Levulinic acid production from lignocellulosic biomass using lignin-catalyst

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Research Article

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Abstract

Purpose: This work developed a new solid carbonaceous acid catalyst on lignin-based and used it to convert cellulose into levulinic acid (LA), resorting a mixture of straw and sugarcane bagasse (1:1 ratio) as reagent and catalyst. **Methods**: Pretreated straw and sugarcane bagasse in dry block reactors. The solid/liquid ratio for all tests was 1:20 at 110°C. Used a 2^2 rotational central composite design to optimize cellulose extraction and lignin precipitation, cellulose extraction: varying NaOH concentration (0.2 to 5 % w/v) and reaction time (3 to 117 min), and lignin precipitation: testing different pH values (3 to 10) and residence time (6 to 69 min). Synthesized the catalysts through an incomplete lignin carbonization followed by sulfonation. Cellulosic pulp was treated with the catalyst at 140 °C (1:8 solid/liquid ratio). **Results**: Under reaction conditions of 110 °C, 117 min, solid/NaOH (4.4 % w/v) and 1:20 ratio, more than 91% of the lignin dissolved into the black liquor, recovering a solid with a cellulose content higher than 78%. Furthermore, lignin was fully recovered as precipitate by reducing the black liquor pH to 3 (11 g of recovered precipitate/liter of liquor) and used to synthesize a 7.3 mmol/g total acid site density catalyst, which converted the cellulose up to 17.11 % and obtained LA yields of up to 38.55 %, when applied an 8:10 catalyst/cellulose ratio (w/w). **Conclusion:** The proposed process has the potential to enable the inclusion of LA among the by-products of the national sugar-energy sector, increasing its competitive potential.

Keywords: lignin-based catalyst, levulinic acid, straw and sugarcane bagasse, sulfonated catalyst.

Graphical abstract
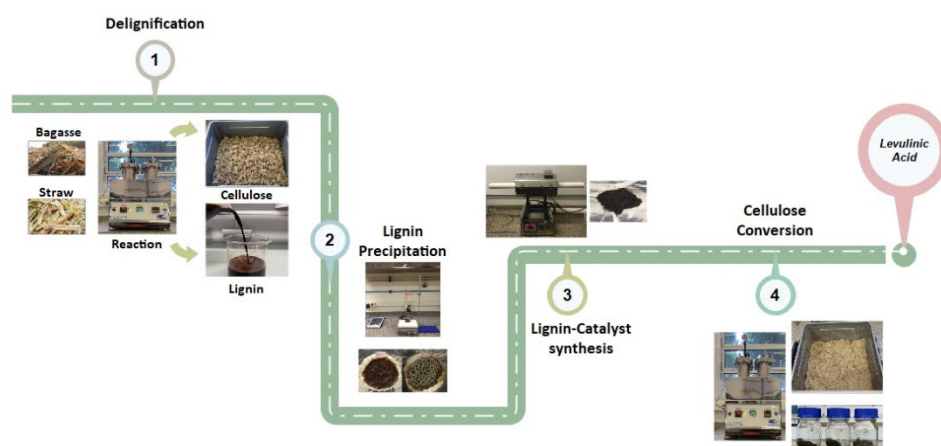
Statement of Novelty

One of the by-products from the ethanol production is the sugarcane biomass, that can be burned to produce energy. However, in the last couple of years researchers are working to obtain high-value products from the sugarcane biomass and one of these products is the levulinic acid, and with that new study fronts have been brought up to optimize levulinic acid yield. In this work, we aimed in the production of a new catalyst made from the same biomass seeking to produce levulinic acid higher yield, we resorted a statistical approach to

49 optimize the experiments results. Thereby, using a solid acid catalyst is able to enhance industrial levulinic acid

50 renewable production and economically strengthen countries that have a biomass-based economy.

51 **Introduction**

52 The global industry in the contemporary context and, therefore, the world economy, depend almost entirely on

53 fossil fuels. These resources are non-renewable and the main contributors to the emission of CO_2 into the

54 atmosphere. The chemical sector, for example, is the industrial sector that most consumes these fuels in the

55 world, accounting for 15% (13 million barrels per day) of the total primary demand for oil on a volumetric basis

56 and 9% (305 billion cubic meters) of natural gas demand [1]. Approximately half of these energy resources are

57 consumed by the sector as an energy source and the other half as raw material to produce the main ingredients

58 that supply the fertilizer industry, the cosmetics and pharmaceutical industries, and the plastics and synthetic

59 textiles industry [1, 2]. This cascading reliance on fossil fuels, however, can be mitigated.

60 Lignocellulosic agro-industrial by-products and residues, also called lignocellulosic biomasses, are important

61 and abundant sources of renewable carbon that can be converted into biofuels and chemical building blocks that

62 are often analogous to those supplied by the traditional or conventional chemical sector. In this sense, biomasses

63 such as forest residues, sugarcane bagasse and straw, grain husks, corn cob and straw and many others, represent

64 a concrete and viable alternative to fossil raw materials. Incorporation of technologies for biomass fractionation

65 in the industrial plants where these residues are generated it is essential, increasing the exploitation of the

66 potential of these materials and creating biorefineries. The term biorefinery refers to industrial complexes that

67 integrate and correlate the different processes necessary for the conversion of biomass into various chemicals

68 and into energy in the form of fuels, electricity, or heat [3, 4]. As the bio-based product market is still in the early

69 stages, there are few companies that control it and the North American countries are among the most important

70 consumers of levulinic acid, the challenge of creating a biomass-based economy relies on the vast number of

71 stakeholders, however if this challenge is overcome the biomass-based industries will be able to create market

72 competitiveness for countries rich in biomass and have the potential to create jobs in the areas where they are

73 implanted [5].

74 The fractionation of biomass is a set of pre-treatments aimed at separating the main constituents of

75 lignocellulose: the polysaccharides cellulose and hemicellulose, the phenolic polymer lignin, and the extractives

76 [3]. Polysaccharides and lignin represent more than 90% of the lignocellulosic matrix and have aroused the

77 interest of researchers for industrial applications. Polysaccharides, after depolymerization reactions, release the

78 sugars that constitute them, such as glucose (6 carbons), the unit that forms cellulose, and xylose (5 carbons) and

79 arabinose (5 carbons), sugars from hemicellulose. These six- and five-carbon sugars from lignocellulosic

80 biomass can also be converted into other chemicals such as 5-hydroxymethylfurfural (5-HMF) and levulinic acid

81 (from glucose) and furfural (from xylose). These compounds are examples of bio-based platform chemicals as

82 they act as precursors in other synthetic routes to produce other chemicals of industrial and commercial value

83 [6]. Lignin is a natural phenolic polymer with a crosslinked and chemically complex structure [7, 8] that has

84 been studied, among many purposes, as an adhesive agent, as a reinforcement in the rubber industry and as a

85 precursor to carbon fibers [9].

86   The first industrial process was developed by the Biofine Renewables LCC CO, which consists of a 2 steps
87   process using diluted sulfuric acid as a catalyst, the first step of the process is the depolymerization of
88   polysaccharides into their soluble monomers, then the second step is developed in another reactor, where the
89   product mixture from the first reactor is recovered and produces the LA, however the lack of industrial product
90   quality is strongly related with the production of by-products and undesired products, obtaining a maximum
91   yield of 71,5% [10, 11].

92   Despite advances in research to improve pretreatment techniques, the recalcitrance of lignocellulosic biomass, or
93   how cellulose, hemicellulose and lignin are strongly associated in the lignocellulosic matrix, still represents a
94   significant challenge for the implementation of biorefineries that are, in fact, economically competitive. Another
95   problem faced is the fact that the polysaccharides of the biomass are better hydrolysed under acidic conditions
96   and the homogeneous acid catalysis using mineral acids, such as sulfuric acid, has been the most used technique
97   for this purpose. Homogeneous systems with mineral acids, although efficient, suffer from problems of product
98   and catalyst separation, reactor corrosion, acid recycling and treatment of residual effluents, limiting the
99   commercialization of levulinic acid and other chemical compounds derived from biomass sugars, when this
100 system is adopted [12, 13].

101 It is in this context that solid acid catalysts come in, which are indicated as good alternatives to liquid mineral
102 acids. Its main advantages over homogeneous acid catalysis systems are the absence of corrosion damage, easy
103 separation and recyclability of the catalysts [13, 14]. Because of these qualities, the interest in using this type of
104 catalysis for the conversion of cellulose into glucose and chemicals derived from its dehydration has grown, as
105 well as the variety of catalysts that have been tested for this purpose. Acid resins, metal oxides, H-zeolites,
106 functionalized silicas, supported metals, solid magnetic acids and sulfonated carbonaceous acids are some of the
107 most used [13, 14]. Among those mentioned, the solid sulfonated catalysts have shown superior catalytic
108 activity, showing promise for the hydrolysis of cellulose due to the synergistic effect of the COOH groups,
109 phenolic OH groups and SO_3H groups present in their structure, where the first two act in the adsorption of
110 cellulose by means of hydrogen bonds and the third acts in the cleavage of β-1,4-glycosidic bonds [13-16].

111 Therefore, based on the considerations mentioned so far and intending contribute to the improvement of
112 technologies that will enable the implantation of biorefineries, the central idea of this work was: developing a
113 process to produce a solid carbonaceous acid catalyst based on lignin and using it in the reaction of converting
114 cellulose into levulinic acid, with the particularity that both reagent and catalyst come from the same biomass, a
115 mixture of straw and sugarcane bagasse. Sugarcane straw and bagasse were chosen because they have similar
116 chemical compositions and because of their strategic potential in the Brazilian industrial scenario.

117 **Material and Methods**

Raw material

The sugarcane bagasse and straw were provided by the ethanol-sugar mill Santa Cruz-São Martinho, Americo Brasiliense, SP, Brazil. The raw material was dried at the sun until reaching a moisture of around 10%. Then, this biomass was milled in a knife mill (Forage Crushers TRF-400) to obtain fractions of smaller granulometry (particles around 5 mm) and stored in hermetically sealed plastic recipients avoiding moisture gain.

Pre-treatment of sugarcane bagasse and straw mixture

Mixtures of sugarcane straw and bagasse (mass ratio 1:1) were pre-treated in dry-block reactor with a volumetric capacity of 1000 ml. The solid/liquid ratio for all tests was 1:20 and the reaction temperature was 110°C. Achieving the optimization of this step, different values of NaOH concentration and reaction time were tested using a rotational central composite design 2^2.

The solid fraction obtained after the pre-treatment step was washed with water until the wash water reached neutral pH. After this procedure, the cellulosic fraction was dried at room temperature and characterized in terms of cellulose, hemicellulose, and lignin content. Analysis of variance and multiple regression were also performed using the STATISTICA software version 10.0 StatSoft Inc., 2015.

Chemical characterization of biomass

The sugarcane straw and bagasse mixture and the solid fractions (cellulose pulp) recovered after the alkaline pretreatment were characterized as to their cellulose, hemicellulose, lignin, ash and extractives contents, according to standard characterization methodology of structural carbohydrates and lignin from biomass from National Renewable Energy Laboratory – NREL [17].

The acid hydrolysate was also used for the analysis of carbohydrates and organic acids from the hydrolysis of cellulose and hemicellulose (polyoses) using high performance liquid chromatography (HPLC). Determining the concentrations of glucose, xylose and arabinose, and acetic, formic and levulinic acids, 20 μL of samples previously filtered through a Sep Pak C18 filter were injected into a Shimadzu liquid chromatography system coupled to a RID refractive index detector, using the BIO-RAD AMINEX HPX-87H analytical column (300 X 7.8 mm). The mobile phase used was 0.01 N H_2SO_4, under a flow rate of 0.6 mL/min and a working temperature of 60°C. The same steps (except for the extractives step) were followed for the characterization of the cellulose pulp obtained after pre-treatment of the biomass with sodium hydroxide. All the tests described, both for in natura biomass and for cellulosic pulp, were carried out in triplicate.

Determination of soluble lignin in liquid fraction (black liquor) recovered after pre-treatment

The soluble lignin content in the black liquor obtained from the alkaline pre-treatment step was determined by measuring the absorbance at 280 nm in a spectrophotometer based on the methodology adapted from Gouveia et al. [18]. The calculation of soluble lignin was calculated according to Equation 1:

$$C_{Lignin} = 4.187 \times 10^{-2} \times (AT - Apd) - 3.279 \times 10^{-4} \tag{1}$$

Where: C_{Lignin} = concentration of soluble lignin in g/L; AT = absorbance of lignin solution together with the degradation products at 280 nm; $Apd = (c_1 * \varepsilon_1) + (c_2 * \varepsilon_2)$ is the absorbance, at 280 nm, of the decomposition products (furfural and 5-HMF), whose c_1 and c_2 concentrations were previously determined by HPLC (high performance liquid chromatography) and ε_1 and ε_2 are the absorptivity and are worth, respectively, 146.85 and 114.00 L g^{-1} cm $^{-1}$ [18].

The samples for determination of furfural and 5-Hydroxymethylfurfural (5-HMF) concentrations (c_1 and c_2, respectively) were first filtered with Minisart syringe filters and then injected into a liquid chromatography system with a Shimadzu chromatograph coupled to an ultraviolet UV-VIS (280 nm). The column used was an Agilent Zorbax Eclipse Plus C18, (4.6mm x 100mm, 3.5 microns), maintained at a temperature of 30 ºC, injection volume of 10 μL, mobile phase acetonitrile/water (1:8) and flow rate of 0.8 ml/min.

Lignin Precipitation

The lignin precipitation was carried out using a rotational central composite design, testing different pH values and rest time of the acidified liquor, arriving at the best condition for lignin precipitation. Stopping the initial tests, 50 mL of black liquor obtained from the optimal condition of biomass pre-treatment had its pH (initially above 12) decreased by titration with 6M sulfuric acid solution, under constant agitation, until reaching a value of pre-defined pH. After reaching the desired pH, the agitation was stopped, and the acidified liquor was left to rest for a pre-established time to verify if the time would exert any influence on the precipitation. At the end of this time, the acidified liquor was filtered, and the filter cake was washed with acidified distilled water (pH = 3), and dried in an oven at 50 °C until constant weight. The mass of recovered precipitate was weighed after drying.

After precipitation tests, analyses of variance and multiple regression were performed using the STATISTICA software version 10.0 StatSoft Inc., 2015 to define the best conditions for pH and rest time, using as a variable response, the dry mass of precipitate recovered.

Catalyst synthesis

The solid acid catalysts were synthesized through an incomplete carbonization step of the lignin followed by a sulfonation step of the carbonized solid (methodology adapted from Hu et al. [14]). The temperatures of 600 ºC and 800 ºC were studied as carbonization temperatures (Tc) in the synthesis of these catalysts. The experiments also included a test of chemical activation simultaneously with carbonization, in which NaOH(s), the activating agent, was mixed with lignin in a lignin/NaOH mass ratio of 2:1. The sulfonation of the precursor materials, that is, the functionalization of the carbonized solids obtained from the mentioned tests, was carried out with concentrated sulfuric acid. All the tests were carried out in duplicate.

In synthesis, the incomplete pyrolysis (or incomplete carbonization) of lignin was carried out in a tubular pyrolytic furnace with a controlled atmosphere system (FTR-1100, FORNOS INTI). The solid lignin precipitates were manually ground using a crucible and pestle and then sieved, recovering the solids that passed through a

184   sieve with 1 mm holes (18 mesh). Samples of 1 g of this lignin, with or without NaOH(s) of the same

185   granulometry for chemical activation, were taken to the oven and initially kept at 120 ºC for 30 min to remove

186   residual moisture. Then, the material was heated up to 600 °C or 800 °C and kept at one of these temperatures

187   for 30 min for carbonization to occur. The temperature increased at a rate of 10 °C/min and the samples were

188   kept under a constant flow of N_2 of 120 mL/min during the entire carbonization process and during the furnace

189   cooling (the samples were taken when the internal temperature of the furnace was between 120 and 130°C).

190   After carbonization, the carbonized solids were washed with distilled water until pH of the wash water, initially

191   alkaline, was close to neutral. The washes were performed by centrifugation (Centrifuge 5810 R, Eppendorf)

192   with repeated cycles of 5 min, at 5 ºC and relative centrifugal force (FCR) equal to 8228 g. After washing, the

193   materials were dried at 105 °C for 12 h.

194   The sulfonation of carbonized solid was carried out in dry-block reactors with a volumetric capacity of 1L. The

195   technique chosen was the impregnation of the carbonized solids with concentrated sulfuric acid (98%) in a

196   solid/liquid ratio (g/mL) of 1:40. The reaction was kept at 180 °C for 12 h under constant stirring at 70.4 rpm

197   and the solids obtained at the end of the process were called acid sulfonated solids (SCS_{Tc-x}) or activated

198   carbonaceous solids sulfonated ($SCAS_{Tc-x}$), where Tc-x refers to the carbonization temperature used. At the end

199   of reactions, the catalysts were washed with distilled water until pH of the wash water approached neutral, thus

200   removing residual sulfuric acid not fixed to the precursor solid during the reaction. The washings were also

201   performed by centrifugation with repeated cycles of 5 min, at 5 ºC and relative centrifugal force (FCR) equal to

202   3214 g. Finally, the catalysts were dried at 95 °C around 8 and 12 h.

Characterization of solid carbonaceous acids synthesized

204   Tests to characterize the chemical properties of the solid sulfonated acid catalyst based on lignin were carried

205   out. The total acid density was defined by conductometric titration using a conductivity meter model 912

206   Conductometer, Metrohm, and a standardized 0.02 M NaOH solution as the titrant, according to a methodology

207   adapted from Hu et al. [14] (titrations were performed in triplicate for each of the two replicates of each

208   condition). Equation 2 was used to calculate the total acid density (σ), in mmol/g, of the solid acid catalyst,

209   where "c" is the NaOH concentration (in M), "m" is the catalyst mass (in g) and "V" is the volume of NaOH

210   titrant (in mL) used on neutralizing the sulfonic acid groups and other acid groups present in the catalyst. The

211   catalyst also had its chemical structure analyzed by total reflectance spectroscopy attenuated in the infrared with

212   Fourier transform (ATR-FTIR) in the wavelength range from 4000 to 400 cm^{-1}.

213    $$\sigma = \frac{c \times V}{m} \hspace{10cm} (2)$$

Cellulose into levulinic acid conversion

215   For comparison purposes and determining which condition would be tested in heterogeneous catalysis, the

216   homogeneous catalysis of cellulose in levulinic acid was first investigated. The first step was to grind the

217   cellulosic pulp in a knife mill (Forage Crushers TRF-400) decreasing its crystallinity, reducing the size of its

218   fibers to approximately 5 mm. The ground cellulose was taken to 100 mL Duran flasks together with sulfuric

219   acid solution. These flasks were closed and taken into cylindrical dry-block reactors containing 200 mL of

220   distilled water for the reaction to take place in a water bath. The fixed reaction conditions were the cellulose pulp

221   /H_2SO_4 (aq) (2% v/v) ratio of 1:8 (g/mL) and the reaction temperature at 140 ºC. The reaction times analyzed

222   were 2, 4 and 6 h and three replications were performed for each of these conditions. The best condition defined

223   was chosen to be tested for heterogeneous catalysis.

224   For heterogeneous catalysis, an amount in grams of the best catalyst obtained was used, equivalent to the

225   normality (N) of the H_2SO_4 solution (2% v/v). Therefore, 1g of cellulosic pulp, 0.8430 g of catalyst and 8 mL of

226   distilled water were reacted in the same way and under the same condition as the homogeneous catalysis that led

227   to the highest yield of levulinic acid. Yield calculations were performed based on the equations described by

228   Chen et al. [19] and Pyo et al. [20]. For the calculation of theoretical yield of levulinic acid, the formation of

229   degradation products, such as humins, was not considered since the mechanism of formation of these products is

230   not completely elucidated. Calculating the theoretical (or real) yield, that is, estimating the yield of product that

231   would be obtained if the reaction were perfect, it was considered 100% conversion of cellulose into glucose and

232   100% conversion of glucose into levulinic acid in a stoichiometry reaction of 1 mol of reactant to 1 mol of

233   products (1 mol of glucose → 1 mol of levulinic acid: 1 mol of formic acid: 1 mol of water). The cellulose to

234   glucose conversion factor is 1.11 and the estimate for the theoretical yield of levulinic acid is 0.64 g of levulinic

235   acid per gram of glucose.

236   Finally, the percentage of cellulose converted in the reactions, the levulinic acid yield and the reaction efficiency

237   were calculated according to Equations 3, 4 and 5 respectively:

238      $$Cellulose\ Coversion\ (\%) = \left(\frac{Consumed\ Glucose\ (g)}{Initial\ Glucose\ (g)}\right) \times 100 \tag{3}$$

239      $$Real\ Levulinic\ Acid\ Yield\ (\%) = \left(\frac{Levulinic\ Acid\ (g)}{Consumed\ Glucose\ (g)}\right) \times 100 \tag{4}$$

240      $$Efficiency\ (\%) = \left(\frac{Real\ Levulinic\ Acid\ Yield}{Theorical\ Levulinic\ Acid\ Yield}\right) \times 100 \tag{5}$$

241   Where the *Consumed Glucose = Initial Glucose (g) –Final Glucose (g)*.

242   **Results and Discussion**

243   ***Pre-treatment of sugarcane biomass***

244   Considering the amount of cellulosic pulp or the treated material recovered at the end of each treatment (Table

245   1), it is noted that assay 2 (NaOH 5% w/v and 20 min) resulted in the highest cellulose yield with the respective

246   lowest amount of residual lignin. Under this condition, for every 100 g of fresh biomass, 36.7 g of treated

247   material are obtained, whose chemical composition was 8.19% lignin, 12.57% hemicellulose and 79.02%

248   cellulose. In terms of mass, therefore, the recovered material contained approximately 3 g of lignin, 4.6 g of

249   hemicellulose and 29 g of cellulose. These amounts, when related to the respective original amounts in the

250   untreated biomass, show that 69.19% of the cellulose was recovered at the end of the process, leaving only

251   10.68% and 18.16% of the original fractions of lignin and hemicellulose, respectively. This means that 89.32%

252   of lignin, 81.84% of hemicellulose and only 30.81% of cellulose were dissolved/removed from the biomass

253   under this condition, integrating the liquid fraction obtained from the reaction, the black liquor.

254   **Table 1** - Matrix for 2^2- central composite rotatable design (CCRD) with three repetitions at the central point to

255   study the influence of the variables NaOH concentration and reaction time in the delignification process of the

256   straw and bagasse mixture (1:1). Levels encoded in parentheses.

257   Quite the opposite, assay 5, that employed the lowest concentration of NaOH (0.2% w/v), is the only one among

258   the alkaline treatments that did not result in a material that can be considered as cellulosic pulp, since it led to a

259   percentage of lignin extraction lower than 50% and hemicellulose extraction lower than 27%. These values are

260   much lower than those observed for all other treatments (removals above 81 and 47% of lignin and

261   hemicellulose, respectively), which, corroborated by the low loss of mass during the process (< 30%), indicates

262   that the lignocellulosic matrix in the original biomass was practically unchanged under assay 5. The mass losses

263   during the other pre-treatments, therefore, were basically due to the removal of lignin and hemicellulose to the

264   reaction medium.

265   Ávila et al. [21] evaluated the chemical composition of a 1:1 (w/w) mixture of sugarcane bagasse and straw after

266   different pre-treatments, including treatments with the ionic liquid [Emim] [Ac] in a thermostatic bath at 100

267   °C, and in an ultrasonic bath at 25 °C. For the respective treatments, the authors found values of 45.62%, 26.75%

268   and 18.48% and 44.27%, 28.48% and 18.77% of cellulose, hemicellulose, and lignin. Therefore, lower cellulose

269   contents and higher residual hemicellulose and lignin contents in the cellulosic pulp than those reported for all

270   treatments tested here. The authors also attributed 47.30% and 50.25% of solubilized mass during these

271   treatments to the removal of lignin [21].

272   Liu et al. [22] performed the pre-treatment of sugarcane bagasse with 0.5 M NaOH at 80 °C, under stirring for 2

273   h, with a solid/liquid ratio of 1:20. Although the reaction temperature is lower, the NaOH concentration used by

274   these authors was 2-fold the concentration used in assays 1 and 3 of this work, and the reaction time is also

275   longer. The chemical composition for the material pre-treated by the authors (63.43% cellulose, 26.11%

276   hemicellulose and 8.26% lignin), however, did not show significant differences compared to the chemical

277   composition of the pre-treated materials under the assays 1 and 3 of this study (56-59% cellulose, 28%

278   hemicellulose and 10-11% lignin).

279   Also, according to Table 1, it can be observed from assays 1 to 6 that the change from the lowest level to the

280   highest level in the NaOH factor, keeping the same level in the time factor, resulted in an increase in the removal

281   of lignin from the biomass. The same fact was observed for changes of level increase in the time factor, fixing

282   one of the NaOH factor levels, when observed assays 1 to 4. Such relationships, however, are not so simple to

283   observe when considering the cellulose yields and become even more complex when trying to relate an eventual

284   maximum extraction of lignin with a minimum loss of cellulose. A slightly more than three percentage point

285   increase in lignin removal by assay 4 compared to assay 2, for example due to the level increase in the time

286   factor, resulted in an increase of more than ten percentage points in cellulose removal or loss to the reaction

287   medium.

288 This relationship that the highest cellulose yield, the highest possible lignin extraction, is of central importance

289 in determining the path to be taken in the fractionation of biomass, since a black liquor rich in lignin and a solid

290 fraction rich in cellulose are sought. The higher lignin concentration in the black liquor, the greater the yield of

291 lignin precipitate in the precipitation step, which is the base material for making the solid carbonaceous acid

292 catalyst which, in a later step of acid hydrolysis, will convert cellulose into glucose and, therefore, into levulinic

293 acid. Consequently, the higher degree of purity of cellulosic pulp, which is also obtained from the alkaline pre-

294 treatment step, the better the yield of acid hydrolysis reaction, since the recalcitrant character of the material will

295 be reduced.

296 The analysis of the estimation of the effects for the removal of lignin from the biomass after alkaline

297 pretreatment is elucidated using the Pareto graph (Figure S1, supplementary material), with terms whose p

298 values were less than or equal to 0.05 being considered significant. It was observed that the variable NaOH

299 concentration had the greatest influence on the percentage response of lignin extraction. Based on the results

300 obtained with the analysis of variance (Table S1, supplementary material), the adjusted regression model was

301 found to be significant (p-value < 0.05) and capable of explaining about 77% of all the variability in the data set.

302 Furthermore, it was observed that the lack of fit test is not statistically significant (p-value > 0.05), which

303 indicates that the proposed model correctly specifies the relationship between the variables independents and the

304 response variable. The mathematical model, with the real variables to describe the response within the studied

305 region, can be presented in the form of Equation 6.

306 $\%Removal\ of\ Lignin = 55.21 + 18.13X_1 - 2.19X_1^2 + 0.00035X_2^2$ (6)

307 Where X_1 is the NaOH concentration (% w/v) and X_2 is the time reaction (min).

308 Optimizing the response, lignin extraction, the independent variable NaOH concentration would ideally be in the

309 range of 4.0 to 5.0 % w/v, while for the time variable the optimal range would be between 100 and 120 min

310 (Figure S2, supplementary material). Under such conditions, the extraction efficiency would be above 90%.

311 Equation 6 was optimized using the desirability function (FD) of the STATISTICA program, which estimates

312 the best combination of factors that results in the best possible response within the studied region. From the

313 optimization, a value of percentage of lignin extraction from the biomass of 97.3% was obtained under the

314 conditions of 4.4% (w/v) of NaOH concentration and reaction time of 117 min, at 110 ºC. This condition was

315 experimentally tested in triplicate and a lignin extraction efficiency of 91.76 ± 1.85% was verified, thus

316 validating the model. The cellulosic pulp resulting from this treatment contained 6.9 ± 1.87% of lignin, 10.81 ±

317 1.50% of hemicellulose and 78.08 ± 1.82% of cellulose. The cellulose yield in relation to the original biomass

318 was also quite significant, totaling 63.34 ± 4.9%. The removal of hemicellulose from the biomass was 85.60 ±

319 1.3%.

320 ***Recovering soluble lignin from black liquor***

321 The volumes of black liquor recovered for each treatment were similar, with an average recovery of 0.9 ± 0.02 L

322 of liquor per treatment. On the other hand, soluble lignin differed from all tests conditions (Table 1), with the

323   lowest soluble lignin concentration for assay 5 (0.2% w/v NaOH for 60 min), which used the lowest NaOH

324   concentration, and the highest soluble lignin concentration for assay 10 (3% w/v NaOH for 60 min). The

325   treatment with the optimal condition (NaOH 4.4% w/v, 117 min and 110 ºC) resulted in 0.91 L of liquor with a

326   soluble lignin concentration of 11.01 g/L.

327   With the best condition for extracting lignin from the biomass defined, the next step consisted of recovering the

328   lignin present in the black liquor. This recovery was carried out by precipitating the soluble lignin present in the

329   black liquor (pH > 12) with its acidification using 6 M sulfuric acid. Many authors reported the reduction of the

330   degree of solubilization of lignin through acidification of black liquor with mineral acids, especially sulfuric,

331   hydrochloric and phosphoric acid [23-25].

332   Table 2 shows the results of experimental design, and it is possible to observe that the amount of precipitate

333   increases when the pH decreases, in addition to the fact that a longer rest time improves precipitation. The best

334   precipitation condition (0.57 g) was achieved when the pH of solution was at 3 and rest time 38 minutes.

335   **Table 2** Matrix for 2^2- central composite rotatable design (CCRD) with three repetitions at the central point to

336   study the influence of the variables, pH and rest time in the recovery process of lignin using the liquid fraction

337   obtained by delignification process. Levels encoded in parentheses.

338   In the Pareto chart (Figure S3, supplementary material), the analysis of the estimated effects for the parameter

339   grams of lignin precipitate recovered is presented. Thus, based on the information in the graph, the pH variable

340   had the greatest influence on the response. Based on the results obtained with the analysis of variance (Table S2,

341   supplementary material), the model that best fitted the data set is a quadratic model that includes only the

342   coefficients for the linear and quadratic terms referring to the pH factor and the coefficient for the quadratic term

343   referring to the time factor. Therefore, at 95% confidence, the regression obtained is significant (p< 0.05), its

344   represented approximately 89.5% of the variability in the data set and not presented a lack of fit (p > 0.05). The

345   mathematical model, with the real variables to describe the response within the studied region, are presented in

346   form of Equation 7.

347    $g\ precipitate/50\ mL\ liquor = 1.233 - 0.249X_1 + 0.014X_1^2 - 2.870 \times 10^{-7}X_2^2$ (7)

348   Where X_1 is the pH of precipitate and X_2 is the rest time (min).

349   Through the response surface graph (Figure S4, supplementary material), it can be seen that the dependent

350   variable precipitation pH would have to be close to its lower level, pH=3, to obtain higher yields of precipitated

351   material, with no dependence on the resting time variable, which could be in the range of 6 to 69 min. Obtaining

352   the best pH condition in the studied range, to precipitate the lignin, the desirability function (FD) of the

353   STATISTICA program was used, which provided as the best pH = 3 and rest time 69 min. The predicted

354   response was 0.616 g of precipitate when acidified a volume of 50 mL black liquor obtained from the

355   delignification of a 1:1 mixture of straw and sugarcane bagasse (ratio 1:20 of biomass to 4.4 (%w/v) NaOH for

356   117 min at 110°C). The condition provided by the desirability function of the STATISTICA program was then

357 experimentally tested in triplicate and a value of 0.55 ± 0.006 g of precipitate was obtained. This result,
358 considering the 90% confidence level, therefore validates the model.

359 It can be concluded, therefore, that the decrease in pH improves the lignin precipitation. The yield of lignin
360 precipitation tends to increase with the reduction of pH and temperature, as well as with the increase of the ionic
361 strength of the liquor, with pH, however, being the predominant factor among the three [26]. However, when the
362 precipitation pH is very acidic, other components of the black liquor, other than lignin, can precipitate and
363 increase the amount of precipitate, as stated by Domínguez-Robles et al. [23], that found the decrease in
364 precipitation pH from 4 to 2, for example, had no effect on the amount of lignin recovered in the precipitate,
365 although the amount of precipitate was higher for pH 2.

366 Zhang et al. [27] researched the effect of the pH in the precipitation of lignin from fermentation processes
367 residues from a sugarcane bagasse biorefinery, they analyzed the precipitation of lignin at pH of 9.0, 7.0, 5.0, 3.0
368 and 0.3 and obtain the highest precipitation yield with the solution at pH of 5.0 and they also found a difference
369 in the contents of hydrogen, carbon, oxygen contents in the samples with pH at 3.0 and 0.3 from the others.

370 Santos et al. [24], when testing hydrochloric and sulfuric acid to precipitate lignin at pH 2, 4 and 6, reported that
371 the amount of solid recovered at pH 4 was higher, regardless of the acid used for precipitation. For pH 4,
372 according to the authors, almost all the lignin precipitated, in addition to salts and hemicelluloses. According to
373 the authors, at lower pH, colloids that are unstable at pH 4 can become stable again at pH 2 and resolubilize.

Density of total acids and chemical structure of catalysts

375 The sulfonated carbonaceous solids (SCS) and the sulfonated activated carbonaceous solids (SCAS), synthesized
376 through the sulfonation of incompletely carbonized lignin, have their total acid densities described in Table 3.
377 These catalysts are presented in their structures of polycyclic sheets of carbon, both sulfonic acid ($-SO_3H$) and
378 carboxylic acid (-COOH) groups. The sulfonic groups, as expected, came from the functionalization of solid
379 precursors with concentrated sulfuric acid. The carboxylic acids probably resulted from the oxidation reaction of
380 free hydroxyl groups (reducing ends) present in the precursor material, which occurs during sulfonation [14].
381 These groups, therefore, constitute the acidic sites responsible for the catalytic activity of these solids.

382 The densities of acid sites (or total acids) were measured using the conductometry technique. The system's
383 behavior during the titration, that is, the variation of electrical conductivity (μS/cm) as a function of the volume
384 of standard NaOH(aq) solution added, was the same in all tests. As the analyte is titrated with NaOH(aq), the
385 sulfonic acid and carboxylic acid groups present on the surface of the catalysts are neutralized. However, once
386 the equivalence point is reached, the OH^- ions from the titrant accumulate in the medium, and the conductivity
387 starts to assume an ascending character. The intersection of the two formed lines (representations of
388 measurements taken before and after the equivalence point) provides the point of discontinuity in the
389 conductance-volume curve [28] used to calculate the density of total acids in mmol/g, summarized in Table 3.

Table 3 Total Acid Density (mmol/g) and Yield (%) of synthesized of catalysts sulfonated carbonaceous solids (SCS) and sulfonated activated carbonaceous solids (SCAS) according to pre-established carbonization temperatures (Tc) and sulfonation temperatures (Ts)

The yields on a dry basis of the synthesized solid catalysts were in the range of 22 to 32 % (w/w), and the increase in the carbonization temperature from 600 ºC to 800 ºC resulted in a yield drop of ten percentage points. These yields were calculated based on the dry mass of catalyst recovered at the end of the synthesis process, which includes all post-carbonization and post-sulfonation washing and drying steps. However, the lignin carbonization step was responsible for the highest mass loss during the process, about 67.63% to 74.64%. Thus, since there was practically no change in the amount of mass after sulfonation, except for minimal losses due to the final washing of the catalyst, carbonization can be considered the most significant step of the process in terms of the synthesized catalyst yield.

These yields were very close to what has been reported for solid acid catalysts synthesized through similar processes employing different biomass. For example, Mar, Somsook [29] synthesized solid sulfonated carbonaceous catalysts using a vermicelli-type pasta made from mung bean starch as raw material. The authors carbonized this mass at 200, 300, and 400 ºC and obtained yields of sulfonated solids of about 25, 29, and 23% m/m. Araujo et al. [30] report a carbon yield of around 35% after carbonizing açaí seed residues for 1 h at 400 °C, 500 °C, and 600 °C. Thanh et al. [31] obtained a yield of 31.4% after carbonization of acacia wood sawdust at 500 ºC for 40 min.

The total acid density was higher for the catalyst prepared from carbonized lignin at the lowest temperature (SCS$_{Tc-600}$). The total acid density for this catalyst was double that observed for the SCAS$_{Tc-800}$ catalyst, which was prepared from carbonized lignin and activated at 800 ºC (1.48 mmol/g and 0.72 mmol/g, respectively); and more than double that observed for the SCS$_{Tc-800}$ catalyst (0.58 mmol/g), carbonized at 800 ºC, but without activation (Table 3). These results agree with what has been reported in the literature since the higher the carbonization temperature, the less efficient the functionalization [30]. Broader and more rigid carbon structures are produced at higher temperatures as a result of the growth and stacking of the carbon sheets, which makes it difficult for the -SO$_3$H functional groups to anchor during the sulfonation step, thus leading to a low total acid density [14, 29, 30].

On the other hand, less rigid carbon structures are obtained with lower carbonization temperatures, and sulfonation is facilitated. Ozsel et al. [32], for example, observed this phenomenon in the carbonization and sulfonation tests. When testing the temperatures of 300 ºC, 400 ºC, and 500 ºC in the carbonization of wheat straw for 3h and subsequent sulfonation at 150 ºC for 16 h, the authors found that although the increase in the carbonization temperature from 300 ºC to 500 ºC resulted in greater surface area, volume and pore size, the catalyst prepared from the carbonized precursor at 300 ºC showed a higher density of acidic sites -SO$_3$H concerning the others (1.76 mmol/g compared to 1.71 and 1.29 mmol/g for the catalysts made with carbonized biomass at 400 ºC and 500 ºC, respectively).

The effect of carbonization temperature on the subsequent sulfonation step can also be observed in the work of Hu et al. [14]. They performed carbonization tests for ultrafine fibers prepared by electrospinning a lignin solution and poly(ethylene oxide) (9:1 w/w) without and with NaOH for activation. According to the authors, the carbonized fibers at 600 ºC had a total acid density of 1.4 mmol/g when sulfonated at 110 ºC and 1.9 mmol/g when sulfonated at 150 ºC, surpassing the sulfonated carbonized fibers at 900 ºC under the same temperature conditions and the fibers were simultaneously carbonized and activated at 900 ºC and sulfonated at 110 ºC. However, the density of total acid sites for fibers carbonized at 600 ºC, according to the authors, was only lower than that of fibers simultaneously carbonized and activated at 900 ºC that were sulfonated at 150 ºC (2.43 mmol/g), which was the result of the combined effect of alkaline activation and the increase in sulfonation temperature.

In the present work, the catalyst prepared from the activated precursor, $SCAS_{Tc-800}$, showed an increase of 24.13% in the density of acid sites compared to the non-activated catalyst SCS_{Tc-800} (0.58 to 0.72 mmol/g). However, the positive effect of alkaline activation on functionalization was not enough for the $SCAS_{Tc-800}$ catalyst to outperform the total acid density of the SCS_{Tc-600} catalyst, which was carbonized at the lowest temperature. The 2:1 lignin/NaOH ratio was likely a limiting factor in activation. A mass ratio with a higher NaOH ratio would have led to better results. The increase in the impregnation rate of NaOH, as shown in some works, increases the specific surface area of the carbonized material [33, 34], and with a more excellent specific surface, it is inferred that more acid groups -SO_3H would be more likely to bind to the precursor in the sulfonation step.

Given that the SCS_{Tc-600} catalyst presented the highest density of acid sites among the conditions tested, it was chosen to catalyze the conversion reaction of cellulose into levulinic acid. Thus, the synthesis process of this catalyst was repeated to obtain it in sufficient quantities for this next step. However, a specific change was made in the execution of the process, which included the post-sulfonation washing and drying steps. It was decided not to rewash the catalyst until the pH of the wash water was neutral (pH ≈ 6), removing only excess residual sulfuric acid (washes were performed until the pH of the supernatant increased from 0 to 2), and the drying temperature was reduced from 95°C to 60°C. This decision was taken to use part of the residual sulfuric acid in the first batch of cellulose conversion into levulinic acid and to reduce the large amount of wastewater generated in the washing step. During catalyst washing, the sulfuric acid that has not reacted with the solid precursor is ionized, forming hydronium ions (H_3O^+) and hydrogen sulfate (HSO_4^-), which also dissociates with increasing dilution, forming more hydronium ions. (H_3O^+) and sulfate ions (SO_4^{2-}) [35]. The HSO_4^- ions are eliminated along with the washing water. However, if the material is not washed enough to remove them altogether, these ions remain adsorbed, probably through hydrogen bonds. Based on this assumption, new conductometry tests were carried out to verify if the total acid density of the synthesized catalysts in the best-adjusted condition would be greater than what was observed in the preliminary trials. After drying, the catalyst obtained from the repetitions of the best condition (Tc= 600 ºC and Ts= 180 ºC) had a moisture content of 9% (b.u.), and its density of total acid sites in mmol/g was 7.27 ± 0.727. Therefore, this value was five times higher than recorded for the preliminary test under the same condition (1.46 mmol/g), which corroborates the hypothesis that residual sulfuric acid is not covalently bound to the catalyst structure.

In the preliminary sulfonation tests, 150 mg of carbonized lignin at 600 ºC were impregnated with 6 mL of 98% sulfuric acid (1:40 m/v) in a cylindrical reactor with a volumetric capacity of 1 L and a base area of 56.7 cm^2. Then, in the repetition step of this same condition, 5 g of carbonized lignin at 600 ºC were impregnated with 200 ml of 98% sulfuric acid (same proportion of 1:40 m/v) in a reactor with the same volumetric capacity and base area. In the first case, there was a distribution of 0.10 mL of sulfuric acid per cm^2 of the base area of the reactor, while in the second case, this distribution was 3.5 mL per cm^2. This means that it is possible that by increasing the load inside the reactor under the mentioned conditions, the submersion of the precursor in sulfuric acid was more efficient, which may have favored the impregnation and, therefore, the functionalization of the solids. It is likely, thus, that more -SO$_3$H groups were anchored to carbonized lignin during repetitions of the sulfonation condition. The presence of sulfonic groups in the structure of the catalysts was confirmed by employing the spectra obtained by the Fourier transform infrared spectroscopy technique - FTIR-ATR (Figure S5, Supplementary material).

Cellulose hydrolysis

SCS$_{Tc-600}$ [Adj.] catalyst was used to convert cellulose into levulinic acid. Before proceeding with the heterogeneous catalysis tests, it is necessary to investigate the homogeneous catalysis of cellulose in the product of interest. These tests were carried out with an H$_2$SO$_4$ solution (2% v/v) and were made to compare the performance of the two types of catalysis. Table 4 shows the conditions tested and the results for the hydrolyzed cellulose and levulinic acid yield, which showed that the performance of the homogeneous catalysis reaction improved with increasing time. Therefore, the condition that employed the longest reaction time (6h) was chosen for the catalyst test.

Table 4 Yields for homogeneous and heterogeneous catalysis reactions of cellulose from sugarcane biomass to levulinic acid

About 17.46% of the cellulose was hydrolyzed for 6h using a homogeneous catalysis reaction, resulting in a levulinic acid yield of 52.55% and an efficiency of 81.53% (Table 4). Thereby 17.46% of the cellulose was converted into glucose, which followed the catalytic route of dehydration and rehydration into levulinic acid, resulting in 0.52 g of this product per gram of glucose consumed. This yield was equivalent to 81.53% of the expected theoretical value. The test for heterogeneous catalysis (Table 4), which used an amount in grams of catalyst that was equivalent to the normality (N) of the H$_2$SO$_4$ solution (2% v/v) and which was carried out under the same conditions of time, temperature and cellulose:H$_2$O ratio (m/v), showed levulinic acid yield of 38.55%, hydrolyzed cellulose of 17.11% and efficiency of 59.81%. These results, therefore, demonstrate that the reaction with the sulfonated solid catalyst achieved performances equivalent to 98% and 73% of those obtained by homogeneous catalysis in terms of cellulose conversion and levulinic acid yield, respectively.

Hu et al. [14] obtained approximately two times cellulose conversion (33.4%) after hydrolyzing semicrystalline cellulose from rice straw using a catalyst made from sulfonated activated carbon fibers (0.88 mmol/ g of total acids). These authors employed a catalyst/cellulose ratio of 4:1 (g/g) at 150 °C for 24 h representing more extreme reaction conditions than those used in the hydrolysis test carried out in this study. Chen et al. [19]

499   synthesized a carbonaceous solid catalyst based on activated carbon (180 ºC, 24h and 0.38 mmol/g of -SO$_3$H

500   groups) and used it to catalyze the hydrolysis of microcrystalline cellulose at 170 ºC for 12h higher than those

501   used in this study. According to these authors, cellulose conversion of 34.6% was observed after the reaction,

502   with selectivities for glucose and levulinic acid of 37.3% and 3.4%, respectively. Zuo et al. [36] synthesized a

503   solid acid catalyst (1.69 mmol/g of -SO$_3$H groups) by partially replacing the chloro groups (-Cl) of a

504   chloromethyl polystyrene resin with sulfonic groups (-SO$_3$H) and used this catalyst in the direct conversion of

505   microcrystalline cellulose in an aqueous medium to obtain levulinic acid, achieving a conversion of 100% of the

506   cellulose and a levulinic acid yield of 33%. The authors, however, used a catalyst load of five times that of the

507   cellulose at 170 °C for 10 h.

508   Cheng et al. [37] carried out an study on the conversion of cellulose into levulinic acid when it was catalyzed by

509   a Brønsted–Lewis acidic Ionic Liquids, in their study they obtained the maximum levulinic acid yield of 49.1%

510   when the [HO$_3$S-(CH$_2$)$_3$-py]Cl-FeCl$_3$ catalyst was used at 180 °C and reaction time of 10 h. In another study, the

511   researchers studied the effect of sulfolane as solvent in sulphuric acid catalyzed levulinic acid production, the

512   authors found that as they increased the sulfolane, the glucose conversion and the levulinic acid yield also

513   increased and because of that the increase in the sulfolane concentration results in a shorter reaction time,

514   however the increase in sulfolane concentration didn't affect the selectivity of levulinic acid, as the highest

515   concentration obtained was closely over 50% [11].

516   Based on the similarity between the cellulose conversions obtained for the homogeneous and heterogeneous

517   catalysis (17.46% and 17.11%) and based on the mentioned literature examples, it is evident that although the

518   SCS$_{Tc-600}$ catalyst [Adjust.] has shown catalytic activity to break the β-1,4-glycosidic bonds of cellulose

519   degradation products, such as low molecular weight glucose oligomers and cellobiose, the initial step of

520   cellulose depolymerization depends mainly on the reaction conditions in the system. Due to solid-solid

521   interaction and, consequently, resistance to mass transfer between the cellulose and the catalyst [38]. The

522   substrate-catalyst interaction occurs predominantly on the catalyst's surface. It depends on the increase in the

523   amount and size of the amorphous regions of the cellulose, which is provided by the action of water

524   (autoionization) and temperature. The contact between the acidic sites of the catalyst and the β-1,4-glycosidic

525   bonds accessible in the less crystalline regions of the cellulose, therefore, determines how efficient the hydrolysis

526   of cellulose to glucose will be susceptible following the levulinic acid synthesis pathway.

527   An alternative to solve the mass transfer problem would be to mill the cellulose with the catalyst. Su et al. [38],

528   for example, hydrolyzed microcrystalline cellulose using a carbonaceous solid catalyst prepared from cow dung

529   and reported an increase in glucose yield from 3.6% to 59.3% after grinding the cellulose and catalyst together in

530   a balls mill. Qiu et al. [15] synthesized a solid carbonaceous acid catalyst having −SO$_3$H and –Cl groups. After

531   mixing it with cellulose, also by ball milling, obtained a glucose yield of 52.8% in a reaction conducted with

532   water at 200 °C for 1 h.

533   Another problem is the thermal stability and the deposition of humins in catalyst pores. In a study carried out by

534   Araujo et al. [30], thermogravimetric analyzes showed that the degradation of sulfonic groups strongly bound to

535   the surface of carbonaceous catalysts occurs in the range of 120 ºC to 260 ºC, which suggests, therefore, that the

thermal stability of the acid sites of the catalyst used in this work is compromised during the cellulose conversion reaction at 140 °C. On the other hand, Zuo et al. [36] showed that the loss of catalytic activity of the catalyst is mainly caused by the deposition of humines in its structure, suggesting that it is possible to remove them completely and recover the catalytic activity of the catalyst through of its washing with H_2O_2 solution. Therefore, more studies taking these factors into account need to be carried out.

Conclusions

The biomass (1:1 straw and sugarcane bagasse mixture) was successfully fractionated in two stages: (1) pre-treatment with NaOH (delignification process) to obtain cellulose and black liquor and (2) recovery of the lignin soluble in the black liquor by precipitation with H_2SO_4. The optimal pretreatment conditions were 4.4 % (w/v) NaOH, 117 min at 110 ºC with 1:20 solid-liquid ratio. Under this condition, it was possibly extracted from the biomass 91.76 ± 1.85% of the lignin, thus obtaining a solid fraction with a high cellulose content (78.08 ± 1.82%) and black liquor lignin concentrated (11.01 g/L). The best condition for the recovery of soluble lignin in black liquor consisted of precipitation using acid until reaching pH equal to 3 and a rest time of 69 min. This condition makes it possible to obtain an average of 0.55 ± 0.006 g of precipitate per 50 mL of liquor. With the lignin precipitate, a carbonaceous catalyst with a total acid density of 7.27 mmol/g ± 0.727 was synthesized under a carbonization temperature of 600 °C (30 min) and a sulfonation temperature of 180 °C (12h). This catalyst showed catalytic activity for the direct conversion of cellulose into levulinic acid, employing a cellulose/catalyst ratio of 10:8 (w/w) at 140 ºC for 6h. Under this condition, the yield of levulinic acid was 38.55%. The hydrolyzed cellulose was 17.11%, equivalent to 73% and 98% of the conversion and yield achieved by the homogeneous catalysis with $H_2SO_{4(aq)}$ under the same reaction conditions. Finally, it was observed that the main bottleneck in the reaction of direct conversion of cellulose into levulinic acid using the catalyst synthesized in this work is the resistance to mass transfer between substrate and catalyst. The reaction parameters need to be better studied to work around this problem. Thus, the factors: temperature, reaction time, and catalyst/cellulose/water mass ratio need to be optimized to obtain the condition that will lead to the maximum cellulose conversion into levulinic acid. Therefore, the process proposed here can enable the inclusion of levulinic acid, a high-value chemical compound, among the by-products of the national sugar-energy sector, increasing the competitive potential of that sector.

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Statements & Declarations

Data availability: The authors declare that all data supporting the findings of this study are available within the article and its supplementary information files.

Conflict of interest: The authors declare that they have no conflict of interest.

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