

A KINETIC STUDY OF SACCHARIFICATION OF JACKFRUIT RESIDUE (*Artocarpus heterophyllus*). CHEMICAL AND ENZYMATIC PATHWAY

José Uriel Medina-Morales

Biological-Agricultural Sciences Area,
Universidad Autónoma de Nayarit, Ciudad
de la Cultura “Amado Nervo”, México

Leticia Mónica Sánchez-Herrera

Biological-Agricultural Sciences Area,
Universidad Autónoma de Nayarit, Ciudad
de la Cultura “Amado Nervo”, México

Ana Alejandra Vargas-Tah

University of Chemical Engineering,
Universidad Michoacana de San Nicolás de
Hidalgo, Ciudad Universitaria, México

Agustín Jaime Castro-Montoya

University of Chemical Engineering,
Universidad Michoacana de San Nicolás de
Hidalgo, Ciudad Universitaria, México

Miguel Angel Espinoza-Rodríguez

Area of Basic Sciences in Engineering,
Universidad Autónoma de Nayarit, Ciudad
de la Cultura “Amado Nervo”, México

Antonio Hidalgo-Millán

Area of Basic Sciences in Engineering,
Universidad Autónoma de Nayarit, Ciudad
de la Cultura “Amado Nervo”, México

Raúl Delgado-Delgado

Area of Basic Sciences in Engineering,
Universidad Autónoma de Nayarit, Ciudad
de la Cultura “Amado Nervo”, México

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Abstract: In this work, the obtaining of sugars from the lignocellulosic residue of the jackfruit fruit was proposed. (*Artocarpus heterophyllus*). Acid hydrolyses were carried out with sulfuric acid (H₂SO₄) at 90 and 105°C for concentrations of 2, 4, 6, 8 and 10%; enzymatic hydrolyses were performed with cellulases and hemicellulases (*Aspergillus niger*) con R_{s/l} of 7.5, 10 and 12.5% at 30 and 40°C. The highest production of reducing sugars in acid hydrolysis was 10.012 g/L in 180 min, 10% and 105°C. In enzymatic hydrolysis, the maximum production was 30.4436 g/L in 12 h with a R_{s/l}=7.5% to 40°C.

INTRODUCTION

There is great interest in the use of lignocellulosic biomass as a source of fermentable sugars to obtain compounds of commercial importance in the context of biorefineries [1]. These sugars can be obtained from the cellulose and hemicellulose of lignocellulosic residues, which can be generated by various activities such as agriculture. One of the sources of lignocellulosic residues in the State of Nayarit is the cultivation of jackfruit (figure 1). Where 88% of the national production is produced (18,611 tons/year) [2]. It has been reported that approximately 40% of the fruit is discarded as waste [3].



Figure 1. Jackfruit fruit.

In addition, jackfruit contains 21.29% cellulose on a dry basis [4]; therefore, the use of this material has the potential to be used, due to its production volume and its

content of holocellulose material, as a sugar producer.

Hydrolysis processes are used to obtain reducing sugars from lignocellulosic residue.

The reaction catalyzed by acids is called acid hydrolysis in which the H⁺ cations attack the bonds of the polymer chains that make up these residues, releasing the monosaccharides in their structure [5]. The process of enzymatic hydrolysis in lignocellulosic residues is carried out when selective, coordinated and synergistic activity of hydrolytic enzymes (protein or protein-like substance with catalytic properties) is carried out on the bonds of these substrates [6]. In the case of cellulose, the endoglucanases break the internal bonds of the network, then the exoglucanases release cellobiose from the non-reducing ends of the chain and, finally, the glucosidases release the glucose units [5]. The objective of this study is to obtain reducing sugars through the hydrolysis of lignocellulosic residues from the jackfruit fruit.

METHODOLOGY

BIOLOGICAL MATERIAL

The jackfruit fruit was collected in a state of physiological maturity. The pulp and seeds were removed from the fruit. The rest of the fruit was dried at 50°C for 96 hours. Next, the dry product was ground, bringing it to a particle size of less than 1.4 mm.

Cellulose and hemicellulose were determined using the methodology proposed in the ANSI/ASTM standard (1997) [7]. 1 g of holocellulose was weighed into an Erlenmeyer flask, 5 mL of NaOH 17.5% (p/p) was added. 2.5 mL of 17.5% NaOH was added gradually until a volume of 12.5 mL was completed. It was placed in a water bath with constant agitation at 30°C. 16.5 mL of distilled water was added. The reaction was carried out for one hour under constant stirring. After this

period, the content was vacuum filtered with 50 mL of NaOH 8.3% (p/p) and subsequently with distilled water. The vacuum was deactivated and 7.5 mL of 10% acetic acid was added, leaving it in contact for 5 min. It is brought to neutrality with distilled water and vacuum filtered once more. The material that remains on the filter paper is cellulose (α -cellulose) and the material degraded in the reaction is hemicellulose (β -cellulose).

The determination of soluble and insoluble lignin was carried out with the methodology proposed by Sluiter et al. (2012) [8]. 0.3 g of dry sample was weighed into a test tube. Hydrolysis was performed by adding 3 mL of H₂SO₄ at 72% (w/w) concentration for one hour at 30°C under constant agitation in a shaking bath (Memmert). Then, 84 mL of distilled water was added to reduce the acid concentration to 4% and it was heated to 121°C for one hour in an autoclave (Biobase BKQ-B7511). At the end of this process, the filtered liquid was filtered and recovered for the determination of soluble lignin, which was carried out in a spectrophotometer (Jenway 7305) where the absorbance reading was made at 320 nm. For insoluble lignin (it remains as a solid on the filter paper), washings were carried out with 750 mL of distilled water. The paper was dried for 24 h. Weight was recorded. The difference of the total weight and the filter paper is the insoluble lignin [8].

Extraction of water solubles was performed by placing 5 grams with 200 ml of water, in a Kimax® Kimble flask, at 96°C in a BIOBASE® BKQ-B75II autoclave. This material was named substrate.

ACID HYDROLYSIS

5 grams of substrate was weighed, which was placed in a Kimax® Kimble bottle. Next, an acid solution of sulfuric acid was added in a fraction of 10 ml of acid per gram of solid substrate.

Sulfuric acid concentrations were 2%, 4%, 6%, 8% and 10% (w/w). The reactions were carried out in an autoclave at temperatures of 90°C, 105°C and 120°C and the reaction time was 30 min, 60 min, 120 min and 180 min.

ENZYMATIC HYDROLYSIS

The enzymatic hydrolysis reaction was carried out in 500 mL Erlenmeyer flasks. A solid load was implemented ($R_{s/l}$) of 7.5%, 10% and 12.5% for a volume of 75 mL of dibasic sodium citrate at a pH of 4.8. 6% (weight of cellulase and hemicellulase relative to cellulose and hemicellulose) concentration of *Aspergillus niger* Sigma-Aldrich® cellulase and hemicellulase enzymes was added. It was carried out in a transverse agitation bath at 150 rpm and temperatures of 30°C, 40°C and 50°C. Sampling was carried out at 0h, 2h, 4h, 8h and 12h.

DETERMINATION OF REDUCING SUGARS

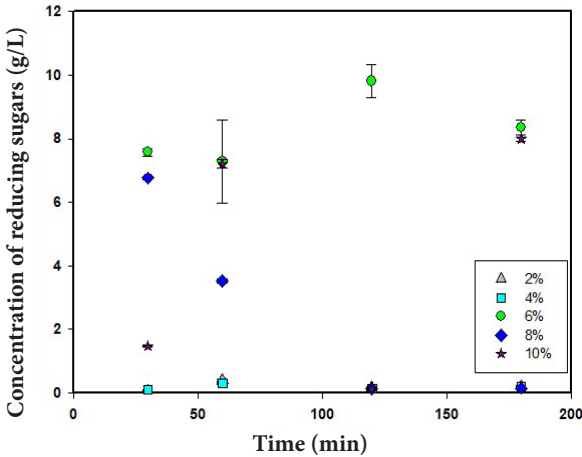
The determination of reducing sugars was carried out using the DNS technique proposed in Bello-Gil et al. (2006) [9]. 0.5 mL of liquid sample of the hydrolyzate was taken. 0.5 mL of DNS reagent was added. The reaction was carried out for 5 min at the boiling point. 5 mL of distilled water was added. It was cooled in a 20°C water bath for 1 min. The absorbance (ABS) reading was performed with a spectrophotometer (Metash) at a wavelength of 540 nm [10].

RESULTS

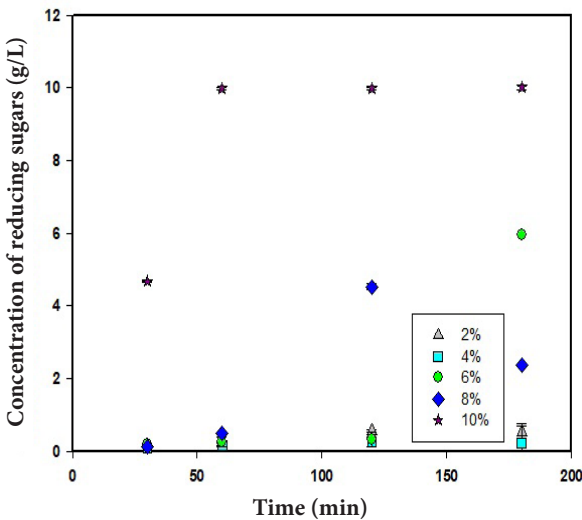
The jackfruit substrate was determined to contain 25.3633% cellulose, 13.2462% hemicellulose and 9.2943% total lignin.

At 90°C, a maximum concentration of reducing sugars of 9.81 g/L is reached at 120 min with an acid concentration of 6% (figure 2a). On the other hand, at 105°C and using

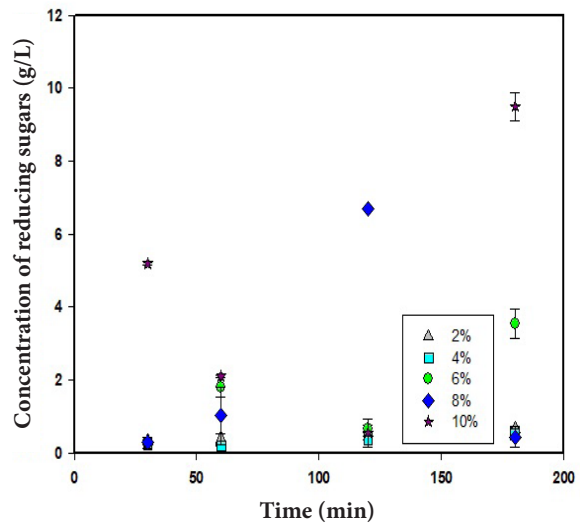
10% acid, a high concentration of sugars is observed from 60 min with a production of 9.98 g/L and is sustained up to 180 min until reaching 10.01 g/L (figure 2b). Regarding the kinetic results carried out at 120°C, a maximum concentration of reducing sugars of 9.49 g/L is obtained at 180 min with an acid concentration of 10% (figure 2c).



(a)



(b)

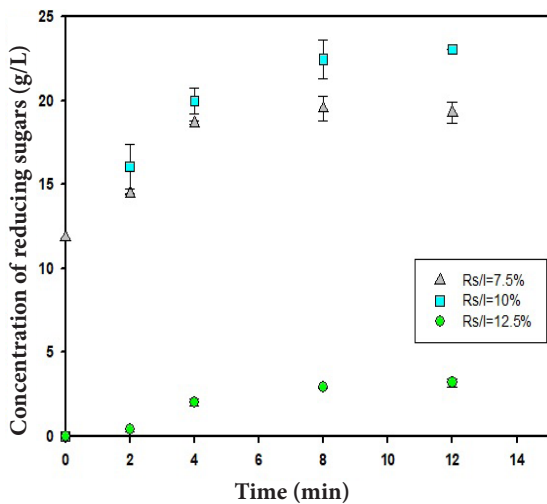


(c)

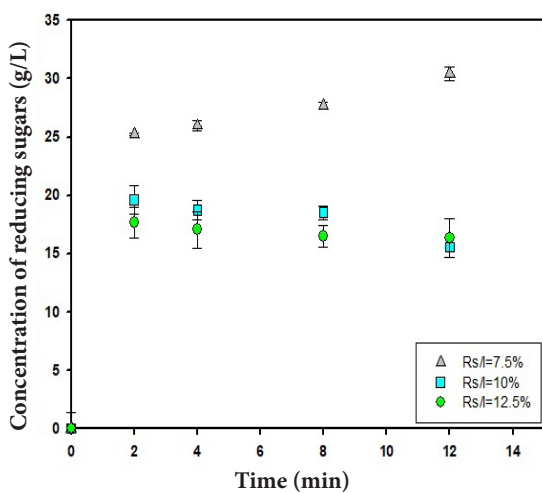
Figure 2. Production of reducing sugars for acid hydrolysis: a) 90°C, b) 105°C and 120°C.

According to what is presented in Figure 2, it can be seen that concentrations of 6% and 10% acid favor the production of reducing sugars. The decreases in concentrations are due to the parallel reactions that occur in the acid hydrolysis reaction, since, when there is a considerable concentration of sugars, the acid reacts with them carrying out a degradation of sugars, producing HMF and furfural from of hexoses and pentoses respectively [11].

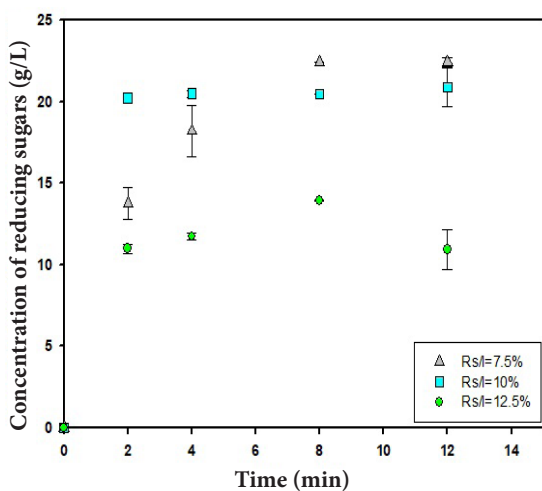
In the kinetics of the enzymatic hydrolysis of the jackfruit residue (figure 3), at 30°C, the maximum concentration of reducing sugars (23.058 g/L) was reached at 12 h with a $R_{s/l}=10\%$; Besides, the kinetics for $R_{s/l}=7.5\%$ has the same behavior as $R_{s/l}=10\%$, but with a lower response (figure 3a). Regarding the kinetics carried out at 40°C, a maximum in the concentration of reducing sugars of 30.4436 g/L is reached at 12 h with a $R_{s/l}=7.5\%$ (figure 3b). On the other hand, the kinetics carried out at 40°C showed a higher concentration of reducing sugars after 8 h of reaction, reaching a maximum of 32.44 g/L at 12 h for a $R_{s/l}=7.5\%$.



(a)



(b)



(c)

Figure 3. Production of reducing sugar for enzymatic hydrolysis: a) 30°C, b) 40°C and 50°C.

Even though the $R_{s/l}=12.5\%$ it is the one that contains the highest concentration of substrate, it is also the one that managed to obtain the lowest concentration of sugars, which can be seen in the kinetics performed at 30°C and 40°C (figure 3). In addition, it can be seen that the kinetics for an $R_{s/l}$ of 10% and 12.5% at 40°C increased their sugar concentrations, but no increase is seen over time. However, the kinetics for an $R_{s/l}$ of 7.5% increases the concentration of reducing sugars. This is due to the fact that, as the load of the solid is greater, the viscosity of the reactive medium increases, causing the thermal resistance to increase. The increase in this intensive property means that the enzyme in the medium does not receive the necessary energy to carry out an efficient reaction [12].

CONCLUSIONS

Due to the content of cellulose and hemicellulose, added to the volume generated, the jackfruit residue can be used as a producer of reducing sugars.

The optimal conditions for the production of reducing sugars by acid hydrolysis were: temperature of 90°C, time of 120 min and catalyst concentration (H_2SO_4) of 6%.

The optimal conditions for the enzymatic hydrolysis of jackfruit residues were: temperature of 40°C, time of 2 h and $R_{s/l}$ of 7.5.

The temperature and solid load factors had a greater effect on the concentration of reducing sugars produced by acid hydrolysis and enzymatic hydrolysis, respectively.

Considering the maximum yields of sugars obtained, enzymatic hydrolysis was 3,041 times more effective than enzymatic hydrolysis. This is because, despite the fact that the material was not subjected to a pretreatment, the enzymatic hydrolysis is not affected by degradation reactions of the desired product.

The results obtained, in both hydrolysis techniques, indicate that for a better use of the material it is necessary to use a pretreatment so that a better interaction of the cellulose and hemicellulose with the acid or the enzymes used for this purpose is carried out.

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