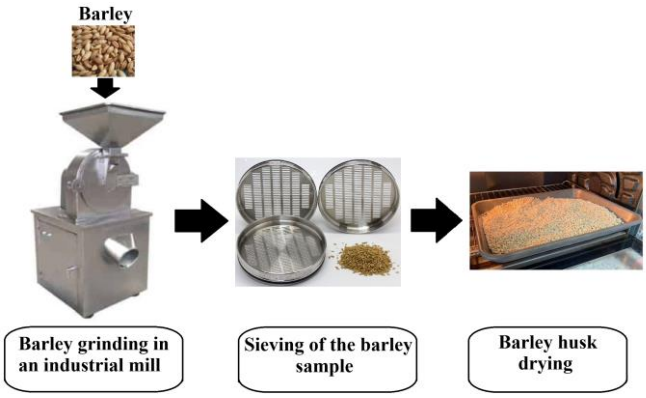

Fig. 6. Barley hull pretreatment

2) Treatment: Acid hydrolysis method

In this stage, the pretreated (dried) sample is added to the reactor (250 gr), followed by sulfuric acid (2500 ml) at different concentrations (0.5, 1.0 and 1.5%), then hydrolyzed at different temperatures (90, 100 and 110 ° C) at the same time varying the times (180, 210 and 240 min) subsequently evaluating the amount of fermentable sugars obtained in each case.

3) Quantification of fermentable sugars

The reduced sugar content is determined by a calibration curve that relates absorbance to concentration. For its construction, standard glucose solutions with concentrations of 0.5, 1.0, 1.5, and 2.0 g/L were prepared. The DNS method was applied to them and their absorbance was measured in a spectrophotometer (Genesys 10 vis) at a wavelength of 540 nm [32]. The DNS method was used to analyze the samples. Each sample (0.5 mL) was mixed with 0.5 mL of DNS reagent and heated in a boiling water bath for 5 min. The reaction was then immediately stopped by placing the samples in an ice-water bath. They were then reconstituted with 5 mL of distilled water, shaken, and allowed to stand for 15 min before measuring their absorbance at 540 nm. The same procedure was performed for the blank, using distilled water instead of a sample.

B. Procedure to determine the optimal fermentation parameters.

1) Regulate the pH.

The hydrolysate with the highest amount of fermentable sugars is taken for the fermentation process, for which it is necessary to regulate the pH, since it is initially at a pH of 2.0, which is not optimal, which is why the pH will rise to 4.8 to be able to ferment, because fermentation must take place in a controlled medium that contains a pH between 4 and 6 [33].

2) Fermentation.

Next, the yeast is activated (5, 10 and 15 g/L) for its addition to the hydrolyzed sample. Then, the activated yeast is added to the hydrolyzed sample to enter the fermentation process at different times and temperatures

3) Reading of fermentable sugars.

Once the samples are fermented, the fermentable sugars are read as fermentation residue using the same method as for the hydrolyzed samples.

C. Procedure to determine degree of purity of ethanol obtained.

100 ml of fermented sample is taken with a lower amount of fermentable sugars and proceeds to distillate with a simple distillation equipment from the beginning of ethanol distillation until reaching the boiling point of water and thus ensure that everything the ethanol has been distilled off. Once the distillation is finished in a 100 ml cylinder, it is mixed with water up to 100 ml. The reading is carried out in the refractometer, for this, ethanol solutions of concentrations were prepared: 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% with a volume of 10 ml, and each of these were measured the refractive index, as well as the sample obtained.

D. Determination of the physicochemical characteristics of ethanol.

1) Density

- Weigh the clean, dry pycnometer on a precision balance and record its mass, called mp.
- Measurement with water (reference fluid): Fill the pycnometer with distilled water, making sure that the liquid reaches the top of the pycnometer capillary. When you close the lid, the water will overflow through the capillary, ensuring that the volume inside the pycnometer is constant and uniform for each measurement. Carefully dry the outside of the pycnometer to remove any water residue before weighing. Measure the mass of the pycnometer filled with water and record it as mp+w (mass of the pycnometer with water).
- Measurement with the test solution: Empty the pycnometer and fill it with the liquid whose density you want to determine, following the same procedure as with water. Dry the outside again and weigh it, recording the mass as mp+s (mass of the pycnometer with the solution).

2) Color

- The color of the liquid is assessed visually using the ASTM D1500 color scale, a standard that classifies liquids in shades from light yellow to dark brown. The procedure involves comparing the color of the sample to reference colors on the ASTM D1500 scale under standard lighting conditions. This allows for a numerical classification of the color of the liquid, which is useful in industries such as oils, fuels, and lubricants to assess their purity and composition.

3) Boiling temperature

- The boiling point of the liquid is obtained by interpolation on a previously constructed graph, in which the boiling point is related to the percentage of ethanol present in the sample. This is because the boiling point varies depending on the composition of the liquid, and by knowing the proportion of ethanol in the sample, it is possible to estimate its boiling point with greater precision.

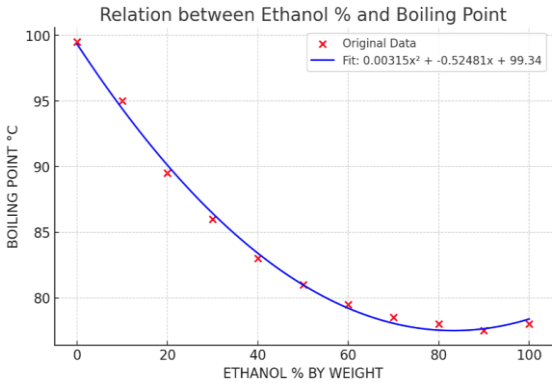

Fig. 7. Boiling points of solutions of ethyl alcohol and water at 20°C

IV. Results

A. Result of the optimal parameters of acid hydrolysis of barley husk

The DNS method was applied and the absorbance (A) of each of the solutions was read in a spectrophotometer at a wavelength of 540 nm to obtain the glucose standard curve. The standard glucose curve (see Fig. 8) is prepared to read the hydrolyzed and fermented samples.

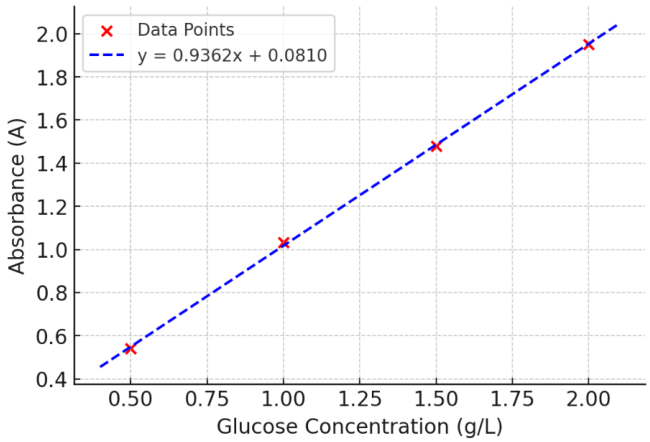

Fig. 8 Glucose pattern curve

Table 4 and Fig. 9 show the results of fermentable sugar concentration where the acid concentration is varied to find the optimum hydrolysis time and temperature.

TABLE IV
RESULTS OF THE HYDROLYSIS PROCESS

Acid concentration (%p/p)	0.5%		1.0%		1.5%	
Temperature (°C)	90	100	110	90	100	110	90	100	110
Time (min)	GLUCOSE CONCENTRATION (g/L)								
180	1.02	1.75	4.58	5.52	8.17	10.68	7.08	7.43	5.98
210	3.50	4.17	6.72	7.64	9.49	12.06	8.13	8.64	6.37
240	4.97	5.08	7.59	9.02	10.31	12.35	9.21	9.42	7.49

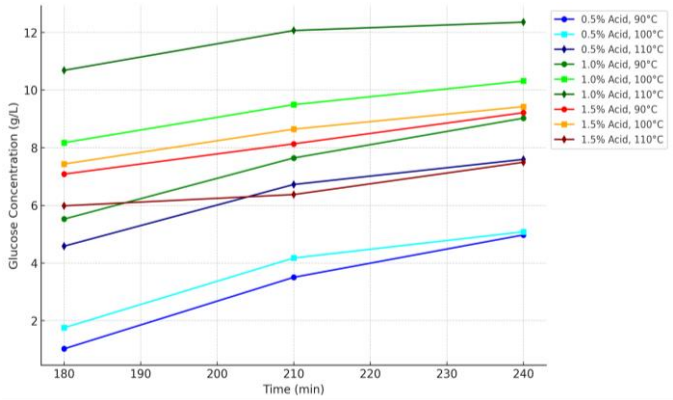

Fig 9 Concentration of fermentable sugars after hydrolysis

B. Result of the optimal parameters of hydrolyzed fermentation

Table 5 and Fig. 10 show the results of fermentable sugar concentration where the yeast concentration is varied to find the optimum fermentation time and temperature.

TABLE V
RESULTS OF THE FERMENTATION PROCESS

YEAST CONCENTRATION (g/L)	5		10		15	
TEMPERATURE (°C)	20	25	20	25	20	25
TIME (h)	GLUCOSE CONCENTRATION (g/L)					
36	9.24	8.01	6.07	5.15	6.98	5.83
48	8.15	6.71	4.04	3.22	6.51	5.58
60	6.49	5.26	3.92	3.87	5.46	5.19

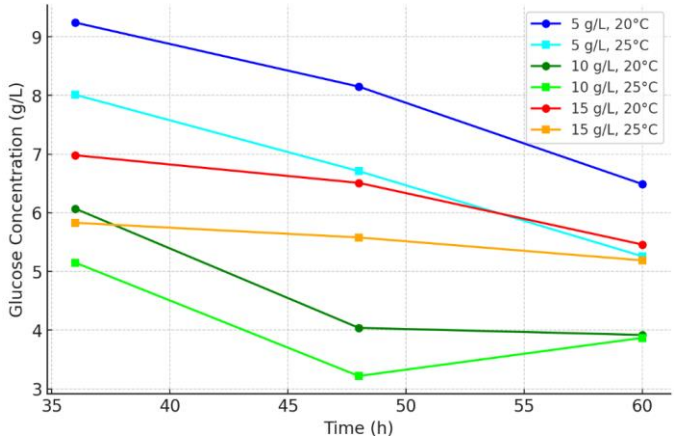

Fig 10 Concentration of fermentable sugars after fermentation

C. Result of the % purity of ethanol obtained

The reading was processed in the refractometer. For this purpose, ethanol solutions of concentrations were prepared:

10, 20, 30, 40, 50,60, 70, 80, 90 and 100% with a volume of 10 ml. In table 6 and Fig. 11, the percentage of ethanol can be observed by interpolating the data in the standard curve.

TABLE VI
ETHANOL STANDARD CURVE

Concentration (%)	IR (ND)
0	1.333
10	1.338
20	1.343
30	1.348
40	1.351
50	1.356
60	1.358
70	1.362
80	1.363
90	1.365
100	1.364

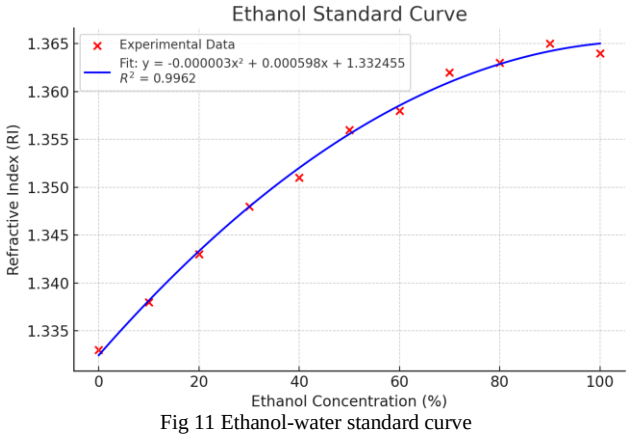

Fig 11 Ethanol-water standard curve

$$y = -0.000003x^2 + 0.000598x + 1.332455$$

The refractive index of the sample is 1.352, this is the result of an ethanol concentration of 39.95%.

D. Result of the physicochemical characteristics of the ethanol obtained

In table 7, Fig. 12 it was determined that the density is in a range of 30-50%.

• Density: Masses of ethanol solutions at different concentrations using the pycnometer.

m_w = 9.7354 g at 20 °C.

TABLE VII
RELATIVE DENSITY OF ETHANOL AT DIFFERENT CONCENTRATIONS

Ethanol concentration (%)	m_p(g)	m_{p+s}(g)	m_s(g)	Relative density
10	18.5814	28.1715	9.5901	0.9842
30	18.5715	27.9227	9.3513	0.9608
50	18.5886	27.6760	9.0874	0.9337
70	18.5709	27.1787	8.6128	0.8851
90	18.5763	26.6169	8.0406	0.8318
Sample	18.5792	27.9121	9.3402	0.9619

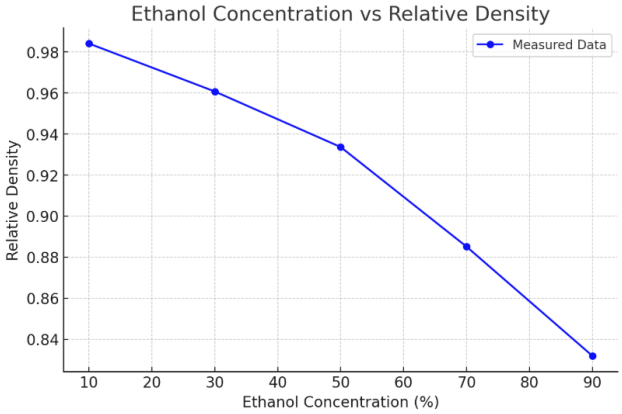

Fig 12 Relative density vs. concentration curve

In Fig. 13 we can see the correlation between the concentration of 30 and 50%.

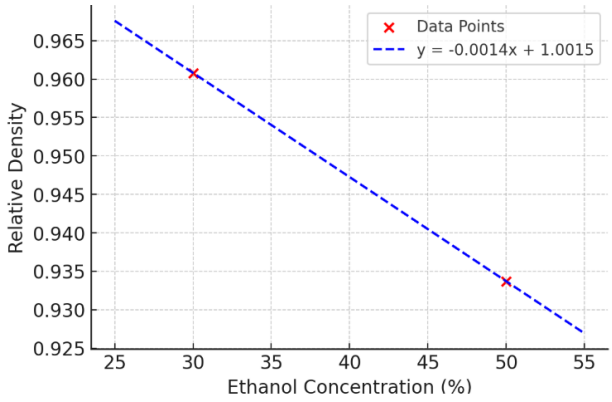

Fig 13. Correlation between the second and third point.

Using regression the Ethanol Concentration is equal to 33.58%.

• Color, the color was determined visually after fermentation is light brown, after distillation it is colorless in appearance.

• Boiling temperature, it was determined by interpolating the graph of Fig. 14.

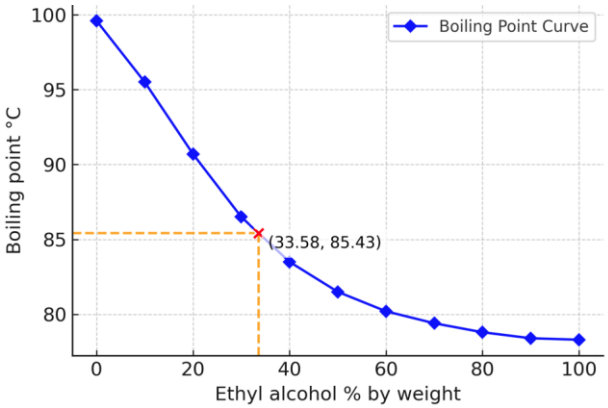

Fig 14. Boiling Points of Ethyl Alcohol and Water Sample

According to the graph, the boiling temperature is 85.43°C.

E. Estimated cost for 1000 liters

The total estimated cost is 177.93 USD as can be seen in table 8, to calculate the cost per liter of ethanol, it must be taken into account that the concentration of ethanol is equal to 33.58%, so 335.8 L of diluted ethanol is obtained from which 113 L of pure ethanol is obtained.

TABLE VIII
ESTIMATED COST FOR 1000 LITERS

Item	Scaled Quantity	Unit Cost	Estimated total (USD)
Barley husk	25 kg	0.055 USD/kg	1.37
Sulfuric acid 1%	250 L (~2.5 L H_2SO_4)	2.7 USD/L (Concentrated)	6.84
Active dry yeast	1 kg	0.041 USD/g	41.06
Distilled water	1000 L	0.082 USD/L	82.12
Electric energy	Approx. 60 kWh	0.19 USD/kWh	11.50
Laboratory materials/ equipment	Equipment depreciation	___	5.47
Labor	1 technician x 3 days	6 h/day x 1.64 USD/h	29.56

The values for total cost and pure ethanol are replaced in the following equation:

Cost per liter = Estimated total cost/Pure ethanol (3)

It is obtained that the cost per liter of bioethanol produced with barley husk is 1.5 USD/L

V. CONCLUSIONS

The maximum percentage of bioethanol was obtained by determining the optimal parameters of acid hydrolysis and fermentation, whose result was 33.58% with physicochemical characteristics: relative density equal to 0.9608 g/L, colorless and with the same boiling temperature at 85.43 °C, all belonging to said percentage of purity.

The optimal acid hydrolysis parameters are: acid concentration 1%, temperature 110 °C and hydrolysis time equal to 240 min, where the maximum glucose concentration equal to 12.35 g/L was obtained.

The optimal fermentation parameters are: yeast concentration 10 g/L, temperature 25°C and fermentation time equal to 48 hours, where the minimum concentration of fermentable sugars equal to 3.22 g/L was obtained.

It is concluded that temperature and acid concentration have a positive effect on the hydrolysis of the compound but there is a point where additional increases may not be as beneficial. To optimize the process, the cost-benefit of using higher acid concentrations and temperatures must be evaluated.

It is concluded that the cost per liter in this research is still somewhat high, due to the lower yield of barley hulls, as can be seen in Figure 2. However, this can be reduced by using renewable energy to operate the equipment. Furthermore, by using waste from the brewing industry, no raw materials need to be spent, thus adopting a sustainable approach.

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