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Dilute sulfuric acid hydrolysis of tropical region biomass

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Although ethanol can be produced from a wide range of biomass materials, biomass, from the tropical region, like mango (skin or bagasse) is a crop residue readily available today as a non-conventional crop for the saccharification process that has had little attention. It has the benefits to be found in large quantities and in an industrial level is completely separated into its components with a free access for acid or enzymatic hydrolysis. These agro-industrial waste as well as other materials like sugarcane bagasse and pine wood are abundant enough, and in virtue of their high carbohydrate content hold tremendous potential for large-scale bioethanol production. The objective of this work is to develop a comparative analysis using dilute acid hydrolysis process between mango (skin and bagasse), sugarcane bagasse, and pine wood. The biomass was subjected to pretreatments like alkaline hydrolysis using calcium sulfate and sodium hydroxide, water immersion, and water autoclaved at 121 °C. Experimental results showed that the maximum percentages of sugar recovery were for sugarcane bagasse—56.62%, pine wood—82.36%, mango skin—97.37%, and mango bagasse—202.91%. From the tested biomass materials, only mango bagasse has a considerable fraction of already digestible sugar that does not undergo a pretreatment + hydrolysis process. © 2012 American Institute of Physics. [doi:10.1063/1.3663878]

I. INTRODUCTION

Ethanol can be produced from renewable resources such as agricultural waste or forestry waste.¹ In addition, the conversion of cellulosic materials into ethanol requires no energy input from fossil fuels. Lignin, which is a by-product, can be burned to provide the energy required. The carbon dioxide released during the production and the use of ethanol can be converted back to biomass in the cultivation of energy crops (cellulosic materials) to provide new raw material for the production.² Consequently, the contribution to the content of greenhouse gases is negligible. Raw material such as lignocelluloses can be considered as an infinite resource. Another advantage gained by using ethanol as fuel is the reduction in hydrocarbon emissions.¹

Mango is one of the most important tropical fruits accounting for nearly 50% of total with a global production estimated by The Food and Agriculture Organization of the United Nations (FAO) of 30.7 million tons during 2010. As mango is a seasonal fruit, it is processed into various products, and during its processing, huge amount of peel and bagasse (composed by fibers, peel, and pulp) is generated as a by-product, and its disposal is a major problem. The peel constitutes about 15%–20% of the fresh fruit, which is mainly composed of cellulose, hemicellulose, and lignin. These agro-industrial waste from other materials such as sugarcane bagasse and pine wood are abundant enough and, in virtue of their high carbohydrate content, hold tremendous potential for large-scale bioethanol production in which the mango peel and bagasse could be an excellent feedstock from the tropical region that have had little attention, with the benefits to be found in large quantities and in an industrial level (e.g., concentrate fruit plants)

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are completely separated from all the other components with a free access for acid or enzymatic hydrolysis.^{3,4}

A number of processes for hydrolyzing cellulose into glucose have been developed over the years. The vast majority of processing schemes utilizes either cellulolytic enzymes or sulfuric acid of varying concentration. Historically, enzymes have been too expensive for economical production of fuel ethanol from biomass.⁵ Acid hydrolysis leads to fission of the glycosidic bonds and occurs in three stages: first, the proton of the acid catalyst interacts rapidly with the glycosidic oxygen that bonds two sugar units, forming the so called conjugated acid; second, there is a slow cleavage of the C-O bond, resulting in an intermediate cation of cyclic carbon; third, the carbon cation starts a rapid addition of a water molecule, resulting in a stable final product and in the proton release.⁶

Dilute sulfuric acid hydrolysis is a chemical hydrolysis for either the pretreatment before enzymatic hydrolysis or the conversion of lignocellulose to the corresponding sugars. In dilute acid hydrolysis, the hemicellulose fraction is depolymerized at lower temperature than the cellulose fraction. If higher temperature or longer retention times are applied, the formed monosaccharides will be further hydrolyzed to other compounds. It is therefore suggested that the hydrolysis process be carried out in at least two stages, the first stage at relatively milder conditions during which the hemicellulose fraction is hydrolyzed and a second stage can be carried out by enzymatic hydrolysis or dilute acid hydrolysis at higher temperatures during which the cellulose is hydrolyzed.⁷

High reaction rates (compared with enzymatic process), low acid consumption, and low cost of sulfuric acid (compared with base-catalyzed pretreatments) are some of the advantages of the dilute sulfuric acid pretreatment.⁸ Avoiding degradation of monosaccharides is important not only to improve the yield of hydrolysis but also to avoid the problems associated with the inhibition of sugars fermentation to ethanol.⁹ The most accepted model considers that the hemicellulose decomposition occurs in two stages. First, the hemicellulose chain is decomposed into xylo-oligosaccharides and second into xylose monomers.⁶ The most important inhibitors are furans, carboxylic acids, and phenolics compounds. Furfural and 5-hydroxymethyl furfural (HMF) are the most important furans. They are formed by decomposition of pentoses and hexoses, respectively. Acetic acid is formed from hydrolysis of the acetyl groups in the hemicellulose, as a consequence of deacetylation of acetylated pentosan.^{6,7}

The hemicellulose fraction can be easily extracted and hydrolyzed by dilute acid treatment. Alkaline treatment digests the lignin matrix and makes cellulose and hemicellulose available to enzyme degradation. Alternatively, biological delignification of agricultural residues is possible using selected strains of *Panus tigrinus*, a white rot fungus.¹⁰ Also, the swelling of the cellulosic material has been demonstrated as an effective pretreatment to increase the possibility of attach of chemical compounds to monomeric carbohydrates in their structure, increasing the accessible surface area.¹¹

Lignocellulosic materials and hydrolyses processes are very complicated. Factors influencing the yields of the lignocellulose to the monomeric sugars and the by-products are, e.g., particle size, liquid to solid ratio, type and concentration of acid used, temperature, and reaction time, as well as the length of the macromolecules, degree of polymerization of cellulose, configuration of the cellulose chain, association of cellulose with other protective polymeric structures within the plant cell wall such as lignin, pectin, hemicellulose, proteins, and mineral elements.¹² However, since these variables are not identical in different types of lignocellulosic materials, it would be difficult to extent the data on a type of lignocellulose to another as well to test pretreatment sequences to obtain an improvement in the overall efficiency using the dilute acid process alone or in combination.¹³

The present study develop a comparative analysis using dilute acid hydrolysis processes between mango (skin and bagasse), sugarcane bagasse, and pine wood with the effect of some hydrolysis parameters in production of monomeric sugars using sulfuric acid. The variables considered were the effects of hydrolysis temperature, time, and acid concentration. Also, the biomass was subjected to pretreatments such as alkaline hydrolysis using calcium sulfate and sodium hydroxide, and water immersion for a period of time, and water autoclaved at 121 °C.

Hydrolysis of biomass resulted in percentage of sugar recovery reported and compared with the data obtained through identical experimental designs and statistical analysis techniques of all cellulosic feedstock.

II. METHODOLOGY

A. Cellulosic materials

All biomass used in all the experiments were obtained in southwest Mexico; sugarcane bagasse was supplied from the sugar plant Ingenio El Molino de Menchaca (Tepic, Nayarit) as a byproduct of sugar production. The mango skin and bagasse were obtained as a byproduct of a local production company of fruit concentrates (MexiFrutas). Finally, pine sawdust was obtained from a local sawmill (Tepic, Nayarit), which was used as test material. All materials received the same treatment, were dried at $45 \pm 5^\circ\text{C}$ for 2 days to less of 10% moisture before use, partly knife-milled and screened having particle size between 10 mesh and 35 mesh (0.5 mm and 2.0 mm) for uniformity, and the moisture of the material was determined by a moisture analyzer IR-200 (Denver instrument Co., Denver, USA) dry scale (105°C , 20 min). The ground and screened biomass samples were stored in seal plastic bags at room temperature, prior to hydrolysis. All experiments were carried out with a minimum of three replicates, and the mean values are reported.

B. Dilute acid hydrolysis

The dilute acid hydrolysis was executed according to the methods and specification of those used with other lignocelluloses substrates.^{14–16} Three hot plate stirrers were utilized for these experiments heated in a reflux system. The operation variables examined were (a) hydrolysis retention time (0–300 min), and (b) acid concentration of 1–5% (w/w) H_2SO_4 ; in a 1/10 relation (dry weight/dilute acid volume) with a speed of agitation of 250 rpm and constant temperature of 100°C . The focus of the experiments included the analysis of all cellulosic materials to evaluate, which is performed in triplicate. Time zero of all reactions corresponded with biomass injection. The experimental approach included for each biomass species, run in triplicate, equaling 36 experiments in total with $2 \times 2 \times 2$ full factorial design. Each reaction was terminated by placing a 5 ml aliquot into cold water. After cooling, the pretreated biomass was neutralized using 2N NaOH. The neutralized mixture was filtered through Whatman filter paper-42 and keeps it under 6°C for sugar analysis.

C. Sugar analysis

Analysis of glucose production reported as total reducing sugars (TRS) was carried out using the 3,5-dinitro salicylic acid (DNS) method. Two milliliter of appropriately diluted sample and 8 ml of DNS solution were placed into a sampling bottle. The mixture was then heated up in boiling water for 5 min. Then, the sample was cooled to room temperature and a UV-Vis spectrophotometer Cary 100 (Varian Co., Palo Alto, CA, USA) was used to measure its absorbance. A blank solution consists of distilled water and DNS reagent was used to zero the spectrophotometer at 540 nm before analyzing the samples. A calibration curve was prepared by determining the absorbance of standard glucose solutions of known concentrations. The glucose concentration in the withdrawn sample was obtained by comparing the absorbance to the calibration curve. The linear relationship between absorbance and concentration of an absorbing species known as Beer-Lambert law was assumed applicable to the whole range of concentrations (from 0 to 2.33 mol m^{-3}). The experimental results were fitted to a straight line by least square method using EXCEL. The straight line fitted well the experimental data in the range considered with R^2 value approaching one or at least 0.995, and the best fit equations were used in subsequent analysis to determine the concentration of TRS for any measured value of absorbance. About the determination of the reducer nature samples were taken during the hydrolysis. It was proceeded in the same form as in the preceding paragraph, for the preparation of the calibration line, applying the analysis method to 1 ml sample of the hydrolyzed and doing the

analysis by triplicate. Due to the relatively low specificity, one must run blanks diligently if the colorimetric results are to be interpreted correctly and accurately. The rate of formation of these soluble reducing sugars (i.e., glucose, cellobiose, and higher soluble oligodextrins) is taken as a measure of the rate of lignocellulose hydrolysis,^{17–19} to finally represented as the percentage of sugar recovery, defined as the weight of total reducing sugars found in the liquid divided by the weight of cellulose and hemicellulose contained in the biomass.

D. Pretreatments

Pretreatment evaluated agents were two different classes each one containing two different processes, and their characteristics were determined by each class with alkaline hydrolysis using calcium sulfate and sodium hydroxide, water immersion for a certain time, and water autoclaved at 121 °C, taking into consideration high yields of biomass resemblances in acid hydrolysis. The experiment approach included for the biomass species, run in triplicate, equaling 30 experiments in total.

In the NaOH (delignification) and water (swelling) pretreatments, biomass was pretreated at 121 °C in an autoclave. A total of 4 g of biomass sample and 40 ml of NaOH solution of 1% were placed in a serum bottle and mixed using a glass rod, forming a slurry at a solid/liquid ratio of 0.1 g/ml during an hour. All serum bottles were sealed and crimped before pretreatment.^{20,21}

In the calcium sulfate (delignification) pretreatment, 0.10 g per gram of biomass dissolved in distilled water, a certain amount of calcium sulfate remained insoluble, although this continued dissolving during pretreatment; biomass (4 g) was treated with 100 ml of the pretreatment solution in 500 ml flasks in an orbital shaker agitated at 150 rpm at 60 °C during 24 h (Ref. 22). In order to produce swelling of the material, biomass samples were immersed in water, using a liquid/solid ratio equal to 5/1, at room temperature for 1 h. After this time, the material was washed three times with distilled water, filtered, and dried in an oven at 45 °C for 24 h (Ref. 11).

E. Statistical analysis

Results were subjected to the analysis of variance (ANOVA) using DESIGN-EXPERT software version 8.06, Inc., Minneapolis, USA. The experimental data were fit using a low-order polynomial equation to evaluate the effect of each independent variable (acid concentration and time) to the response (% sugar recovery). In this study, a polynomial quadratic equation, as shown in Eq. (1), was employed:

$$y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \sum \beta_{ij} X_i X_j, \quad (1)$$

where y is the response, x_i and x_j are independent variables, β_0 is the offset term, β_i are linear coefficients, β_{ii} are quadratic coefficients, and β_{ij} are cross-product coefficients.

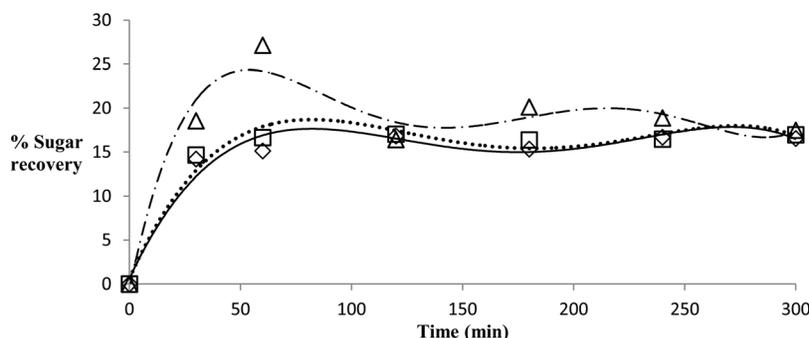


FIG. 1. Percent sugar recovery of sugarcane bagasse with dilute acid hydrolysis at different times and concentration of acid (◇ = 1% w/w, □ = 2% w/w and △ = 5% w/w).

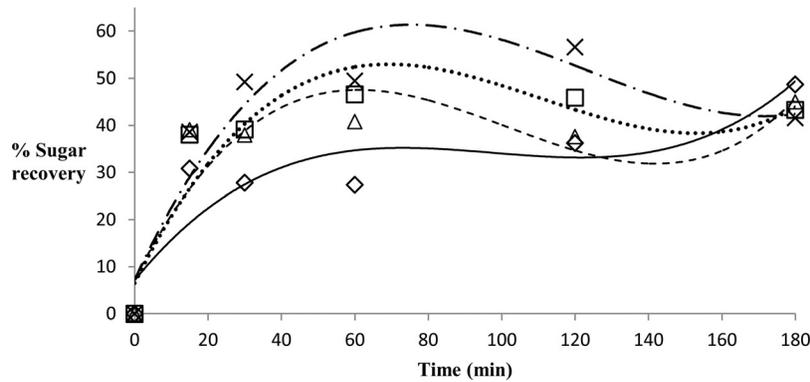


FIG. 2. Achieved percent of sugar recovery of sugarcane bagasse during dilute acid hydrolysis with 5% by weight of acid subjected to different pretreatments (\diamond = immersion in water for 24 h, \square = autoclaved water for 1 h, Δ = autoclaved 1% NaOH for 1 h, and \times = alkaline hydrolysis).

III. RESULTS AND DISCUSSION

Acid hydrolysis of all cellulosic materials was performed using dilute H_2SO_4 , as the most widely used and tested approach. Cellulose and hemicellulose content of all biomass materials was based upon the next references sugarcane bagasse,²² mango skin and mango bagasse,²³ and pine wood,⁵ which is used to calculate the percent of recovered sugars in the experiments. Fig. 1 shows the results of acid hydrolysis of sugarcane bagasse treated with H_2SO_4 at a concentration of 1%-5% (w/w). An increase in the amount of acid solution from 1% to 5% correlated with an increased saccharification, with a maximum peak of 27.14% (5% acid—60 min) and a total accumulated of sugar recovery of 20% to 25% higher than 1% and 2% acid concentration.

The experimental data shown in Fig. 1 were used to determine the regression coefficients of the second-order polynomial equation using DESIGN-EXPERT software and the following models that describe the percent sugar recovery in terms of actual parameter were obtained in the following equation:

$$\begin{aligned} \% \text{ Sugar recovery} = & 4.20793 + 0.15296 * \text{Time} + 0.45746 * \text{Acid} - 0.002432 * \text{Time} * \text{Acid} \\ & - 0.00039 * \text{Time}^2 + 0.12000 * \text{Acid}^2. \end{aligned} \quad (2)$$

To test the significance of the developed model as well as analyzing the effects of variables, ANOVA was performed. A model is considered significant if its p-value (also known as the Prob > F value) is lower than 0.05, indicating only a 5% chance that a “Model F-value” could occur because of the noise. The Prob > F values were also used to evaluate the significance of

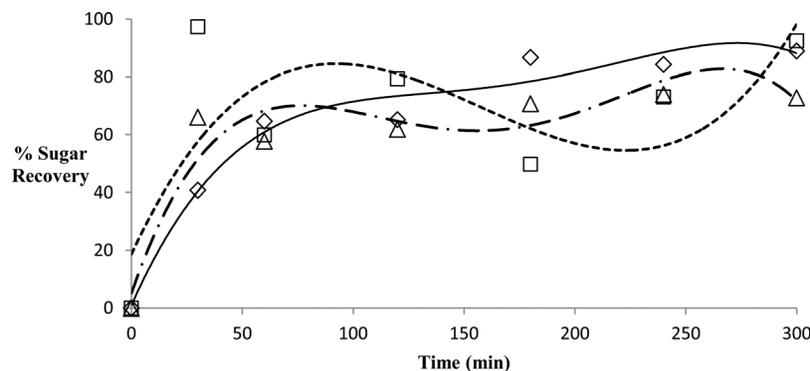


FIG. 3. Percent sugar recovery of mango skin with dilute acid hydrolysis at different times and concentration of acid (\diamond = 1% w/w, \square = 2% w/w, and Δ = 5% w/w).

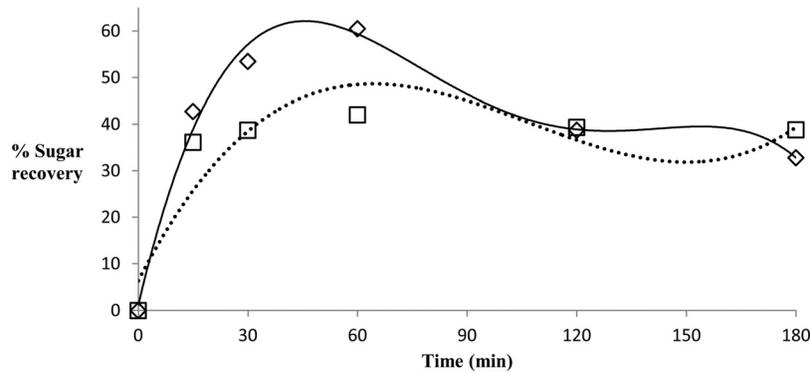


FIG. 4. Achieved percent of sugar recovery of released of mango skin during dilute acid hydrolysis with 2% by weight of acid subjected to different pretreatments (\diamond = immersion in water for 24 h and \square = autoclaved water for 1 h).

the effects of each linear, quadratic, and interaction term on the response. Because the $\text{Prob} > F$ values for the model is low (less than 0.02), the model equation adequately describes the response with 5 degrees of freedom.

Sugarcane bagasse was also hydrolyzed with dilute acid using a 5% by weight (because with this concentration was obtained the maximum sugar recovery in the acid hydrolysis process) and subjected to different pretreatments (immersion in water for 24 h, autoclaved water for 1 h, autoclaved 1% NaOH for 1 h and alkaline hydrolysis) and the results are presented in Fig. 2. All pretreatments presented a higher sugar recovery than just with the acid hydrolysis process, and the maximum peak is presented with alkaline hydrolysis (56.62% at 120 min). Statistically significant differences were determined by ANOVA when the pretreatments and sugar recovery were studied as an effect of the kind of pretreatment where the $\text{Prob} > F$ values for the model was 0.002, and statistically no significant differences were found.

Fig. 3 shows the results of acid hydrolysis of mango skin treated with H_2SO_4 at a concentration of 1%-5% (w/w). Due to the material characteristics and composition differences compared as such as sugarcane bagasse, the highest yield occurred within an acid concentration of 2%, with a maximum peak of 97.37% at 30 min. The equation that best describe the process with the actual parameter is presented as follows:

$$\begin{aligned} \% \text{ Sugar recovery} = & 7.64965 + 0.53169 * \text{Time} + 16.73778 * \text{Acid} - 0.00531 * \text{Time} * \text{Acid} \\ & - 0.00012 * \text{Time}^2 + 2.67535 * \text{Acid}^2. \end{aligned} \quad (3)$$

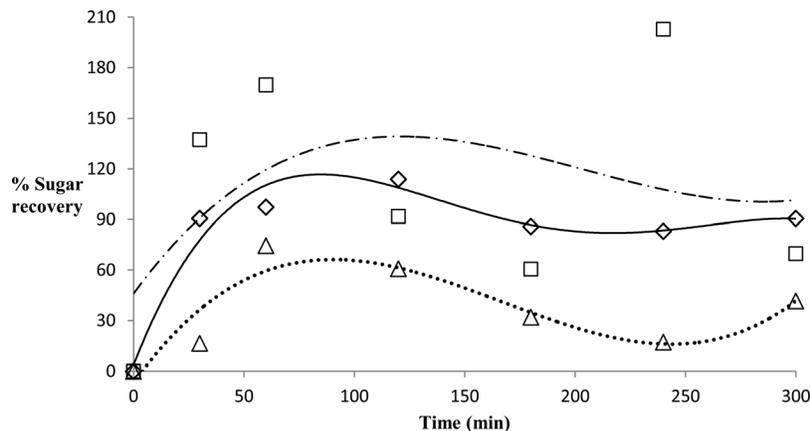


FIG. 5. Percent sugar recovery of mango bagasse with dilute acid hydrolysis at different times and concentration of acid (\diamond = 1% w/w, \square = 2% w/w, and Δ = 5% w/w).

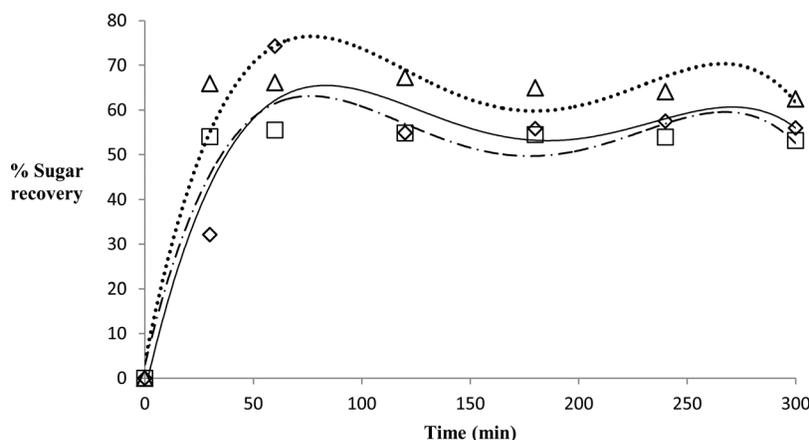


FIG. 6. Percent sugar recovery of pine wood with dilute acid hydrolysis at different times and concentration of acid (\diamond = 1% w/w, \square = 2% w/w, and Δ = 5% w/w).

This model with 5 degrees of freedom and a Prob > F values significant of 0.0242, with values for each model term, suggests that A, B, B², C², and AB are the model terms that have significant effects on the percent of sugar recovery. However, the lack of fit test, which was used to determine the adequacy of the model, indicates a not significant Prob > F value of 0.0833.

Mango skin was also hydrolyzed with dilute acid using a 2% by weight (because with this concentration, the maximum yield in the acid hydrolysis process was obtained) and subjected to different pretreatments (immersion in water for 24 h and autoclaved water for 1 h), and the results are presented in Fig. 4. All pretreatments presented a lower sugar recovery compared with those with only the acid hydrolysis process, and the maximum peak is presented with immersion in water for 24 h (60.52% at 60 min). Besides no statistically significant differences were found comparing the pretreatments with sugar recovery in the model with a low Prob > F value (0.0486).

Acid hydrolysis of mango bagasse sugar recovery percent results are presented in Fig. 5 and presented a higher level of sugar recovery with a maximum peak of 202.91% (2% acid—240 min), when compared to either mango skin and sugarcane bagasse (97.37% and 56.62%, respectively). These results higher than the theoretical maximum could be explained with a specific fraction of the material with lots of readily digestible sugars in the form of saccharose or other sugars in which this material does not need a specific hydrolysis procedure and could be fermented immediately. The mathematical model of the process is shown as follows:

$$\begin{aligned} \% \text{ Sugar recovery} = & 14.95652 + 0.81545 * \text{Time} + 65.39142 * \text{Acid} - 0.02324 * \text{Time} * \text{Acid} \\ & - 0.00228 * \text{Time}^2 - 12.23725 * \text{Acid}^2. \end{aligned} \quad (4)$$

Using 5 degrees of freedom, this model is described as not significant with a Prob > F value of 0.0710, with a lack of fit describe as well as not significant with a Prob > F value of 0.2384.

In Fig. 6, the sugar recovery (%) of acid hydrolysis process of pine wood is presented. It can be noticed from Fig. 6 that acid concentration of 1% shows the best result compared to 2% and 5% with a maximum peak occurred at 1% acid and 60 min (74.30% sugar recovery). The mathematical model of the process is shown as follows:

$$\begin{aligned} \% \text{ Sugar recovery} = & 15.02568 + 0.50983 * \text{Time} + 2.61101 * \text{Acid} - 0.01107 * \text{Time} * \text{Acid} \\ & - 0.00151 * \text{Time}^2 - 0.14812 * \text{Acid}^2. \end{aligned} \quad (5)$$

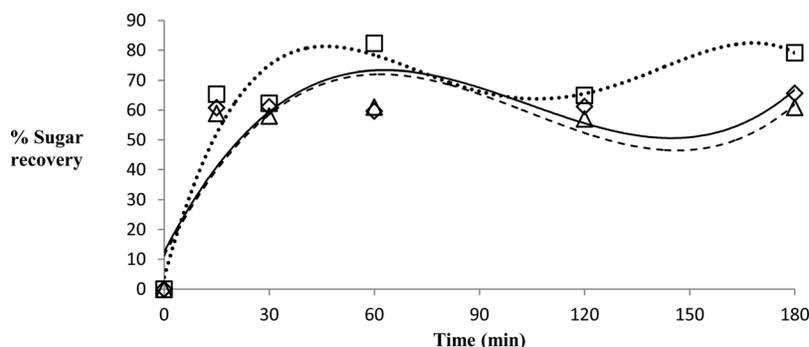


FIG. 7. Achieved percent of sugar recovery of pine wood during dilute acid hydrolysis with 1% by weight of acid subjected to different pretreatments (\diamond = immersion in water for 24 h, \square = autoclaved water for 1 h, Δ = autoclaved 1% NaOH for 1 h, and \times = alkaline hydrolysis).

Using 5 degrees of freedom, this model is described as significant with a $\text{Prob} > F$ value of 0.0139, with a lack of fit described as well as not significant with a $\text{Prob} > F$ value of 0.0893.

Pine wood was also hydrolyzed with dilute using a 1% by weight (because with this concentration, the maximum yield in the acid hydrolysis process was obtained) and subjected to different pretreatments (immersion in water for 24 h, autoclaved water for 1 h, autoclaved 1% NaOH for 1 h and alkaline hydrolysis) and the results are presented in Fig. 7. All pretreatments presented a higher sugar recovery (%) than just with the acid hydrolysis process, and the maximum peak is presented with autoclaved water for 1 h (82.36% at 60 min). No statistically significant differences were found by ANOVA when the pretreatments and sugar recovery were studied as an effect of the kind of pretreatment used in the model with a $\text{Prob} > F$ value of 0.007.

From all the studied materials, the pine wood was the one with minimum overall sugar recovery efficiency in both dilute acid hydrolysis and pretreatments processes lower than 60%; besides just the mango bagasse was the only material that exceeded the theoretical maximum, even where there is a peak on the 200% probably due to readily digestible sugars that does not need any pretreatment + acid hydrolysis to obtain all the sugars from the cellulose-hemicellulose fraction, which can subject this material immediately to a fermentation process.

Table I shows the approximate amount of theoretical production of ethanol in liters per ton of each cellulosic material evaluated in this study. The maximum percent of sugar recovery obtained in the experiments (sugarcane bagasse—56.62%, mango skin—97.37%, mango bagasse—202.91%, and pine wood—82.36%) was used according to the preferred method.²⁴ The percent conversion is calculated by dividing the reducing sugars in solution by the theoretical amount of sugars and multiplying by 100. The theoretical amounts of sugars are calculated based upon the hemicellulose and cellulose content (dry basis).²⁵

TABLE I. Theoretical ethanol production using highest experimental sugar recovery percent from cellulosic materials (% in dry weight).

	Sugarcane bagasse	Mango skin	Mango bagasse	Pine wood
Amount of biomass (kg)	1000	1000	1000	1000
Cellulose + hemicellulose content (kg)	664	563	649	616
Sugar conversion and recovery efficiency	0.5662	0.9737	2.0291	0.8236
Ethanol stoichiometric yield	0.51	0.51	0.51	0.51
Glucose fermentation efficiency	0.75	0.75	0.75	0.75
Ethanol yield from glucose (kg)	144	209	503	194
Total ethanol yield (liter)	165	241	579	223

IV. CONCLUSIONS

Mango (skin and bagasse), sugarcane bagasse, and pine wood are renewable, cheap, and widely available resources. The hydrolysates obtained from all used biomass materials can be used to produce hydrogen and methane by anaerobic fermentation process. Therefore, this study can be conceived as the first stage of an integrated strategy for materials from the tropical region utilization.

The data presented here supports the use of different tropical biomass materials for saccharification processes to obtain valuable fermentation products. In particular, the use of mango skin and bagasse waste residues from the fruit concentrate industry would provide an excellent source of a cellulosic material easily hydrolyzed to a glucose rich, xylose sparse product, comparable to other biotechnologically important bioresources, like the ones also studied and well known sugarcane bagasse and pine wood. In the present study, experimental data demonstrates with the maximum percentage of sugar recovery from the conversion of sugarcane bagasse—56.62% using alkaline hydrolysis, mango skin—97.37% using 2% dilute acid hydrolysis, mango bagasse—202.91% using 2% dilute acid hydrolysis, and pine wood—82.36% by autoclaved water treatment. It should be noted that mango bagasse from all the cellulosic material evaluated is the only one that does not need a pretreatment + hydrolysis step to produce sugars from lignocelluloses, because although it contains a specific fraction of fiber it also contains another one with readily digestible sugars that can be taken directly into a fermentation process. Besides, with a theoretical production of ethanol comparable with other common sources of ethanol from the highest recovery from all biomass materials being sugarcane bagasse (165 l), mango skin (241 l), mango bagasse (579 l), and pine wood (223 l).

The distribution of the corresponding cultivars is geographically distinctive, but their combined distribution covers an important proportion of Mexican land as well in all the tropical region of the world like India, Brazil, and China. Future studies should be proposed to employ techniques such as HPLC/HPAEC to identify specific comprehensive sugars to developed kinetic measurements as well as enzymes like *Trichoderma reesei* and *Aspergillus niger*; and finally going all through fermentation experiments to obtain actual ethanol production measurements.

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