

# Obtaining Bioethanol through Hydrolytic Treatment of Agro-Industrial Banana Residues

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## Abstract

The banana is a food of great importance and it is consumed in almost the entire world. However, its harvest generates large quantities of mostly lignocellulosic waste, which can be used for the production of biofuels such as bioethanol. In this work, the potential for bioethanol production from agro-industrial plantain crop residues was evaluated with different operating conditions. A 2<sup>4</sup> experimental design was used, having as study variables: time of hydrolysis, pH of hydrolysis, concentration time, and fermentation time. The samples used were scraps consisting of a mixture of stems, leaves, and banana peels. The bioethanol obtained was characterized by physicochemical properties such as density, refractive index, and FTIR. As a result, it was obtained that the volume of bioethanol represented higher yields; using NaOH as a hydrolyzing agent, with hydrolysis time of 30 minutes, high fermentation time, and low concentrations. The chemical characterization of banana agro-industrial waste indicated that, the raw material could be considered as a potential source for bioethanol production, since it has a high content of cellulose.

## Keywords

Hydrolysis, Lignocellulosic Waste, Banana, Bioethanol, Experimental Design

## 1. Introduction

Banana is one of the most abundant crops in the world, mainly in South America. Each year production increases, as it is part of the daily food of many regions. Currently the banana production worldwide amounts to 145 Million tons per

year, and it is commercially grown in many varieties in about 120 countries. On the other hand, commercial banana production generates a large proportion of waste. Bello *et al.* [1] report that for every ton of bananas produced approximately 3 tons of pseudostem, 160 kg of stems, and 480 kg of leaves are generated. Thus, an established commercial use for these residues (such as obtaining bioethanol, as well as generating extra remuneration for regional farmers,) would help to reduce environmental pollution which is currently caused by fossil fuels [1]. The burning of these fuels is the main cause of climate change. CO<sub>2</sub> emissions from fossil fuel combustion are largely responsible for global warming [2], which is why biofuels appear as an alternative solution, since the percentage of polluting gases emissions they produce is reduced during their production cycle [3]. One solution is the use of biofuels such as Ethanol that is currently used as liquid non-pollutant fuel or as a gasoline enhancer in many countries [4]. Banana waste has the potential to produce ethanol with a low-cost and sustainable production method [1].

Bioethanol serves as a liquid fuel or gas additive in many countries in response to public policy, market pressure, and depletion of the world's energy resources. The production of bioethanol from inexpensive and widely available raw materials is a highly attractive option [4]. Bioethanol is adaptable to 5 or 10% blended feed systems and it is mainly obtained from biomass, which is competitively given its price, quality and origin [5] [6] [7]. The production of bioethanol based on sugars and starches has been a subject of great controversy due to the food competition that is generated, as well as to the emerge of more sustainable sources from agricultural byproducts, forest residues or energy crops denominated as lignocellulosic biomass or also known as second generation [8] [9]. However, these raw materials present drawbacks due to the high content of lignin and hemicellulose, high cellulose crystallinity and low surface area, so it is necessary to perform a physical, chemical or biological type pretreatment to facilitate the production of biofuel [10] [11].

A large amount of agro-industrial waste generated annually, throughout the world, contains high lignocellulosic levels and starch [12] [13] [14] [15]. Lignocellulose is the main component of biomass, which comprises about half of the plant material produced by photosynthesis and represents the most abundant renewable organic resource in the soil [15]. Since the composition of the lignocellulosic materials depends on various factors [16], it is necessary to adjust certain parameters for each raw material, thus making the structural sugars accessible to the fermentation [15]. One of the most well-known pretreatments is acid hydrolysis, which presents drawbacks such as corrosion of the equipment and the need for neutralization. However, it has a high efficiency in the conversion of hemicellulose to monosaccharides and increases the cellulose digestibility in the solid residues obtained [17]. As for the basic pretreatment, it produces a rupture of the structure of the lignin increasing the internal surface, thus reducing the degree of polymerization and crystallinity [18].

Recent studies about the use of various lignocellulosic residues include fruits

such as grapes, apples, melons, bananas and, coffee, etc. [19]-[24], to produce bioethanol. In so much as the composition of lignocellulosic materials depends on several factors [16], it is necessary to adapt certain parameters for each raw material, thus making structural sugars accessible to fermentation and obtain bioethanol [15]. One of the most known pretreatments is acid hydrolysis, which has drawbacks such as equipment corrosion and neutralization is needed. However, it has a high efficiency in conversion of hemicellulose into monosaccharides and increases the digestibility of cellulose in the solid waste obtained [17]. As for the basic pretreatment, the degree of polymerization is reduced [18]. In regard to the enzymatic pretreatment, it is a promising topic that is still under development [19] [20], considering that despite reporting high yields of ethanol, it represents high costs due to the adequacy of the systems.

In order to provide added value to banana agro-industrial residues, in this study acid and basic hydrolytic pretreatments were evaluated in order to identify the most appropriate bioethanol production process. The novelty of the present study lies in the use of the design of experiments, particularly solving multiple responses simultaneously. Although the bioethanol obtaining is a topic, which has been extensively studied, hydrolytic treatment of agro-industrial does not frequently use the design of experiments for optimization of their processes. Parts of this work were presented in the event organized by the World Academy of Science, Engineering and Technology and focuses on the optimization of multiple responses in a bioethanol obtaining process [25].

## 2. Material and Methods

The crop waste from banana is generated by management practices in stages such as defoliate, deschante, acorn removal, clearing and dethroning [25]. In this study, the samples used were debris consisting of a mixture of stems, leaves and husks produced by a plantain crop located in the Guayabetal village in the municipality of Yopal, department of Casanare-Colombia.

### 2.1. Characterization of the Raw Material

The most used raw materials for bioethanol production can be classified into three main types: sugars, starches, and cellulose materials [4]. Because banana residues are made up of lignocellulosic fibers, they could be used as raw material for obtaining cellulose or in obtaining bioethanol, in this way, an added value would be provided to say residues.

Initially the raw material was washed with tap water, then dried at 60°C for 48 h, and finally mechanical milled using a conventional mill (Corona brand) to obtain a particle size between 1 - 5 mm. Subsequently, the determination of the percentage of cellulose, hemicellulose and lignin was carried out, in establishing the potential for the raw material in bioethanol production [20].

### 2.2. Experimental Design

The experimental variables evaluated were fermentation time, hydrolysis pH,

hydrolyzing agent, concentration and hydrolysis time [3] [21]. This work was carried out in duplicate by a 2<sup>4</sup> experimental factorial design, where two levels and four factors were obtained, thus having an arrangement of 16 combinations that are observed in **Table 1**.

### 2.3. Fermentation and Separation

Initially, a pretreatment to the raw material was carried out, as described in **Figure 1(a)**. In the fermentation process, the reducing sugars were converted to alcohol using yeast *Saccharomyces cerevisiae* as the fermenting agent, which was activated prior to use (see **Figure 1(b)**). Finally, the raw material subjected to pretreatment with yeast activated was mixed and kept in an amber bottle in the incubator for 7 or 15 days at a temperature of 25 °C [22]. The fermented product was manually filtered [23], and fractional distillation was performed according to ASTM method D2892-16.

### 2.4. Physical and Chemical Characterization of Ethanol Obtained

The different bioethanol samples obtained were characterized by the refractive index using a PZO Warszawa Poland refractometer with temperature control of  $\pm 0.1$ , specific gravity (20 °C) and infrared spectrum through a Shimadzu FTIR spectrophotometer, model Prestige-21, following the procedure established in the equipment manual for volatile liquid samples.

### 2.5. Statistical Analysis

In order to evaluate the simple and combined influence of critical variables in the production of bioethanol, a statistical ANOVA factorial analysis was performed using the Statgraphics Plus program, through graphical representation with a Pareto diagram.

## 3. Results and Discussion

### 3.1. Characterization of the Raw Material

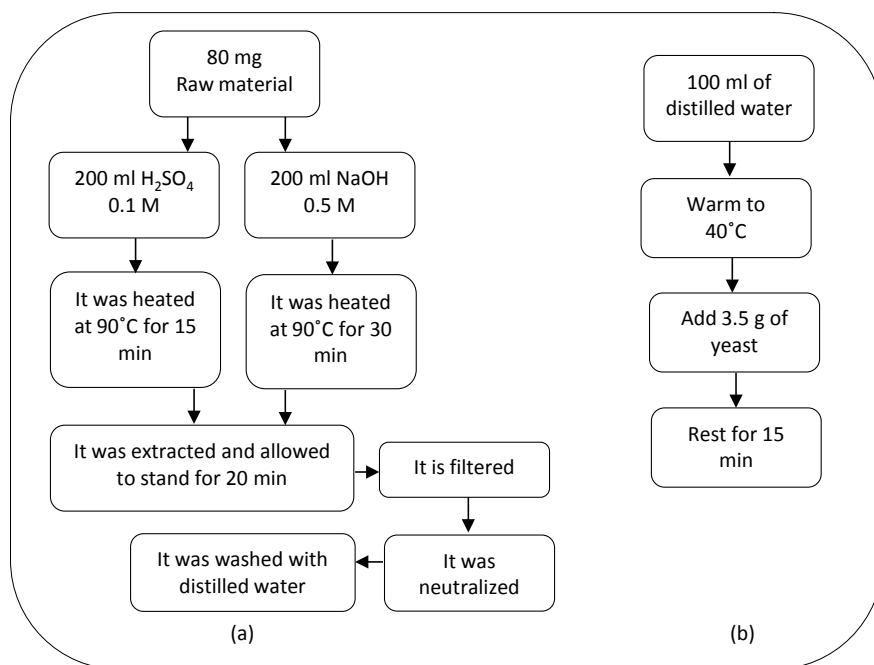
The chemical characterization of banana agro-industrial waste (**Table 2**) indicated that, the raw material can be considered as a potential source for the production of bioethanol as it has a high content of cellulose. When comparing its composition with lignocellulosic materials, studies in the literature find a low percentage of hemicellulose and lignin, which increases the production of reducing sugars, and that the optimal hydrolytic pretreatment could include hydrolyzing agents in relatively low concentrations [3] [6] [24].

### 3.2. Density-Concentration-Refractive Index of Bioethanol Obtained

**Figure 2** shows that the density values closest to anhydrous ethanol (0.789 mg/L to 20 °C) come from samples obtained through basic hydrolytic pretreatment with 30 minutes hydrolysis time. The lignin degradation of the lignocellulosic

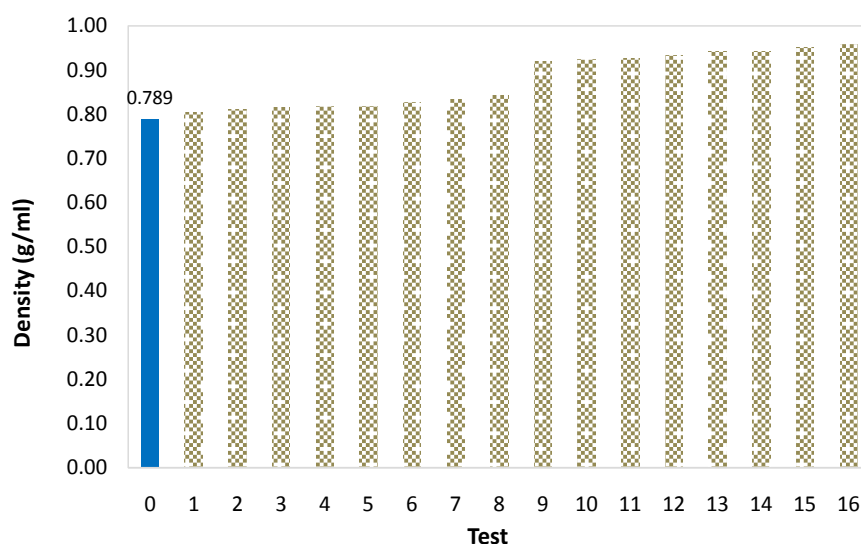
**Table 1.** Experimental design—study variables.

Tests	Reagent (pH)	Concentration (M)	Fermentation (Days)	Time (min)
1	NaOH	0.1	7	30
2	H <sub>2</sub> SO <sub>4</sub>	0.5	7	30
3	H <sub>2</sub> SO <sub>4</sub>	0.1	15	30
4	NaOH	0.5	7	30
5	NaOH	0.1	15	30
6	NaOH	0.5	15	30
7	H <sub>2</sub> SO <sub>4</sub>	0.5	15	30
8	H <sub>2</sub> SO <sub>4</sub>	0.1	7	30
9	H <sub>2</sub> SO <sub>4</sub>	0.1	7	15
10	H <sub>2</sub> SO <sub>4</sub>	0.1	15	15
11	H <sub>2</sub> SO <sub>4</sub>	0.5	7	15
12	NaOH	0.1	7	15
13	NaOH	0.1	15	15
14	NaOH	0.5	7	15
15	NaOH	0.5	15	15
16	H <sub>2</sub> SO <sub>4</sub>	0.5	15	15

**Figure 1.** Flow chart for: (a) Chemical pretreatment of organic matter; (b) Activation of the yeast.

**Table 2.** Percentage composition of agro-industrial banana residues.

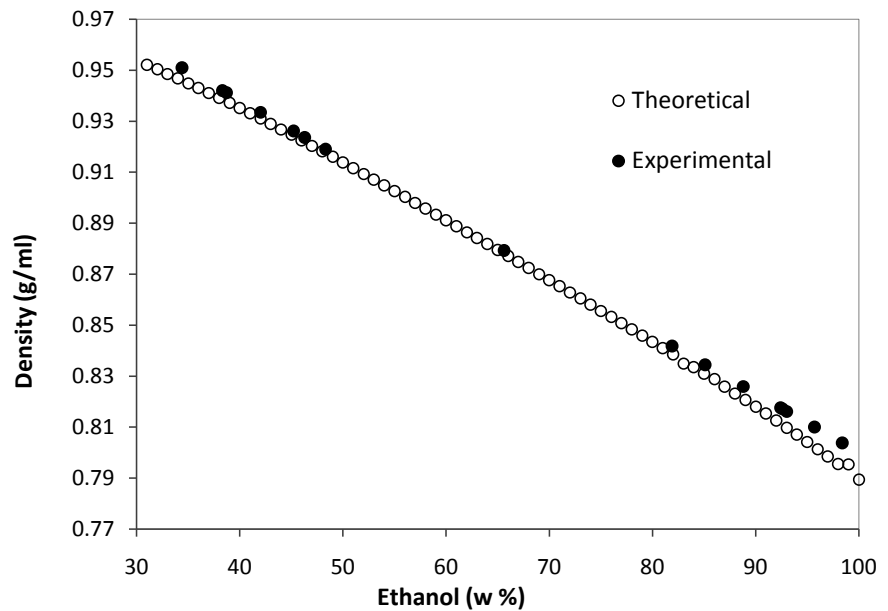
Organic fraction	Experimental	Theoretical
Lignin (%)	11.1	12.2
Cellulose (%)	50.4	28.3
Hemicellulose (%)	6.70	15.7

**Figure 2.** Sample densities of experimental bioethanol.

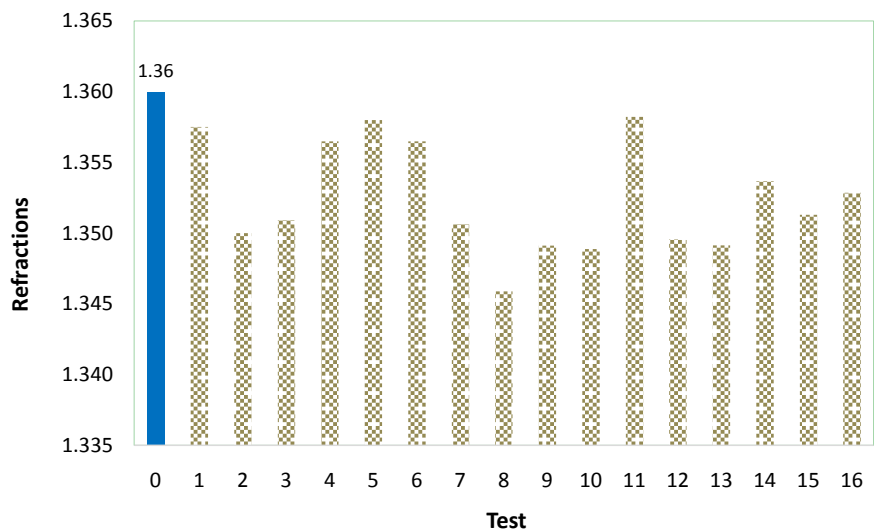
biomass allows the conversion of cellulose and hemicellulose into simpler sugars more easily digested by the fermentation agent, further decreasing the crystallinity of the cellulose and increasing the surface area [5]. In contrast, the samples obtained with 15 minutes hydrolysis time or acidic hydrolyzing agents represent densities close to the one from water, indicating a composition which water percentage is higher, and therefore, the existence of hydrogen bridges intra and intermolecular [26]. That fact is corroborated when establishing the concentration of bioethanol in each of the samples through the interpolation of the experimental data in theoretical curves of ethanol-water mixtures [27]. **Figure 3** shows the increase in the percentage of ethanol as the density of the distillate decreases and approaches the one from pure alcohol.

The refractive index can indicate the purity of a substance or quantify the amount of a component in a binary mixture [28]. **Figure 4** shows that the pre-treatment with NaOH showed that the bioethanol had less water, which is reflected in refractive indices closer to the value registered in the literature for ethanol [29].

The experimentally obtained bioalcohol can contain traces of compounds specific to the fermentation process, as well as water, given the characteristic formation of the ethanol-water azeotrope [30] [31]. This bioalcohol cannot be destroyed through separation methods such as fractional distillation.



**Figure 3.** Concentration ethanol-water mixtures.

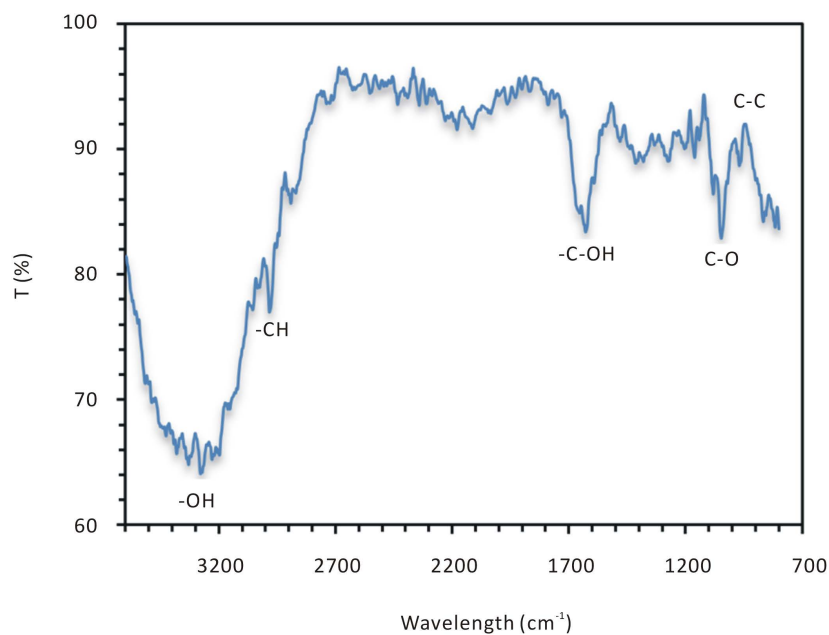


**Figure 4.** Refractions samples of experimental bioethanol.

**Figure 5** shows the differences with respect to the anhydrous compound showing existence of absorption bands, characteristic of the organic alcohol functional group. For the particular case, they correspond to ethanol, and are located between  $3050 - 3600 \text{ cm}^{-1}$  for O-H bond,  $2950 - 3000 \text{ cm}^{-1}$  C-H bond and  $1000 - 1100 \text{ cm}^{-1}$  C-O bond [32] [33].

### 3.3. Statistical Analysis

The ANOVA statistical analysis showed significant differences between treatments when combinations of AB, AD, BC and CD variables were performed (**Table 3**) because the P-Value was higher than the  $\lambda$  set at 0.05. However, it should be mentioned that the individual influence of variables (A, B, C and D)



**Figure 5.** FTIR spectra of bioethanol obtained.

**Table 3.** Abstract ANOVA treatments bioethanol production.

Source	Sum of squares	Degrees of freedom	Middle square	F-ratio	P-value
A: Fermentation time	8.30281	1	8.30281	11.81	0.0026
B: pH	79.1911	1	79.1911	112.67	0
C: Concentration	40.2753	1	40.2753	57.3	0
D: Time of hydrolysis	99.7578	1	99.7578	141.93	0
AB	0.945313	1	0.945313	1.34	0.2596
AC	1.55761	1	1.55761	2.22	0.1522
AD	2.07061	1	2.07061	2.95	0.1015
BC	0.227813	1	0.227813	0.32	0.5755
BD	48.2653	1	48.2653	68.67	0
CD	0.73813	1	0.73813	1.05	0.3177
Blocks	0.01362125	1	0.01362125	0.02	0.8907
Total error	14.0568	20	0.702842		
Total	295.402	31			
Description	Value				
R-Square	95.2412				
Standard error	0.838357				
Absolute error	0.492891				

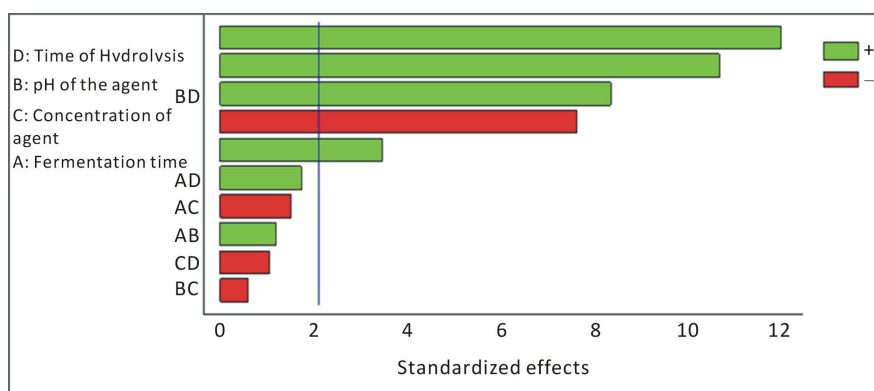


does not prove to be significant between treatments, which would indicate that these alone would not have a significant influence on the production of bioethanol.

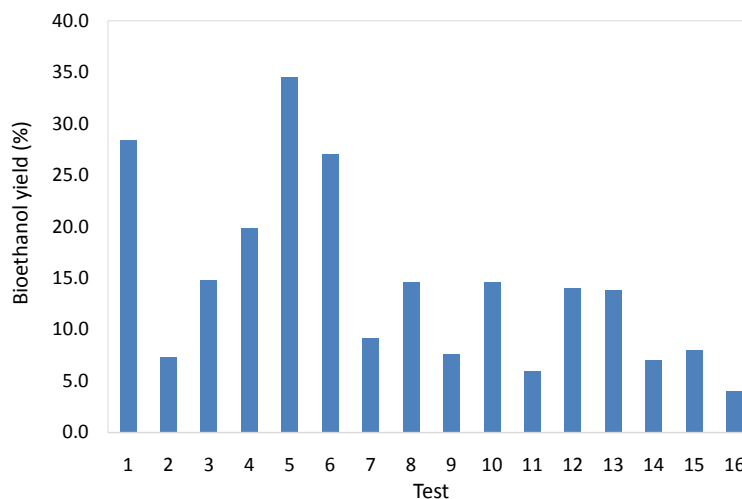
**Figure 6** represents the Pareto diagram obtained through the statistical program Statgraphics. The most influential variable in the production of bioethanol is the pH of hydrolysis, since the best results in general were obtained with a basic pre-treatment (NaOH) because as it was mentioned before, it produces a rupture of the structure of the lignin increasing the internal surface, thus reducing the degree of polymerization and crystallinity [10]. The second most consequential variable was the concentration, as the experimental conditions using a 0.1M concentration represented a higher yield. At a higher concentration, the cellulose might convert to other molecules that may not be fermentable [22] [34]. The combination of fermentation time and concentration of the hydrolyzing agent showed a greater influence than the independent variable of fermentation time, although this last one proved to have a positive effect, directly proportional to the yield of ethanol. This result could be attributed to the essential role of hydrolysis in the release of cellulose and formation of bioethanol [35].

### 3.4. Bioethanol Yield

**Figure 7** shows the yield of bioethanol obtained under different hydrolytic pre-treatment conditions. It is seen as a general tendency that NaOH as a hydrolyzing agent represented better results; tests with hydrolysis time of 30 minutes at basic pH (NaOH), with high fermentation time and low concentrations tend to have higher yields, the most representative being the test carried out with 15 days of fermentation in a 0.1 M concentration. The aforementioned behavior could be attributed to the fact that hydrolysis at high concentrations may form inhibitory agents that affect the fermentation process, which for the particular case would be present in greater proportion when the pH of hydrolysis is acidic and for short periods [25]. Although, in the present study the bioethanol obtained is not higher than 35% in yield, which is low compared with recent researches on banana residues that report ranges from 40% to 80% of bioalcohol



**Figure 6.** Pareto diagram simple and combined effects ( $P < 0.05$ ).



**Figure 7.** Experimental bioethanol yield under different hydrolysis conditions.

[22] [23] [24]. It is important to take into account that the analysis conditions play a fundamental role since the aforementioned investigations evaluate enzymatic hydrolysis, involving strongly acid pretreatments and high temperatures, while this study was carried out with acid-base hydrolysis, using temperatures and moderate times, which facilitates the use of waste byproducts as part of the solution to the energy demand in remote rural areas and in the development process, giving the population the possibility of using a versatile and much less polluting biofuel [36] [37].

#### 4. Conclusion

Banana production is affected by climate change. Nevertheless, these residues can be used to reduce the climate change that affects the production of these same bananas through the production of biofuel such as bioethanol. Benefits in the use of these residues for bioethanol production include the reduction of the environmental impact generated by fossil fuels and the waste of crop residues. Plantain agro-industrial residues represent a raw material with important potential for the production of bioethanol, since they contain a high percentage of cellulose and do not require complex pretreatments to decompose the lignin fraction. In this work, different operating conditions were studied for obtaining bioethanol. A  $2^4$  experimental design was used, having as study variables: hydrolysis time, pH of hydrolysis, concentration and fermentation time. It was determined that the most suitable conditions for higher bioalcohol yields correspond to basic pretreatments, 15 days of fermentation and 0.1 M concentration of hydrolyzing agent. The chemical characterization of banana agro-industrial waste indicated that the raw material could be considered as a potential source for bioethanol production.

#### Competing Interests

The authors declare that there is no conflict of interests regarding the publica-

tion of this paper.

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