

Article

Kinetic Study of Acid Hydrolysis of the Glucose Obtained from Banana Plant

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Abstract: The biomass of crops in rotation, such as that generated by the banana plant, is an interesting source of lignocellulose due to its composition and availability. This research aimed to compare the amount of glucose obtained from different parts of the banana plant (leaves, rachis, and pseudostem) by hydrolysis with sulfuric acid at 100 °C. This reaction was analyzed to determine the amount of water and reagents consumed versus the glucose obtained. The optimal time and acid concentration were studied between 0–30 min and 3–5% *v/v*, respectively. The best results were obtained with the pseudostem of 13.02 gL⁻¹ of glucose in a reaction time of 20 min and an acid concentration of 5%. In addition, the kinetic study of hydrolysis was carried out. The adjustment to the Saeman model was R² 0.96, which represents a first-order reaction and kinetic constants K₁ = 0.5 and K₂ = 0.3 min⁻¹. This study has shown that these residues can be used as raw materials to generate value-added products due to their high glucose content.

Keywords: biomass-banana-plant; kinetic; hydrolysis; glucose



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1. Introduction

Bananas are a tropical, herbaceous, and perennial crop of the Musaceae family; each banana plant is harvested once during its lifetime. Therefore, a large amount of plant residues are left on the ground after cropping [1,2]. Bananas are among the most produced, marketed, and consumed fruits worldwide, with over 1000 varieties. The most commercialized is the Cavendish banana, which represents approximately half of the world's production, with an estimated annual volume of 50 million tons. Major exporting countries include Ecuador and the Philippines, Ecuador being the largest exporter, according to FAO's 2021 preliminary report. The harvested area of bananas in Ecuador was 160.6 thousand hectares, with an approximate annual production of 6.0 million tons in 2020 [3]; 92% of production was concentrated in the provinces of Los Ríos (41%), Guayas (26%) and El Oro (25%) [4].

In Ecuador, around 5 million tons of waste are generated annually, consisting of leaves, rachis, pseudostem, and product rejections [5]. This is used at the community level for various purposes. Terminal inflorescence is used as a salad or can be cooked, pseudostem is a source of starch, traditional medicine benefits, and other potential uses are animal feed and handicrafts. Residues left on the soil contribute to maintaining humidity and adding nutrients. Nevertheless, they also constitute a potential risk for disease dissemination, besides generating greenhouse gasses (i.e., methane) during decomposition. Figure 1 shows the different parts of the plant considered for this study.

Banana biomass is an important source of organic matter that can be used in various ways: (1) Banana biomass can be used as raw material to produce compost, enriching the soil with nutrients and improving its quality; (2) Non-commercial banana leaves, stems, and fruits can be used as animal feed, especially for livestock and poultry; (3) Banana

biomass can also be a renewable energy source. It can be converted into biogas through anaerobic digestion, generating electricity and heat [6]; (4) The fibers of the banana biomass can be used to produce paper since they have properties similar to wood pulp; (5) Banana biomass fibers can be turned into strong and durable yarns and fabrics [6,7]. Typically, banana biomass is used as compost. However, allowing large amounts of biomass to rot in the ground to obtain compost can have several disadvantages, such as soil nitrogen depletion, because biomass can consume a lot of nitrogen in the decomposition process. This can reduce the amount of available nitrogen in the soil, negatively affecting plant growth; biomass can generate bad odors during decomposition, unpleasant for people in the area; biomass can attract pests and create diseases that can affect nearby plants; nutrient loss occurs if biomass rots in the soil because without being properly processed there may be a loss of valuable nutrients that could be used to improve soil quality, and groundwater contamination occurs if large amounts of biomass are added to the soil near aquifers and groundwater sources [8,9]. It is important to analyze the components of this biomass to generate products of higher value and, in turn, combat the negative effects of leaving this residue on the ground.

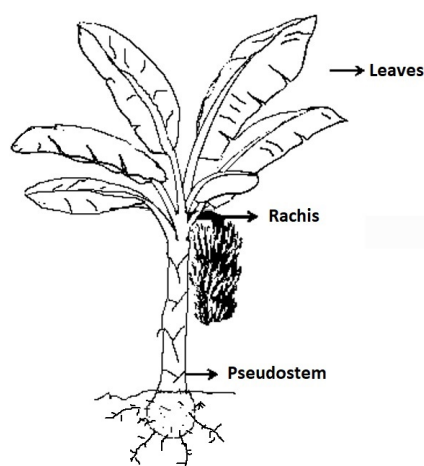


Figure 1. Parts of the banana plant.

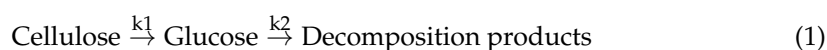
The residues contain a high content of cellulose, hemicellulose, and lignin that various processes can modify, such as bacterial fermentation and anaerobic degradation, and to obtain bioplastics, organic fertilizers, and biofuels, such as ethanol, biogas, hydrogen, and biodiesel [8]. In general, lignocellulosic biomass consists mainly of a large fraction of cellulose (40–50%), followed by hemicellulose (25–30%) and lignin (15–25%). Other minor components are represented by pectin, proteins, extractables, and ashes. Cellulose is the main structural component of plants. The degree of cellulose polymerization varies among plant species, ranging from 500 to 15,000 glucose units, the latter being the most favorable substrate for biofuel production. The cellulosic chains are bound together by intra- and intermolecular hydrogen bonds [10,11]. Hemicellulose is a heteropolysaccharide whose role is to form a flexible adhesive between lignin and cellulose and between individual cellulose fibrils. It comprises polymers of different monomeric sugar units such as glucose, mannose, galactose, xylose, arabinose, O-methyl-glucuronic acid, and galacturonic acid. The hemicellulose polymers are often interconnected by covalent and hydrogen bonds. In addition, hemicellulose is hygroscopic and hydrophilic. Lignin, conversely, is a complex structure with a high molecular weight that contains cross-linked polymers of phenolic molecules. Due to its strength and flexibility, it can adapt to compressive forces and protect the plant against microbial invasion; however, this property also complicates the transformation process of lignocellulosic biomass into biofuels. Biomass with high cellulose and low lignin content is preferred for these processes [12,13].

Biomass is currently the most widespread form of renewable energy; its exploitation is increasing due to concerns about the devastating impacts of fossil fuel consumption, climate change, and global warming [14]. Using lignocellulosic biomass to produce value-added chemicals essentially involves two steps: hydrolysis of the hemicellulosic and cellulosic biomass fractions, followed by fermentation of the reducible sugars in the hydrolysate. Commonly applied hydrolysis processes are acidic, alkaline, and enzymatic. However, one of the leading and most common technologies for biomass pre-treatment is acid hydrolysis due to its low cost and high effectiveness. This chemical technique breaks the lignocellulosic matrix by cleaving the glycosidic bonds. This process primarily solubilizes the hemicellulosic portion of biomass but also solubilizes part of the lignin, making cellulose more open to enzymatic attack [15,16]. The most used acids are sulfuric, hydrochloric, and nitric, although the latter two to a lesser extent. Treatments with high acid concentrations generate corrosivity problems, and their manipulation is dangerous. Therefore dilute acid treatment is applied more due to lower acid consumption, and, in general, the obtained hydrolyzate can be used directly after neutralization [17,18]. Glucose, as a building unit of cellulose, is the most widely used monosaccharide with wide applications in the pharmaceutical and food industries. Glucose conversions to various value-added chemicals and biofuels have been studied extensively, such as the production of alcohols, gluconic acid, 5-hydroxymethylfurfural (HMF), lactic acid, pentanoic acid esters, and 2,5-dimethylfuran (2,5-DMF). HMF can be converted into industrially important chemicals, such as furan and its derivatives, levulinic acid, and formic acid, currently produced from petroleum resources [19]. Table 1 shows the results obtained from different hydrolysis processes using biomass from the banana plant and cane bagasse currently used for bioethanol production.

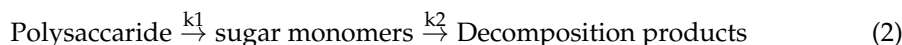
Table 1. Results of acid hydrolysis with sulfuric acid of sugarcane bagasse or biomass of the banana plant.

Biomass	Hydrolysis Process Conditions [Temperature, Time and H ₂ SO ₄ Concentration]	Monosaccharide Performance, gL ⁻¹	Reference
Sugarcane bagasse	90 °C, 3 h, 4% w/v	Fermentable sugars = 10.26	[20]
Sugarcane bagasse	130 °C, 0.25 h, 2% v/v	Xylose = 9.26	[21]
Sugarcane bagasse	90 °C, 1 h, 3% v/v	Fermentable sugars = 0.32–5	[22]
Banana stem	90 °C, 1.5 h, 0.5 M	Glucose = 5.61	[23]
Banana pseudostem	177 °C, 5 min, 2.2%	Glucose = 38.90	[24]
Banana rachis	198 °C, 5 min, 1.5%	Glucose = 3.40	[24]

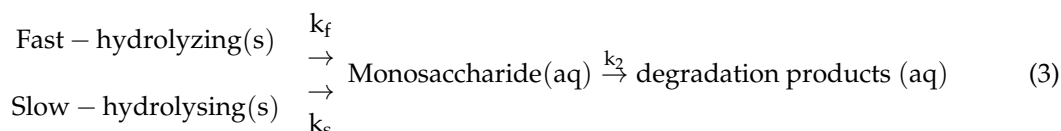
The hydrolysis reaction rate for monosaccharide production depends on certain variables: temperature, reaction time, acid concentration, substrate concentration, and substrate composition, in addition to physical factors, such as porosity or surface area of the biomass, the crystallinity of the cellulosic fraction, and the hemicellulosic and lignin content of the biomass [25]. The objective of the study of reaction kinetics is, firstly, to optimize the process and, secondly, to obtain equations to predict results and economic estimates. In general, for batch hydrolysis, the kinetic models reported can be summarized into three types: the Saeman model, the two-fraction (biphasic) model, and the “degree of potential hydrolysis” model [26]. The first empirical model for dilute acid-catalyzed saccharification of wood was proposed by Saeman [27], which comprised two consecutive first-order reactions with kinetic constants of similar Equation (1) [21,28].



This model can be generalized for polysaccharides in biomass, as presented in Equation (2).



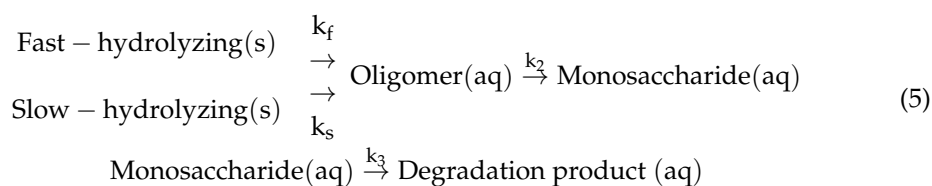
The biphasic hydrolysis model is divided into fast-reacting and slow-reacting parts. This model is shown in Equation (3) [29].



The monosaccharide concentration (M) by this method can be determined with Equation (4). Where P_0 , k_1 , k_2 and α represent the initial concentration of polysaccharide, kinetic constant, and the ratio between the fast and slow fraction of the reaction, respectively.

$$M = \alpha P_0 \left[\frac{k_1}{k_2 - k_1} \right] \left(e^{-k_1 t} - e^{-k_2 t} \right) \quad (4)$$

The “potential hydrolysis degree” model considers the effect of the multilayered structure of the plant cell wall and assumes that total polysaccharides in cell wall layers are not reactive enough to hydrolyze. This model is shown in Equation (5) [26].



In recent years, several studies on the kinetics of the biomass hydrolysis reaction have been carried out. Among them, Gómora-Hernández et al., who analyzed acid hydrolysis with corn cobs and applied the Saeman model, obtained an adjustment of 0.99 [30]. Additionally, Uzoh et al. applied the Saeman and biphasic models to hydrolyze the corn cob, obtaining a fit equal to 1 in both cases [31]. Mensah et al. performed a kinetic analysis of the hydrolysis of cocoa pod shell residues to reducing sugars by applying the Saeman model; in this case, the highest value of R^2 reached was 0.9878 [32].

The objective of this study is to obtain glucose from different residues of the banana plant through the application of one of the most used processes at an industrial level, such as acid hydrolysis; a material balance will be carried out that allows comparing the resources used in the reaction with the results achieved. The glucose obtained from this process can be used as a raw material for the elaboration of value-added products, thus providing options for using banana biomass generated in large quantities in the country that does not have a specific use. In addition, a kinetic study of the reaction will be carried out to determine the reaction parameters, thus improving existing processes and increasing obtaining monosaccharides. The Saeman model will be applied because it is the most widely used in acid hydrolysis reactions, the simplest, and because it presents similar results to the other models mentioned in this study.

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, Lenesa, KS, USA).

2.2. Collection and Preparation of Biomass

The biomass from banana plant waste was collected from the Hacienda María Esperanza-Ginafruit located in the province of Guayas—Ecuador, km 31 Guayaquil—Puerto Inca at coordinates $2^{\circ}12'21.024''$ S, $79^{\circ}54'28.619''$ W (see Figure 2). For this study, the Musa AAA Cavendish banana variety was selected. The sampling area was delimited, and 12 plants in the harvest stage were randomly selected; these were cut to collect the bunch of fruits. Each plant was separated into its different parts: leaves, rachis, and pseudostem. Samples of approximately 300 g were taken from each part and stored in hermetically sealed plastic bags for transport. Once in the laboratory, the samples were cut into sections of approximately 5 mm, then subjected to a drying process on the stove (brand Innotech, model BJPX-Summer) at 105°C for 24 h, ground in a cutting mill (ALQUIMIA, model MC002), and sieved with mesh No. 35. The samples obtained were kept under refrigeration until analysis.



Figure 2. Sampling Farm Location.

2.3. Structural Characterization

The cellulose, hemicellulose, and lignin content of each part of the banana plant were determined by removing the extractives from the biomass, according to the TAPPI T-204 cm-97 standard [33], using the Soxhlet equipment, for which 7 g of each sample were weighed. The toluene-ethanol mixture was used as a solvent (Merck Darmstadt, Germany) in a proportion of 427 mL of toluene per liter of ethanol, according to specifications of ASTM D 1107-21 [34], and the extraction time was 7 h.

For lignin quantification, the TAPPI T-222 om-98 standard [33], also known as the Klason method, was used; this is applied in the extractable-free sample. 1 g of the sample was used and treated with 72% sulfuric acid (Merck, Darmstadt, Germany) for 2 h under constant stirring, diluting the solution with up to 4% acid, and subjecting it to boiling for 4 h. Finally, the obtained solution was filtered, dried, and the insoluble lignin quantified.

To determine the percentage of cellulose and hemicellulose, the holocellulose was extracted using the ASTM D 1104 method [35], and using glacial acetic acid and sodium chlorite (Merck, Darmstadt, Germany) in different stages, as indicated by the standard. Once holocellulose is obtained, the TAPPI 212 standard [36] is applied to obtain cellulose through treatment with sodium hydroxide (Merck, Darmstadt, Germany). Hemicellulose is determined by the difference between holocellulose and cellulose.

The functional groups of lignocellulosic biomass were identified by Fourier Transform Infrared (FTIR) spectroscopy. The spectra offer qualitative and semi-quantitative information suggesting the presence and absence of lignocellulosic compounds. This analysis was performed on the Spectrometer NICOLET IS5 FTIR.

The calorific value of biomass was determined using the method described in the CEN/TS 14918 standard, using a bomb calorimeter. The calorimeter measures the heat released in the combustion of solid biofuel. The samples were burned in an oxygen atmosphere.

2.4. Acid Hydrolysis Process

An acid hydrolysis process was carried out to obtain glucose using 20 mL of sulfuric acid for each gram of biomass. This reaction was performed in a batch reactor (THR 250 high pressure) with a 250 mL capacity, equipped with a pressure gauge, temperature sensor plugs, and a mechanical stirring system. The reaction conditions were obtained employing a literature review, selecting the best results [37] at a constant temperature of 100 °C; acid concentration varied in a range of 3–10%, and the reaction time was 0–30 min. The solution obtained was filtered with Whatman paper N° 54 and neutralized with 30% sodium hydroxide *w/v*.

2.5. Chromatographic Analysis

Glucose of the hydrolyzed banana plant 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 µL. The column used was Nuclogel Sugar 810 Ca, (300 × 7.8 mm) (Macherey-Nagel, Germany). Column temperature, flow rate, and mobile phase were 85 °C, 0.5 mlmin⁻¹ and 0.01% *v/v* sulphuric acid, respectively. Operation of the instrument and data processing were implemented using ChromNAV Ver.2.1.0 (Jasco Corporation, Tokyo, Japan).

2.6. Kinetic Model Development

The simplest kinetic models assume hydrolysis occurs directly from polysaccharides to monosaccharides without forming any intermediate oligosaccharide [38]. For this work, Saeman's kinetic model was used, which proposes that biomass saccharification is a consecutive two-step process where the reaction proceeds so that cellulose is first hydrolyzed to produce glucose, which is broken down into degradation products. This reaction is considered serial and irreversible. The model exhibits first-order dependency upon the sugar concentrations.

The system of differential equations that results from the Saeman kinetic model is given by:

$$\frac{dC_C}{dt} = -k_1 C_C \quad (6)$$

$$\frac{dC_G}{dt} = k_1 C_C - k_2 C_G \quad (7)$$

$$\frac{dC_D}{dt} = k_2 C_G \quad (8)$$

where C_C , C_G , and C_D represent the time (t)-dependent concentrations of cellulose, glucose, and degradation products, respectively. The rate of cellulose scission is described by k_1 , and k_2 represents the rate of glucose degradation.

$$C_G = C_C^0 \frac{k_1}{k_2 - k_1} (e^{-k_1 t} - e^{-k_2 t}) \quad (9)$$

The rate constants k_1 and k_2 were determined by minimizing the sum of the squares of the difference between the experimental and calculated concentrations using Equation (9). The parameters were optimized by simulation using the Microsoft Excel Solver Tool.

2.7. Statistical Analysis

The non-parametric Kruskal Wallis statistical test was applied to determine the median differences in glucose production in different parts of the banana plant at a significance level of 5%. Once the alternative hypothesis was accepted, a post hoc test was applied. Statistical software R version 4.2.3 with R-studio interface was used for statistical analyses.

3. Results and Discussion

3.1. Biomass Characterization

The results obtained from the characterization are presented in Table 2. It is observed that the highest content of holocellulose is present in the pseudostem, 71.75%, which can benefit the obtaining of monosaccharides depending on the reaction conditions since the higher content of holocellulose may create greater degradation to fermentable sugars, such as glucose. The lignin content is also higher for the leaves, 19.84%, implying greater difficulty for the decomposition process by hydrolysis. Comparing this biomass with sugarcane bagasse, which is currently mostly used for producing bioethanol, the holocellulose content of bagasse must be 74.84% [39], a value similar to the pseudostem of the banana plant, which makes this biomass a potential raw material for obtaining chemicals of greater added value, such as biofuels. Additionally, it can be observed that the leaves have the highest calorific value compared to the pseudostem and rachis. Other authors in research with banana peel obtained a calorific value of 18,890 kJkg⁻¹. It should be noted that this type of waste has a calorific value higher than that of other biomasses such as rice husks, corn plant, and barley bagasse with values of 15,324 kJkg⁻¹, 10,790 kJkg⁻¹, and 14,270 kJkg⁻¹, respectively [40–42]. Due to its high production and calorific power, it is an interesting raw material to use in energy production.

Table 2. Structural characterization of biomass from the banana plant.

Biomass	Insoluble Lignin, %	Holocellulose, %	Alpha Cellulose %	Hemicellulose, %	Calorific Value, kJkg ⁻¹
Leaves	19.84 ± 0.13	66.64 ± 0.18	36.98 ± 0.39	29.66 ± 0.20	17,369.0 ± 65.05
Pseudostem	14.12 ± 0.10	71.75 ± 0.06	39.25 ± 0.05	32.496 ± 0.01	12,457.5 ± 71.42
Rachis	7.67 ± 0.01	55.63 ± 0.29	31.40 ± 0.52	24.23 ± 0.52	12,212.5 ± 6.36

Figure 3 shows the results obtained from the FTIR analysis for the three parts of the banana plant. A broad band at 3500–3000 cm⁻¹ is observed, indicating the presence of a hydroxyl group, the OH bond stretching vibration of cellulose, hemicellulose, and pectin [43]. The peak at 2900–2800 cm⁻¹, showed C-H stretching of lignocellulosic components. The presence of peaks at 1700–1500 cm⁻¹, indicated C=O carbonyl group of lignin and hemicellulose, C=C stretching vibration of the aromatic rings of lignin. In the range of 1500 to 1200 cm⁻¹, we can find several peaks which correspond to groups C-H, C-O and C-O-C characteristic in the plane of bending of cellulose I and cellulose II, stretching in lignin and xylan and vibrational stretching of B(1,4)-glycosidic bond ring for cellulose [43,44]. The absorption band of the carboxylic acid group at 1030 cm⁻¹, C-OH stretching vibration of the cellulose and hemicelluloses [45].

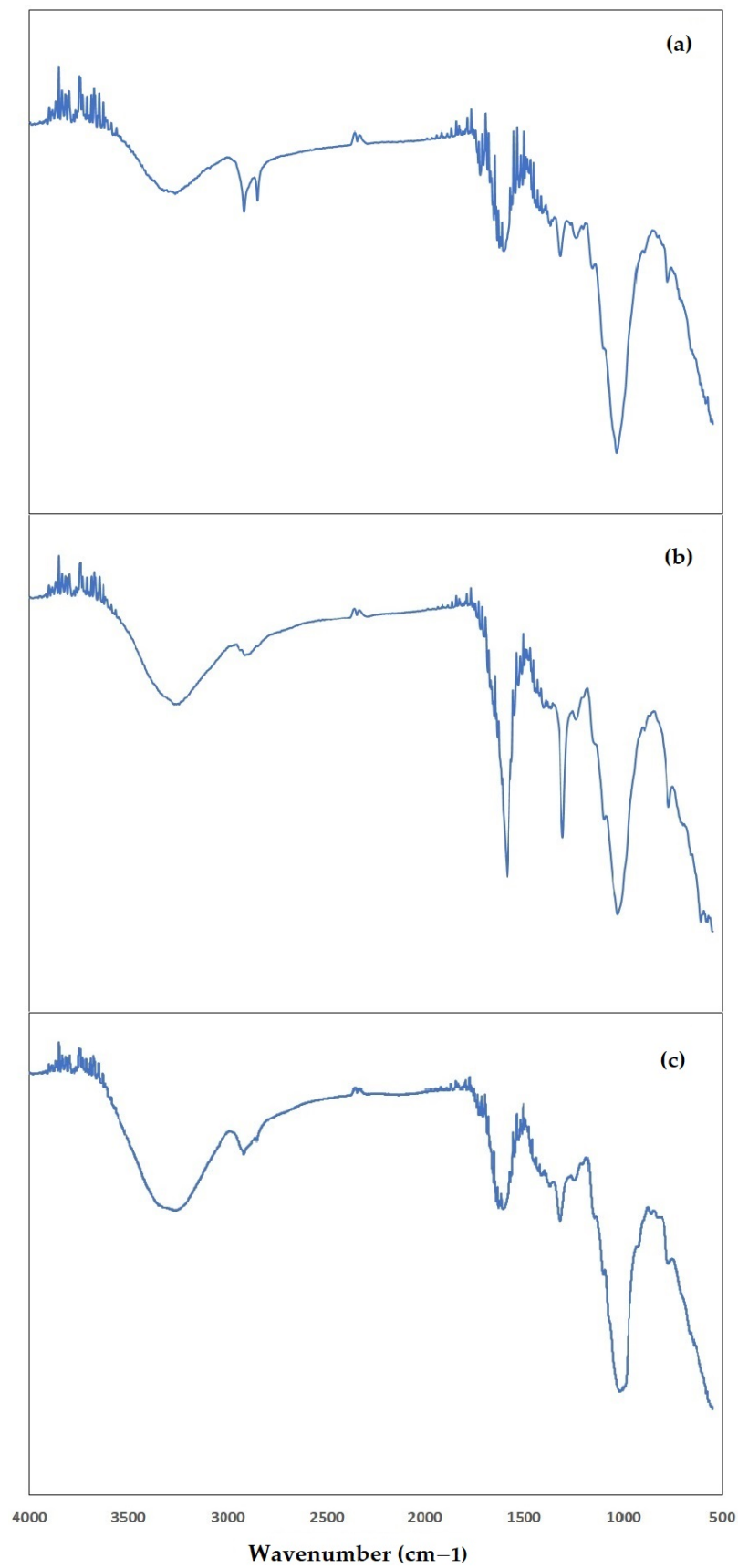


Figure 3. FTIR spectrum of biomass in (a) leaves, (b) rachis, and (c) pseudostem.

3.2. Acid Hydrolysis

After the characterization process, the acid hydrolysis reaction was performed on the three parts of the banana plant. This verified that the portion presenting the highest content of holocellulose was the same as that which showed a better glucose production yield of glucose with a value of 13.02 gL^{-1} , obtained in a concentration of 5% acid. In addition, the leaves that had a higher content of lignin generated the lowest glucose yield being 1.86 gL^{-1} . The results are shown in Figure 4. A Kruskal-Wallis statistical analysis determined that the glucose median concerning each type of banana plant residue presented significant differences with a p -value of 0.03, where the pseudostem generated the highest concentration of glucose, therefore, was chosen for the study of the kinetics of the acid hydrolysis reaction.

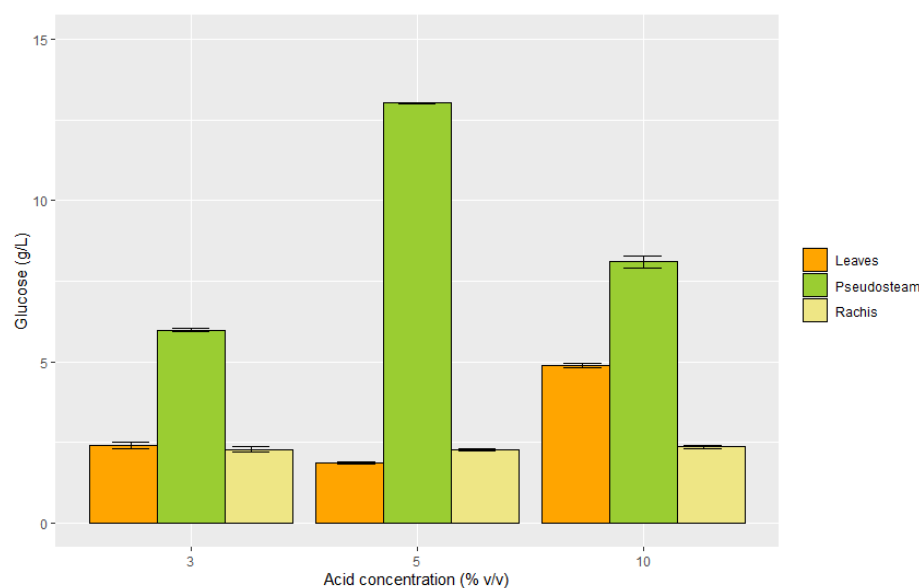


Figure 4. Hydrolysis Results, temperature $100 \text{ }^{\circ}\text{C}$, 20 min.

Comparing the results obtained in this study with the percentage of fermentable sugars of cane bagasse presented in Table 1, we observe that the amount of glucose obtained by pseudostem is higher, making it a competitive raw material in bioproduct manufacturing.

3.3. Reaction Kinetics

For the study of kinetics, the acid hydrolysis reaction was performed, quantifying glucose seven different times; the results are reported in Figure 5. It is observed that the maximum yield was reached after 20 min of reaction; for longer times, the concentrations decrease, representing a breakdown of glucose into by-products that can be furfural and hydroxymethylfurfural. Applying the minimization function in Microsoft Excel Solver for the sum of squares of the standard deviation between the experimental data and calculated by Equation (9), the parameters k_1 and k_2 were obtained as 0.05 and 0.03, respectively. The statistical parameter R^2 of 0.96 corroborates that Saeman's model fits well with the experimental data.

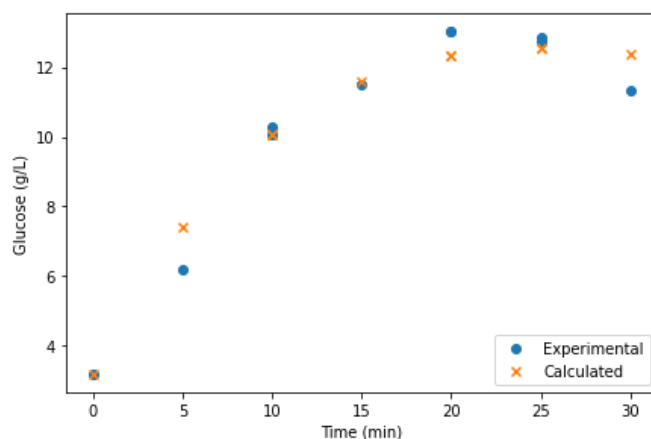


Figure 5. Glucose concentration vs. time plots using experimental data (points) and application of the Saeman model (x) Equation (9).

3.4. Material Balance

In the hydrolysis reaction, the inputs and outputs corresponding to the treatment of 1 kg of biomass on a dry basis were analyzed (Figure 6). For each kg of biomass, 1.83 kg of concentrated sulfuric acid and 18.94 kg of water were used, obtaining 21.08 kg of the hydrolyzed solution with a glucose content of 0.26 kg, a solution that could then be subjected to a fermentation process to obtain biofuels. The amount of solid waste obtained was 0.69 kg, mainly lignin and holocellulose. It should be noted that acid hydrolysis is a process widely used in the treatment of biomass due to the low cost of raw materials. However, it must be considered that the consumption of reagents and water is high, so it is important to venture into the study of new treatment techniques that reduce the consumption of resources and use environmentally friendly materials. Some promising processes are currently under investigation, such as applying ionic liquids and new catalytic methods [46,47].

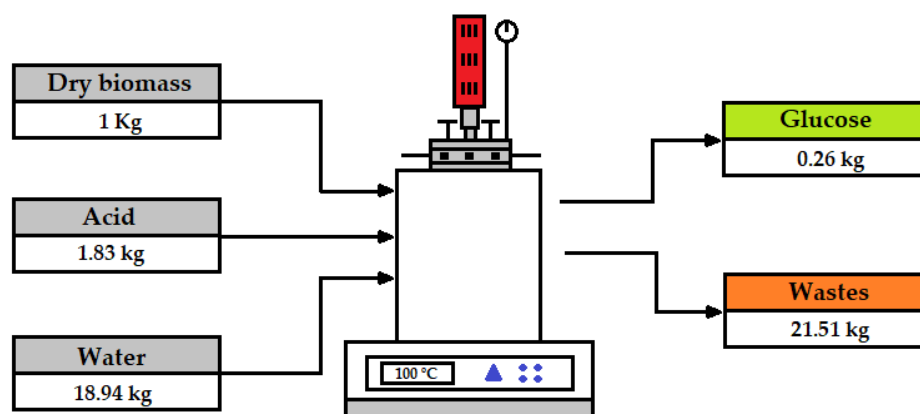


Figure 6. Matter balance of the hydrolysis reaction.

4. Conclusions

Through this study, it was determined that the part of the banana plant that presented the best glucose formation yield was the pseudostem, with a concentration of 13.02 gL^{-1} , the same as in the characterization, and it obtained the highest holocellulose content. In addition, the maximum glucose concentration was reached after 20 min of reaction, with subsequent degradation implying the formation of by-products, such as furfural and hydroxymethylfurfural compounds, typical in the decomposition of this product upon acid attack.

As for the kinetic analysis, a good fit was obtained between the experimental data and Saeman's model, with an R^2 of 0.96, indicating a first-order reaction with a constant kinetic value $k_1 = 0.05$ and $k_2 = 0.03 \text{ min}^{-1}$.

Regarding the material balance in this study, we concluded that the acid and water use was high. Studying new treatment techniques that are more friendly to the environment is recommended. In this regard, the application of ionic liquids and the search for new catalysts and processes will make it possible to take advantage of the lignin present in solid waste. Indeed, more research on the use of biomass must be carried out since its use allows sustainability and waste reduction and helps achieve energy security.

Sustainability: Biomass is a renewable and sustainable energy source that can replace non-renewable fossil fuels, thus reducing fossil fuel dependency and greenhouse gas emissions.

Waste reduction: The recovery of biomass also helps reduce organic and other waste, transforming it into useful products, such as biofuels, chemical products, construction materials, etc.

Employment generation and economic development: The valorization of biomass can create employment opportunities and support the economic development of local communities, especially in rural areas where biomass is abundantly available.

Energy security: Biomass recovery can help diversify energy sources, reduce dependence on imported fossil fuels, and improve energy security.

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Conflicts of Interest: The authors declare no conflict of interest.

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