



OPTIMIZATION OF THE ACID HYDROLYSIS FACTORS FOR OBTAINING GLUCOSE FROM BANANA, CACAO, AFRICAN PALM, AND SUGARCANE BAGASSE RESIDUES

OPTIMIZACIÓN DE LOS FACTORES DE HIDRÓLISIS ÁCIDA PARA OBTENER GLUCOSA DE LOS RESIDUOS DE BANANO, CACAO, PALMA AFRICANA Y BAGAZO DE CAÑA DE AZÚCAR

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Abstract

Biomass is an alternative to provide energy sources that replace fossil fuels. The objective of this investigation is to optimize the conditions of the acid hydrolysis reaction for obtaining glucose from biomass: banana rachis, cacao pod, African palm rachis, and sugarcane bagasse. The conditions were evaluated through a 2^k experimental design. The factors studied were temperature, time, and acid concentration; the minimum and maximum levels correspond to 70 to 120 °C, 20 to 150 min, and 1% to 5% v/v, respectively. In addition, two replicates were made at the center. The best conditions for obtaining glucose were: 120 °C, 150 min and 1% v/v sulfuric acid. Sugarcane bagasse and African palm rachis were the biomasses from which the highest glucose content was obtained, 9,936.48 and 7,745.14 mg L⁻¹, respectively. The influence of biomass composition on the amount of glucose obtained is discussed.

Keywords: Acid hydrolysis, biomass, glucose, structural characterization.

Resumen

La biomasa es una alternativa estudiada para proporcionar fuentes de energía que reemplacen a los combustibles fósiles. El objetivo de esta investigación es optimizar las condiciones de la reacción de hidrólisis ácida para la obtención de glucosa a partir de la biomasa procedente de los residuos de banano, cacao, bagazo de caña de azúcar y palma africana. Las condiciones fueron evaluadas a través de un diseño experimental 2^k . Los factores estudiados fueron la temperatura, el tiempo y la concentración de ácido; los niveles mínimo y máximo corresponden a 70 a 120 °C, 20 a 150 min y 1 % al 5 % v/v, respectivamente. Además, se realizaron dos réplicas al centro. Las mejores condiciones encontradas para la obtención de glucosa fueron: 120 °C, 150 min y 1 % de ácido sulfúrico. El bagazo de caña de azúcar y el raquis de palma africana fueron las biomásas de las que se obtuvo el mayor contenido de glucosa 9.936,48 y 7.745,14 mg L⁻¹, respectivamente. Se discute la influencia de la composición de la biomasa en la cantidad de glucosa obtenida.

Palabras clave: Hidrólisis ácida, biomasa, glucosa, caracterización estructural.

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1 Introduction

The change in energy sources is a real challenge. Aiming for oil consumption reduction, the search for more sustainable materials with less environmental impact during their life cycle leads to selecting renewable raw materials or improving the processes used to obtain the final products. The use of lignocellulosic biomass, mainly derived from agricultural residues, has increased in recent years because these residues are renewable compared to the raw material of fossil origin (Thompson et al., 2017; Yáñez-Iñiguez et al., 2020). However, the main restraint to its use is the lack of low-cost technology that can degrade the polysaccharides present in biomass. Thus, the lignocellulosic material can be converted into soluble sugars (Adsul et al., 2020; Loow et al., 2015; Faba et al., 2014). In Ecuador, there is a wide source of renewable raw material derived from the main permanent crops in this country, as indicated by the data presented in the 2014 Bioenergetic Atlas (Ministerio de Electricidad y Energía Renovable del Ecuador, 2014), and the main four are summarized in Table 1.

Table 1. Waste generated by permanent crops in Ecuador.

Crop	Waste Tn/Year
African palm	6,872,469.27
Banana	4,926,095.60
Cocoa	2,015,352.60
Sugarcane	793,283.38

The waste indicated in Table 1 can be from the field or processing, and it is possible to obtain second generation biofuels that do not involve a risk for food sources.

The lignocellulosic material consists of cellulose, hemicellulose, and lignin. These polymers contain many active functional groups capable of reacting (Odalanowska et al., 2021; Yogalakshmi et al., 2022). Cellulose polymers are glycoside units connected through β -1,4-glycosidic bonds, which are very stable and difficult to hydrolyze by the chemical or enzymatic route. Hemicellulose hydrolyzes more easily than cellulose and produces valuable pentoses, such as xylose and arabinose. Lignin has a very complex polymeric structure (Sun et al., 2016; Okolie et al., 2021). One of the leading and most

common technologies for biomass treatment is acid hydrolysis; this method is preferred due to its low cost and effectiveness in the fractioning of hemicelluloses into monomeric while causing structural changes in the fractions (Loow et al., 2016). In general, sulfuric, hydrochloric, and nitric acids are used, although the last two are to a lesser extent. Treatments with high acid concentrations generate corrosivity problems, and their handling is dangerous. Hence, treatment with diluted acid is more applied (Hoang et al., 2021; Yuan et al., 2022). At an industrial level, biomass hydrolysis is a fundamental stage in obtaining second-generation biofuels, such as the specific case of bioethanol; therefore, the yield improvement towards monosaccharides, especially glucose will positively influence the subsequent manufacturing processes (Duque et al., 2021).

Glucose is the most abundant platform molecule of biomass in nature. Since it is the monomeric unit of the abundant biopolymer cellulose, it is considered a sustainable feedstock for producing carbon-based transportation fuels, chemicals, and polymers (Dutta and Bhat, 2022). Its water-soluble property makes it a promising alternative to produce valuable chemicals and biofuels efficiently. The C6 products obtained from glucose are suitable because their syntheses have a 100% carbon economy (Liu et al., 2015).

Table 2 shows some results of different investigations, verifying that diluted sulfuric acid is the most used; however, the factors that intervene in the reaction of both temperature and time are high, which entails higher energy consumption.

The aim of this research is to take advantage of the residual biomass obtained in large quantities in Ecuador, mainly generated from banana, cocoa, African palm, and sugar cane crops, and to provide alternatives for their use in the production of biofuels or other derived products, which will contribute positively to the energy industry development in the country so that it is economically, technically, and environmentally sustainable. For this, the dilute acid hydrolysis process is studied, through an experimental design that optimizes the values of temperature, time, and acid concentration to maximize glucose conversion. Besides, the interactions between factors are statistically analyzed to determine their influence, which also favors

decision-making regarding reaction conditions. In addition, each biomass is structurally characterized to obtain a database that allows knowing its com-

position and possible applications in industry, as well as its effect on the conversion to glucose.

Table 2. Results obtained from biomass hydrolysis processes

Biomass	Acid	Temperature (°C)	Time (min)	Concentration (%)	Glucose (g·L ⁻¹)	Bibliographic source
Sugarcane bagasse	Sulfuric acid	90	60	0.5	0.65	(Roslan and Salimi, 2020)
Sugarcane bagasse	Sulfuric acid	122	24	1	3	(Dussan et al., 2014)
African palm rachis	Sulfuric acid	210	5	0.8	6.27	(Millati et al., 2011)
African palm rachis	Sulfuric acid	100	240	6	1.43	(Amirkhani et al., 2015)
Banana rachis	Sulfuric acid	170	120	5	0.3	(El-Zawawy et al., 2011)
Banana rachis	Hydrochloric acid	150	280	3	0.4*	(Sathendra et al., 2019)
Cocoa pod	Sulfuric acid	90	240	1M	1.7	(Samah et al., 2011)
Cocoa pod	Hydrochloric acid	20	441.6	8.36	4.09*	(Shet et al., 2018)

* Reduced sugars.

2 Materials and Methods

2.1 Reagents

Analytical grade sulfuric acid was purchased from Fisher Chemical (Center Valley, PA, USA). Analytical standard glucose and sodium chlorite were purchased from Sigma-Aldrich (St. Louis, MO, USA). Analytical grade ethanol, toluene, glacial acetic acid, and sodium hydroxide were purchased from Merck (Merck Darmstadt, Germany). Distilled water was obtained from a Water still WS 8000 (Boeco, Germany). Water type I was obtained from a Barnstead Water Purification System (Thermo Fisher Scientific, Kansas, USA).

2.2 Plant Material and Sample Preparation

The biomass from banana, cacao and oil palm plants were obtained from the crops of "Hacienda San José" located in Los Ríos province – Ecuador, km 8.5 Babahoyo - Febres Cordero at coordinates 1°53'15.7"S, 79°29'18.8"W. The banana variety used was *Musa Acuminata*, Cavendish subgroup of the AAA Group, Cocoa variety was CC N51, and African palm family Arecaceae, Liliopsida subgroup *Commelinidae*.

The sugarcane bagasse was obtained from a farm in the Yunguilla Valley, southwest of Cuenca at coordinates 3°17'58.843"S, 79°17'59.497"W, the variety was Canalpoa. The plants were cut down to collect the fruit bunch during harvest. The harvest area was delimited, and the residues were collected from twelve banana plants randomly distributed within the land area. Each plant was separated into its different parts: rachis banana, cocoa pod, rachis African palm and sugarcane bagasse according to their different physical-chemical characteristics; therefore, different monosaccharides concentrations were expected. Samples of approximately 300 g were taken from each part and were stored in hermetically sealed plastic bags for transportation to the laboratory, where they were cleaned up to remove any chemical residue (e.g., pesticides or insecticides) using pure water. The samples were then cut into sections of approximately 5 mm and dried in the oven (brand Innotech, model BJPX-Summer) at 105 °C until constant weight for approximately 24 hours. Then they were ground using a cutting mill (ALQUIMIA, model MC002) and sieved with a mesh, obtaining a particle size of maximum 5 μm. The prepared samples were stored in plastic bags at 2 °C until analysis.

2.3 Structural Characterization

First, the extractive and non-structural material from the biomass are removed to avoid interference with subsequent analytical steps (National Renewable Energy Laboratory, 2008). The extractives were eliminated according to the ASTM D1107 standard, using ethanol-toluene as solvent and the Soxhlet extraction equipment, the extraction thimble was placed with 7 g of sample and the flask was filled with 150 mL of solvent, the liquid was kept boiling for 7 hours, to later dry the sample to constant weight (ASTM International, 2021).

Once the extractible had been removed, the acid-insoluble lignin content was determined according to the TAPPI 222 standard (TAPPI, 2006). 1 g of the extract-free sample was used and 15 mL of 72 % sulfuric acid was gradually added. The carbohydrates were hydrolyzed and solubilized by sulfuric acid and the insoluble lignin is filtered, dried, and weighed.

To determine the percentage of holocellulose (cellulose and hemicellulose), the ASTM D 1104 standard was applied, which is obtained through the chlorination process eliminating the lignin, 2.5 g of extractive-free sample was used, 80 mL of hot distilled water, followed by 0.5 mL of acetic acid and 1 g of sodium chlorite, this addition was made 4 times (ASTM International, 1956). Cellulose was obtained from the holocellulose fraction, using the TAPPI 212 standard, which consists of treating 2 g of extractive-free holocellulose with 10 mL of 17.5 % sodium hydroxide for the degradation of hemicellulose and its content was determined from the difference between holocellulose and cellulose (TAPPI, 2002).

2.4 Experimental Design for Acid Hydrolysis Reaction

Acid hydrolysis was carried out in a batch reactor (THR 250 high pressure) with a 50 mL capacity, equipped with a pressure gage, temperature sensor plugs and mechanical stirring system. The material of construction is SS304L with Teflon coated to reduce corrosion from acid attack.

Monosaccharides were obtained from 3 g of biomass using 60 mL of acid aqueous solution (v/v).

To study the influence of factors acid concentration, temperature, and reaction time on the amount of recovered glucose in the reaction, a 2^k factorial design was applied for each of the biomasses. Table 3 shows the three-factor design with two levels per factor applied to each biomass. Two replicates were made to the center. The factors: temperature, reaction time and acid concentration were tested in intervals of 70–120 °C, 20–150 min and 1–5 % v/v H_2SO_4 , respectively. These conditions were applied for different biomasses (banana rachis, cocoa pod, african palm rachis and sugarcane bagasse) and the obtained solutions were filtered with Whatman N°54 paper and neutralized with 30 % (w/v) sodium hydroxide solution. After that, the samples were immediately analyzed in HPLC.

2.5 Chromatographic Analysis

Glucose concentration of the hydrolyzed plants residues was analyzed in an HPLC system (Jasco LC4000) equipped with a thermostat (Jasco CO4061), a refractive index detector (IR4030), a quaternary pump (PU4180) and a manual injector. The injection volume was 10 μ L. The column used was VA 300 / 7.8 Nucleogel, Sugar 810 Ca, (300 \times 7.8 mm) (Macherey-Nagel Germany). Column temperature, flow rate and concentration of sulphuric acid (mobile phase) were 85 °C, 0.5 mL/min and 0.01 % v/v sulphuric acid, respectively. Quantification was carried out using calibration curves of mix solutions of the tested monosaccharides in concentrations from 500 to 5,000 mg/L. Operation of the instrument and data processing were implemented using ChromNAV Ver.2.1.0 (Jasco Corporation, Tokyo, Japan).

2.6 Statistical Analysis

To determine the effects and significant interactions, the normal effects plot was used, with the effects found outside the line being considered significant. The individual and interaction effects were determined graphically. To obtain the linear models of each biomass, the assumptions of homoscedasticity and normality were verified through the normal quantile-quantile graphs, boxplot and the Shapiro-Wilk and Levene tests, results not shown; in all cases the assumptions were fulfilled. To determine the difference in means between biomasses, one-way ANOVA was applied. When significant differences

were obtained with a significance level of 0.05, a post hoc test of mean difference was applied using the Bonferroni correction. The error was found through the replicas to the center. The R program version 4.0.4 with the R-studio interface was used for all analysis.

3 Results and Discussion

3.1 Structural Characterization of Biomass

Lignocellulosic material is mainly composed of cellulose, hemicellulose, lignin, ash, and extractable soluble in different solvents and impurities. For this study, the most relevant components in obtaining monosaccharides have been determined. Table 3 shows the results obtained for α -cellulose, hemicellulose, insoluble lignin and ethanol-toluene extractables for each type of biomass. All these analyses were made in triplicate.

As seen in Table 3, when structurally characterizing the biomass, a higher α -cellulose content was obtained in African palm with 48.11 %, followed by sugarcane bagasse with 46.19 %, and banana rachis with 31.40 %; these results are similar to those obtained by Kumneadklang et al. (Kumneadklang et al., 2019) who obtained 42.67 % for African palm while the study presented by Oliveira et al. (Oliveira et al., 2007) for banana rachis, it reaches 28.4 %. For hemicellulose, bagasse predominates with 28.64 %, a value similar to that presented in the Moraes Rocha investigations of 27.6 % (Dussan et al., 2014). The highest lignin content is presented by the cocoa pod, with 23.44 %, found within the range of 14–28 %, established in the studies of Lu et al. (Lu et al., 2018), the rachis of the banana has the minimum value of 7.67 %, compared to 9.6 % presented in the Oliveira studies. These percentages reflect that each biomass has a predominance in different components, which will influence the process towards glucose. Those biomasses with high contents of cellulose and hemicellulose will have a better yield in the conversion to monosaccharides.

Table 3. Structural characterization of banana rachis, African palm rachis, cocoa pod and sugarcane bagasse.

Biomass	Holocellulose (%)	α -Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ext. Eth-Tol (%)
Banana rachis	55.62	31.40 \pm 0.519	24.23 \pm 0.519	7.67 \pm 0.001	3.49 \pm 0.003
African palm rachis	71.00	48.11 \pm 0.449	22.89 \pm 0.449	14.97 \pm 0.003	3.85 \pm 0.004
Cocoa pod	40.90	25.54 \pm 0.291	15.36 \pm 0.291	23.44 \pm 0.003	2.53 \pm 0.004
Sugarcane bagasse	74.84	46.19 \pm 0.290	28.64 \pm 0.290	18.33 \pm 0.007	5.71 \pm 0.004

3.2 Acid Hydrolysis

In the results in Table 4, the highest amount of glucose was obtained with the sugarcane bagasse and the lowest with the banana rachis. Roslan et al. (Roslan and Salimi, 2020) used sugarcane bagasse biomass at a concentration of 1 % sulfuric acid, temperature of 122 °C for 24 minutes of reaction, obtaining a yield of 3 g L⁻¹ of glucose. Dussan et al. (Dussan et al., 2014) also experiment with sugarcane bagasse, under reaction conditions of 90 °C, 60 min and a sulfuric acid concentration of 0.5 %, obtaining a maximum total sugar yield of 0.65 g L⁻¹. The reaction conditions of those investigations with the present study are similar in temperature and concentra-

tion; however, the time variable is greater, being 150 minutes, which may influence the increase in yield obtained in this work. This amount of glucose could be used to obtain various value-added chemicals and biofuels; for instance: alcohols, gluconic acid, 5-hydroxymethylfurfural (HMF), lactic acid, pentanoic acid esters, 2,5-dimethylfuran (2,5-DMF). HMF can be converted into industrially useful chemicals such as furan and its derivatives, levulinic acid and formic acid, which are currently produced from petroleum resources (Liu et al., 2015; Bali et al., 2012).

The analysis of the interaction of the variables is described in Section 3.3.

Table 4. Factorial design 2^3 with two replicas at the center.

Labels	Factors			Glucose (mg/L)			
	a Acid concentration	b Temperature	c Time	Sugarcane bagasse	Banana rachis	Cocoa pod	African palm rachis
(1)	-1	-1	-1	3,800.90	498.19	2,114.87	2,476.84
a	1	-1	-1	3,145.30	424.85	1,472.60	1,017.43
b	-1	1	-1	4,641.81	1,001.65	2,680.75	6,297.29
ab	1	1	-1	5,942.57	1,787.32	1,526.06	6,542.49
c	-1	-1	1	4,355.34	541.77	880.11	2,657.56
ac	1	-1	1	6,417.91	451.76	849.67	3,089.24
bc	-1	1	1	9,936.48	1,670.22	2,719.70	7,745.14
abc	1	1	1	1,800.71	1,552.54	1,701.08	1,427.80
Rep1	0	0	0	7,118.53	1,220.17	1,615.34	5,888.72
Rep2	0	0	0	7,375.05	1,297.92	1,452.47	5,554.95

Comparing the glucose results obtained with the structural characterization of each biomass, both the sugarcane bagasse and the African palm present the highest content of holocellulose, reflecting in a higher conversion to glucose. However, this is not replicated in the case of banana rachis and cocoa pod, since even though the cocoa pod has a higher content of holocellulose, its conversion to glucose is lower. It may be due to different factors in the composition of these biomasses, so additional studies must be carried out to identify these factors.

Nowadays, different pretreatment processes are investigated to enhance the yield with acid hydrolysis; using ionic liquids is among the most promising, since it has proven to facilitate biopolymers decomposition due to removing the structural protection of the hydrolysis centers. The second part of this investigation will consider using these liquids to improve yields and reduce energy requirements.

3.3 Statistical Analysis

In the results in Table 5, the highest amount of glucose was obtained with sugarcane bagasse and the lowest with banana rachis. There are statistically significant differences between the amount of glucose obtained by each biomass $p(2.47e-06) < 0.001$. The differences are found between the glucose value obtained between the sugarcane bagasse and the banana rachis and between the cocoa pod and the sugarcane bagasse with $p(0.000) < 0.001$ in both cases. Also, significant differences are found bet-

ween the African palm rachis and the banana rachis and between the African palm rachis and the cocoa pod with $p(0.001) < 0.01$ and with $p(0.013) < 0.05$, respectively.

Table 5. One-way ANOVA and post hoc test

Biomass	Mean (DE)
Sugarcane bagasse	5,453.46 (2,380.42) ^{a, b}
Banana rachis	1,044.64 (535.91) ^{a, c}
Cocoa Pod	1,701.27 (642.86) ^{b, d}
African palm rachis	4,269.75 (2,391.86) ^{c, d}

For sugarcane bagasse, Figure 1a, only the time factor is statistically significant, and no interaction was significant. Analyzing the effects, glucose obtention is favored with the high levels of temperature and time factors and with the lowest level of acid concentration (see S1).

About double interactions, these were observed between concentration and temperature, where the highest glucose obtained was at low acid concentration and high temperature. Interaction between time and concentration was also found, where the same previous case occurred. The graphs of double interaction can be seen in S2. Acid concentration and the triple interaction have negative coefficients (see Table 6). As seen, the concentration at a high level of the acid reduces the yield and this is increased at higher temperature and/or reaction time.

Table 6. Model coefficients.

Coefficients	Sugarcane bagasse	Banana rachis	Cocoa pod	African palm rachis
Intercept	3,800.9**	498.19*	2,114.9**	2,476.8*
Concentration	-655.6	-73.34	-642.3	-1,459.4
Time	554.4	43.58	-1,234.8*	180.7
Temperature	840.9	503.46*	565.9	3,820.4*
Concentration:Time	2,718.2*	-16.67	611.8	1,891.1
Concentration:Temperature	1,956.4	859.01*	-512.4	1,704.6
Time:Temperature	4,740.2**	624.99	1,273.7	1,267.1
Concentration:Time:Temperature	-12,154.7**	-886.68	-475.8	-8,453.6*
Residual standard error	181.4	54.98	115.2	236

Significant: * $p < 0.1$ and ** $p < 0.05$

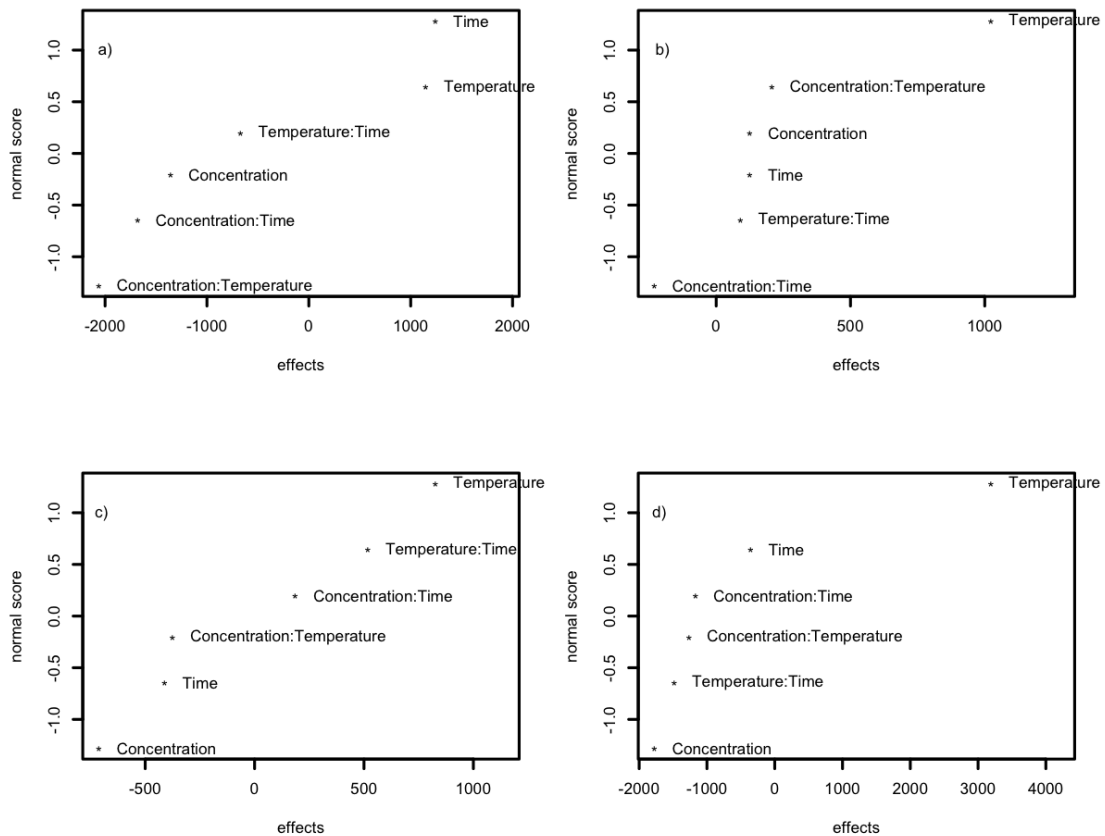


Figure 1. Analysis of interactions a) sugarcane, b) banana, c) cocoa and d) palm.

For banana rachis, Figure 1b, only the temperature factor was significant. Analyzing the effects, obtaining glucose is favored with high levels of the three factors, mainly with the temperature factor, since the other two levels do not vary too much their response between levels (see S1). Weak interactions between concentration-time and between temperature-time are found in both cases.

The best results are obtained with high temperature, and the levels of the other factors do not have significant influence. The acid concentration, the concentration-time interaction and the triple interaction have negative coefficients (see Table 6).

For the cocoa pod, in Figure 1c, neither the factors nor any of the interactions were significant. The simple effects show that obtaining glucose is favored by the high level of the temperature factor and with the low levels of the factor acid concentration and time (see S1). Double interactions were observed between time and temperature, where the highest glucose obtained was at the high temperature level, with no difference in time (see S2). Finally, the acid concentration, the temperature, the concentration-temperature interaction, and the triple interaction have negative coefficients (see Table 6).

In Figure 1d, for the rachis of African palm, only the temperature factor is significant. The simple effects show that obtaining glucose is favored with the high level of the temperature factor and with the low levels of acid concentration and time factors (see S1); being time the factor with the least influence. There is a temperature-time interaction, where the high-temperature factor is the one that produces more glucose. The acid concentration and the triple interaction show negative coefficients (see Table 6).

4 Conclusions

From the results obtained, it can be concluded that, in the case of cocoa pod, African palm rachis, and sugarcane bagasse, a temperature of 120 °C, a reaction time of 150 min and a concentration of 1% sulfuric acid were the conditions that presented the best reaction yields. At these conditions, for the banana rachis a value of 1,670.22 mg L⁻¹ of glucose was obtained, while the best performan-

ce was given at a temperature of 120 °C, reaction time of 20 minutes, and acid concentration of 5%, obtaining a value of 1,787.32 mg L⁻¹, the difference being 117.1 mg L⁻¹. In addition, it is shown that the structural composition of each type of biomass influences the obtaining of glucose; in this case, the bagasse being sugarcane and African palm rachis were those with the highest content of cellulose and hemicellulose.

In the statistical analysis for sugarcane bagasse only, the time factor is significant, while the high-level concentration of the acid impairs performance. For the case of the banana rachis and the African palm rachis, the temperature factor was significant. For the cocoa pod, neither the factors nor any of the interactions were significant.

Finally, the objective of this research was fulfilled, obtaining high yields of glucose conversion that allowed improving the subsequent biofuel manufacturing processes. A technical and economic feasibility study is recommended to achieve a balance between the amount of glucose obtained and manufacturing costs so that the process is profitable and can be applied on a large scale.

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Author’s contribution

M.A.G.: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **A.V.S.:** Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **V.P.V.:** Conceptualization, Formal analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing.

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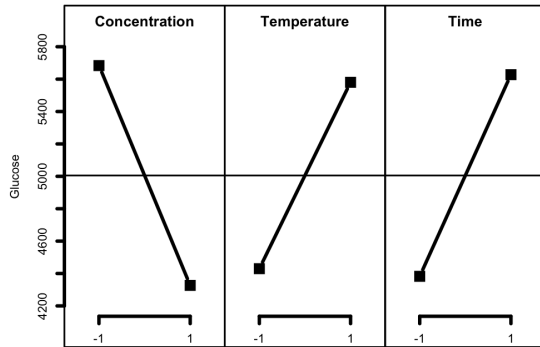
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Appendix

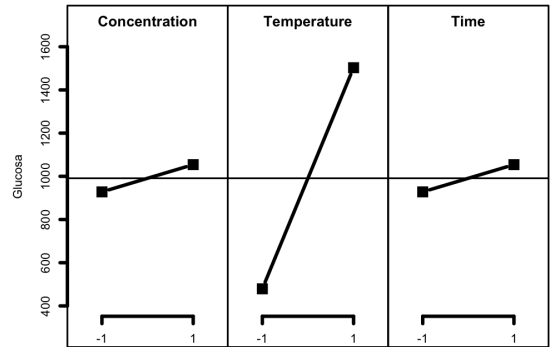
S1. Main effects: a) sugarcane bagasse, b) banana rachis, c) cocoa pod y d) African palm.

Main effects plot for Glucose



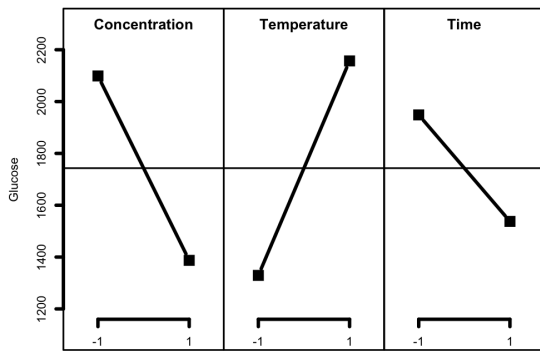
[a]

Main effects plot for Glucosa



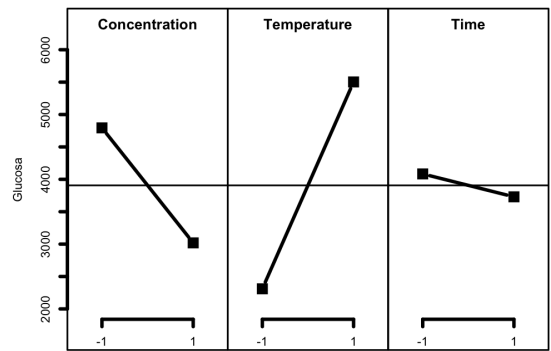
[b]

Main effects plot for Glucose



[c]

Main effects plot for Glucosa



[d]

S2. Interaction effects: a) sugarcane bagasse, b) banana rachis, c) cocoa pod y d) African palm.

