Ultrasound-assisted acid hydrolysis of cassava (*Manihot esculenta*) bagasse: Kinetics, acoustic field and structural effects

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ABSTRACT

Improving the actual acid hydrolysis of cassava bagasse (CB) with the assistance of high-intensity ultrasound (US) was aimed in comparison with mechanical agitation (AG). The kinetics of reducing and total sugar release were mathematically modeled. The acoustic field characterization and apparent viscosity of the suspensions were correlated. Moreover, microscopic analyses (visible, fluorescence and polarized light) were carried out to identify changes produced by the treatments. Both AG and US-treatments showed themselves to be effective at hydrolyzing CB. However, US-experiments reached equilibrium in the reducing sugar release process earlier and obtained slightly higher values of total sugars released. The Naik model fitted the experimental data with good accuracy. A greater loss in the birefringence of the starch granules and the degradation of lignocellulosic matter was also observed in US-assisted hydrolysis. The actual acoustic power applied was reduced after hydrolysis, probably due to the increase in the apparent viscosity of the resulting suspensions.

1. Introduction

Ethanol has been considered one of the most suitable secondary biofuels to replace fossil fuels [1]. It can be produced through the fermentation of the sugars that compose the carbohydrates of some food industry residues, such as those generated from the processing of sugarcane, corn, or potato. In this sense, cassava (*Manihot esculenta*) bagasse represents an abundant source of agro-industrial waste in Brazil. The development and design of alternative treatments for this waste have become a must. Its residual starch content can range from 35 to 85% db as a consequence of the low yield of the cassava starch extraction process [2]. Moreover, this byproduct also includes a higher content of fiber (10–65% d.b).

The release of sugars of low molecular weight from this material is dependent on adequate pretreatments and hydrolysis. So, studies into alternative and eco-friendly processes have become worthy of research [3]. The pretreatment step, commonly carried out by chemical or physical methods or a combination of them, is responsible for breaking

and exposing the structure of lignocellulose and starch granules, making them more accessible to enzymes and chemicals [4,5]. An efficient pretreatment can provoke simultaneous alterations in both the structure and chemical linkages, leading to a partial hydrolysis and enhanced further enzymatic hydrolysis [6]. However, the differences of using one method or other are more accurately evaluated if the mechanisms of sugar dissolution are differentiate from the sugar production during the actual hydrolysis [7].

Of the chemical methods, alkaline treatments aim to solubilize the lignin and increase cellulose digestibility [8]. On the other hand, the use of acids, such as phosphoric acid, has demonstrated to be an effective means of modifying the structure of both lignocellulose and starch granules [7,9–11]. Phosphoric acid is reported to be less aggressive, producing less sugar degradation compounds as furfural and acetic acid at lower costs [12]. Gámez et al. [12] also highlights the potential use of sodium phosphate, obtained after phosphoric acid neutralization with sodium hydroxide, as nutrient for microbial growth during sugar fermentation. Although it can be used in concentrated

solutions, diluted and non-aggressive acids are preferred in this kind of pretreatments to avoid equipment corrosion and make acid recovery easier [13].

During the acid pretreatment, biomass suspensions can be stirred by conventional agitation at controlled temperatures to make it easy to transfer the acid to the internal matrix. On the other hand, alternative physical methods can be applied to enhance the effects of the acid solution on biomass and to allow the use of milder conditions of temperature, catalyst concentration, pressure and processing times commonly applied in literature [14,15]. High-intensity (or power) ultrasound has appeared as an interesting non-conventional technology for this purpose [16]. The sonochemical effects are linked to certain mechanisms produced when ultrasound travels across a medium with enough energy: cavitation, micro-agitation or heating. Cavitation occurs in liquid media caused by the collapse of the cavitation bubbles, creating molecular displacement throughout the successive zones of high and low pressure. The intensity of the collapse is able to cause the erosion of polymeric materials through the formation of micro-jets and enhance the mass transfer between liquid and solid media [17]. Ultrasound can also promote micro-agitation inside the particles and acoustic streaming at interfaces [18]. Moreover, heating is a consequence of the resistance forces against acoustic vibration. This effect is useful for measuring the real acoustic power applied and for determining the proportion of acoustic energy transferred to the medium, this being important information for the purposes of reproducing similar conditions and scaling up the process [19].

Therefore, the main goal of this paper was to study the ultrasonically assisted acid treatment of cassava bagasse under mild conditions of temperature and phosphoric acid concentration to evaluate their influence on the kinetics of sugar release and the structural effects.

2. Materials and methods

2.1. Sample preparation

In this study, the residue from the cassava (*Manihot esculenta* C.) starch extraction process was used. Wet bagasse was provided by TechnoAmido (São Pedro do Turvo, Brazil), a starch extraction industry. Thus, the wet bagasse was dried at 55 °C for 48 h. The resulting coarse powder was milled in a domestic grinder to be, then, sieved to obtain samples with a particle size of under 1 mm. This material presented a moisture content of $4.18 \pm 0.22\%$ (w. b.), determined in triplicate in a vacuum oven at 60 °C until constant weight (about 24 h). The chemical composition of the dried matter was characterized in a previous study [20].

2.2. Experimental plan

The experimental plan included two sets of experiments. The first one studied the dissolution of the free sugars present in cassava bagasse. In the second one, an analysis of the acid hydrolysis treatment was carried out. In the latter, mild conditions were tested in order to avoid the use of corrosive acids and temperatures that might cause the gelatinization of the suspensions, creating difficulties in the mass transfer. For this reason, the temperature of the test was maintained at 50 °C to enhance the acid actuation, but keeping the temperature below the gelatinization temperature of cassava starch [21] without significant impairment in the cavitation activity [22]. All of the experiments as well as the different chemical determinations were performed in triplicate.

2.2.1. Sugar dissolution experiments (SD)

For this set of experiments, 5% cassava bagasse suspensions in distilled water (g of dried cassava bagasse per 100 g of suspension) were prepared. This biomass concentration was established because it was the maximum concentration that allowed an adequate mixing and

coupling between the sample-sonotrode during the ultrasound-assisted process. Above this concentration, the suspensions assume non-Newtonian behavior and deviations from linearity should be also considered [20]. After that, the suspensions (150 g each one) were subjected to conventional agitation for 5 h at 50 °C in order to determine the amounts of total and reducing sugar in the cassava bagasse that were directly soluble (experiments B5AG0). At the preset times, the samples were extracted and total and reducing sugar determinations were carried out to obtain the kinetics of the process.

2.2.2. Acid hydrolysis experiments (AH)

These experiments were carried out in two steps. Firstly, 5% (g of cassava bagasse per 100 g of suspensions) cassava bagasse suspensions were agitated for 4 h at 50 °C in the same way as that described for SD experiments. This was long enough to ensure the total dissolution of the free soluble sugars present. After that, metaphosphoric acid (GPR Rectapur, VWR Chemicals, Lutterworth, UK) was added to the aqueous suspensions to obtain an acid solution of 10% (g of acid per 100 g of solution). This concentration was based on previous studies that concerned the hydrolysis of starchy and lignocellulosic feedstock [7,9], aiming at obtaining interesting effects without acid over-dosage by working below the gelatinization temperatures. These suspensions (150 g by sample) were maintained under conventional agitation (B5AG10-A experiments) or high-intensity ultrasound application (B5US10-A experiments) for 1 h. Samples were extracted at preset times and total and reducing sugar determinations were performed.

2.3. Conventional agitation (AG) system

Acid treatments under conventional agitation were performed in a jacketed vessel using an agitator (Heidolph RZR1; Heidolph Instruments GMBH & Co., Schwabach, Germany). This equipment was adjusted to work at 1000 rpm in order to obtain an intense stirring in the suspensions. The suspensions were maintained at 50 °C during the treatments by placing the reaction vessel into a thermostatic bath (Frigedor, J.P. Selecta, Barcelona, Spain). Fig. 1 shows some shape parameters (F_1, F_2, F_3, F_4 and F_5) that characterize the agitation of the suspension induced by the system.

2.4. High-intensity ultrasound (US) system

A scheme of the experimental set-up used in the ultrasound-assisted hydrolysis experiments is presented in Fig. 2. The equipment consisted of an ultrasonic probe-type system (UP400S, Dr. Hielscher, Teltow,
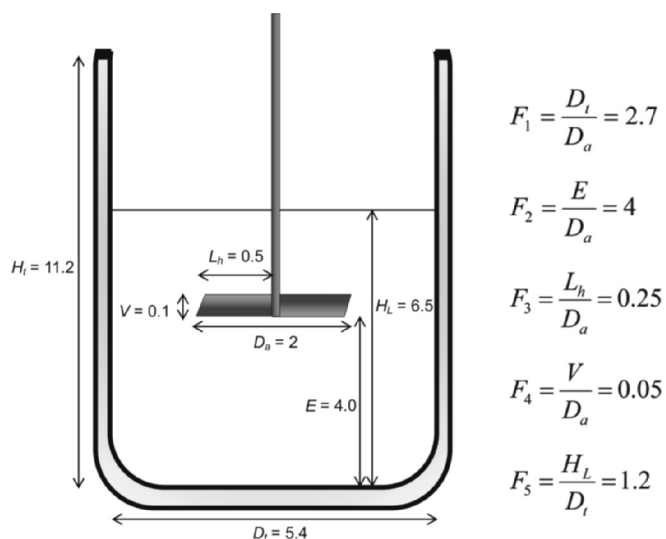

$$F_1 = \frac{D_t}{D_a} = 2.7$$

$$F_2 = \frac{E}{D_a} = 4$$

$$F_3 = \frac{L_h}{D_a} = 0.25$$

$$F_4 = \frac{V}{D_a} = 0.05$$

$$F_5 = \frac{H_L}{D_t} = 1.2$$

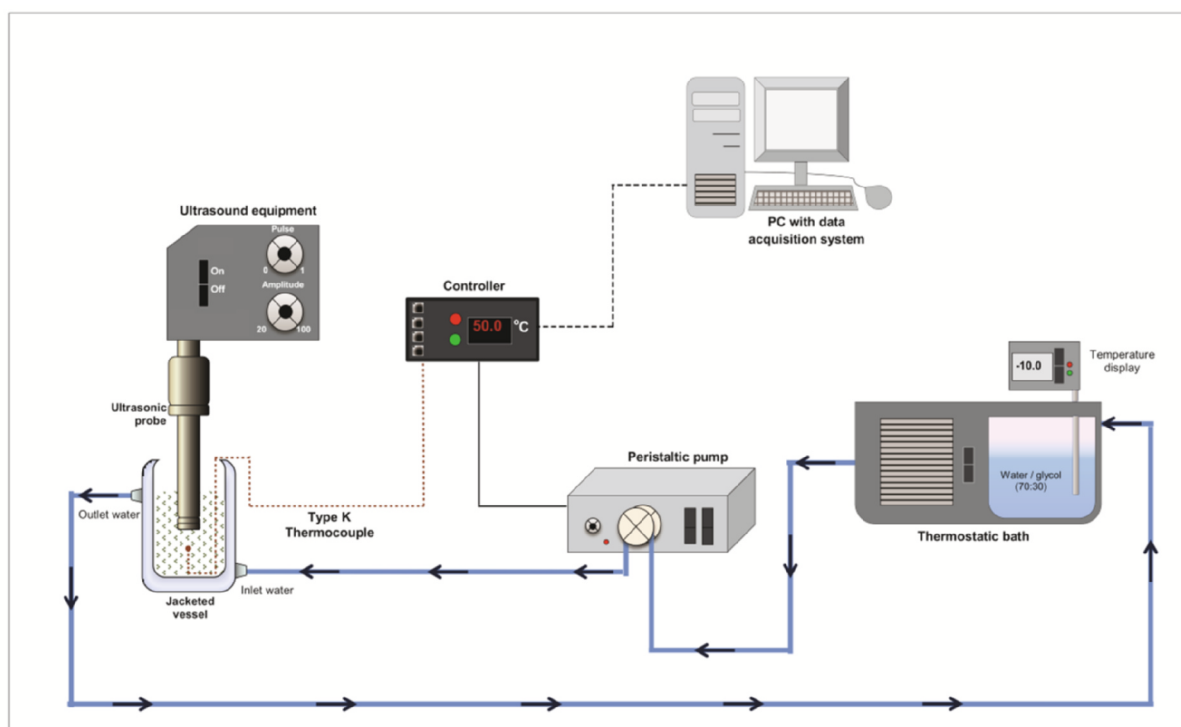**Fig. 1.** Set-up used in the experiments under conventional agitation.

Fig. 2. Scheme of the set-up used in the ultrasound-assisted hydrolysis.

Germany) supplied with a 22 mm diameter emitter probe (irradiation surface of 3.8 cm^2). The probe was immersed 1 cm into the acid suspensions, which were placed in a jacketed vessel. The tests were carried out at the maximum electrical power input of the system (400 W) in pulsed mode (0.6 s on − 0.4 s off). The temperature was maintained constant (50 ± 2 °C) during the experiments to prevent overheating. To this end, the temperature of the suspensions was recorded with a K type thermocouple placed 2 cm below the probe. The thermocouple was connected to a process controller (E5CK, Omron, Hoofddorp, Netherlands) which drove a peristaltic pump (302 S, Watson-Marlow, Postfach, Germany) to recirculate a 30% glycol solution at − 10 °C from a refrigerated bath (Frigedor, J.P. Selecta, Barcelona, Spain) through the jacketed recipient containing the suspensions (Fig. 2).

2.5. Sugar determination

Aliquots of approximately 3 mL were taken every 60 min in the case of the B5AG0 experiments (0, 60, 120, 180, 240 and 300 min), and every 15 min in the case of the B5AG10 and B5US10 experiments (0; 15; 30; 45 and 60 min). The acid aliquots were neutralized with NaOH 2.5 M, to be subsequently centrifuged and filtered in PTFE micro filters (0.45 μm, 25 mm diameter; LabBox, Barcelona, Spain). The supernatant was used for the different sugar content measurements. Thus, the reducing sugars (S_R) were determined in triplicate using the DNS method, as proposed by Kassaye et al [3]. This is a rapid and practical method, which is based on the reduction, in alkaline medium, of 3,5-dinitrosalicylic acid. The absorbance of the resulting compound was measured at a wavelength of 540 nm and the reducing sugar content was quantified by using a standard curve of anhydrous glucose, previously determined. The data were expressed as g of reducing sugar (glucose equivalent) per 100 g of dried cassava bagasse.

The total amount of hydrolysable sugars (S_T) was determined by the same method but including a previous hydrolysis step [15]. Thus, samples were placed into HCl 2 M solution and maintained under boiling for 30 min. After that, they were cooled and neutralized with NaOH 2.5 M. Then, the hydrolyzed samples were submitted to the DNS method to determine the total amount of fermentable sugar (glucose

equivalent) per 100 g of dried cassava bagasse.

The values obtained by both methods were used to calculate the relative sugar release, Y (Eq. (1)):

$$Y = \frac{([S]_t - [S]_{t=0})}{[S]_{t=0}} \tag{1}$$

where $[S]_t$ represents the sugar released at time t and $[S]_{t=0}$ the sugar released at the very beginning of the experiment.

2.6. Mathematical modeling

The Naik model [23], given by Eq. (2), was used to fit the kinetics of sugar release. This model is reported to represent not only the extraction kinetics of food compounds [24] but also the sugar release during hydrolysis of byproducts [7] with good accuracy:

$$Y = \frac{Y_\infty t}{(B + t)} \tag{2}$$

where t is the treatment time (min), Y_∞ is the relative sugar release at equilibrium and B is the time needed (min) to reach half of Y_∞, which means a measurement of the process kinetics. The goodness of the fitting procedure was evaluated by the adjusted determination coefficient (R_{adj}^2) and the root-mean-square error ($RMSE$), given by Equations (3) and (4), respectively.

$$R_{adj}^2 = 1 - \frac{n - 1}{n - (k + 1)}(1 - R^2) \tag{3}$$

$$RMSE = \sqrt{\frac{\sum\limits_{n=1}^{n}(y_p - y_{exp})^2}{n}} \tag{4}$$

where n is the number of observations, k is the number of parameters in the model (excluding the constant), R^2 is the determination coefficient, y_p is the predicted value and y_{exp} is the experimental value.
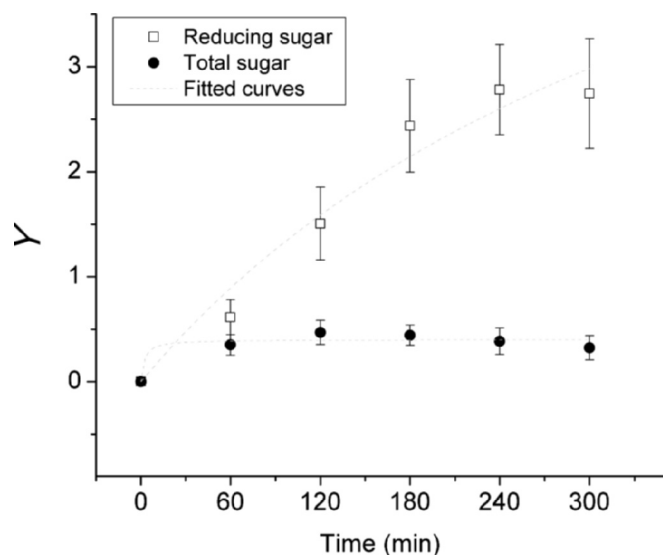
Fig. 3. Relative release of total and reducing sugar concentration over the agitation of cassava bagasse in aqueous medium.

2.7. Apparent viscosity

The apparent viscosity of the suspensions before and after the acid hydrolyses was measured under steady-state flow in a rotational rheometer AR-G2 (TA Instruments, USA) coupled with the Starch Pasting Cell (SPC) geometry to avoid particle sedimentation. The shear stress was obtained over an interval of shear rate between 1 and 265.5 s^{-1}, maintaining the temperature at 50 °C by means of the thermostatic bath of the equipment itself. The flow curves were analyzed to determine the apparent viscosity over the range of shear rate.

2.8. Acoustic field characterization

A calorimetric method was employed to characterize the actual acoustic power applied in each case, as seen as the use of hydrophone for measuring acoustic pressure was unfeasible due to their sensitivity to acids and interference of the suspended particles. For this purpose, the temperature increase in the first 60 s of ultrasound application [22] was recorded with a K type thermocouple placed in the suspensions 2 cm below the probe and connected to a data acquisition system (LabVIEW Run-Time Engine 7.0, National Instruments, USA). The measurements were taken in triplicate before and after 60 min of hydrolysis treatment in order to detect any influence of the treatment on the actual ultrasonic power applied. The temperature records were taken using the same experimental set-up used for US-assisted hydrolysis experiments. So, the acoustic power applied can be calculated by Eq. (5):

$$P = mc_p \frac{dT}{dt} \tag{5}$$

where P is the acoustic power (W), m is the mass of the suspension (kg), c_p is the specific heat capacity of the acid solution (4064.8 J·kg^{-1}·°C^{-1}, determined by differential scanning calorimetry) and dT/dt is the heating rate (°C·s^{-1}). The acoustic intensity (I, W·cm^{-2}) was calculated by considering the acoustic power per area of the emitter surface; the acoustic density per treated volume (D; W·mL^{-1}) and the acoustic load per treated mass of particles (W·g^{-1} of dried biomass).

2.9. Microscopic analysis

The morphology of the raw cassava bagasse and the product obtained after the hydrolysis treatments were microscopically analyzed in order to identify changes attributed exclusively to the acid treatment. To separate and to identify the effects on the fibrous and starchy fraction different methodologies were considered to avoid erroneous conclusions. For this purpose, a Nikon Eclipse E80i (Nikon, Tokyo, Japan) optical microscope was used. To evaluate any possible alterations in the fibrous particles of the bagasse, untreated and treated samples were placed on a glass slide and stained with toluidine blue (0.1%) to be observed using bright field mode. Non-stained samples were observed by bright field, fluorescence and polarized light microscopy in order to evaluate possible alterations to the starch granules' structures and to their birefringence.

All of the images were captured and stored by NIS-Elements F software (Version 4.0, Nikon, Tokyo, Japan).

3. Results and discussion

3.1. Sugar dissolution

The SD experiments allowed the kinetics of reducing and total free sugar release in aqueous medium to be quantified. The results showed significant amounts of free sugars remaining in cassava bagasse, which may be explained because the main goal of cassava industrialization is the extraction of starch. These compounds are easily fermented for the purposes of producing bioethanol. As expected, the amount of total sugars was greater than that of reducing sugars. As regards the kinetics, the maximum total sugar content was reached after 2 h of treatment (Fig. 3). On the contrary, the reducing sugars needed 4 h to achieve asymptotic behavior. Therefore, 4 h of agitation was considered to be enough to attain dissolution equilibrium for both. After this period of time, cassava bagasse released 3.30 ± 0.26 g of reducing sugars per 100 g of dried CB and 4.87 ± 0.04 g of total sugars per 100 g of dried CB, which correspond to a relative release of 2.78 ± 0.43 and 0.39 ± 0.13, respectively (Fig. 3).

3.2. Acid hydrolysis

The results obtained in SD experiments showed that there was a significant amount of soluble sugars, total and reducing, in CB. For this reason, for AH experiments, the CB was previously treated for 4 h in a water suspension. Thus, soluble sugars were extracted from the CB matrix prior to the acid treatment and, consequently, it was possible to quantify the actual effect of acid treatment on the CB. The experimental kinetics of the relative production of reducing and total sugars during AH experiments carried out under agitation (AG) or with ultrasound application (US) are shown in Fig. 4.

Thus, the AH treatment demonstrated that it was relatively efficient at hydrolyzing cassava bagasse, with a continuous release of reducing and total sugars throughout the time tested. Phosphoric acid acts on the α-1,6 bonds when hydrolyzing starch-based feedstock [14], debranching and opening up the starch structure.

The experimental kinetics showed that, for reducing and total sugars, the process was slightly faster in the case of US-assisted experiments (B5US10-A) than when using conventional agitation (B5AG10-A). In fact, after 30 min of hydrolysis, the release of reducing and total sugars was respectively 52.0 and 69.3% higher for the experiments with ultrasound than with mechanical agitation. In addition to the current hydrolysis and possible side effects on the biomass structure, Li et al. [25] found that the ultrasound pretreatment was able to enhance the liquefaction and saccharification of starch-based materials. The enhancement of hydrolysis by US could be attributed to the disruption of the rigid hydrophobic protein matrix and the amylose–lipid complex surrounding the granules [26]. However, the relative release of reducing sugars by US-assisted (B5US10-A) and conventionally agitated (B5AG10-A) experiments reached similar values after 60 min of hydrolysis, around 25% of the initial reducing sugar content for both processes. Although both technologies could hydrolyze cassava bagasse,
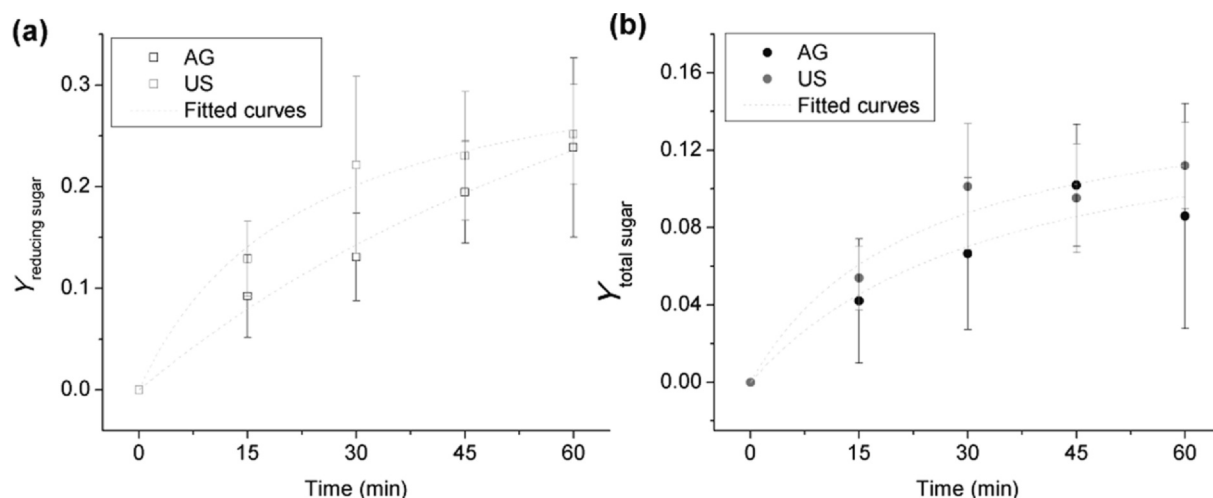
Fig. 4. Relative production of (a) reducing and (b) total sugars during the acid hydrolysis of cassava bagasse (CB) assisted by conventional agitation (AG) or by ultrasound application (US).

ultrasound is considered a useful tool for reducing the processing time and increasing productivity.

In the case of total sugar release after 60 min of treatment, a 30% higher yield was observed in B5US10-A experiments (11.2%) than in B5AG10-A (8.6%) ones. On the contrary, after hydrolyzing yam with 5% phosphoric acid at 50 °C for 300 min, Ramón et al. [9] found a 14% greater release of fermentable matter in experiments carried out in an ultrasonic bath when compared to non-ultrasonically assisted experiments. It is in agreement to the observation that ultrasound accelerated the acid hydrolysis, but prolonging the irradiation cannot be feasible due to increased energetic costs [27].

The values for total sugars are lower than the ones found for reducing sugars. This may be expected because the hydrolysis of the total sugars in solution into reducing sugars is faster than the hydrolysis of the fermentable matter trapped in the cassava bagasse structure into soluble total sugars. The fact that the differences observed between US and AG treatments were not significant is likely to be due to the variability in the sugar content of the raw matter. The post-harvest physiological deterioration of the cassava roots occurs rapidly, within 24 h, which can impair the homogeneity of the resulting samples [28,29].

3.3. Mathematical modeling

The Naik model was fitted to the kinetics of sugar release by dissolution in aqueous medium (SD experiments) and by acid hydrolysis (AH experiments). The fitting parameters for the reducing and total sugars are shown in Table 1. A close correlation between the experimental and those calculated with the model data were found, the R_{adj}^2 being higher than 0.8715 and the low root-mean-square error (RMSE) lower than 0.2074.

As for the parameters identified for the acid hydrolysis experiments, the higher release yield (Y_∞) of the reducing sugars found in the

experiments carried out using suspension agitation (B5AG10-A) indicated that the yield achieved with this kind of treatment could be higher than when using US treatments (B5US10-A) if the process time was prolonged. Mathematical modeling gives insights that ultrasound effects are weakened over the acid hydrolysis likely due to changes occurring in the ultrasound actuating mechanisms and in the product itself. Indeed, such changes were more deeply investigated in Section 3.4, Section 3.5 and Section 3.6. On the contrary, US application increased the hydrolysis kinetics, as showed by the 80% reduction of B value found in US experiments (B5US10-A) when compared to AG (B5AG10-A), which is very interesting from an industrial point of view. As far as the fitting parameters identified for total sugars are concerned, they presented similar values in both US and AG experiments.

3.4. Apparent viscosity

After the hydrolysis experiments, the suspensions passed from liquid-like to paste-like in appearance. Because of this reason, working with higher biomass concentration lead to bad coupling between the sonotrode and the sample and poor ultrasound propagation. During the treatments, some compounds could pass from the biomass structure into the solution. Thus, the solubilization of these compounds may be responsible for the increase in the apparent viscosity of the suspensions. The faster equilibrium attained in US-assisted hydrolysis could also be linked to a reduction in the actual acoustic power throughout the treatment produced by this increase in the apparent viscosity of the suspension. In fact, Polachini et al. [7] observed an enhancement in the ultrasound effects when decreasing the biomass concentration of the suspensions – and, consequently, their viscosity – during acid hydrolysis of artichoke residues. To confirm this hypothesis, rheological measurements were taken to quantify the apparent viscosity before and after the acid treatment under agitation or under ultrasound application (Table 2). Flow curves indicated that apparent viscosity was practically

Table 1
Fitting parameters of the Naik model for the different treatments.

	Reducing sugars			Total sugars		
	B5AG0	B5AG10-A	B5US10-A	B5AG0	B5AG10-A	B5US10-A
Y_∞	7.2051	0.6780	0.3520	0.4009	0.1556	0.1525
B	424.2585	112.4069	22.4413	2.0547	23.2993	35.1955
R_{adj}^2	0.9533	0.9879	0.9819	0.8715	0.9551	0.9171
$RMSE$	0.2074	0.0008	0.0109	0.0498	0.0076	0.0089

Table 2
Apparent viscosity of the acid suspensions before and after acid hydrolysis.

Experiment	Apparent viscosity (mPa·s)	
	Before Hydrolysis	After Hydrolysis
B5AG10-A	1177 ± 9[c]	1341 ± 10[b]
B5US10-A		1746 ± 3 [a]

Means and standard deviation followed by the same lowercase letters signify there are no significant differences between the measurements according to the Fisher test at the 95% confidence level.

constant in the range of shear rate applied ($R^2_{adj} > 0.9986$), characterizing Newtonian behavior in the conditions being studied.

The measurements indicated that the hydrolysis of the suspensions in the presence of ultrasound led to higher viscosity values when compared to those mechanically agitated. This pointed to the fact that changes in the cassava bagasse structure occurred more intensely in US-assisted treatments. Yang et al. [30] reported that ultrasound can increase the surface roughness of the starch granules, which leads to higher friction and, consequently, to an increase in the viscous properties of the suspensions.

In this sense, it is important to state that US-assisted hydrolysis needs to be properly mixed for the suspensions to be able to provide homogenous treatment to the biomass particles throughout a certain period of processing; this mixing energy is supplied by acoustic agitation.

It is useful to characterize the evolution of the apparent viscosity throughout hydrolysis, not only as regards the release of compounds of interest, but also for the purposes of carrying out a correct design of the unit operations and the equipment [31]. As the material becomes more viscous, a greater amount of energy is demanded in order to promote the same degree of agitation that would be provided to less viscous fluids. Additionally, the viscosity of the medium can prevent cavitation from occurring. Therefore, the process should be designed with a greater energy demand in mind if a similar degree of cavitation is required throughout the process.

3.5. Acoustic field characterization

The sonochemical effects of ultrasound are related to the real acoustic power transmitted to the medium. In order to characterize this energy, acoustic parameters were determined by the linear increase in temperature throughout the initial periods of ultrasound application.

Table 3 summarizes the acoustic parameters obtained by the ultrasound-assisted hydrolysis before and after 60 min of the acid treatment. For every studied parameter, the values found before the treatment were higher than those found after. During acid hydrolysis, fermentable matter is leached from the grains and from the lignocellulose. These compounds could be dispersed in the liquid medium without much alteration in the volume of solid biomass, contributing to the increase in the apparent viscosity (Table 2) and to the observed paste-like appearance of the suspensions.

The increase in the flow resistance reflects a greater interaction between molecules and more difficulty in promoting adequate cavitation [18,32]; the milder the cavitation activity, the lower the degree of erosion on the liquid–solid interface. Thus, extending the sonication time throughout the 60 min in this system could reduce the efficiency of the energy transfer, leading to wear in the equipment and sonotrode. Montalbo-Lomboy et al. [27] also stated that longer sonication periods can make the process more onerous, with no big differences in the release of fermentable matter.

Table 3
Acoustic parameters obtained from the determinations of the acoustic fields.

Acoustic parameters	B5US10-A experiment	
	Before	After
Acoustic power (W)	148.202 ± 2.070 [a]	77.129 ± 19.499 [b]
Acoustic intensity (W·cm^{-2})	38.987 ± 0.544 [a]	20.290 ± 5.130 [b]
Acoustic density (W·mL^{-1})	0.988 ± 0.011 [a]	0.514 ± 0.130 [b]
Acoustic load (W·g^{-1} of dried cassava bagasse)	20.586 ± 0.235 [a]	10.714 ± 2.709 [b]

Means and standard deviation followed by the same lowercase letters in the same line signify there are no significant differences between the measurements according to the Fisher test at the 95% confidence level.

3.6. Microscopic analyses

3.6.1. Effects on the fibrous matter

The morphology of cassava bagasse was qualitatively assessed by different techniques of optical microscopy. Firstly, an analysis of the general structure of raw cassava bagasse was performed in order to compare it with the treated samples. Fig. 5 presented the light micrographs of both the raw material and the partially hydrolyzed cassava bagasse, with agitation and with ultrasound application. The staining technique using toluidine blue imparted metachromatic properties to the samples treated using the different technologies. Shades of blue or green indicate the presence of phenolic compounds, such as lignin [33]. It could be easily observed that the larger fibers are mainly made up of cellulose and hemicellulose with the presence of lignin. These blue/green structures apparently exhibited more integrity in the untreated cassava bagasse than in the treated, which could be related to the lower viscosity observed for the untreated suspensions. Moreover, in the US-treated samples, the fibrous structure seemed to have been subjected through a shearing process in which the surface became less irregular when compared to the untreated or the AG treated samples. Small pieces of fibrous matter could also be observed in the bagasse hydrolyzed by US, indicating that ultrasound could have an effect on biomass fragmentation. In fact, it could be responsible for the greater increase in the viscosity of the suspensions treated with US.

Despite the bluish structures, regions could be seen that were purple in color. Santos et al. [33] related these shades of purple with primary structures of cell walls, such as cellulose, hemicellulose and pectin. In the raw sample, these regions appeared dispersed around the fibrous particles, in all likelihood as a consequence of the milling process. This empurpling of the material was not noticed in the bagasse hydrolyzed under agitation to the same degree as occurs in the raw material, which gives an indication that free cellulose and hemicellulose could be hydrolyzed by the acid. On the other hand, bagasse hydrolyzed in the presence of ultrasound exhibited a higher concentration of empurpled materials dispersed in the sample as a consequence of cell wall disruption [34]. As the yield of sugar release was similar to conventionally agitated samples, it is possible to infer that ultrasound was able, not only to hydrolyze the free polysaccharide, but also to retrieve part of the fermentable matter from the lignocellulose structure.

3.6.2. Effects on the starch granules

A high concentration of starch granules could be observed both around and entangled in the fibrous matter (Fig. 5), reflecting the fact that cassava bagasse is a potential source of fermentable matter. It reinforces the need to evaluate the effects of both processes on the structure of starch granules.

In Fig. 6, there are images obtained by bright field, fluorescence and polarized light microscopy of the starch granules before and after acid treatments at 20x of magnitude. Starch granules in raw cassava bagasse presented regular surfaces with the typical morphology of cassava starch: oval, truncated and rounded [35]. In this sample, no significant alterations could be evidenced. The subsequent application of fluorescence and polarized light exhibited the birefringence properties of starch granules. The high light intensity of the Maltese cross in the grains was an evidence of strong birefringence, even after 4 h of prior agitation in aqueous medium.

When cassava bagasse was subjected to acid hydrolysis under conventional agitation, the alterations were not easily detectable by observation under bright field mode. On the other hand, a reduction in the granules' birefringence could be observed, as indicated by white arrows. It may be stated that agitation could promote adequate contact of phosphoric acid with the bagasse, affecting the crystalline structure of the starch granules.

The application of ultrasound during the acid treatment led to a clearer degradation of the starch granules observed through bright field mode. The effects of acid seemed to be intensified by ultrasound
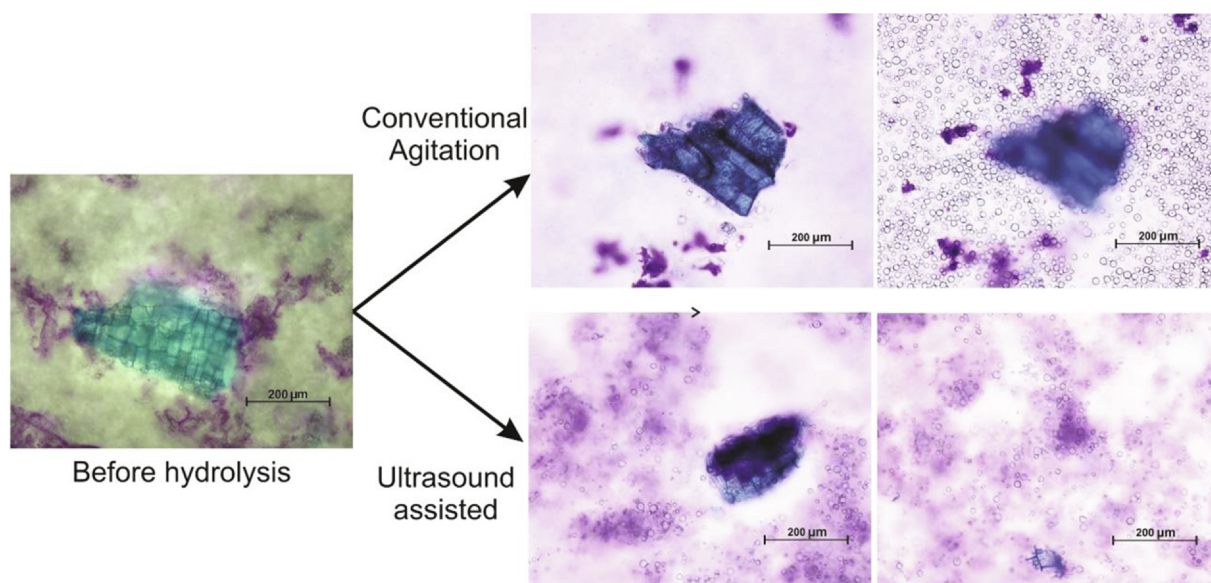
Fig. 5. Bright field micrographs of cassava bagasse before and after acid treatment under conventional agitation or assisted by high-intensity ultrasound (10x).
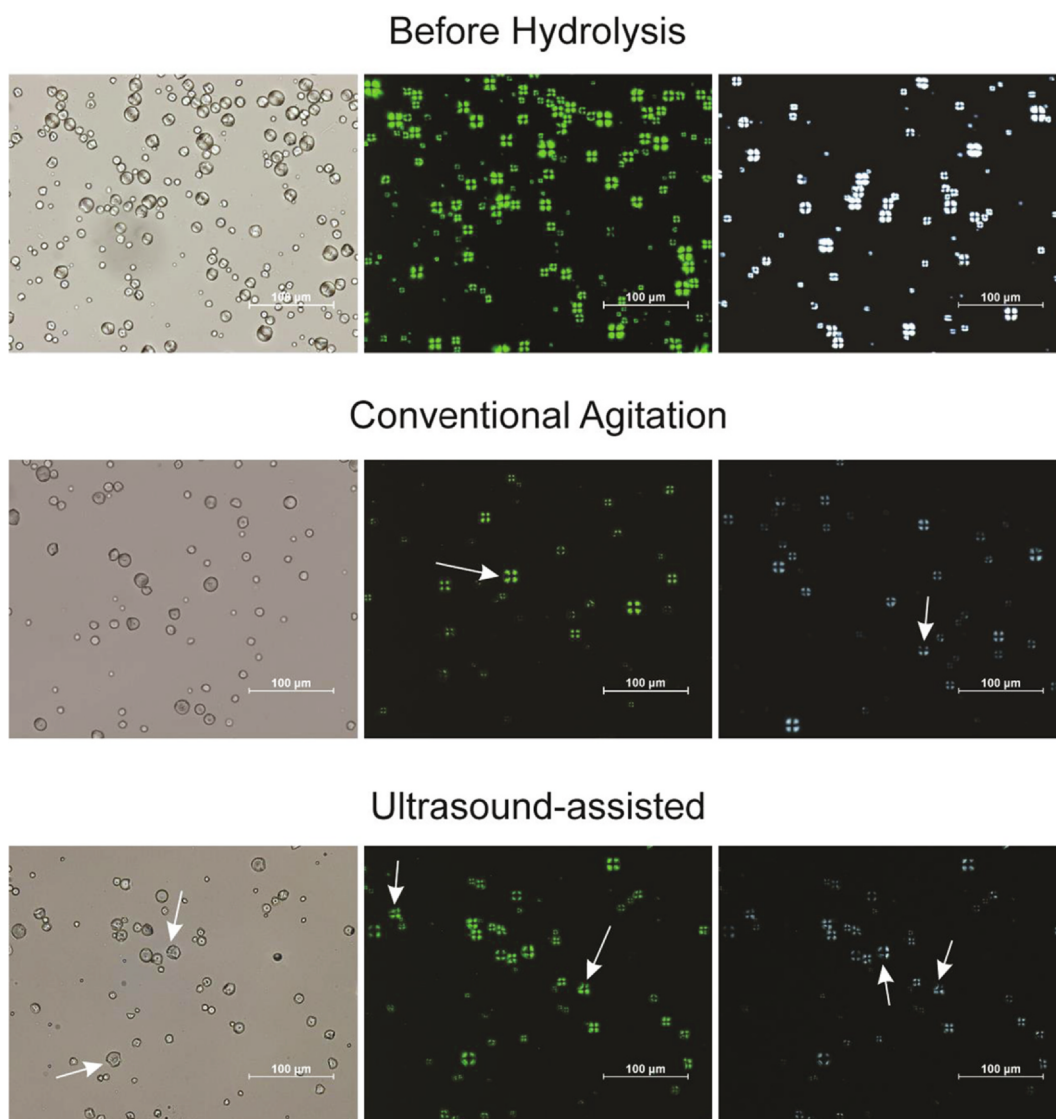

Fig. 6. Bright field (left), fluorescence (middle) and polarized light (right) micrographs of starch granules from cassava bagasse before and after acid treatments under conventional agitation or by high-intensity ultrasound (20x).

application. There were cracks and surface irregularities in the granules, which were not observed in the raw bagasse or in the hydrolyzed samples under mechanical agitation. Monroy et al. [21] reported that ultrasound has a more pronounced effect on the surface roughness of the cassava starch than on the shape itself. The authors attributed these alterations to the collapse of the cavitation bubbles, which cause high pressure gradient and shear forces in the surrounding area to depolymerize amylose and amylopectin the longer the process carries on. This degradation generates micro channels in the granules, which are useful for enhancing digestibility by glucoamylase, for example [36].

Analogously, as the interaction between enzyme and starch was reported to be enhanced by US, the same mechanism could be applied to phosphoric acid in solution. The presence of micro-pores in the granule may be responsible for intensifying acid penetration and promoting structural disorganization. Sujka and Jamroz [37] also observed the depolymerization of different starches, with the consequent changes in morphology and swelling properties caused by the increase in water absorption after ultrasonic treatment.

These alterations are reinforced by a greater decrease in the degree of birefringence of US-treated bagasse when compared to that conventionally agitated, observed in fluorescence and polarized light microscopies. According to Muñoz et al. [38], the granule swelling is preceded by a rapid loss of birefringence and tends to continue even after the complete loss of birefringence. The Maltese cross presented a gradual decrease from the hilum towards the surface as a consequence of the absorption of the acid solution by the granules. It is a result of the greater loss of radial organization of amylose and amylopectin chains [39]. Tao et al. [40] also observed loss of birefringence by the technique of polarized light microscopy throughout the gelatinization of starch granules.

These findings concur with the observations made in the stained fibrous matter and with the kinetics data of sugar release, which demonstrated a more pronounced effect of ultrasound than agitation on hydrolyzing solid particles. The degradation of the starch surface by cavitation indicates that there is a significant release of carbohydrates from the granules and better accessibility to chemicals and enzymes could be acquired in further steps of bioethanol production.

4. Conclusions

Ultrasound application enhanced the hydrolysis of cassava bagasse in the presence of phosphoric acid by increasing the kinetics of the process. Thus, compared to experiments carried out with agitation, the sugar release of ultrasonically assisted experiments was 52–70% higher after only 30 min of processing. The Naik model adequately fitted the experimental kinetics of sugar release, indicating a decrease of ultrasound influence at the end of the process. The reduction in the acoustic power produced by the increase in viscosity could weaken the ultrasound effects.

Conventional agitation and US application provided a good interaction between the catalyst and the biomass, reducing the birefringence of the starch granules in both processes. In addition, US brought about a slight change in the fibrous matter and a more pronounced disorganization of the crystalline structure of the starch granules, which could enhance the later accessibility to chemicals and enzymes in further steps of bioethanol production.

Therefore, ultrasound technology represents an alternative means of treating biomass for the purposes of altering the structure and releasing fermentable matter. It may also account for a shortening of the processing time and a reduction in chemical usage in further steps involved in bioethanol production.

CRediT authorship contribution statement

Tiago Carregari Polachini: Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing,

Visualization. **Isabel Hernando:** Methodology, Formal analysis, Investigation. **Antonio Mulet:** Resources, Writing - review & editing, Supervision, Funding acquisition. **Javier Telis-Romero:** Conceptualization, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Juan A. Cárcel:** Conceptualization, Methodology, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2020.105318.

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