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Challenges to Levulinic Acid and Humins Valuation in the Sugarcane Bagasse Biorefinery Concept

Emília Savioli Lopes¹ · Jean Felipe Leal Silva¹ · Elmer Ccopa Rivera^{1,2} · Alana Petrina Gomes³ · Melina Savioli Lopes⁴ · Rubens Maciel Filho¹ · Laura Plazas Tovar³

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Abstract

Levulinic acid (LA) is currently one of the most promising chemicals derived from biomass. However, its large-scale production is hampered by the challenges in biomass hydrolysis and the poor selectivity due to the formation of humins (HUs). This study addresses these challenges using the biorefinery concept of biomass fractionation. A three-step process (pretreatment, delignification, and acid-catalyzed conversion) was optimized to produce LA from SCB considering the yield (Y_{LA}), efficiency (E_{LA}), and concentration of LA (C_{LA}) as functions of temperature, reaction time, acid concentration, and solids loading. By means of a multi-response optimization, values of Y_{LA} (20.9 ± 1.25 g/100g_{ISF-D}), E_{LA} (37.5 ± 2.24 mol%), and C_{LA} (25.1 ± 1.50 g/L) were obtained at 180 °C, 75 min, 7.0% w/v H₂SO₄, and 12.0% w/v of solids loading. Six scenarios for production of LA were analyzed in terms of yields of LA, HUs, lignin, and other sugar-derived products considering one-, two-, or three-step processes. The economic analysis indicated that the three-step scenario delivers better economic figures given that other valuable biomass fractions (hemicellulosic sugars and lignin) are better used and contribute to the overall economic performance of the process. The results also demonstrate the burden of HUs in the economics of the process because it was shown that the largest production of LA is also linked to the largest formation of HUs, which does not necessarily yield the best economic results. These findings indicate the importance of added value by-products for the profitable production of LA in biorefineries.

Keywords Biomass · Biorefinery · Humins · Levulinic acid · Optimization · Sugarcane bagasse

Introduction

Many solutions have been developed by the academy and industry to mitigate the consequences of the climate crisis, including the development of more sustainable processes [1]. The concept of biorefinery has been developed to fulfill

this purpose: in biorefineries, the biomass feedstock is transformed into many chemicals, fuels, and solid products depending on biomass composition [2]. Today, nearly 170 billion metric tons of biomass is produced annually, contributing to 10% of the primary energy market [3]. Brazil is one of the world leaders in the biomass market, and part of this success is attributed to the sugarcane industry. One of the products obtained from sugarcane is sugarcane bagasse (SCB), with a production of 180 million metric tons in the harvest of 2019–2020 [4]. Although today SCB is used mainly as a boiler fuel in sugarcane mills, it can be potentially used in the production of chemicals and fuels [5].

One of these chemicals is levulinic acid (LA, CH₃(CO)CH₂CH₂COOH), a γ -keto acid obtained via hydrolysis sugars with applications in the chemical, food, agriculture, and fuel industry [6, 7]. When biomass hydrolysis is carried out in an environment with severe oxidation strength, cellulose is hydrolyzed to glucose, whose dehydration yields in 5-hydroxymethylfurfural (5-HMF), which in turn can be decomposed in LA and formic acid (FA). Among the possible applications of LA, fuels and fuel additives deserve special

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attention because they present a vast market and demand low prices, which is possible for LA [8]. The first large-scale endeavor on the production of LA was based on the Biofine process, which consists of two reactors, each focused on different biomass fractions [9]. Fractionation of biomass for the production of LA is being investigated to create a better understanding of the advantages of this approach: Pradipta et al. [10] attained a yield of 43% from Indonesian SCB, Ji et al. [11] obtained a yield of 58% from poplar wood, Jeong et al. [12] obtained 16.5% from Mongolian oak, and Liang et al. [13] obtained 39% after 7 fed-batch steps. These results exemplify many approaches to biomass decomposition to sugars and its subsequent conversion to dehydration productions such as LA, FA, and furfural. However, little attention is given to the lignin fraction of biomass, which is usually discarded with the humins (HUs) produced during the hydrolysis process.

HUs are a solid condensation polymer resulting from the reactions between furans (furfural and 5-HMF) and sugars [11]. Other negative impacts are possible as well: HUs may deposit in the inner reactor wall (thus clogging the reactor) and reduce heat transfer efficiency. Another important factor to be considered is that cellulosic carbon is largely wasted as HUs, resulting in poor use of biomass sources in biorefineries focusing on LA [14]. Thus, it is important to define a compromise between the extent of these reactions to LA and HUs to maximize biomass conversion without losing selectivity to its many valuable products, which also include lignin, furfural, and other sugar derivatives.

This investigation proposes a multi-stage process for the production of LA and HUs from SCB. The yield (Y_{LA}), efficiency (E_{LA}), and concentration of LA (C_{LA}) were optimized considering the following parameters: temperature, reaction time, concentration of sulfuric acid, and solids loading. With the definition of the optimized operating conditions to produce LA, a multi-step strategy consisting of six scenarios was discussed in terms of selectivity and economic feasibility. This strategy addresses the biorefining of SCB to define opportunities to produce LA as well as HUs. Moreover, the obtained HUs were purified to provide a better understanding of its chemical composition and discuss the current challenges in making this solid material profitable in an economically attractive and integrated process.

Materials and Methods

Materials

SCB provided by “Usina São João” (Araras, São Paulo, Brazil) was dried under environmental conditions for 5 days until reaching an average moisture content of 5.0%. The SCB without size reduction was homogenized in a single batch and

stored in polyethylene bags. The composition of SCB was determined according to the standard analytical procedure detailed in “[Characterization of Solid Fractions](#)” and “[Quantification of Carbohydrates, Organic Acids, and Furans](#).” Type I ultrapure water and deionized water were prepared using a filter (Megapurity, Brazil, resistivity > 18.2 MΩ cm). Suppliers and purities of reagents and chromatographic standards are listed in Table S1—Supplementary Information.

Processing Steps for Obtaining LA

Figure 1 shows a scheme of the experimental setup and the trial sequence described in this section of the methodology.

Pretreatment with Sulfuric Acid (PT)

SCB was subjected to dilute sulfuric acid (1.0% w/v) with a solids loading of 20.0% w/v. The process was carried out in a vertical autoclave at 121 °C for 80 min. After PT, the resulting slurry was submitted to filtration. The insoluble solid fraction (ISF) was neutralized with approximately 1 L of distilled water until the pH of the resulting liquid was near 6. With 7.8% of moisture, the ISF was stored in polyethylene bags to be submitted to delignification. The ISF was characterized according to the method described in “[Characterization of Solid Fractions](#).” The resulting liquid, named acid hydrolysate-I (AH-I), was characterized according to the methods described in “[Characterization of Liquid Fractions](#)” and “[Quantification of Carbohydrates, Organic Acids, and Furans](#).”

Delignification (DL)

ISF was mixed to solution of NaOH (0.5% w/v) with solids loading of 20.0% w/v for 90 min. DL was carried out for 90 min at 80 °C, with temperature-controlled by a thermostatic bath Marconi MA 108/9 (Marconi, Brazil). After DL, the insoluble solid fraction—delignified (ISF-D) was neutralized with approximately 1 L of distilled water until the pH of the resulting liquid was 6. With 6.2% of moisture, ISF-D was stored in polyethylene bags until being submitted to a catalyzed conversion with an acid catalyst. The ISF-D was characterized according to the method described in “[Characterization of Solid Fractions](#).”

Acid-catalyzed conversion (ACC)

Experiments were made in a bench system composed of two vessels of stainless steel of 300 mL each, with an internal diameter of 70 mm and a wall thickness of 5 mm. Each vessel contains a pressure gauge, a temperature controller, and a mica band heater of 1 kW. The reactor has limits of operation of

Fig. 1 Schematic of the experimental setup and the trial sequence

200 °C and 20 bar. After turning on, the reactor demands from 10 to 20 min to reach temperatures from 150 until 190 °C.

First, a sample of ISF-D was weighted according to the equivalent solids loading desired (8.0–14.0% w/v). The samples were transferred to the bench reaction system with the

acid solution of H_2SO_4 (1.0–9.1%) w/v. After each ACC run, the reactor was turned off and quenched in water. The resulting slurry was filtered, and the solid fraction, named insoluble solid fraction (ISF-ACC), was neutralized with approximately 2–4 L of distilled water, depending on the

concentration of the acid solution, until the pH of the resulting liquid was near 6. Quantification of total solids and composition of liquor (named acid hydrolysate-II, AH-II) followed the procedures mentioned in “[Characterization of Solid Fractions](#),” “[Characterization of Liquid Fractions](#),” and “[Quantification of Carbohydrates, Organic Acids, and Furans](#).”

Characterization of Solid Fractions

The composition of SCB was determined via analysis of extractives [15], structural carbohydrates, acetyl groups [16], soluble and insoluble lignin [17], and ash [18]. The compositions of the other fractions, ISF, ISF-D, and ISF-CCA were determined in the same way, except by the analysis of extractives. All analyses were carried out in triplicate.

Analysis of Extractives

Approximately 3 g of milled *in natura* SCB was contacted with cyclohexane and ethanol (v/v, 1:1) for 48 h in Soxhlet extractor, followed by aqueous extraction (using deionized water) for 20 h. After the analysis of extractives, SCB was dried at 105 °C for 24 h, milled, and homogenized before structural carbohydrate analysis.

Structural Carbohydrates and Acetyl Groups

Samples from extracted SCB and ISF, ISF-D, and ISF-CCA were analyzed by two-step acid hydrolysis. Samples (300.0 ± 10.0 mg) were hydrolyzed with 72% sulfuric acid (3 mL) at 30 °C for 1 h. Deionized water was added to dilute the acid to a 4% concentration (addition of 84 mL). The mixture was transferred to laboratory bottles (GL 45 screw top, Schott Duran, Germany) and heated to 121 °C for 1 h in a vertical autoclave. After that, the hydrolysates were quenched before the removal of the caps. The hydrolysates were filtered through a previously weighed dry filter paper. An aliquot was filtered through Millipore membranes (mixed cellulose esters, 0.22 µm, 13 mm, white, plain filter), and the filtrates were stored at −2 °C for analysis via chromatography. The solids were washed to a pH of 6 and oven-dried (105 °C) for 48 h to a constant weight.

Structural Lignin

Acid-insoluble lignin was determined by subtracting the ash content from the mass of dried material at 105 °C as described in previous literature [17]. The acid-soluble lignin in the filtrate was determined by measuring the absorbance at 280 nm and using a correlation described in previous literature [17]. For this procedure, a 1 mL sample of the filtrate was pipetted into a 25 mL volumetric flask and alkalinized to pH 12 with

6.5 M NaOH, and the volume was completed with deionized water. The total lignin content in the SCB, ISF, ISF-D, and ISF-CCA was calculated as the sum of acid-insoluble and acid-soluble lignin.

Structural Ash

Total ash was determined by gravimetry: the dried solids were transferred to a weighed crucible and heated in a muffle furnace to 300 °C for 1 h and then heated to 800 °C for 2 h.

Characterization of Liquid Fractions

The chemical composition of AH-I and AH-II was determined via chromatography [19]. For each sample, the pH was measured and recorded to the nearest 0.01 pH unit, and aliquots of 5 mL of each sample were pipetted in triplicate into glass tubes. The volume of 72% sulfuric acid required to bring the acid concentration to a final concentration of 4% was then calculated to treat the samples in the first step [19]. Acid hydrolysis was carried out using 4% H₂SO₄ and the samples were autoclaved for 1 h at 121 °C. Then, the hydrolysates were cooled to room temperature. The hydrolysates were filtered through Millipore membranes (mixed cellulose esters, 0.22 µm, 13 mm, white, plain filter), and the filtrates were stored at −2 °C for analysis.

Quantification of Carbohydrates, Organic Acids, and Furans

The composition was determined in an HPLC system (Agilent 1260 Infinity II, Agilent Technologies, USA) equipped with a quaternary pump G1311B, a refractive index detector G1362A, and diode array detector G4212B. Cellobiose, glucose, xylose, arabinose, acetic acid, FA, and LA were separated in a Bio-Rad® column HPX-87H (9 µm, 300 × 7.8 mm) operated at 35 °C, in isocratic elution using 5 mM of H₂SO₄ at a flow rate of 0.6 mL/min, with a sample injection of 15 µL and a run time of 25 min. For these chemicals, the signal was measured using the refractive index detector. Chemicals were identified based on retention time and elution of the standards. Quantification was performed by the external standard method. Calibration curves (plotted with 11 different concentrations) were established using a mixture of cellobiose, glucose, xylose, and arabinose standards, at concentrations ranging from 0.1 to 4 g/L. Concentrations of standards for organic acids (LA and FA) ranged from 0.1 to 10 g/L.

Furfural and 5-HMF were separated in Nova-Pak C18 column (4 µm, 4.6 mm × 150 mm) at 30 °C with the diode array detector at 280 nm using an eluent composed of 1:8 v/v solution of acetonitrile-water and 1% w/w of acetic acid in a flow rate of 0.8 mL/min. Furfural and 5-HMF were quantified by the external standard method.

Calibration curves (plotted with 16 different concentrations) were established using a mixture of furfural and 5-HMF standards, at concentrations ranging from 0 to 1 g/L. Data were processed using the software OpenLAB CDS ChemStation Edition C.01.05 (Agilent Technologies, USA, 2013). Some examples of chromatograms are displayed in the Supplementary Information—S2.

Definition of Yield and Efficiency of LA

The theoretical yield ($Y_{T,LA}$) and the LA yield based on the fraction of ISF-D (Y_{LA}) were calculated according to Eqs. (1) and (2), respectively.

$$Y_{T,LA}(\%) = \text{Cellulose content (g)} \times 0.715 / \text{substrate(g)}. \quad (1)$$

$$Y_{LA}(\text{g}/100 \text{ g}) = [\text{LA(g)}/\text{substrate(g)}] \times 100. \quad (2)$$

where “substrate” is understood as follows: in the three-step fractionation, the substrate is ISF-D; in the two-step fractionation, the substrate is ISF; and in the one-step fractionation, the substrate is SCB.

Efficiency (E_{LA}) is the theoretical yield based on cellulose content, expressed by Eq. (3) [20].

$$E_{LA}(\text{mol}\%) = [Y_{LA}/Y_{T,LA}] \times 100. \quad (3)$$

Experimental Design and Statistical Analysis

When considering a fractionation of SCB in three steps, the ACC runs were performed for the production of LA and the experiments were based in a 2^4 central composite design with three replicates in the central point and 8 axial points totalizing 27 trials (all experiments were performed in triplicate). The effects of solids loading (S), reaction time (t), temperature (T), and acid concentration (A) were analyzed in 5 codified levels (Table 1). The concentration of LA (C_{LA}), the yield regarding the fraction ISF-D (Y_{LA}), and the efficiency (E_{LA}) were used as response variables. The software StatSoft Statistica 7.0 was used on the analysis of the data obtained. The significance of variables and their interactions were determined with a 98% confidence interval.

Table 1 Coded and real variables chosen in the design of experiments

Variables (coded parameters)	Levels				
	−2	−1	0	1	2
S (% w/v) (X1)	6.0	8.0	10.0	12.0	14.0
t (min) (X2)	35	55	75	95	115
T (°C) (X3)	150	160	170	180	190
A (% w/v) (X4)	1.0	3.0	5.0	7.0	9.0

Statistical Model Development

The statistical model for each response (C_{LA} , Y_{LA} , and E_{LA}) was approximated by the second-order polynomial model presented in Eq. (4):

$$\begin{aligned} Z_i = & \alpha_0 + \alpha_1 \times (S) + \alpha_2 \times (t) + \alpha_3 \times (T) + \alpha_4 \times (A) \\ & + \alpha_{11} \times (S \times S) + \alpha_{22} \times (t \times t) + \alpha_{33} \times (T \times T) \\ & + \alpha_{44} \times (A \times A) + \alpha_{12} \times (S \times t) + \alpha_{13} \times (S \times T) \\ & + \alpha_{14} \times (S \times A) + \alpha_{23} \times (t \times T) + \alpha_{24} \times (t \times A) \\ & + \alpha_{34} \times (T \times A). \end{aligned} \quad (4)$$

where Z_i correspond to each predicted response (C_{LA} , Y_{LA} , and E_{LA}); α_0 is a constant; α_1 , α_2 , α_3 , and α_4 are linear coefficients; α_{11} , α_{22} , α_{33} , and α_{44} are quadratic coefficients; and α_{12} , α_{13} , α_{14} , α_{23} , α_{24} , and α_{34} are the coefficients of the interaction terms. The regression coefficients were obtained, and the response surfaces of the significant binary interactions were plotted. The validation of the statistical models was determined by Fisher’s distribution test (F test), and the quality of the fitted surface was assessed with the squared correlation coefficients (R^2). The statistical model presented in Eq. (4) was used to predict the optimized hydrolysis conditions and, which were then validated experimentally.

Multi-response Optimization Approach

Following Derringer’s desirability function, responses C_{LA} , Y_{LA} , and E_{LA} were simultaneously optimized [21]. This function transforms each response in d_i values between 0 and 1 (from response completely undesired to response completely desired). Global desirability index D is given by Eq. (5), which is the geometric mean from the combination of each one of the transformed responses:

$$D = (d_1^{r_1} \times d_2^{r_2} \times d_3^{r_3})^{1/\sum r_i} \quad (5)$$

where d_i is the response of desirability of each response and r_i is the importance of each variable regarding the others, with values between 0 and 1 and $r_1 + r_2 + r_3 = 1$. Considering that C_{LA} , Y_{LA} , and E_{LA} have the same importance, thus $r_1 = r_2 = r_3 = 1/3$. In order to maximize the responses, d_i assumes values of 1, and when all responses reach their maximum value, D is equal to 1 [22].

After analyzing the multi-response optimization as a function of C_{LA} , Y_{LA} , and E_{LA} , it was needed to evaluate if the use of a three-step process is feasible for the conversion of SCB to LA taking into consideration the production of HUs. The definition of the strategy adopted is presented in the next section.

Multi-step Strategies for Production of LA and HUs

This section aims to investigate the production of LA from SCB in different process steps and the consequent formation

of HUs. When evaluating different treatment steps (e.g., hydrothermal decomposition and hydrothermal decomposition catalyzed by acid), it is possible to ally the primary and secondary biorefining to make a process more or less selective to LA. In order to evaluate the efficiency of the process proposed in this study, PT, DL, and ACC were combined in different scenarios. PT operating conditions were 120 °C, 80 min, 20.0% w/v of solids loading, and 1.0% w/v H₂SO₄, and DL operating conditions were 80 °C, 90 min, 2.0% w/v of solids loading, and 0.5% w/v NaOH. Operating conditions for ACC were the optimized conditions obtained according to “[Multi-response Optimization Approach](#).”

In the first scenario (named Cn1), SCB was submitted to a three-step conversion: PT followed by DL and then ACC. The second scenario (Cn2) consisted of PT followed by ACC. The third scenario (Cn3) consisted of a single-step ACC. The other three scenarios (named Cn4, Cn5, and Cn6) consisted of replacing ACC by a hydrothermal step with no addition of H₂SO₄. After executing the experiments, the resulting slurry was filtered through a Buchner funnel. The hydrolysate was analyzed according to the method described in “[Characterization of Liquid Fractions](#)” and “[Quantification of Carbohydrates, Organic Acids, and Furans](#),” whereas the insoluble solid fractions (ISF-ACCs) were analyzed according to the method described in “[Characterization of Solid Fractions](#).”

HUs Purification and Structural Characterization

The HUs were determined according to the methodology described by Hoang et al. [23]. To remove the residual sugar, the residual solid (ISF-ACC) was contacted with deionized water for 48 h in a Soxhlet extractor. After that, the solid was oven-dried at 105 °C to constant weight. Subsequently, the total solid material was dispersed on 150 mL of 1% NaOH solution and kept at 95 °C for 1 h at constant stirring. The solids were thrice washed in a 10% acetic acid solution in a proportion of 4:1 (liquid to solid) and then washed with water at 80 °C to reach a pH of 6. Finally, the material (filter paper and retained solids) was oven-dried at 105 °C to constant weight. After treatment with NaOH, the resulting filter paper and solids were contacted with acetone in a Soxhlet extractor (six

refluxes per hour for 18 h). Then, the solids were washed and oven-dried at 105 °C to constant weight.

The resulting solid samples from the analysis of the six scenarios (referred to as HUs) and the SCB were analyzed in a Fourier transform infrared spectrometer (Thermo Scientific Nicolet 6700, Madison, USA). The measurement was conducted twice, using the accessory snap-in baseplate (KBr method). The detected spectra are the absorbance of the samples in the wave number range of 4000–400 cm⁻¹, resolution of 4 cm⁻¹, and scan of 32.

Economic Analysis of Multi-step Conversion

A brief economic analysis was carried out to analyze the impact of the multi-step strategy in the production of LA from SCB. The analysis considered the process feedstock (SCB), other required process inputs, and the obtained products (LA, FA, furfural, and HUs) to calculate the earnings before interest, taxes, depreciation, and amortization (EBITDA). The EBITDA was used to estimate the EBITDA margin, which is the ratio between EBITDA and revenue. The following assumptions were considered in the analysis: (i) SCB was available with 50% moisture; (ii) the sugars removed during PT were converted into ethanol via fermentation with 90% efficiency for glucose, 80% efficiency for xylose, and a steam demand of 9.8 kg/kg for recovery [24]; (iii) a steam demand of 9.1 kg/kg was assumed in the recovery of LA, FA, and furfural

Table 3 Chemical composition of SCB, ISF, ISF-D, and recovery yield of pretreatment fractions. Data represent the mean ($\pm$ standard deviation)

	SCB	ISF	ISF-D
Content (w/w)			
Cellulose	40.5 $\pm$ 2.17	62.5 $\pm$ 1.33	78.0 $\pm$ 0.51
Hemicelluloses	26.5 $\pm$ 2.33	12.5 $\pm$ 1.70	6.4 $\pm$ 0.45
Acetyl groups	3.6 $\pm$ 1.11	—	—
Lignin	19.1 $\pm$ 3.18	23.2 $\pm$ 1.89	7.4 $\pm$ 0.45
Extractives	8.9 $\pm$ 0.06	—	—
Ash	2.5 $\pm$ 0.12	1.8 $\pm$ 0.03	2.1 $\pm$ 1.18
Total	101.1 $\pm$ 1.64	100 $\pm$ 2.04	93.9 $\pm$ 1.47
Composition (%)			
Cellulose		36.7 $\pm$ 0.11	36.7 $\pm$ 0.16
Hemicelluloses		7.3 $\pm$ 1.17	3.0 $\pm$ 0.14
Lignin		13.6 $\pm$ 1.25	3.5 $\pm$ 0.14
Solid recovery		58.8 $\pm$ 1.34	47.1 $\pm$ 0.46
Recovery ^a (%)			
Cellulose		90.8 $\pm$ 1.40	90.8 $\pm$ 0.49
Hemicelluloses		27.6 $\pm$ 1.10	13.2 $\pm$ 2.40
Lignin		72.1 $\pm$ 1.12	16.7 $\pm$ 1.97

^a The recovery is defined as the fraction of the cellulose, hemicelluloses, or lignin remaining in the solid fraction (ISF or ISF-D) after treatments compared to what was available in SCB

Table 2 Prices of feedstock and products considered in the economic analysis. All values were updated to December 2019 using appropriate indexes and exchange rates [8, 26, 27]

Feedstock or product	Price (\$/t)
SCB (50% moisture)	26
Lignin	301
Ethanol	602
LA	8906
Formic acid	496
Furfural	1591

Table 4 Concentration of residual sugar (glucose (GLC)) and intermediary byproducts (furfural (FUR), 5-hydroxymethylfurfural (5-HMF) and formic acid (FA)) obtained in the 27 trials of the design of experiments

Trial	S (% w/v)	t (min)	T (°C)	A (% w/v)	Concentration (g/L)			
					GLC	FUR	5-HMF	FA
1	14.0	75	170	5.0	0.4 ± 0.03	0.5 ± 0.05	0.2 ± 0.02	2.3 ± 0.03
2	8.0	55	180	3.0	0.1 ± 0.02	0.3 ± 0.09	0.1 ± 0.01	1.3 ± 0.03
3	12.0	95	180	3.0	0.1 ± 0.05	0.2 ± 0.02	0.0 ± 0.02	1.7 ± 0.04
4	12.0	95	180	7.0	0.0 ± 0.00	0.0 ± 0.01	0.0 ± 0.00	2.8 ± 0.04
5	8.0	95	160	7.0	0.1 ± 0.03	0.2 ± 0.05	0.1 ± 0.02	1.7 ± 0.07
6	10.0	35	170	5.0	0.3 ± 0.06	0.5 ± 0.19	0.0 ± 0.00	2.5 ± 0.03
7	10.0	75	170	5.0	0.2 ± 0.01	0.4 ± 0.02	0.1 ± 0.01	2.3 ± 0.04
8	6.0	75	170	5.0	0.0 ± 0.00	0.2 ± 0.03	0.0 ± 0.00	1.7 ± 0.07
9	10.0	115	170	5.0	0.1 ± 0.01	0.1 ± 0.03	0.0 ± 0.00	1.3 ± 0.02
10	10.0	75	190	5.0	0.0 ± 0.00	0.1 ± 0.02	0.0 ± 0.00	1.8 ± 0.12
11	10.0	75	150	5.0	1.8 ± 0.08	0.7 ± 0.10	0.1 ± 0.03	1.6 ± 0.06
12	10.0	75	170	5.0	0.0 ± 0.01	0.3 ± 0.03	0.0 ± 0.00	2.3 ± 0.10
13	12.0	95	160	7.0	0.1 ± 0.01	0.2 ± 0.01	0.0 ± 0.00	2.7 ± 0.03
14	8.0	55	160	7.0	0.2 ± 0.02	0.5 ± 0.06	0.1 ± 0.03	1.1 ± 0.04
15	12.0	55	160	3.0	3.4 ± 0.01	1.1 ± 0.05	0.2 ± 0.01	2.0 ± 0.04
16	10.0	75	170	5.0	0.3 ± 0.02	0.5 ± 0.07	0.1 ± 0.02	2.4 ± 0.03
17	12.0	55	180	7.0	0.0 ± 0.00	0.1 ± 0.03	0.0 ± 0.00	2.9 ± 0.15
18	12.0	95	160	3.0	0.8 ± 0.01	1.0 ± 0.25	0.3 ± 0.11	1.1 ± 0.12
19	12.0	55	160	7.0	0.5 ± 0.06	0.6 ± 0.01	0.1 ± 0.01	1.8 ± 0.04
20	8.0	95	180	3.0	0.0 ± 0.01	0.2 ± 0.04	0.0 ± 0.00	1.4 ± 0.12
21	8.0	55	180	7.0	0.0 ± 0.00	0.2 ± 0.06	0.0 ± 0.00	2.3 ± 0.07
22	8.0	55	160	3.0	1.8 ± 0.12	0.8 ± 0.06	0.1 ± 0.05	1.5 ± 0.04
23	12.0	55	180	3.0	0.0 ± 0.00	0.5 ± 0.10	0.0 ± 0.00	2.7 ± 0.06
24	8.0	95	180	7.0	0.0 ± 0.00	0.0 ± 0.01	0.0 ± 0.00	2.2 ± 0.16
25	10.0	75	170	1.0	1.0 ± 0.04	1.1 ± 0.11	0.4 ± 0.08	0.8 ± 0.02
26	10.0	75	170	9.0	0.0 ± 0.00	0.1 ± 0.01	0.0 ± 0.00	1.8 ± 0.09
27	8.0	95	160	3.0	0.6 ± 0.01	0.7 ± 0.04	0.1 ± 0.02	0.8 ± 0.02

[25]; (iv) residues after ACC (humins and residual cellulignin) were used as supplementary boiler fuel capable of providing half of the steam provided by SCB; (v) prices were based on previous literature and updated to December 2019 (Table 2) [8, 26]; (vi) lignin is a product only in scenarios with DL, and is sold as soda-lignin [27]. It is important to mention that LA is currently commercialized in the specialties markets with a market price of nearly \$10/kg [28]. Nevertheless, this price is incompatible with the commodities market, and much lower prices are possible [29]. Therefore, besides calculating the EBITDA margin with the price for the specialties market, a minimum selling price (MSP) of LA was determined to attain an EBITDA margin of 30% which is acceptable in the chemical industry [8].

Results and Discussion

PT of SCB yielded the ISF, also called cellulignin. According to the chemical composition of ISF (Table 3), the recovery of

solid material after PT was 58.8%, indicating that nearly one-third of SCB was solubilized. The hemicelluloses were the most solubilized fraction, from 26.5% they were reduced to 12.5% after PT. DL removed 75.4% of lignin with a recovery of solid material of 80.0%. The total mass obtained after DL is equivalent to 44.2%, representing a recovery of 75.0% of cellulignin in this second step. Analyzing ISF-D, it is possible to note a high recovery yield of cellulose (90.8%) and the content of hemicelluloses is low (6.4%) due to the previous PT.

Table 4 reports the concentrations of residual glucose, 5-HMF, furfural, and FA obtained in the liquid fraction of the 27 runs of ACC of the design of experiments, whereas the C_{LA} , Y_{LA} , and E_{LA} are reported in Table 5. Minimum amounts of arabinose were found. Relatively low yields of intermediary byproducts were observed: furfural yields were between 0.04 and 1.2 g/100g_{ISF-D}, and 5-HMF yields between 0.0 and 0.5 g/100g_{ISF-D}. Also, in the cellulose depolymerization-catalyzed system, H₂SO₄ influenced residual glucose, 5-HMF and furfural yields, and their composition. The highest residual

Table 5 Results of the design of experiments used in process optimization. In the responses, the value predicted by the fitted model is indicated between parentheses

Trial	Coded variables				Responses		
	(X1)	(X2)	(X3)	(X4)	C_{LA} (g/L)	Y_{LA} (g/100g _{ISF-D})	E_{LA} (mol%)
1	+2	0	0	0	24.9 ± 0.26 (26.8)	17.8 ± 0.18 (20.8)	30.9 ± 0.33 (36.8)
2	−1	−1	+1	−1	10.2 ± 0.35 (14.9)	12.7 ± 0.01 (18.1)	22.8 ± 0.03 (32.6)
3	+1	+1	+1	−1	12.8 ± 0.11 (10.9)	10.7 ± 0.09 (7.8)	19.1 ± 0.17 (14.0)
4	+1	+1	+1	+1	27.3 ± 0.70 (26.1)	22.8 ± 0.58 (21.7)	40.7 ± 1.05 (38.8)
5	−1	+1	−1	+1	15.3 ± 0.76 (15.0)	19.2 ± 0.95 (18.4)	34.3 ± 1.70 (32.9)
6	0	−2	0	0	22.4 ± 0.42 (20.1)	22.4 ± 0.41 (19.6)	40.1 ± 0.75 (35.3)
7	0	0	0	0	24.4 ± 0.47 (23.3)	24.4 ± 0.47 (23.3)	43.6 ± 0.85 (41.8)
8	−2	0	0	0	14.9 ± 0.61 (14.1)	24.9 ± 1.02 (22.4)	44.7 ± 1.83 (40.0)
9	0	+2	0	0	10.3 ± 0.13 (13.7)	10.3 ± 0.13 (13.6)	18.4 ± 0.24 (24.5)
10	0	0	+2	0	15.5 ± 1.10 (19.2)	15.5 ± 1.09 (18.9)	27.7 ± 1.97 (34.0)
11	0	0	−2	0	13.4 ± 0.81 (10.8)	13.4 ± 0.81 (10.5)	24.1 ± 1.46 (19.0)
12	0	0	0	0	22.5 ± 1.41 (23.3)	22.5 ± 1.41 (23.3)	40.4 ± 2.53 (41.8)
13	+1	+1	−1	+1	24.3 ± 0.25 (20.1)	20.3 ± 0.20 (16.4)	36.3 ± 0.38 (29.3)
14	−1	−1	−1	+1	7.5 ± 0.38 (9.9)	9.4 ± 0.48 (13.7)	16.8 ± 0.86 (24.7)
15	+1	−1	−1	−1	17.3 ± 0.48 (17.5)	14.5 ± 0.40 (15.3)	25.9 ± 0.73 (27.8)
16	0	0	0	0	23.2 ± 0.07 (23.3)	23.2 ± 0.07 (23.3)	41.5 ± 0.14 (41.8)
17	+1	−1	+1	+1	28.4 ± 1.49 (27.1)	23.7 ± 1.24 (22.3)	42.4 ± 2.22 (39.8)
18	+1	+1	−1	−1	7.1 ± 1.04 (8.9)	5.9 ± 0.86 (7.1)	10.7 ± 1.55 (12.7)
19	+1	−1	−1	−1	15.4 ± 0.45 (18.2)	12.9 ± 0.37 (14.4)	23.1 ± 0.67 (25.7)
20	−1	+1	+1	−1	11.1 ± 1.09 (6.6)	13.9 ± 1.36 (10.1)	24.9 ± 2.44 (18.2)
21	−1	−1	+1	+1	19.8 ± 0.59 (16.3)	24.8 ± 0.73 (21.4)	44.4 ± 1.32 (38.4)
22	−1	−1	−1	−1	13.0 ± 0.21 (12.5)	16.3 ± 0.26 (15.1)	29.1 ± 0.47 (27.1)
23	+1	−1	+1	−1	23.9 ± 0.9 (22.5)	19.9 ± 0.75 (18.5)	35.7 ± 1.35 (33.1)
24	−1	+1	+1	+1	18.2 ± 0.68 (18.5)	22.8 ± 0.85 (23.5)	40.7 ± 1.53 (42.1)
25	0	0	0	−2	5.0 ± 0.32 (4.4)	5.1 ± 0.32 (4.1)	9.12 ± 0.58 (7.5)
26	0	0	0	+2	15.1 ± 1.07 (17.0)	15.2 ± 1.07 (16.6)	27.1 ± 1.93 (29.9)
27	−1	+1	−1	−1	5.3 ± 0.18 (7.1)	6.7 ± 0.23 (9.6)	11.9 ± 0.41 (17.2)
Optimized condition (predicted by desirability function)					28.2	23.7	42.3
Optimized condition (experimental data)					25.1 ± 1.50	20.9 ± 1.25	37.5 ± 2.24

glucose yield (2.8 g/100g_{ISF-D}) was achieved in run 15 (12% w/v of solids loading, 55 min, 160 °C and 3.0% w/v H₂SO₄). No residual glucose was observed in runs 4, 8, 10, 12, 17, 20, 21, 23, 24 and 26 (0.0 g/100g_{ISF-D}). In these cases, temperatures were in the range of 170–190 °C, indicating that temperature has a high effect on glucose conversion, which was already observed by other investigators [30]. This fact is also confirmed by the maximal LA concentration that was obtained at a relatively higher T of 180 °C while the lower concentration of LA was achieved at a lower T of 170 °C.

The maximum FA yield (2.4 g/100g_{ISF-D}) was verified in trial 17, and the lowest value was 0.9 g/100g_{ISF-D} in trial 25. Comparing concentrations of LA and FA on a molar basis, it was observed that less FA was produced than the stoichiometric proportion. This same behavior was verified by Galletti et al. [31] when analyzing the production of LA with homogeneous acid catalysts in poplar sawdust, paper mill sludge, tobacco

chops, wheat straw, and olive tree pruning, with 11.5 mEq HCl and 83 mEq H₂SO₄, at 200 °C and 1 h. Authors justify the smaller concentrations of FA regarding LA due to the possibility of reactions secondary to the process, such as the formation of HUs. The same phenomenon was observed by Fleig et al. [32] when converting rice husk into LA in a three-step process, being the last one (catalytic depolymerization of cellulose) run at 175 °C, 5.0% w/v H₂SO₄ and 75 min.

Main Effects on the Statistical Analysis of LA Production

The effects of S , t , T , and A , according to responses C_{LA} , Y_{LA} , and E_{LA} , were investigated (Fig. 2). Regarding the solids loading, it was observed a negative effect for the first-order effect (or linear) of Y_{LA} and E_{LA} and positive regarding C_{LA} , having high significance (p value of 0.0035) (Fig. 2a). In the quadratic

Fig. 2 Standardized effects at 98% confidence interval: **a** main and quadratic effects; **b** interaction effects. (X1): coded variable for S ; (X2): coded variable for t ; (X3): coded variable for T ; (X4): coded variable for A

effect of this one (Fig. 2a) and in the interaction with reaction time (Fig. 2b), the effect was negative. The effect with interactions of temperature and concentration of acid is positive, while the effects of interactions between the factors are not significant for any response (p value >0.02) (Fig. 2b). The solids loading has a slightly more pronounced effect when allied to reaction time than when compared to the other variables (Fig. 2b), which is evident by the results of Table 5. It is still possible to verify by Fig. 2 that solids loading is the variable with the least effect. This means that a higher solids loading would not result in higher amounts of cellulose decomposition. Kim et al. [33] also reported that the concentration of substrate had the least effect on the conversion of glucose to LA and FA.

When analyzing the reaction time, only its interaction with temperature has a positive effect on all responses (Fig. 2b), although not significant in the range evaluated. It is possible to notice from trials 4 and 17 (Table 5) that under the same

Fig. 3 Response surfaces for the effect of $t \times A$ (only significant interaction at 98% confidence level) for **a** C_{LA} , **b** Y_{LA} , and **c** E_{LA} . Filled circles represent experimental data

operating conditions (180 °C, 7.0% w/v H_2SO_4 and 12% solids loading), a reaction time ranging from 55 to 95 min does not seem to impact in the response, with only 1 g/L of LA more being produced (which is insignificant considering

the standard deviation). However, the only significant interaction term is the interaction of reaction time and acid concentration (p value of 0.0077 for C_{LA} and of 0.0084 for Y_{LA} and E_{LA}) (Fig. 2b). The combination of these interactions seems to generate a response similar to a parabola, indicating the presence of optimal conditions.

It is possible to analyze by the response surfaces (Fig. 3) that reaction times higher than 90 min associated to low concentrations of acid (<3.0% w/v) have no positive influence for any of the responses, indicating that for LA formation it is necessary the use of concentrations of acid higher than 3.0% w/v. When reaction times between 60 and 80 min and high concentrations of acid (5.0–7.0% w/v) are combined, it may be seen from Fig. 3a–c that it is possible to reach a C_{LA} near 25.1 g/L (Fig. 3a) equivalent to a Y_{LA} of 20.9 g/100g_{ISF-D} (Fig. 3b) and an E_{LA} of 37.5 mol% (Fig. 3c). Jeong [34] stated that reaction time impacts positively on LA yields, although the author only analyzes reaction times below 50 min. However, the use of longer reaction times favors the formation of HUs, thus causing a decrease in the yield of LA [35, 36], and this should be accounted for in process development.

Regarding temperature, the first-order effect has a positive effect on all responses (Fig. 2a), as in the interaction of temperature with acid concentration (Fig. 2b). However, the quadratic term has a negative effect (Fig. 2a). The p value is significant both for first and quadratic effects for all responses (Fig. 2a). This occurs because the temperature has an important effect in the production of LA facilitating the hydrolysis of cellulose by breaking its rigid structure, living it susceptible to the formation of 5-HMF and in the sequence of LA and FA [37].

Analyzing the responses C_{LA} , Y_{LA} , and E_{LA} , those have their higher values when the temperature of 180 °C was used. On the other hand, a decrease in the analyzed responses was observed when using 190 °C, reaching 15.5 g/L of LA, which is possibly correlated with the simultaneous formation of HUs and poorer selectivity. Park et al. [38] also reported an optimal temperature of 180 °C, for the production of LA from *Gracilaria verrucosa*. Also, Kim et al. [33] found 181 °C as the optimal temperature in the production of LA from glucose. Generally, when considering the operating conditions applied to hydrolysis, the most significant of them is the temperature, influencing drastically on the hydrolysis yields. Earlier studies proved that the temperature applied during hydrolysis is important to modify the rigid structure of lignocellulosic materials since the rate of hydrolysis can be increased when using higher temperatures [30].

The most significant effect, the acid concentration (p value of 0.0036), demonstrated also to be the one with the highest effect for all responses (Fig. 2a). Acid catalysis is considered one of the most important variables in LA synthesis. The first-order term has a positive effect, while the quadratic term has a negative effect on all responses (Fig. 2a). This indicates that for the concentration of acid the responses have a maximum

point (Fig. 3a–c); the same behavior was verified when the production of LA from rice husk was analyzed [28]. In other words, the axial points used for the concentration of acid, 1.0 and 9.0% w/v H_2SO_4 (trials 25 and 26; Table 5) have low concentrations of LA, 5.0 and 15.1 g/L respectively, indicating that those extremes that were analyzed do not favor the production of LA. Analogously with temperature, the concentration of acid was also positively correlated with the responses analyzed for LA: the higher the concentration of sulfuric acid was, the greater were the LA responses (with increased rates of carbohydrate hydrolysis to organic acids). The same behavior was verified by Fang and Hanna [39]: even though they worked with a concentration of H_2SO_4 up to 8%, they did not find a decrease in the yield at a high concentration of catalyst.

Reliability Analysis of the Statistical Model

The experimentally obtained values (Table 5) were analyzed through multiple regression. The adequacy of the developed models was analyzed by means of the analysis of variance (ANOVA) for responses C_{LA} , Y_{LA} and E_{LA} (Supplementary Information—S3). Coefficients of the surrogate models were considered significant if the p value was less than 0.02. Having a low p value ($p < 0.02$) implies that the respective coefficient was statistically significant at a confidence level of 98%, meaning that the probability of noise caused in the correlation between one factor and the response is inferior to 0.02. Analyzing responses C_{LA} , Y_{LA} , and E_{LA} , the linear coefficient of T (α_3); the three quadratic coefficients t , T , and A (α_{22} , α_{33} , α_{44}); and the interaction coefficient $t \times A$ (α_{24}) were determined as having significance at the confidence level of 98% (Supplementary Information—S3). According to this analysis, t , T , and A were significant, indicating that they have a high influence in the responses analyzed during the conversion of LA.

For C_{LA} , according to ANOVA (Supplementary Information—S3), the model finds the condition of adjustment for the F test. It may be observed that the value of the F test calculated for regression analysis (6.103) is higher than the tabulated value (3.401), satisfying the test. The model also has a good adjustment, because the value of the F test calculated is 16.934, which is smaller than the tabulated value of 49.398. For Y_{LA} and E_{LA} , the test is also satisfied: 3.964 and 3.874 for F test for regression analysis and 20.039 and 20.409 for the adjustment of the model, respectively.

The fit of regression models for each response (C_{LA} , Y_{LA} , and E_{LA}) was verified by the coefficient of determination (R^2). Values of 0.876, 0.822, and 0.818 were obtained for C_{LA} , Y_{LA} , and E_{LA} , respectively. These results indicate that only 12.4%, 17.8%, and 18.2% of the total variation could not be explained by the model due to high non-linearity between the studied variables. This is confirmed when analyzing the proximity

between most of the experimental values and predicted by the model (values between parentheses in Table 5). The value for the relative error of the experimental and predicted values for C_{LA} was 14.8%, whereas for Y_{LA} , it was 16.3%, and for E_{LA} , it was 16.5%.

Multi-response Optimization for LA Production

Analyzing each response individually, it was found that for C_{LA} , the maximum experimental value was 28.4 g/L (trial 17—Table 5) besides trial 4 which has a value of 27.3 g/L. On the other hand, for Y_{LA} and E_{LA} , the maximum values are found in different trials. For Y_{LA} is the maximum value of 24.9 g/100g_{ISF-D} (trial 8—Table 5), besides trials 7 and 21 that have close values (24.4 and 24.8 g/100g_{ISF-D}, respectively). Analogously, for E_{LA} , the maximum value was 44.7 mol% (trial 8—Table 5), besides trials 7 and 21 that have close values (43.6 and 44.4 mol%, respectively). The desirability function (D) approach in the case of the nominal-the-best was used to incorporate the multiple responses of LA production into a single response in the form of the composite desirability function. Supplementary Information—S4 shows the results of the predicted responses after random shuffles at each level of each factor holding all other factors constant.

In this way, the optimization allowed the elaboration of profiles for the predicted values of responses and the functions of desirability (Eq. 5). The maximum value of desirability (D) for the optimized process was 0.953 (Fig. 4). The optimized condition of ACC that satisfies simultaneously the responses C_{LA} , Y_{LA} , and E_{LA} , was found to be 12.0% w/v of solids loading, 75 min, 180 °C, and 7.0% w/v H_2SO_4 . This resulted in the following predicted values: 28.2 g/L of C_{LA} , 23.7 g/100g_{ISF-D} of Y_{LA} , and 42.3 mol% of E_{LA} . Experiments in triplicate were made in the optimized condition of operation and obtained a C_{LA} of 25.1 g/L, a Y_{LA} of 20.9 g/100g_{ISF-D}, and an E_{LA} of 37.5 mol%. This indicates an error of at least 10.9% between the predicted and observed values for C_{LA} , 11.8% for Y_{LA} , and 11.3 mol% for E_{LA} . Similarly, Park et al. [38] found an optimized operating condition in 180 °C, 10% of biomass, 0.5 M of methanesulfonic acid and 20 min of reaction for the production of LA from *Gracilaria verrucosa*. Those authors obtained a yield of 22.0% based on biomass weight. Kang et al. [35] used a batch reactor to produce LA from marine biomass *Gelidium amansii*. They obtained, in the optimized condition, a yield of 42.8% at 180 °C, 3% w/w H_2SO_4 and 48 min. Ramli and Amin [36] found an optimized condition at 154.5 °C, 3.7 h, 0.18 g starting from oil palm fronds and [SMIM][FeCl₄] as catalyst achieving 24.8% of LA yield.

Fig. 4 Predicted response profiles (C_{LA} , Y_{LA} , and E_{LA}) and the desirability function when considering a 98% confidence level ($p < 0.02$, horizontal continuous line) by analyzing S , t , T , and A . Vertical dotted lines indicate values below optimal values

Fig. 5 Scheme showing the six scenarios of the multi-step strategy for SCB biorefining to LA, FA, and HUs (dry basis)

Pradipta et al. [10] also proved that multiple-stage acid-catalyzed conversion of carbohydrates into LA is crucial to increase the LA yield. Nevertheless, the researchers do not consider a DL step. This process design is valid if a solvent is used for lignin and hemicelluloses break up. In this sense, the authors used HCl, which can also remove lignin.

After considering process optimization, it is necessary to analyze and provide the efficiency of the biomass fractionation in a multi-step process for LA production. Thus, the following session discusses the evaluation of six conceptual scenarios (ranging from one to three steps) in the process of LA production.

Multi-step Conceptual Scenarios for LA Production

Fractionation of SCB was analyzed through six conceptual scenarios as described in “Multi-step Strategies for Production of LA and HUs” and based on the concepts of integrated use of biomass. The results are reported in Fig. 5 considering 100 kg SCB (dry basis). Cn1 and Cn4 consist of SCB being submitted to three steps of biorefining: for Cn1, PT is followed by DL and then ACC (corresponding to the optimized values found in this study), and for Cn4, PT is followed by DL, and then one step on the same operating conditions of ACC but only involving a hydrothermal conversion (no H₂SO₄ added).

The first step of fractionation had a recovery (ISF) of 58.8 kg indicating that 41.2 kg was solubilized. ISF consists mainly of 36.7 kg of cellulose (90.8% recovery of cellulose from SCB), 13.6 kg of lignin (72.1% recovery of cellulose

from SCB), and 1.1 kg of ash. This fraction was then submitted to DL, which resulted in the removal of 91.3% of ash and partial solubilization of lignin, nearly 82.0% of the raw SCB. ISF-D, a solid resulting from DL, had a mass of 44.2 kg, equivalent to 47.0% of the initial SCB. These processes increase the accessibility to cellulose (78% of cellulose in ISF-D; Table 3) with 90.8% of cellulose recovery regarding the cellulose content in SCB, favoring the production of LA. Then, the third step was executed to hydrolyze the remaining cellulose to produce LA. After ACC, the remaining solid was separated by filtration, resulting in 13.8 kg for Cn1 and 43.0 kg for Cn4, which was equivalent to 31.5% and 93.3% of ISF-D for Cn1 and Cn4, respectively. After the extraction process according to Hoang et al. (2015) [23], nearly 13.3 kg for Cn1 and 7.1 kg for Cn4 of HUs were recovered. For Cn1, the amount of cellulose in the solid waste was null (complete conversion), and in Cn4, it was 35.3 kg.

Concentrations of LA and FA were high when using H₂SO₄ in the last step (Cn1) and undetectable when considering the hydrothermal process (Cn4). In scenario Cn1, the mass of LA obtained after the third step was 9.8 kg, with equivalent values of Y_{LA} and E_{LA} of 21.5 g/100g_{ISF-D} and 38.5 mol%, and the mass of FA was 1.2 kg. LA yields regarding the initial raw material represent 24.3% of the cellulose in SCB, 9.8% of raw SCB, and 34.0 mol% based on theoretical yield from cellulose in SCB. The concentration of LA was 20.9 g/L. After analysis of results of Cn1 and Cn4, it becomes evident that the use of a strong acid catalyst in the third step is fundamental for the production of LA: when no strong acid is present, there is no production of LA

and the amount of cellulose in the solid waste is preserved, thus making Cn4 unfeasible.

Scenarios Cn2 and Cn5 were comprised of two steps: in the case of Cn2, PT was followed by ACC (considering the optimized conditions described in this work: 180 °C, 75 min, 12.0% w/v solids loading and 7.0% w/v H₂SO₄), whereas for Cn5, PT was followed by a step under the same operating conditions of ACC but involving a hydrothermal. After ACC, the solid waste was separated by filtration, resulting in 27.1 kg for Cn2 and 50.6 kg for Cn5, which is equivalent to 45.3% and 86.8% of ISF for Cn2 and Cn5, respectively. After the extraction process was performed on the solid residue of ACC, 5.3 kg of HUs were obtained in Cn2 and 2.6 kg in Cn5. In Cn2, the amount of cellulose in the solid waste was 15.5 kg (38.3% recovery of cellulose from SCB), and in Cn5, it was 34.7 kg (85.6% recovery of cellulose from SCB). The concentrations of LA and FA after the ACC step were considered as relatively high when using H₂SO₄ (Cn2) and low with the hydrothermal conversion (Cn5). It was possible to obtain from ISF 0.2 kg of FA in Cn2 and 0.1 kg in Cn5. The mass of LA obtained after the ACC step was of 6.6 kg in Cn2 and 0.4 kg in Cn5, with equivalent values of Y_{LA} of 10.9 and 0.7 g/100g_{ISF} and E_{LA} of 24.5 and 1.6 mol%, respectively. LA yields regarding the initial raw material represent 16.2% and 1.1% of cellulose in SCB for Cn2 and Cn5, respectively; 6.6% and 0.4% of SCB; and 22.6 mol% and 1.5 mol% based on theoretical yield from cellulose content of SCB. The concentration of LA was 11.2 g/L and 0.7 g/L for Cn2 and Cn5, respectively. Scenarios Cn2 and Cn5 indicate that the use of acid catalysis favorable for the production of LA. Regarding the decomposition of cellulose, Cn2 kept 15.5 kg of this fraction, while Cn5 kept 34.7 kg, indicating a small yield when compared with Cn1 and Cn4 (0.0 and 35.3 kg, respectively). Therefore, when comparing Cn1 and Cn2, it becomes evident that the DL step (present on Cn1) increases the concentrations of LA and FA.

Literature reports other results for biomass processing in two steps. Kang et al. [35] studied the optimized conditions for LA production from *Gelidium amansii* using a two-step treatment. Those authors reached a maximum yield of 42.8% as a function of the initial amount of biomass at 180 °C, 3% w/w of sulfuric acid, and 48 min of reaction. On the other hand, Wang et al. [40] analyzed a two-step process to produce furfural and LA using a hydrothermal treatment combined with a solid acid catalyst in SCB. After the second step, they reached a yield of 62.1% at 180 °C and 3 h of reaction. The high yield found by the authors may be due to the use of a solid catalyst, which keeps the cellulose and hemicelluloses in the first step. Besides, the authors do not consider the fraction of hemicelluloses in the calculation of the yield, although LA may also be produced from it [41]. Authors also do not report about the formation of HUs, which is related to the selectivity of the solid acid catalyst. Nevertheless, the use of heterogeneous

catalysts in SCB conversion is a cumbersome task: their catalytic sites may be deactivated by the formation of HUs [14] and its recovery is not reported.

Scenarios Cn3 and Cn6 consisted of a single step of ACC. Cn3 used the optimized operating conditions of ACC, whereas Cn6 used the same operating conditions but with no addition of H₂SO₄. After the reaction, the solid residue was separated by filtration, resulting in 36.8 kg for Cn3 and 64.4 kg for Cn6, equivalent to 37.5% and 71.5% of the raw SCB for Cn3 and Cn6, respectively. After the extraction performed on this solid residue, 30 kg in Cn3 and 18.3 kg in Cn6 of HUs were obtained. For Cn3, the amount of cellulose in the solid residue was 0.6 kg (1.6% recovery of cellulose from SCB), and in Cn6, it was 32.3 kg (79.9% recovery of cellulose from SCB). Concentrations of LA and FA were higher using when using acid in ACC (Cn3) than when using only water (Cn6). This way, it was obtained 0.5 kg of FA in Cn3 and 0.4 kg of FA in Cn6 for each 100 kg of SCB. The mass of LA obtained was 16.9 kg and 3.8 kg for Cn3 and Cn6, respectively, with equivalent values of Y_{LA} of 17.0 and 3.9 g/100g_{SCB}. LA yields regarding the initial raw material represented 41.8% and 9.3% of cellulose in SCB for Cn3 and Cn6, respectively; 16.9% and 3.8% of raw SCB; and 58.4 mol% and 13.0 mol% based on the theoretical yield from cellulose on SCB. The concentration of LA was 16.9 g/L and 3.8 g/L for Cn3 and Cn6, respectively. It was possible to observe that, as in the previously discussed scenarios, the use of H₂SO₄ increases the yield of LA: Cn3 produced a higher concentration of LA (16.9 g/L) than Cn6 (3.8 g/L). Nevertheless, the formation of HUs was also higher (30.0 kg in Cn3 and 18.3 kg in Cn6).

Regarding Cn3 and Cn6, it is also possible to attribute the high formation of HUs with the presence of hemicelluloses, an observation that was already reported in the literature. Chamnankid et al. [41] demonstrated the synthesis of LA starting from xylose under hot-compressed water using as catalyst an alkaline-treated zeolite. The treatment created new strong acid sites over the surface of the zeolite and improved the LA yield. The maximum LA yield was 30.0% after 3 h of reaction. Although high reaction temperatures (190 °C) led to high conversion, they also led to an increased formation of by-products. The increase of temperature from 170 to 190 °C reduced the LA yield by 8.0%, while the furfural yield increased from 28.0 to 30.0%. The authors reported that under higher temperatures the formation of HUs was increased due to the dehydration of xylose to furfural. It was proposed by the authors that xylose was mostly converted into intermediaries that, eventually, were transformed in desirable products with the formation of insignificant amounts of HUs under 130 °C. The catalytic conversion of hemicellulosic sugars from biomass to produce LA was conducted by Jeong et al. [42] using zeolite Y modified via NaOH treatment in different concentrations. The best LA yield was 4.6 g/100g_{biomass} (conversion of pentoses to LA: 42.7%, based on theoretical yield) of the

liquid hydrolyzed when using the zeolite treated with 0.25 M NaOH, at 190 °C and 180 min. The authors reported that at 210 °C, the yield of LA decreased to 3.9 g/100g_{biomass} due to a large hydrolysis rate, which increased the rate of condensation reactions that produce HUs.

It was evident that the higher concentrations of LA and FA were obtained in scenarios that use H₂SO₄ in ACC (Cn1, Cn2, and Cn3) (Fig. 5). It was also observed that the LA concentration was higher in scenario Cn1 than in Cn2. This result was attributed to the high accessibility of cellulose after the removal of lignin via DL, which is a step available only in scenario Cn1. Analyzing the three scenarios with hydrothermal conversion (Cn4, Cn5, and Cn6, no H₂SO₄ added), it was observed that Cn6 (1 step) has the higher concentrations of LA when compared with Cn5 (2 steps) and Cn4 (three steps). This occurs because hemicelluloses contain acetyl groups which are easily hydrolyzed by water at high temperature [43]. The product of this hydrolysis, acetic acid, though at low concentrations, increases the oxidizing strength of the medium, thus leading to a small yield of LA.

Allied with the process, it is also possible to analyze the formation of HUs. Although Cn3 with a single step of ACC produced considerable amounts of LA (16.9 kg), there was also the abundant simultaneous formation of HUs, 30.0 kg—the greatest amount found on the different scenarios studied. This may be explained by the fact that the hemicelluloses present on SCB become potential sources for the formation of HUs. As in Cn1, those fractions were completely removed, the formation of HUs in Cn1 (13.3 kg) is smaller than in Cn3. Thus, it becomes evident that a high yield of LA is also related to an increased yield of HUs. Therefore, thinking of biorefinery strategies and the need to provide uses for any by-product of biomass fractionation, a better comprehension of the molecular structure of HUs is necessary.

Analysis of FTIR of Solid Residue from the Evaluation of the Conceptual Scenarios

The FTIR spectra of SCB and HUs of the different conceptual scenarios are presented in Fig. 6. The main stretching range regarding the presence of groups hydroxyl (bonds O–H) is reported in a wave number between 3422 and 3410 cm⁻¹ and in 2880 cm⁻¹ [13]. On the other hand, the weak contributions corresponding to aliphatic and aromatic bands between 3000 and 2900 cm⁻¹ and near 2850 cm⁻¹ correspond to the bending and stretching of C–H, as well as the vibration of the aromatic ring, from residual lignin, respectively [44].

Although the region of the spectrum encompasses the band with wave number from 4000 to 500 cm⁻¹, the region of the fingerprint area from 1800 to 500 cm⁻¹ is generally the one with more interest, because it has most of the spectral information regarding the molecular structure. Similar observations were made by Acquah et al. [44]. The spectra of HUs

Fig. 6 FTIR spectra of sugarcane bagasse (SCB) and HUs from scenarios Cn1, Cn2, Cn3, Cn4, Cn5, and Cn6

of scenarios Cn1, Cn2, and Cn3 (HUs-Cn1, HUs-Cn2, and HUs-Cn3, respectively) (Fig. 5) had absorption patterns very similar when compared with the ones reported in the literature. The band in 1735 cm⁻¹, which is a characteristic band of raw SCB, was strongly reduced in HUs-Cn4, HUs-Cn5, and HUs-Cn6 (Fig. 6). On the other hand, the bands between 1800 and 1000 cm⁻¹ significantly increase from SCB to HUs.

The oxygenated functional groups may also be observed from the spectra (Fig. 6). The peak between 1708 and 1697 cm⁻¹, for HUs-Cn1, HUs-Cn2, HUs-Cn3, HUs-Cn5, and HUs-Cn6, was attributed to the stretching of C=O [23], and its strong intensity suggests abundant aldehydes, ketones, and carboxyl groups [45]. Observing the presence of these organic functions is in accordance with the chemical structure of humins already suggested by other investigators [46]. As a relevant characteristic, spectra of HUs-Cn4, HUs-Cn5, and HUs-Cn6 (Fig. 6) clearly showed the presence of a furanyl

structure [47] that was not observed in HUs-Cn1, HUs-Cn2, and HUs-Cn3, denoting that the conversion process involving the ACC suppresses the interaction C=C or C=O. The peaks between 1612 and 1605 cm^{-1} and between 1520 and 1512 cm^{-1} were attributed to the stretch C–C of the furan ring [23], which corresponds to poly-substituted furans due to the furan structure present in the solid matrix of HUs [23, 46]. It was also observed in the spectra for HUs-Cn4, HUs-Cn5, and HUs-Cn6 a specific peak between 668 and 608 cm^{-1} , resulting from flexion in C=O.

The absorption at 1420 cm^{-1} corresponds to the stretch C–O–C of furanylic ether [48]. Besides the previous one, in these samples, the bands near 1215 and 1170 cm^{-1} correspond to the phenolic ring or phenolic hydroxide regarding the vibrations of functional groups of residual lignin. It is also important to highlight that the presence of a relevant phenolic structure, which was confirmed by the absorption band at 1204 cm^{-1} , predominant in HUs-Cn1, HUs-Cn2, and HUs-Cn3 [49]. When compared to SCB, the weaker peak between 1168 and 1161 cm^{-1} corresponds to the stretching of C–O in alcohols and aliphatic ethers [49], and the peak near 1450 cm^{-1} may be attributed to vibrations of deformation CH_2 in aliphatic chains present in HUs [23].

Inside the region of the fingerprint, the peaks resulting due to polysaccharides include the peak in 897 cm^{-1} corresponding to deformation C–H [44] that disappears in HUs-Cn1, HUs-Cn2, and HUs-Cn3 (Fig. 6). Besides that, absorptions in 1260, 1161, and 1034 cm^{-1} were attributed to the stretch of C–O of aliphatic alcohols and ethers [47], the stretch of C–O in polysaccharides [44], or deformation of the furan ring being characteristic of HUs produced from sugars [45]. The peak in 1117 cm^{-1} occurs due to the skeletal aromatic stretching of C–O of residual lignin [44]. The band near 834 cm^{-1} corresponds to the aromatic deformation of C–H. The same observation was made by Rasrendra et al. [47], where it corresponded to the interval between 850 and 700 cm^{-1} .

In general, a comparison between the FTIR spectra of the HUs obtained via the catalytic process with H_2SO_4 and the HUs formed through the hydrothermal process suggests that the two sets have different distributions of substituents in their unities of furanyl, phenolic, and hydroxyl. This is due to the difference in the oxidizing strength of the hydrolysis environment. Thus, the HUs obtained in Cn1, Cn2, and Cn3 are much more oxidized than the HUs obtained in scenarios Cn4, Cn5, and Cn6.

Economic Analysis of Scenarios

The results of the economic analysis are presented in Table 6. Considering the LA price for the specialties market, the EBITDA margin of the scenario Cn1, Cn2, Cn3, and Cn6 attains figures of 80–90% for even low yields of LA (scenario

Table 6 Results of the economic analysis. In scenario Cn4, MSP is unavailable because no LA was produced

Scenario	EBITDA margin	MSP (\$/t)
Cn1	91%	98
Cn2	88%	479
Cn3	94%	236
Cn4	29%	n.a.
Cn5	30%	5379
Cn6	81%	363

Cn6) because of its high added value. Nevertheless, it should be noted that the LA suitable for applications in the specialties market requires high purity. Hence, the recovery process should include an additional separation step (e.g., crystallization). Moreover, the specialties market for LA is limited in size: about 20 kt/y in 2020 [28]. This market volume could be supplied by a single state-of-the-art autonomous Brazilian sugarcane mill processing 4 million tons of sugarcane per year using only 28% of its surplus bagasse [24]. Therefore, this price is not suitable for large-scale production of LA.

Literature reports several other uses for LA outside the specialties market granted that its price is low enough [8]. In this context, the analysis of the MSP for an EBITDA margin of 30% led to a price of \$98/t in scenario Cn1 (Table 6), which is compatible with previous price estimates for large-scale LA production [29]. Such low MSP for LA is a result of the biomass fractionation strategy because part of the revenue is also obtained from selling ethanol (fermentation of sugars from PT) and lignin (recovered after DL). This price is low enough to enable the conversion of LA to γ -valerolactone or dibutyl ketone, both of which can be blended into gasoline or used in the production of other liquid hydrocarbon fuels [25]. Still, to enable production at large-scale, it is necessary to address the challenge of HUs and how to safely use them as supplementary boiler fuel in biomass boilers or to develop new applications for them.

The Challenge of the Current Circular Economy in Valuing HUs

Besides the already known environmental advantages of products obtained from renewable and/or biodegradable sources, the use of several sources of renewable raw materials (such as SCB for example), as well as the intensive use of their products and by-products (ethanol, sugar, lignin, and others), might help biorefineries in maximizing their profit margins [32]. Therefore, with the desire of having an attractive circular economy, the conversion of SCB into chemical products, materials, and fuels emerges nowadays as a promising alternative for the replacement of fossil resources. In this context, it is fundamental to discuss the possible applications of HUs.

The diverse range of formation/production of HUs depends upon the catalytic conditions of conversion of the biomass and the source of the substrate or agro-industrial waste. For instance, Weingarten et al. [50] reported a HUs yield of approximately 31.6% from D-glucose using acid catalysis with HCl for 2.5 h. Other examples include the conversion of cellulose [51] and fructose [52] having a yield of 49.6% and 16.0% respectively. In an alternative approach, using a biphasic system, Ordonsky et al. [53] obtained typical HUs yields during the reaction of dehydration of glucose of almost 50.0% using ZrPO in an aqueous medium. Thus, HUs were observed as a residue (or by-products) occurring in reaction systems with acid aqueous media or heterogeneous catalysts, both in water and in organic solvents [54].

The formation of HUs is inevitable during the production of LA. Preliminary investigations are being made and forwarded to the valuation of HUs approaching the structure and its formation mechanism [14, 46]. Recently, it was demonstrated that HUs generally contained a carbon content of 60–80 wt% [55], which indicated that more than 30% of the initial carbon in cellulose was wasted as solid carbonaceous residue. As a material with high carbon content, Kang et al. [55], showed that about 74.4% of the initial carbon of cellulose was transferred into value-added products, (47% carbon in LA), and also 16.1% in carbon adsorbents, especially for wastewater treatment. HUs represent a matrix rich in carbon and furanic structures with hydroxyl and carbonyl functional groups. Thus, it has a high potential as a biobased thermoset polymer to be employed in composites applications. Nevertheless, these applications need to be further studied before any added-value applications for HUs are proposed. In the meantime, HUs can be considered as supplementary boiler fuel in the biorefinery, which is suitable for the production of LA which demands a considerable amount of steam [25].

Conclusions

This investigation evaluated the optimization of the levulinic acid (LA) production process from sugarcane bagasse (SCB). Fractionation of SCB was performed in three steps (acid pretreatment, PT; delignification, DL; and acid-catalyzed conversion, ACC) for the depolymerization of cellulose and conversion to LA. By employing a multi-response optimization strategy, optimized operating conditions were obtained for the third step: 180 °C, 7.0% w/v sulfuric acid, 75 min of reaction, and 12% of solids loading. In these conditions, the response variables of the study were of 25.1 ± 1.50 g/L for C_{LA} , 20.9 ± 1.25 g/100g_{ISF-D} for Y_{LA} , and 37.5 ± 2.24 mol% for E_{LA} . Based on these optimized conditions, six conversion scenarios were proposed, where it was confirmed the efficiency of the fractionation of biomass in three steps for production of LA in the biorefinery concept. In the three-step fractionation scenario,

LA and formic acid (9.8 and 1.2 kg, respectively per 100 kg of dry SCB) were obtained allied with the inevitable production of HUs (humins, 13.3 kg, based on 100 kg of dry SCB). Even though this was not the best LA yield among the studied scenarios, the biomass fractionation strategy of biorefineries allowed for the separation of other added-value components, which allow a smaller minimum selling price for LA, fundamental for many possible market applications. The chemical composition of HUs was analyzed and discussed. Considering the biorefinery concept in which all products and by-products can be used to integrate value to biomass, HUs still represent a challenge because it has a chemical structure that is not yet fitted for many applications besides burning as supplementary boiler fuel in biorefineries. Therefore, other potential uses for HUs can add more value to the LA production chain.

Author Contributions

Emília Savioli Lopes: conceptualization, methodology, validation, formal analysis, data Curation, writing—original draft, visualization; Jean Felipe Leal Silva: conceptualization, methodology, writing—reviewing and editing, visualization; Elmer Ccopa Rivera: writing—original draft, writing—reviewing and editing, visualization; Alana Petrina Gomes: visualization, methodology, validation, investigation, writing—original draft; Melina Savioli Lopes: methodology, formal analysis; Rubens Maciel Filho: conceptualization, validation, resources, writing—reviewing and editing, supervision, project administration, funding acquisition; Laura Plazas Tovar: conceptualization, validation, writing—original draft, writing—reviewing and editing, resources, visualization, supervision, project administration, funding acquisition.

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