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ORIGINAL PAPER

Sugarcane bagasse enzymatic hydrolysis: rheological data as criteria for impeller selection

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Abstract The aim of this work was to select an efficient impeller to be used in a stirred reactor for the enzymatic hydrolysis of sugar cane bagasse. All experiments utilized 100 g (dry weight)/l of steam-pretreated bagasse, which is utilized in Brazil for cattle feed. The process was studied with respect to the rheological behavior of the biomass hydrolysate and the enzymatic conversion of the bagasse polysaccharides. These parameters were applied to model the power required for an impeller to operate at pilot scale (100 l) using empirical correlations according to Nagata [16]. Hydrolysis experiments were carried out using a blend of cellulases, β-glucosidase, and xylanases produced in our laboratory by *Trichoderma reesei* RUT C30 and *Aspergillus awamori*. Hydrolyses were performed with an enzyme load of 10 FPU/g (dry weight) of bagasse over 36 h with periodic sampling for the measurement of viscosity and the concentration of glucose and reducing sugars. The mixture presented pseudoplastic behavior. This rheological model allowed for a performance comparison to be made between flat-blade disk (Rushton turbine) and pitched-blade (45°) impellers. The simulation showed that the pitched blade consumed tenfold less energy than the flat-blade disk turbine. The resulting sugar syrups contained 22 g/l of glucose, which corresponded to 45% cellulose conversion.

Keywords Rheology of biomass slurries · Biomass enzymatic hydrolysis · Biomass reactor impellers · Power consumption

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List of symbols

du/dy	Shear rate
N	Rotation (r.p.m)
D	Impeller diameter
H	Height helpful
T	Tank diameter
w	Thickness pitched blade
P	Power
η	Apparent viscosity
ρ	Fluid density
Θ	Angle pitched blade
g	Gravitation constant
N_p	Power number
N_{Re}	Reynolds number

Introduction

Currently, there is a scientific consensus that the present trends and patterns of energy production and consumption are unsustainable. This poses to the worldwide scientific community a challenge to implement technological solutions to protect the environment and society from the adverse impacts caused by the continuous and intensive use of oil for power generation. In this context, the production of second-generation ethanol is an attractive alternative [1]. The lignocellulosic material sugarcane bagasse is an ideal raw material for ethanol production due to its abundant availability in Brazil [5]. There are three technological routes for the conversion of lignocellulosic materials into fermentable sugars: (1) concentrated acid hydrolysis, (2) dilute-acid hydrolysis, and (3) enzymatic hydrolysis after biomass pretreatment. The enzymatic route is advantageous for energy consumption as it operates under mild temperature, pH, and pressure conditions. Moreover, the selective reaction conditions avoid the formation of sugar degradation products such as furfural and hydroxymethylfurfural, which decrease the sugar concentration in the resulting biomass syrups and inhibit the fermentation process [3, 13, 27].

The development of technology for the enzymatic hydrolysis of sugarcane bagasse is of paramount importance for the production of second-generation ethanol in Brazil. Amongst the main parameters to be studied, the rheological behavior of the hydrolysis suspension stands out as a major determinant of process efficiency. In particular, this knowledge would enable the selection of an impeller type to minimize energy consumption during the homogenization and enzymatic hydrolyses of the biomass suspensions. Indeed, mixing is an important operation parameter in chemical plants to achieve a uniform suspension, particularly when the components are in different phases (liquid, solid, or gas) [4, 17]. In addition, heat and/or mass transfer and the size reduction of agglomerated particles, all of which are promoted by the impeller, help to initiate a chemical reaction and assist its progress [10].

The present work focused on the rheological behavior of sugarcane bagasse hydrolysates. The rheological data guided the selection of an efficient impeller to be used in stirred reactors for the development and scale-up of the enzymatic hydrolysis of sugarcane bagasse.

Materials and methods

Pretreated sugarcane bagasse

Pretreated sugarcane bagasse was kindly provided by the sugar and ethanol industry, Usina Vale do Rosário (Santa Elisa Vale Conglomerate), located in Morro Agudo, São Paulo State, Brazil. This plant processes 250 tons of bagasse per day, during the harvest season, to be used as cattle feed. The bagasse is pretreated with steam at 200°C for 7 min (average pressure of 15 atm), followed by gradual decompression. In our laboratory, the bagasse was washed with warm distilled water to remove residual soluble sugars and dried overnight at 60°C. This material was washed with distilled water and ground to a particle size smaller than 2 mm using a grinder with knives coupled to a 2 mm diameter sieve at the sample outlet.

The determination of the carbohydrate content of the pretreated bagasse was done according to the Standard Biomass Analytical Procedures from the National Renewable Energy Laboratory, USA [19]. The total hydrolysis of pretreated bagasse polysaccharides was performed by incubating the material with 72% sulfuric acid at 30°C with agitation for 1 h, followed by neutralization to a pH value around 6 with calcium carbonate. This procedure was followed by the determination of glucose and total reducing sugars in the hydrolysate.

Sourcing of enzymes

Hydrolysis experiments were carried out using a blend of endo- and exo-glucanases, β-glucosidase, xylanases and accessory enzymes produced in our laboratory by *Trichoderma reesei* RUT C30 and *Aspergillus awamori* 2B.361 U2/1 [2, 9, 22]. The blended culture supernatants presented a FPU:β-glucosidase ratio of 1:1 due to the very high β-glucosidase production by *A. awamori,* which was coupled to the production, at lower levels, of other necessary enzymes also produced by *A. awamori*. Enzyme activities were measured as previously reported [8, 9, 22].

Enzymatic hydrolysis of pretreated sugarcane bagasse

The enzymatic hydrolysis experiments were carried out using 100 g (dry weight)/l of steam-pretreated sugarcane bagasse and an enzyme load of 10 FPU/g dry biomass. The suspensions were incubated at 200 rpm, 50°C, and pH 4.8 (sodium citrate buffer, 50 mM) for 36 h with regular sampling every 2 h. The glucose concentration was determined using a YSI 2,700 Select Biochemistry Analyzer, and the total reducing sugar content was determined according to the method of Miller [15].

Tank and impeller geometries

A stirred reactor of 1 l capacity (Biostat B-plus, Sartorius), equipped with a Rushton turbine, was used for sugarcane bagasse hydrolysis. The equipment presented the same geometry as a conventional full-scale stirred reactor. The featured geometries of the reactor and impeller used at laboratory scale where: 0.1084 (T); 0.1084 (H); 0.045 (D) and 0.009 (w).

Rheological assays

The rheological behavior of the hydrolyzed samples was evaluated as a function of the hydrolysis time, using a concentric cylinder rheometer (AR-G2; TA Instruments). The apparent viscosity was determined with regular sampling at 2-h intervals, as a function of shear rate (100–1,200 s^{-1}) at 50°C, which was the same temperature used in the enzymatic hydrolysis experiments. The parameters of the exponential model were estimated from experimental points at a 0.05 significance level, using the software Statistica 7.0.

Density assays

The relative density (ρ_r) of the sugarcane bagasse particles was determined by picnometry.

Dimensionless equations

A dimensionless correlation provides the relationship between the power number (N_p) and the Reynolds number (N_{Re}) [6, 14, 23]. A pitched blade promotes an axial flow and is mainly used in operations involving the mixing of miscible liquids or the dissolution of solids. The power number for a pitched blade, according to Nagata [16], is presented below:

$$N_p = \frac{A}{N_{Re}} + B\left(\frac{10^3 + 1.2N_{Re}^{0.66}}{10^3 + 3.2N_{Re}^{0.66}}\right)^p \left(\frac{W}{T}\right)^{\left(0.35+\frac{W}{T}\right)} (\sin\theta)^{1.2} \quad (1)$$

$$A = 14 + \frac{W}{T}\left\{670\left(\frac{D}{T} - 0.6\right)^2 + 185\right\} \quad (1a)$$

$$B = 10^{\left[1.3-4\left(\frac{W}{T}-0.5\right)^2-1.14\left(\frac{D}{T}\right)\right]} \quad (1b)$$

where

$$N_{Re} = \frac{D^2 N\rho}{\eta} \quad (2)$$

$$N_p = \frac{g_c * P}{\rho * N^3 * D_a^5}. \quad (2b)$$

For non-Newtonian liquids, the shear rate can be estimated by Eq. (3) [13].

$$\frac{du}{dy} = 10 * N. \quad (3)$$

The Rushton turbine promotes a radial flow that is employed in mass transfer between a gas phase and a liquid phase. These agitators are suitable for turbulent flow mixing with high power consumption. The power number for a Rushton turbine, according to Nagata [16], is presented below (Eq. 4):

$$N_p = \frac{11.39 * 10^4}{(N_{Re})^2} + \frac{717}{N_{Re}} + 0.2. \quad (4)$$

The geometric ratios used to calculate the mixing power were as follows: 0.42 (D/T); 1.00 (H/T); 0.22 (w/D). The tank and impeller geometries for a 100-l tank (scale-up) used to calculate the mixing power by a pitched blade (45°) impeller without baffles were: 0.5 (T); 0.21 (D); 0.5 (H); 0.05 (w); 45° (θ); 2 impellers. The corresponding calculation for a Rushton turbine without baffles is simpler in comparison to the pitched-blade turbine, and the tank and impeller geometries employed were 0.5 (T); 0.21 (D).

Results

Pretreated sugarcane bagasse characterization

Data for the normalized bagasse composition (cellulose 43.6%; hemicellulose 8.75%; lignin and ashes 33.75%) indicated, as expected for steam-pretreated material, a low hemicellulose content. The densities of the sugarcane bagasse and that of the mixture of sugarcane bagasse suspended in the enzymatic solution were 1.42 and 1.03 g/cm^3, respectively.

Hydrolysis kinetic data

The kinetics curves for the enzymatic hydrolysis are shown in Fig. 1. The maximum conversion of cellulose to glucose was 45% after a reaction time of 36 h. At this point, the concentrations of glucose and total reducing sugars reached 22 and 27 g/l, respectively.

Rheology data

This work studied biomass conversion using a 10% suspension of pretreated sugarcane bagasse. Nevertheless, the operational cost of biomass conversion to ethanol can be reduced by increasing the dry matter concentration above this level. However, the apparent viscosity of

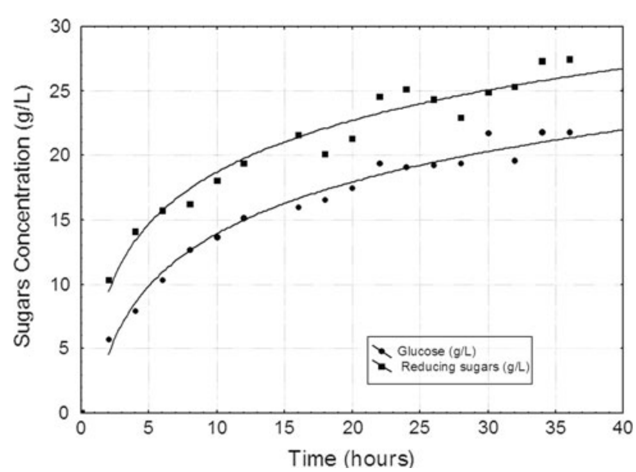

Fig. 1 Glucose concentrations during enzymatic hydrolysis

steam-pretreated sugarcane bagasse increased drastically—by at least one order of magnitude—when its concentration was increased from 10 to 20%, and such a high viscosity hinders mass transfer in the stirred tank reactor. To overcome this limitation and improve the economic performance of the enzymatic biomass conversion, Geddes et al. [7] suggested the blending of fresh acid-pretreated sugarcane bagasse (10% dry matter) with a slurry (10% dry matter) previously subjected to partial hydrolysis with cellulolytic enzymes. According to these authors, under specific conditions, the viscosity of this mixture did not increase significantly. The addition of chemical additives to reduce the yield stress and viscosity of pretreated corn stover slurries was evaluated by Knutsen and Liberatore [11]. A preliminary economic analysis suggested that the price of modifiers must be lower than $0.10 per kilogram to justify their use in this application.

As expected, the apparent initial viscosity of the mixture ($t = 0$) of 3.5 Pa s was very high. However, a sharp viscosity decrease was observed as the hydrolytic reaction progressed (Fig. 2). After 22 h, the viscosity of the hydrolyzed biomass presented asymptotic behavior and approached 0.007 Pa s. It was observed that the apparent viscosity of the mixture decreased as the shear rate increased. This behavior is characteristic of a pseudoplastic or Herschel–Bulkley fluid and can be explained by changes in the structure of long-chain molecules as the shear rate increases. The fibers of bagasse tend to align parallel to current lines, thereby reducing the flow resistance [25] and generating behavior typical of a Newtonian fluid. The same rheological behavior was reported by Pimenova and Hanley [18] for corn stover suspensions (5–30% dry matter). However, when considering a constant 10% dry matter concentration, the steam-pretreated sugarcane bagasse slurries presented a very high apparent viscosity (2.6 Pa s at 100 s^{-1}) as compared to acid-pretreated corn stover suspensions (0.02 Pa s at 100 s^{-1}). This difference could be related to the characteristics of the acid-pretreated material and to the small particle size of the corn stover fibers (125 μm). Viamajala et al. [24] attribute the considerable reduction in viscosity of corn stover slurries after acid pretreatment to the removal of hydrophilic components of biomass such as xylan and pectin.

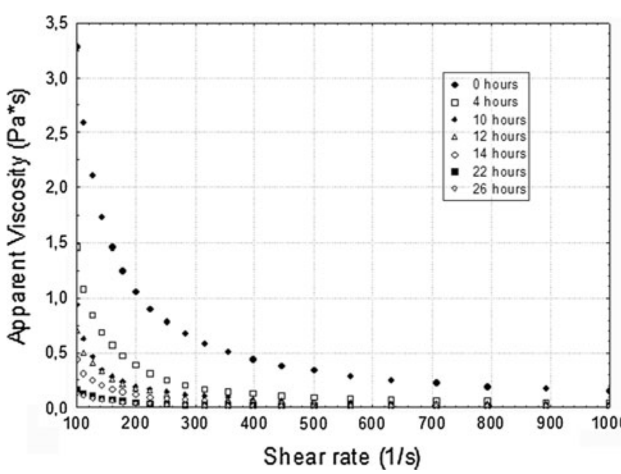

Fig. 2 Apparent viscosities of the enzymatic hydrolysis mixture at different shear rates

The sugarcane bagasse suspension presented pseudoplastic behavior in the region of intermediate shear rates (100–800 s^{-1}). This result is consistent with that of Stickel et al. [21] for corn stover suspensions (15% dry matter) at shear rates between 100 and 600 s^{-1}. According to Steffe [20], this region is the most significant when evaluating the mixing performance of a reactor.

As shown in Fig. 3, fitted curves show that an increase in rotation rate promotes a significant decrease in the apparent viscosity of the hydrolyzed material. At a fixed rotation rate, it was observed that the apparent viscosity decreased with the progress of the enzymatic reaction. These results show that the apparent viscosity of the mixture is dependent on the shear rate and strongly dependent on the hydrolysis time. This time-dependence of sugarcane bagasse slurry viscosity is closely related to carbohydrate hydrolysis and is consistent with rheological data obtained from acid-pretreated sugarcane bagasse [7].

The above results indicate that the rotational speed of the blender should be reduced continuously during the hydrolysis of sugarcane bagasse, according to an empirical exponential model. This control is important to minimize energy consumption in this step of the process.

The curves plotted in Fig. 4 show the effect of biomass conversion on the apparent viscosity of the mixture. It is apparent that the viscosity behavior is similar at rotation speeds within the range of 600–1,200 rpm. Within 8 h of starting the reaction, 26% of the initial cellulose content had hydrolyzed concomitant to an apparent viscosity decrease of around 68%. These results compare well to those reported by Geddes et al. [7], as the viscosity of acid-pretreated fiber slurries was reduced by 99% after 6 h of hydrolysis, corresponding to 17.6% of conversion.

Similarly to our data for sugarcane bagasse, Viamajala et al. [24] reported pseudoplastic behavior for corn stover biomass slurries. Nevertheless, the mechanism leading to pseudoplasticity is unknown. It is generally advantageous to have Newtonian fluid behavior for dilute slurries as the separation of particles avoids the effects of mutual interaction.

Table 1 shows the variation of Reynolds number as a function of hydrolysis time at 600 rpm, which was the velocity used in this study. The Reynolds number was estimated for a 100-l tank and a paddle diameter of 21 cm. The scaled-up mixing reactor dimensions were calculated

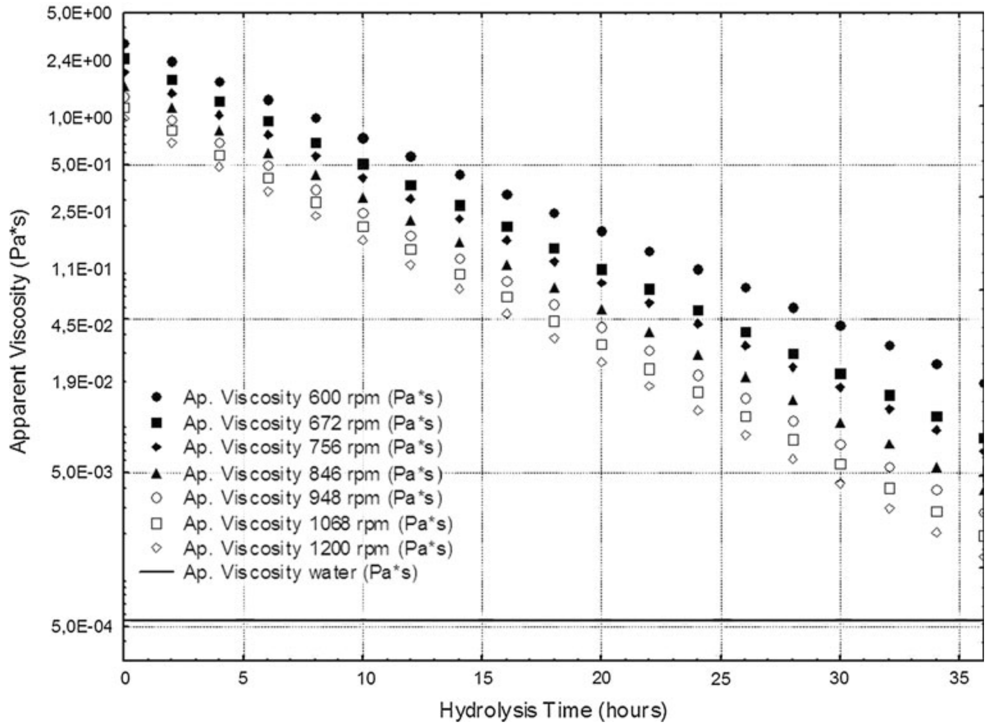

Fig. 3 Evolution of apparent viscosity as a function of the hydrolysis time

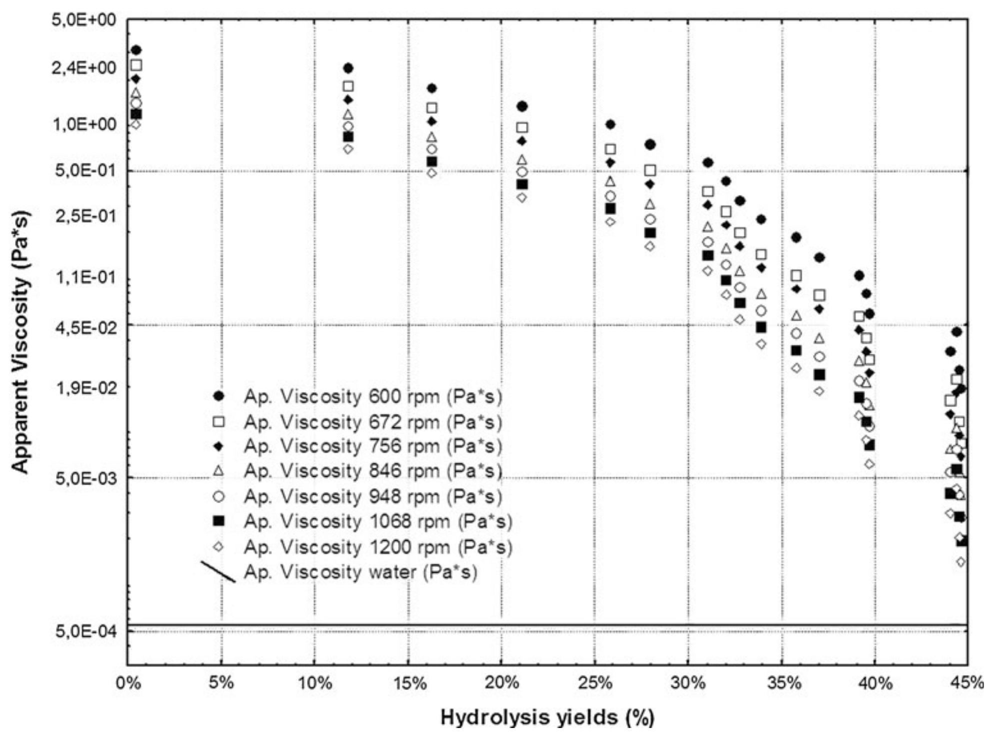

Fig. 4 Profile of apparent viscosity as a function of the hydrolysis yield

by geometric similarity. This analysis shows that at the beginning of the hydrolysis reaction, the bulk mixture presents a transition regime behavior. For a Reynolds number below 10,000 (e.g., at 600 rpm), the turbulent regime is reached after 30 h of hydrolysis. According to Wu et al. [26], a decreased Reynolds number produces an increased chance for energy and mass transport improvements.

Based on empirical correlations and experimental data regarding the apparent viscosity of sugarcane bagasse, the predicted power necessary to reach a homogeneous mixture using a pitched blade (45°) at 600 rpm, at the

Table 1 Evolution of Reynolds number as a function of reaction time

Hydrolysis time (h)	Reynolds number	Regime
0	144	Transition
2	190	
4	253	
6	336	
8	445	
10	591	
12	785	
14	1,042	
16	1,383	
18	1,835	
20	2,436	
22	3,234	
24	4,292	
26	5,697	
28	7,562	
30	10,037	Turbulent
32	13,323	
34	17,684	
36	23,472	

Table 2 Power consumption, at 600 rpm, of the selected mixing paddles

Hydrolysis time (h)	Cellulose conversion (%)	Power of mixing (W)–600 rpm	
		Pitched blade (45°)	Rushton turbine
0	0	446	4,512
2	12	408	2,988
4	16	375	2,026
6	21	344	1,408
8	26	315	1,003
10	28	288	731
12	31	261	546
14	32	235	418
16	33	209	327
18	34	184	263
20	36	160	216
22	40	138	182
24	39	118	157
26	40	100	139
28	40	84	125
30	45	70	115
32	45	58	107
34	45	49	101
36	45	41	97

beginning of the reaction, was ten times lower in comparison to the use of a Rushton turbine (Table 2). These empirical models were based on dimensionless Reynolds numbers and allowed for the power consumption of a stirred reactor tank to be estimated [16]. This result is consistent with previous reports in which the Rushton turbine demands more power than other mixing paddles. Biomass hydrolysis on an industrial scale is carried out at low rotation speeds. In this case, the apparent viscosity of the reaction slurry is high, and better performance may be expected from a pitched-blade (45°) impeller.

In addition, Wu et al. [26] concluded, in his work about the energy efficiency of axial flow impellers, that it is possible to improve the efficiency of impellers operating in a highly shear-thinning, viscous non-Newtonian fluid. As such, it was possible to achieve increased velocities at a given power input and tank diameter by optimizing impeller geometrical parameters. However, Kuzmanic and Ljubicic [12] showed that upon the increase of both the impeller diameter and the blade angle, the value of dimensionless mixing time decreased, concomitant, however, to the increase in power consumption. On the other hand, by increasing the off-bottom impeller clearance, a reduction of power consumption is commonly observed.

A Rushton turbine promotes a radial flow, which is appropriate for a turbulent mixing regime and for high power consumption, as shown in previous results (Table 1). However, besides requiring less power for mixing, the pitched blade (45°) produces an axial flow, which is usually required for the flow control of mixtures containing suspended solids. Furthermore, as suggested by Kuzmanic and Ljubicic [12], the use of an up-pumping pitched blade impeller may be a viable option for the maintenance of long processes with complete suspension of floating solids.

In the continuation of this work, the pitched-blade (45°) impeller will be tested for the enzymatic hydrolysis of sugar cane bagasse pretreated by milling and steam explosion with the aim of improving the hydrolysis yields. The membrane process of separating the sugar syrups from the lignin residue will also be studied.

Conclusions

The rheological behavior of the enzymatic hydrolysis mixture of steam-treated sugarcane bagasse at 100 g (dry weight)/l, using an enzyme load of 10 FPU/g of substrate, was characteristic of a pseudoplastic fluid. A pitched-blade (45°) paddle, when used to homogenize the sugarcane bagasse suspension, showed an energy consumption that was ten times lower than that for a Rushton turbine. After 36 h of enzymatic hydrolysis it was obtained sugar syrups presenting 22 g/l of glucose, which corresponded to 45% cellulose conversion.

Knowledge of the degree of conversion and the rheological characteristics of the mixture facilitated the selection of a suitable agitator for a reactor to be used in the enzymatic hydrolysis of sugarcane biomass.

Experimental data was fitted to an exponential model to evaluate the time-dependence of substrate viscosity, and this made it possible to predict the rotation speed at a given Reynolds number. As such, the rotation speed can be continuously reduced, using a process control tool, to minimize energy consumption.

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