# Comparative Study of Acid Hydrolysis of Lignin and Polysaccharides in Biomasses

Danila Morais de Carvalho,[a,b,*] and Jorge Luiz Colodette [a]

Effects of different acid hydrolysis conditions were studied relative to the chemical transformations of lignin in eucalyptus, sugarcane bagasse, and sugarcane straw, and on the transformations of multiple polysaccharides in eucalyptus. The acid hydrolysis using 12 mol/L sulfuric acid followed by acid hydrolysis using approximately 0.41 mol/L sulfuric acid was used as the reference for the lignin and sugar analysis. During acid hydrolysis, the relative amount of lignin increased with longer reaction times and/or greater acid concentrations for all biomasses. The overestimation of lignin in harsher acidic conditions resulted from the summation of lignocellulosic-derivatives (pseudo-lignin) together with lignin itself. Lignin reactions (dissolution/deposition) for bagasse and straw occurred in a greater extent than for eucalyptus, considering similar conditions of acid hydrolysis. The sugar transformation during acid hydrolysis was also investigated for eucalyptus. The sugar content quantified in eucalyptus decreased as the acid concentration and/or reaction time in the second hydrolysis increased. Glucose, galactose, and mannose were more resistant to harsher acidic conditions than xylose and arabinose. However, the most severe conditions (121 ºC, 90 min, and 6.15 mol/L $H_2SO_4$) caused complete sugar degradation.

*Keywords: Acid concentration; Eucalyptus wood; Klason lignin; Lignocellulosic biomasses; Sugarcane bagasse; Sugarcane straw*

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## INTRODUCTION

Acid hydrolysis is a classic method used to assess the lignin and sugar contents of lignocellulosic biomass. The classic method involves two sequential hydrolyses. The first hydrolysis is performed at ambient temperature (30 ºC) for 60 min using 12 mol/L sulfuric acid ($H_2SO_4$) (~72%) and a solid/liquid ratio of 100:1 mg/mL. The second hydrolysis is performed at 121 ºC for 60 min using a $H_2SO_4$ dilution of approximately 0.41 mol/L (~3%) and a solid/liquid ratio of approximately 3.4:1 mg/mL (adapted from TAPPI T222 om-11 (2011)). During the first hydrolysis, the concentrated acid disrupts the crystalline cellulose structure. During the second hydrolysis, the lignin and polysaccharides are hydrolyzed, and sugars are obtained from polysaccharides hydrolysis.

Acid hydrolysis is not exclusively applied to chemical analyses. The production of more advanced products from lignocellulosic chemical components, such as second-generation ethanol (Taherzadeh and Karimi 2007; Dawson and Boopathy 2008; Zhou *et al*. 2014; Carvalho *et al*. 2015), cellulose nanocrystals (Rosli *et al*. 2013; Dong *et al*. 2016; Tian *et al*. 2016), lignin hydrogel (Sun *et al*. 2016), and adhesives (Yu *et al*. 2016), also involve acid treatments.

Various chemical reactions occur in lignocellulosic biomass during acid hydrolysis, resulting in a chemical transformation of the biomass. Recent studies have demonstrated the formation of pseudo-lignin and pseudo-extractives from lignin and polysaccharide degradation products during acid and hot water pretreatments (Carvalho *et al*. 2015; Chen *et al*. 2015; Yang *et al*. 2015; Wang *et al*. 2016).

The formation of pseudo-lignin has been demonstrated in biomasses, such as eucalyptus wood, aspen wood, poplar wood, corn stover, corn stem, sugarcane bagasse, and sugarcane straw (Sannigrahi *et al*. 2011; Carvalho *et al*. 2015; Chen *et al*. 2015; Liu *et al*. 2015; Yang *et al*. 2015; Wang *et al*. 2016). Pseudo-lignin is a chemical component generated under specific conditions, and it exhibits similar chemical properties to those of lignin in the biomass. Lignin and polysaccharide re-polymerization reactions performed in acidic conditions are the most probable origins of pseudo-lignin (Li *et al*. 2005, 2007; Sannigrahi *et al*. 2011; Hu *et al*. 2012). Recent studies have demonstrated pseudo-lignin formation from holocellulose samples from poplar and pure xylose, demonstrating that pseudo-lignin generation occurs more from polysaccharide degradation products than from lignin during acid pretreatment of the biomass, particularly under severe conditions (higher temperature, longer reaction time, and greater acid concentration) (Sannigrahi *et al*. 2011; Kumar *et al*. 2013). The formation of pseudo-lignin during pretreatments should be avoided because of its established role in inhibition of the enzymes involved in the enzymatic hydrolysis of the biomass (Hu *et al*. 2012).

A recent study demonstrated that polysaccharide degradation/condensation reaction products with low molecular weight were involved in the formation of pseudo-extractives in eucalyptus (*Eucalyptus urophylla* × *Eucalyptus grandis*). These new structures generated during chemical treatments were quantified together with extractives because of their similar solubility (Carvalho *et al*. 2015).

In this study, the effects of different conditions of acid hydrolysis on lignin (Klason lignin and acid-soluble lignin) were examined and compared for eucalyptus, sugarcane bagasse, and sugarcane straw. For eucalyptus, which has been shown to form pseudo-lignin and pseudo-extractives during acid hydrolysis, the effect of acid hydrolysis on polysaccharide degradation was also investigated. The classic method (adapted from TAPPI T222 om-11 (2011)) for lignin and sugar determination was used as the reference because the extent of degradation for the chemical components was expected to be minimal. This method involves two sequential hydrolysis reactions. The first reaction was performed using 12 mol/L $H_2SO_4$, at 30 ºC for 60 min, and the second reaction was performed using the classic method (0.41 mol/L $H_2SO_4$, at 121 ºC for 60 min), and using modified conditions to evaluate different reaction times (15 min to 90 min) and $H_2SO_4$ concentrations (0.41 mol/L to 6.15 mol/L).

## EXPERIMENTAL

### Materials

Wood chips from a 7-year-old clonal eucalyptus hybrid (provided by a Brazilian pulp mill located in Mogi Guaçu, SP, Brazil) (*Eucalyptus urophylla* × *Eucalyptus grandis*) and five-month-old sugarcane bagasse (Oratórios, MG, Brazil) and sugarcane straw (Oratórios, MG, Brazil) (cultivar RB867515) were tested under different conditions of acid hydrolysis. The eucalyptus, sugarcane bagasse, and sugarcane straw specimens were converted to sawdust (40/60-mesh) with a Wiley mill bench model (Marconi, Piracicaba,

SP, Brazil), dried at room temperature (23 ºC ± 1 ºC and 50% ± 2% relative humidity), and stored inside airtight containers. The moisture content was determined according to TAPPI T264 cm-07 (2007).

Extractive removal was performed in the sawdust using a sequential extraction process as follows: 1:2 ethanol/toluene (5 h), followed by 95% ethanol (4 h) in a Soxhlet extractor (Marconi, Piracicaba, SP, Brazil), and hot water (1 h). Following the extractions, the sawdust was washed with hot water and dried at room temperature (23 ºC ± 1 ºC and 50% ± 2% relative humidity).

## Methods

*Acid hydrolysis*

The extractive-free sawdust samples were subjected to two sequential acid hydrolyses (adapted from TAPPI T222 om-11 (2011)). For the first acid hydrolysis, 3 mL of 12 mol/L $H_2SO_4$ was added to 300 mg of sawdust in a glass beaker. The hydrolysis was performed in a water bath at 30 ºC for 60 min, with frequent stirring using a glass rod (solid/liquid ratio of 100:1 mg/mL). For the second hydrolysis, multiple conditions were tested, including the classic method for Klason lignin and for acid-soluble lignin (Goldschimid 1971; Gomide and Demuner 1986). The products of the first hydrolysis were diluted to different $H_2SO_4$ concentrations ranging from 0.41 mol/L to 6.15 mol/L in a penicillin flask. The post-dilution solid/liquid ratio was approximately 3.4:1 mg/mL. The penicillin flask was closed and placed into an autoclave at 121 ºC for 15 min to 90 min, as shown in Table 1.

**Table 1.** Conditions for the Second Hydrolysis

| Treatments | $H_2SO_4$ (mol/L) | Time (min) | Temperature (ºC) |
|---|---|---|---|
| Classic Method (4)* | 0.41 | 60 | 121 |
| 1-6** | 0.41 | 15; 30; 45; 60; 75; and 90 | 121 |
| 7-12 | 0.82 | 15; 30; 45; 60; 75; and 90 | 121 |
| 13-18 | 2.05 | 15; 30; 45; 60; 75; and 90 | 121 |
| 19-24 | 4.10 | 15; 30; 45; 60; 75; and 90 | 121 |
| 25-30 | 6.15 | 15; 30; 45; 60; 75; and 90 | 121 |

* Classic method corresponds to treatment number 4 (Goldschimid 1971; Gomide and Demuner 1986)

** Treatment 1 indicates acid hydrolysis using 0.41 mol/L $H_2SO_4$ for 15 min at 121 ºC, treatment 2 indicates acid hydrolysis using 0.41 mol/L $H_2SO_4$ for 30 min at 121 ºC, *etc.*

After the reaction, the penicillin flask was opened, and the products of the hydrolysis were filtered in a sintered-glass crucible with hot water. The insoluble fraction was recovered for Klason lignin determination, and the hydrolysates were used for the determination of acid-soluble lignin and, only for eucalyptus, the sugar content.

*Determination of lignin content in eucalyptus, sugarcane bagasse, and sugarcane straw*

Klason lignin was measured gravimetrically using the acid-insoluble fraction recovered after filtration. The silica content in the biomass was determined according to TAPPI 244 cm-11 (2011). The overestimation of Klason lignin, as a result of the co-precipitation of silica in bagasse and straw, was deducted from the results presented in the discussion according to the procedure described by Carvalho *et al*. (2015).

The acid-soluble lignin (ASL) content was measured in the hydrolysates using ultraviolet spectroscopy (Cary 50 Probe, Varian, Mulgrave, VIC, Australia) at 215 nm and 280 nm, and was calculated according to Eq. 1. Total lignin content was calculated as the sum of the Klason lignin and the acid-soluble lignin contents,

$$ASL = [4.538 \times (B - C) \times 1.1] \quad (1)$$

where *ASL* is the acid-soluble lignin content (%), *B* is the absorbance read at 215 nm wavelength in the hydrolysates (%), and *C* is the absorbance read at 280 nm wavelength in the hydrolysates (%).

*Determination of sugar content in eucalyptus*

The glucose, xylose, mannose, galactose, and arabinose contents were determined from the hydrolysates of the extractive-free eucalyptus obtained after the acid hydrolyses. Neutral sugars were analyzed according to the method described by Wallis *et al*. (1996) using ion chromatography (IC) with a Dionex ICS3000 (Thermo Fisher Scientific, Taboão da Serra, SP, Brazil) using a pulsed amperometric detector, a CarboPac PA1 column (Thermo Fisher Scientific, Taboão da Serra, SP, Brazil), and a 25-µL injection volume at a flow rate of 1 mL/min. The external sugar standards used for the calibrations were glucose (Merck, Darmstadt, Germany), xylose (Merck, Darmstadt, Germany), galactose (Vetec, Rio de Janeiro, RJ, Brazil), mannose (Vetec, Rio de Janeiro, Brazil), and arabinose (Sigma, St. Louis, MO, USA). Fucose (Sigma, Banská Bystrica, Slovakia) was used as an internal standard. The neutral sugar content was reported as anhydrosugar.

*Models estimation*

The results of the Klason lignin, soluble lignin, total lignin, glucose, xylose, galactose, mannose, and arabinose contents were used to generate second-order polynomial models (Eq. 2). The models contained the following regression terms: coefficients for the main effects, coefficients for quadratic main effects, and coefficients for factor interaction effects. Statistically significant coefficients were used for the models (*i.e*., coefficients that did not exceed the significance level of 0.05 in the Student's t-test < 2 and that had a 95% confidence interval that excluded zero). Models were estimated considering $H_2SO_4$ concentration in mol/L.

$$Y = a_0 + \sum_{i=1}^{n} b_i * X_{ni} + \sum_{i=1}^{n} c_i * X_{ni}^2 + \sum_{\substack{i=1 \\ j=1}}^{n} d_{ij} * X_{ni} * X_{nj} \quad (2)$$

where *Y* is the dependent variable (%), $a_0$, $\sum_{i=1}^{n} b_i$ , $\sum_{i=1}^{n} c_i$ , and $\sum_{\substack{i=1 \\ j=1}}^{n} d_{ij}$ are the constants obtained from experimental data after the mathematic adjustment for independent term, linear term, quadratic term and interaction factor term, respectively, $X_{ni}$ is the independent variable in linear term, $X^2_{ni}$ is the independent variable in quadratic term, and $X_{ni} * X_{nj}$ is the interaction factor between independent variables.

## RESULTS AND DISCUSSION

### Lignin Hydrolysis in Eucalyptus, Sugarcane Bagasse, and Sugarcane Straw

Thirty acid hydrolysis conditions for the second hydrolysis of the classic method to quantify lignin and polysaccharides were performed in eucalyptus, sugarcane bagasse, and sugarcane straw. The various acid conditions were evaluated regarding the effect of

different $H_2SO_4$ concentrations (0.41 mol/L, 0.82 mol/L, 2.05 mol/L, 4.10 mol/L, and 6.15 mol/L) and reaction times (15 min, 30 min, 45 min, 60 min, 75 min, and 90 min) on the chemical transformations of the biomasses, particularly as they affected the quantifiable lignin. The results for Klason lignin, acid-soluble lignin, and total lignin after acid hydrolysis are shown in the Supplementary Data (Table S1, S2, and S3). Based on these results, second-order polynomial models were generated, where the errors between the observed and calculated values were lower than 14%, and the coefficient of determination was above 76% for all of the models (Table 2).

The models for Klason lignin, soluble lignin, and total lignin showed negative and positive signals for the coefficients associated with acid concentration in linear and in quadratic terms, respectively. The model generated for Klason lignin in eucalyptus produced a negative signal for the coefficient associated with acid concentration in the quadratic term, along with a non-significant effect of acid concentration on the linear term. The reaction time was associated with low coefficient values in all of the significant terms in which it appeared in the models (linear, quadratic, and interaction terms).

**Table 2.** Second-order Polynomial Models Adjusted for Klason Lignin, Acid-soluble Lignin, and Total Lignin Measured under Different Conditions of Acid Hydrolysis for Eucalyptus, Sugarcane Bagasse, and Sugarcane Straw

| Eucalyptus | | $R^2$ | F-Snedecor |
|---|---|---|---|
| 3A | $Y_{KL} = 23.78011 - 0.05809\ X_C X_C + 0.00789\ X_C X_t$ | 76.5 | 44 |
| 4A | $Y_{SL} = 5.14892 - 1.28556\ X_C + 0.22547\ X_C X_C + 0.00856\ X_C X_t - 0.00017\ X_t X_t$ | 95.8 | 144 |
| 5A | $Y_{TL} = 28.94656 - 1.38809\ X_C + 0.18657\ X_C X_C + 0.01591\ X_C X_t - 0.00014\ X_t X_t$ | 94.7 | 111 |
| Sugarcane Bagasse | | $R^2$ | F-Snedecor |
| 3B | $Y_{KL} = 20.80946 - 2.09566\ X_C + 0.29975\ X_C X_C - 0.03041\ X_t + 0.01767\ X_C X_t$ | 97.3 | 232 |
| 4B | $Y_{SL} = 2.42913 - 1.26090\ X_C + 0.25906\ X_C X_C + 0.00795\ X_C X_t - 0.00006\ X_t X_t$ | 99.2 | 756 |
| 5B | $Y_{TL} = 23.37050 - 3.36019\ X_C + 0.55881\ X_C X_C - 0.03651\ X_t + 0.02568\ X_C X_t$ | 99.2 | 732 |
| Sugarcane Straw | | $R^2$ | F-Snedecor |
| 3C | $Y_{KL} = 14.76649 - 2.10698\ X_C + 0.30624\ X_C X_C + 0.1814\ X_C X_t - 0.00028\ X_t X_t$ | 97.8 | 278 |
| 4C | $Y_{SL} = 2.55253 - 1.29636\ X_C + 0.25274\ X_C X_C + 0.00805\ X_C X_t - 0.00007\ X_t X_t$ | 98.8 | 511 |
| 5C | $Y_{TL} = 17.31903 - 3.40334\ X_C + 0.55898\ X_C X_C + 0.02619\ X_C X_t - 0.00035\ X_t X_t$ | 99.0 | 628 |
| Description of equations: A- eucalyptus; B- sugarcane bagasse; C- sugarcane straw; 3- $Y_{KL}$: Klason lignin (%); 4- $Y_{SL}$: acid-soluble lignin (%); 5- $Y_{TL}$: total lignin (%); Independent variables: $X_C$: sulfuric acid concentration; $X_t$: reaction time | | | |

The highest coefficient was associated with acid concentration in the models. This result showed that the quantifiable amounts of Klason and soluble lignin were more sensitive to a variation in the acid concentration than to a variation in the reaction time during the acid hydrolysis. The classic method for lignin quantification, which promote only minimal degradation of the chemical components in the biomass, were used as the reference (60 min and 0.41 mol/L $H_2SO_4$). The increased acidity in the acid hydrolysis affected the quantifiable amount of lignin in two ways: 1) it intensified the lignin hydrolysis and its dissolution, and 2) generated pseudo-lignin structures, which were deposited on the

acid-insoluble components. When the acid concentration was increased to 2.05 mol/L $H_2SO_4$, the lignin hydrolysis was remarkably intensified as a result of a larger hydrolysis rate of Klason lignin and soluble lignin, irrespective to the biomass (Fig. 1). For acid concentrations greater of equal to 4.10 mol/L $H_2SO_4$, the lignin hydrolysis occurred at a rate lower than the formation and deposition of lignin and polysaccharide derivatives. As a consequence, the quantifiable amount of lignin (Klason lignin and soluble lignin) increased compared to the reference. Liu *et al*. (2015) observed a reduction in the lignin dissolution rate compared to its deposition for eucalyptus subjected to hot water extraction in different conditions (150 °C to 180 °C and 40 min to 110 min). Hot water extraction is a milder acid condition in which the only sources of acids are acidic species from hemicelluloses (Lee *et al*. 2009).

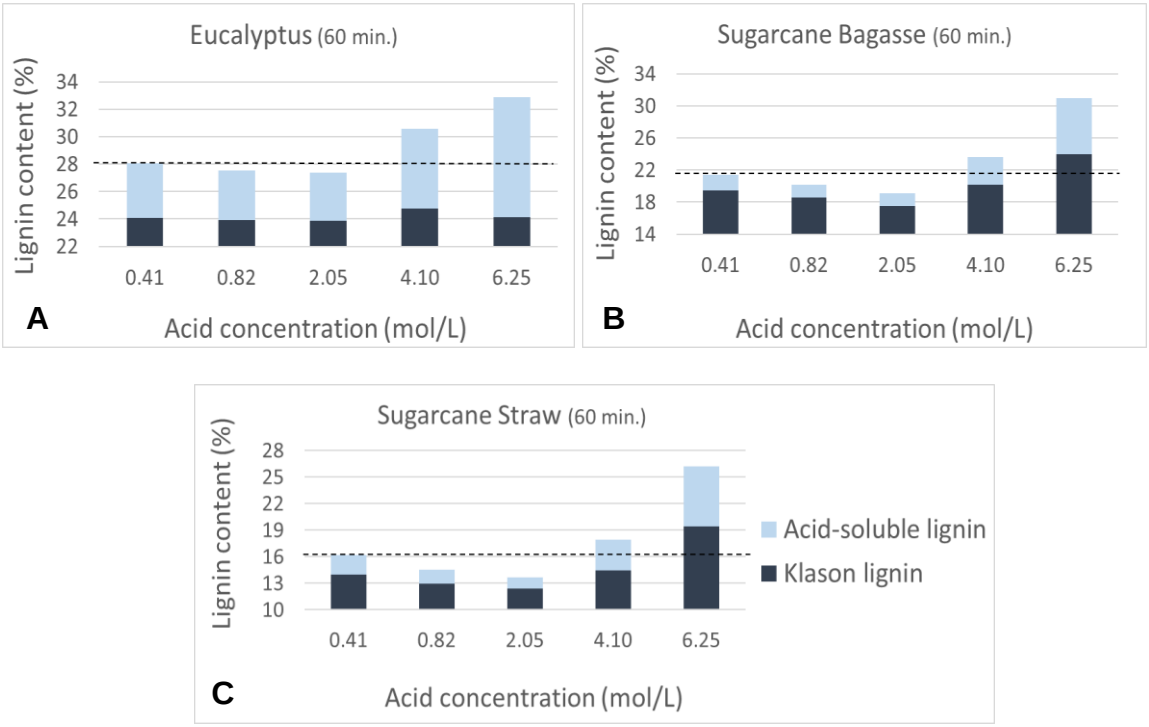


**Fig. 1.** Effect of acid concentration on the quantifiable lignin in eucalyptus (A), bagasse (B), and straw (C) after 60-min acid hydrolysis. The total lignin value (Klason lignin + acid-soluble lignin) represented under the dashed line indicates that the hydrolysis of lignin occurred to a greater extent than the formation of pseudo-lignin. The total lignin value represented above the dashed line indicates that lignin hydrolysis occurred to a lesser extent than the pseudo-lignin formation.

Lignin deposition and pseudo-lignin formation are possible reasons for the increased lignin content after certain conditions of acid hydrolysis (Liu *et al*. 2015). Although a small amount of pseudo-lignin may have been measured as acid-soluble lignin, as demonstrated by Kumar *et al*. (2013), its main fraction is quantified as Klason lignin (Sannigrahi *et al*. 2011). Pseudo-lignin is generated through lignin condensation and xylan degradation with re-condensation and precipitation onto fibers (Lora and Wayman 1978). Recent studies have demonstrated that the polysaccharide contribution to pseudo-lignin formation is more significant than that of lignin itself (Sannigrahi *et al*. 2011). In fact, apparent lignin can be generated from pure xylose during acid hydrolysis (Kumar *et al*. 2013).

The acid-soluble lignin is typically composed of low-molecular-weight products formed during lignin degradation (soluble in diluted $H_2SO_4$, *i.e.*, 0.41 mol/L $H_2SO_4$) and the hydrophilic lignin derivatives that are generated during the first hydrolysis (*i.e.*, 12 mol/L $H_2SO_4$) (Yasuda *et al*. 2001). In the present study, the chemical transformation of acid-soluble lignin occurred to a larger extent than the transformation of Klason lignin, for all of the biomasses. In the most severe conditions (90 min and 6.15 mol/L $H_2SO_4$), the amount of acid-soluble lignin was 2.2-, 4.4-, and 3.6-fold greater than that observed in the classic method (60 min and 0.41 mol/L $H_2SO_4$), for eucalyptus, bagasse, and straw, respectively. Under similar conditions, the values for quantifiable Klason lignin were only 1.1, 1.4, and 1.5 times higher compared with the classic method, for eucalyptus, bagasse, and straw, respectively (Table S1, S2, and S3).

The total lignin content was obtained mathematically as the sum of the acid-soluble lignin and Klason lignin contents. The behavior of the total lignin transformation in the various acid hydrolysis conditions was similar among the biomasses (Fig. 2).

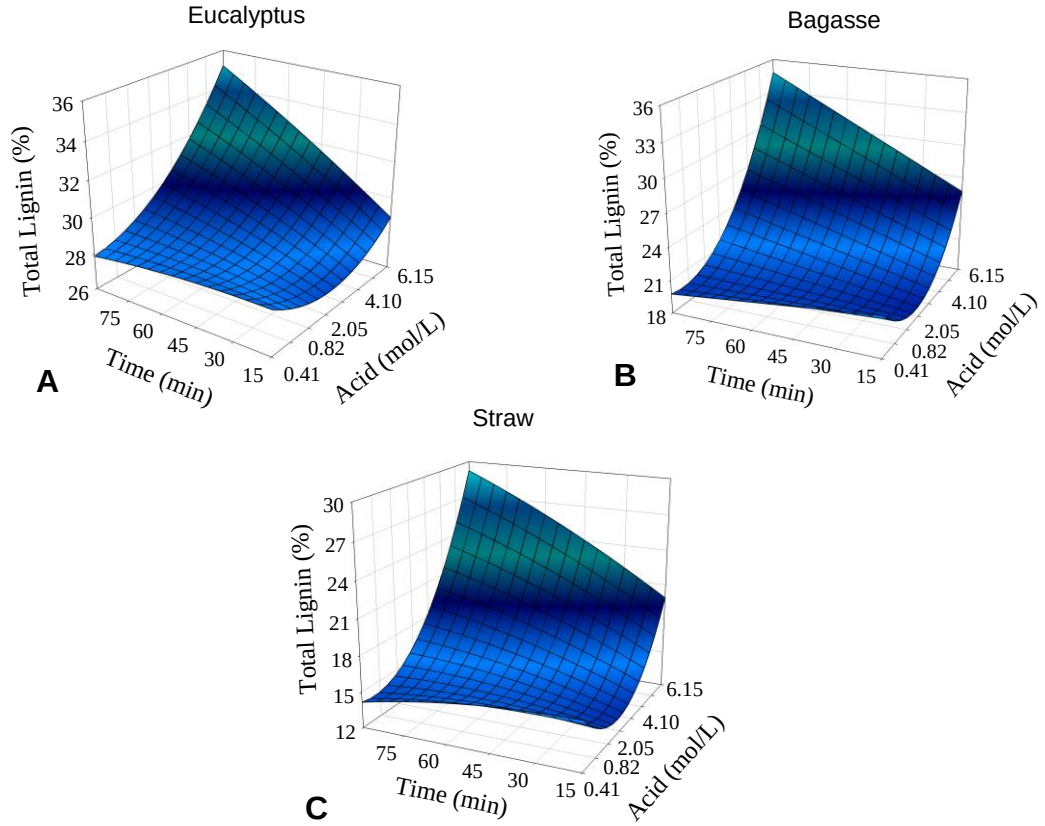


**Fig. 2.** Effect of acid concentration and reaction time on the total lignin content in eucalyptus (A), bagasse (B), and straw (C) after acid hydrolysis

The extent of the dissolution/deposition reactions was more remarkable for bagasse and straw than for eucalyptus. The values of total lignin varied in the range of 27% to 35%, 19% to 35%, and 14% to 29% for eucalyptus, bagasse, and straw, respectively. These results confirmed those from literature, in which the lignin content increased more remarkably in bagasse and straw than in eucalyptus, after acid pretreatments for second-generation ethanol production (Carvalho *et al*. 2017). The differences in the dissolution/deposition reactions most likely were caused by the different structures of the

chemical components present in these biomasses, *e.g.*, the lignin structure. The lignin in eucalyptus contains guaiacyl (G) and syringyl (S) units, while in bagasse and straw, the p-hydroxyphenyl (H) unit is also present. A previous study demonstrated S/G ratios of 2.7, 1.1, and 0.5 for lignin in eucalyptus, sugarcane bagasse, and sugarcane straw, respectively (Carvalho *et al*. 2015). The higher susceptibility of H and G lignin to condensation reactions, compared to S lignin, has been demonstrated elsewhere (Santos *et al*. 2011; Brandt *et al*. 2013). The results of this study confirmed those from the literature, showing that for bagasse and straw, which contain large amounts of H and G lignins, the deposition during acid hydrolysis was 63% and 79%, respectively (compared with the classic method). For eucalyptus, which presents a more favorable S/G ratio, the maximum deposition was only 24%. The increased acid concentration and reaction time in acid hydrolysis promoted an increased amount of quantifiable total lignin. This was due to the larger formation and deposition of lignin degradation products (quantified as Klason lignin) and hydrophilic lignin derivatives (quantified as acid-soluble lignin).

## Chemical Transformation of Polysaccharides in Eucalyptus

During acid hydrolysis, eucalyptus potentially generates pseudo-lignin and pseudo-extractives from polysaccharide degradation products as demonstrated elsewhere (Carvalho *et al*. 2015). The dynamic for the formation of these new structures depends on the conditions of the acid hydrolysis. The effect of acid hydrolysis, in various acid concentrations, on polysaccharide degradation was investigated for eucalyptus. Identical conditions of acid hydrolysis were used to assess the lignin and sugar contents in eucalyptus ($H_2SO_4$ concentrations of 0.41 mol/L, 0.82 mol/L, 2.05 mol/L, 4.10 mol/L, and 6.15 mol/L and reaction times of 15 min, 30 min, 45 min, 60 min, 75 min, and 90 min). The results for the glucose, xylose, galactose, mannose, and arabinose contents, along with the total sugar content and lignin + sugars content for the different conditions of acid hydrolysis are shown in the Supplementary Data (Table S4). Based on these results, second-order polynomial models were generated, with the error between the observed and calculated values set to below 13% and the coefficient of determination set to above 94% for all of the models (Table 3).

The chemical transformations of the polysaccharides through acid hydrolysis were affected by the acid concentration and reaction time. Similar to the results observed for lignin, the hydrolysis reactions were more affected by acid concentration than by reaction time, proved by the highest coefficient values for terms containing the acid concentration variable, particularly in linear terms (Table 3). In addition, a negative interaction between acid concentration and reaction time was observed in most of the models. This result indicated that a longer reaction time, combined with a higher acid concentration, increased the rate of polysaccharides hydrolysis.

A negative effect of acid concentration on sugar degradation was observed for all of the sugars evaluated. Taking into consideration the second hydrolysis of classic method for sugar quantification (60 min and 0.41 mol/L $H_2SO_4$), the increasing acid concentration, for the same reaction time, promoted intense polysaccharide degradation (Fig. 3). This result is probably explained because during hydrolysis, especially in harsher acid conditions, polysaccharides are likely fragmented into low-molecular-weight degradation products such as furfural, hydroxymethyl furfural (HMF), acetic acid, *etc*. (Li *et al*. 2005). These structures probably were either dissolved in the acid solution or deposited on acid-insoluble structures, which remained after acid hydrolysis.

**Table 3.** Second-order Polynomial Models Adjusted for Glucose, Xylose, Galactose, Mannose, Arabinose, Total Sugar Content, and the Total Contents of Lignin + Sugars Measured under Different Acid Hydrolysis Conditions for Eucalyptus

| Eucalyptus | | $R^2$ | F-Snedecor |
|---|---|---|---|
| 6 | $Y_{Glc} = 47.50919 - 1.29277\ X_C - 0.42411\ X_CX_C - 0.04785\ X_CX_t$ | 99.1 | 989 |
| 7 | $Y_{Xyl} = 16.73465 - 5.21243\ X_C + 0.43812\ X_CX_C - 0.02520\ X_t$ | 98.5 | 585 |
| 8 | $Y_{Gal} = 0.795849 - 0.172486\ X_C + 0.018318\ X_t - 0.000766\ X_CX_t - 0.000134\ X_tX_t$ | 95.6 | 134 |
| 9 | $Y_{Man} = 0.724449 - 0.117548\ X_C + 0.007971\ X_t - 0.000575\ X_tX_t - 0.000068\ X_CX_t$ | 94.5 | 107 |
| 10 | $Y_{Ara} = 1.605607 - 0.154392\ X_C - 0.036216\ X_t + 0.001437\ X_CX_t + 0.000234\ X_tX_t$ | 95.5 | 133 |
| 11 | $Y_{TS} = 65.58355 - 6.52135\ X_C - 0.05429\ X_CX_t$ | 99.2 | 1686 |
| 12 | $Y_{L+S} = 91.31399 - 9.03315\ X_C + 0.27473\ X_CX_C - 0.05534\ X_t - 0.02768\ X_CX_t$ | 99.7 | 1031 |
| Description of equations: 6- $Y_{Glc}$: glucose (%); 7- $Y_{Xyl}$: xylose (%); 8- $Y_{Gal}$: galactose (%); 9- $Y_{Man}$: mannose (%); 10- $Y_{Ara}$: arabinose (%); 11- $Y_{TS}$: total sugars (%); 12- $Y_{L+S}$: total lignin + sugars (%); Independent variables: $X_C$: sulfuric acid concentration; $X_t$: reaction time | | | |

Although in the present study the composition of polysaccharides degradation products was not assessed, interesting results were observed for sugars content. Considering 60 min acid hydrolysis, by increasing the acid concentration from 0.41 mol/L to 0.82 mol/L, the hydrolysis of the sugars intensified, but at varying rates for the different sugars (Fig. 3).

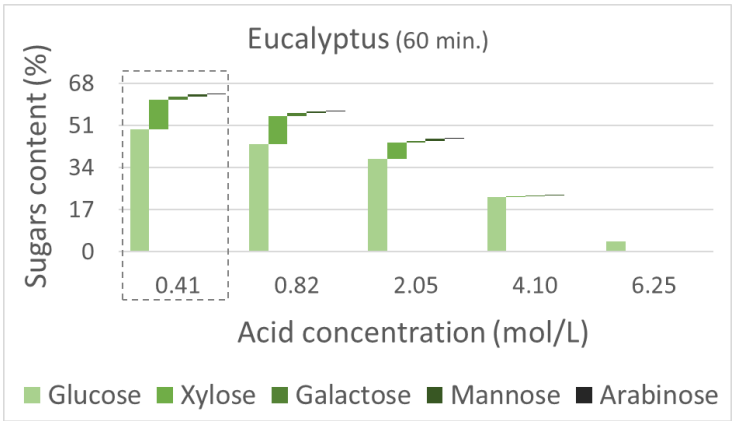


**Fig. 3.** Sugar degradation in eucalyptus during treatments performed for 60 min using different concentrations of sulfuric acid (0.41 mol/L to 6.15 mol/L); reference in dashed box

In contrast, acid concentrations greater than 0.82 mol/L $H_2SO_4$ preferentially intensified the degradation of xylose and arabinose (C5 sugars) over the degradation of glucose, mannose, and galactose (C6 sugars). Consequently, approximately 97.5% of xylose and 100% of arabinose were degraded using 4.10 mol/L $H_2SO_4$ in acid hydrolysis. Using 6.25 mol/L $H_2SO_4$, glucose was the only sugar quantified, but the amount recovered was only 8.4% of the glucose content measured using the classic method. Considering the same acid concentration, variation in time resulted expressive variation in sugars content only when combined with greater acid concentrations (> 2.05 mol/L $H_2SO_4$) (Fig. 4).

Glucose was the sugar that was least affected by the acid hydrolysis conditions, even when the variation in acid concentration was considered. Under severe conditions of acid hydrolysis, glucose was the only sugar remaining (Fig. 4). Even so, the glucose was completely degraded at the most extreme acid hydrolysis conditions (90 min and 6.15 mol/L $H_2SO_4$) along with all of the other sugars.

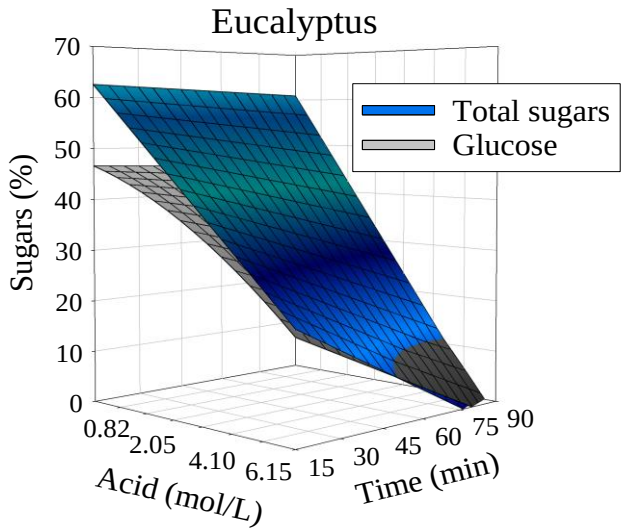


**Fig. 4.** Effect of the acid concentration and reaction time on the total sugar content and the glucose content in eucalyptus after acid hydrolysis

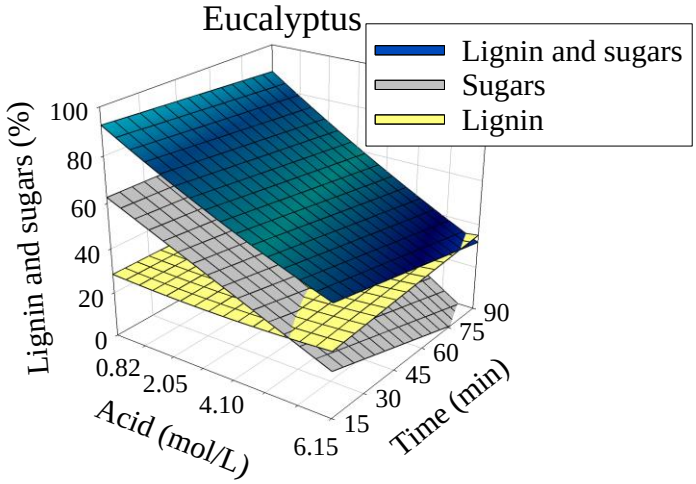


**Fig. 5.** Effect of acid concentration and reaction time on the total lignin + sugar content, sugar content, and lignin content in eucalyptus after acid hydrolysis

During acid hydrolysis, by increasing the acid concentration and reaction time, a strong sugar loss associated with the increasing lignin content was observed for eucalyptus. These chemical transformations could have been linked to complete polysaccharide hydrolysis and to reactions that involved rearrangements and/or the condensation of lignin and sugars, which resulted in the formation of new structures, such as pseudo-lignin. Thus, the increase in the acid concentration and reaction time increased the overall loss of eucalyptus during the acid hydrolysis. The classic method (0.41 mol/L $H_2SO_4$ and 60 min in second hydrolysis) recovered 89.7% of the total lignin and sugar contents in eucalyptus.

By increasing the $H_2SO_4$ concentration from 0.41 mol/L to 6.15 mol/L, the maximal total mass recovery decreased to 37.0% (from which 32.9% was lignin and 4.1% was sugars), using an identical reaction time (60 min). Only lignin (including pseudo-lignin) was observed after hydrolysis when 6.15 mol/L $H_2SO_4$ was used for 90 min (Fig. 5).

## CONCLUSIONS

1. The quantifiable amounts of Klason and soluble lignin were more affected by variation in the acid concentration than to the reaction time during acid hydrolysis.
2. Using identical acid hydrolysis conditions, the extent of the dissolution/deposition reactions for lignin were more remarkable for bagasse and straw than for eucalyptus.
3. The quantifiable sugar content in eucalyptus decreased with increasing acid concentration and reaction time until the most severe condition of acid hydrolysis (121 ºC, 90 min, and 6.15 mol/L $H_2SO_4$) was reached, in which all of the polysaccharides were degraded.
4. Xylose and arabinose (C5 sugars) were highly degraded under severe acid hydrolysis conditions (higher acid concentrations and longer reaction times) compared to glucose, galactose, and mannose (C6 sugars).

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## APPENDIX

### SUPPLEMENTARY DATA

**Table S1.** Results of Klason Lignin, Acid-soluble Lignin, and Total Lignin for Eucalyptus Treated under Different Acid Hydrolysis Conditions

| Treatments | $H_2SO_4$ (mol/L) | Time (min) | Klason Lignin (%) | Soluble Lignin (%) | Total Lignin (%) |
|---|---|---|---|---|---|
| 1 | 0.41 | 15 | 24.33 | 4.39 | 28.72 |
| 2 | 0.41 | 30 | 24.30 | 4.63 | 28.93 |
| 3 | 0.41 | 45 | 23.17 | 4.60 | 27.77 |
| 4* | 0.41 | 60 | 24.07 | 3.97 | 28.04 |
| 5 | 0.41 | 75 | 24.00 | 4.35 | 28.35 |
| 6 | 0.41 | 90 | 24.67 | 3.84 | 28.51 |
| 7 | 0.82 | 15 | 23.81 | 4.34 | 28.15 |
| 8 | 0.82 | 30 | 23.93 | 5.02 | 28.95 |
| 9 | 0.82 | 45 | 23.70 | 4.20 | 27.90 |
| 10 | 0.82 | 60 | 23.93 | 3.58 | 27.51 |
| 11 | 0.82 | 75 | 24.10 | 3.86 | 27.96 |
| 12 | 0.82 | 90 | 24.30 | 3.51 | 27.81 |
| 13 | 2.05 | 15 | 23.27 | 3.60 | 26.87 |
| 14 | 2.05 | 30 | 24.10 | 3.51 | 27.61 |
| 15 | 2.05 | 45 | 25.23 | 3.90 | 29.13 |
| 16 | 2.05 | 60 | 23.87 | 3.53 | 27.40 |
| 17 | 2.05 | 75 | 25.17 | 3.30 | 28.47 |
| 18 | 2.05 | 90 | 25.04 | 3.88 | 28.92 |
| 19 | 4.10 | 15 | 23.10 | 3.68 | 26.78 |
| 20 | 4.10 | 30 | 22.80 | 5.19 | 27.99 |
| 21 | 4.10 | 45 | 24.47 | 4.96 | 29.43 |
| 22 | 4.10 | 60 | 24.78 | 5.78 | 30.56 |
| 23 | 4.10 | 75 | 25.33 | 5.44 | 30.77 |
| 24 | 4.10 | 90 | 25.60 | 5.43 | 31.03 |
| 25 | 6.15 | 15 | 22.87 | 6.17 | 29.04 |
| 26 | 6.15 | 30 | 22.99 | 7.22 | 30.21 |
| 27 | 6.15 | 45 | 23.76 | 7.99 | 31.75 |
| 28 | 6.15 | 60 | 24.13 | 8.73 | 32.86 |
| 29 | 6.15 | 75 | 25.29 | 8.77 | 34.06 |
| 30 | 6.15 | 90 | 26.13 | 8.57 | 34.70 |
| * Classic method used as the reference | | | | | |

**Table S2.** Results of Klason Lignin, Acid-soluble Lignin, and Total Lignin for Sugarcane Bagasse Treated under Different Acid Hydrolysis Conditions

| Treatments | $H_2SO_4$ (mol/L) | Time (min) | Klason Lignin (%) | Soluble Lignin (%) | Total Lignin (%) |
|---|---|---|---|---|---|
| 1 | 0.41 | 15 | 19.30 | 1.92 | 21.22 |
| 2 | 0.41 | 30 | 19.17 | 1.99 | 21.16 |
| 3 | 0.41 | 45 | 18.90 | 2.00 | 20.90 |
| 4* | 0.41 | 60 | 19.50 | 1.87 | 21.37 |
| 5 | 0.41 | 75 | 18.10 | 2.04 | 20.14 |
| 6 | 0.41 | 90 | 17.90 | 1.91 | 19.81 |
| 7 | 0.82 | 15 | 18.54 | 1.77 | 20.31 |
| 8 | 0.82 | 30 | 18.87 | 1.77 | 20.64 |
| 9 | 0.82 | 45 | 18.70 | 1.71 | 20.41 |
| 10 | 0.82 | 60 | 18.57 | 1.58 | 20.15 |
| 11 | 0.82 | 75 | 18.30 | 1.77 | 20.07 |
| 12 | 0.82 | 90 | 17.94 | 1.71 | 19.65 |
| 13 | 2.05 | 15 | 18.37 | 1.55 | 19.92 |
| 14 | 2.05 | 30 | 17.97 | 1.48 | 19.45 |
| 15 | 2.05 | 45 | 18.10 | 1.24 | 19.34 |
| 16 | 2.05 | 60 | 17.50 | 1.58 | 19.08 |
| 17 | 2.05 | 75 | 17.54 | 1.44 | 18.98 |
| 18 | 2.05 | 90 | 18.20 | 2.12 | 20.32 |
| 19 | 4.10 | 15 | 18.70 | 1.73 | 20.43 |
| 20 | 4.10 | 30 | 18.37 | 2.66 | 21.03 |
| 21 | 4.10 | 45 | 19.04 | 3.05 | 22.09 |
| 22 | 4.10 | 60 | 20.17 | 3.48 | 23.65 |
| 23 | 4.10 | 75 | 20.44 | 3.86 | 24.30 |
| 24 | 4.10 | 90 | 20.70 | 4.22 | 24.92 |
| 25 | 6.15 | 15 | 20.30 | 4.91 | 25.21 |
| 26 | 6.15 | 30 | 21.27 | 6.19 | 27.46 |
| 27 | 6.15 | 45 | 22.40 | 6.85 | 29.25 |
| 28 | 6.15 | 60 | 23.95 | 7.04 | 30.99 |
| 29 | 6.15 | 75 | 25.54 | 7.76 | 33.30 |
| 30 | 6.15 | 90 | 26.53 | 8.30 | 34.83 |
| * Classic method used as the reference | | | | | |

**Table S3.** Results of Klason Lignin, Acid-soluble Lignin, and Total Lignin for Sugarcane Straw Treated under Different Acid Hydrolysis Conditions

| Treatments | $H_2SO_4$ (mol/L) | Time (min) | Klason Lignin (%) | Soluble Lignin (%) | Total Lignin (%) |
|---|---|---|---|---|---|
| 1 | 0.41 | 15 | 13.50 | 2.12 | 15.62 |
| 2 | 0.41 | 30 | 14.16 | 1.97 | 16.13 |
| 3 | 0.41 | 45 | 13.63 | 2.14 | 15.77 |
| 4* | 0.41 | 60 | 14.00 | 2.17 | 16.17 |
| 5 | 0.41 | 75 | 13.23 | 2.11 | 15.34 |
| 6 | 0.41 | 90 | 12.93 | 1.86 | 14.79 |
| 7 | 0.82 | 15 | 13.53 | 1.85 | 15.38 |
| 8 | 0.82 | 30 | 12.90 | 1.85 | 14.75 |
| 9 | 0.82 | 45 | 13.46 | 1.90 | 15.36 |
| 10 | 0.82 | 60 | 12.93 | 1.64 | 14.57 |
| 11 | 0.82 | 75 | 12.36 | 1.67 | 14.03 |
| 12 | 0.82 | 90 | 11.93 | 1.87 | 13.80 |
| 13 | 2.05 | 15 | 12.63 | 1.43 | 14.06 |
| 14 | 2.05 | 30 | 12.93 | 1.39 | 14.32 |
| 15 | 2.05 | 45 | 12.86 | 1.18 | 14.04 |
| 16 | 2.05 | 60 | 12.40 | 1.30 | 13.70 |
| 17 | 2.05 | 75 | 12.63 | 1.49 | 14.12 |
| 18 | 2.05 | 90 | 12.46 | 1.84 | 14.30 |
| 19 | 4.10 | 15 | 12.66 | 1.75 | 14.41 |
| 20 | 4.10 | 30 | 13.40 | 2.57 | 15.97 |
| 21 | 4.10 | 45 | 14.23 | 2.93 | 17.16 |
| 22 | 4.10 | 60 | 14.43 | 3.53 | 17.96 |
| 23 | 4.10 | 75 | 15.62 | 3.71 | 19.33 |
| 24 | 4.10 | 90 | 15.66 | 4.3 | 19.96 |
| 25 | 6.15 | 15 | 15.06 | 4.67 | 19.73 |
| 26 | 6.15 | 30 | 16.03 | 5.51 | 21.54 |
| 27 | 6.15 | 45 | 17.30 | 6.72 | 24.02 |
| 28 | 6.15 | 60 | 19.44 | 6.79 | 26.23 |
| 29 | 6.15 | 75 | 20.42 | 7.34 | 27.76 |
| 30 | 6.15 | 90 | 21.18 | 7.79 | 28.97 |
| * Classic method used as the reference | | | | | |

**Table S4.** Results of Glucose, Xylose, Galactose, Mannose, Arabinose, Total Sugars and Lignin + Sugars for Eucalyptus Treated under Different Acid Hydrolysis Conditions

| Eucalyptus | | | | | | | |
|---|---|---|---|---|---|---|---|
| Treatments * | Glucose (%) | Xylose (%) | Galactose (%) | Mannose (%) | Arabinose (%) | Total sugars (%) | Lignin + sugars (%) |
| 1 | 46.61 | 14.29 | 0.69 | 0.66 | 1.60 | 63.85 | 92.57 |
| 2 | 44.03 | 12.80 | 1.25 | 0.79 | 0.63 | 59.50 | 88.43 |
| 3 | 46.66 | 13.40 | 1.31 | 0.85 | 0.32 | 62.53 | 90.30 |
| 4** | 49.4 | 12.00 | 1.20 | 0.90 | 0.30 | 61.68 | 89.72 |
| 5 | 44.26 | 12.62 | 1.36 | 0.85 | 0.27 | 59.37 | 87.72 |
| 6 | 46.44 | 13.13 | 1.25 | 0.95 | 0.28 | 62.05 | 90.56 |
| 7 | 45.58 | 12.38 | 0.62 | 0.61 | 1.52 | 60.72 | 88.87 |
| 8 | 44.91 | 12.71 | 1.26 | 0.84 | 0.59 | 60.32 | 89.27 |
| 9 | 44.40 | 12.40 | 1.29 | 0.87 | 0.28 | 59.24 | 87.14 |
| 10 | 43.48 | 11.27 | 1.08 | 0.91 | 0.25 | 57.00 | 84.51 |
| 11 | 42.60 | 11.16 | 1.15 | 0.79 | 0.19 | 55.89 | 83.85 |
| 12 | 41.91 | 10.78 | 1.22 | 0.74 | 0.21 | 54.86 | 82.67 |
| 13 | 41.61 | 7.51 | 0.47 | 0.48 | 1.23 | 51.30 | 78.17 |
| 14 | 41.18 | 7.20 | 0.82 | 0.64 | 0.49 | 50.33 | 77.94 |
| 15 | 38.95 | 8.01 | 1.07 | 0.74 | 0.19 | 48.96 | 78.09 |
| 16 | 37.44 | 6.43 | 0.90 | 0.73 | 0.18 | 45.68 | 73.08 |
| 17 | 34.69 | 5.52 | 0.92 | 0.44 | 0.15 | 41.73 | 70.20 |
| 18 | 34.92 | 4.95 | 0.90 | 0.50 | 0.13 | 41.40 | 70.32 |
| 19 | 32.13 | 2.35 | 0.32 | 0.33 | 0.50 | 35.64 | 62.42 |
| 20 | 30.11 | 2.60 | 0.43 | 0.36 | 0.12 | 33.61 | 61.60 |
| 21 | 24.89 | 1.34 | 0.71 | 0.36 | 0.08 | 27.39 | 56.82 |
| 22 | 22.05 | 0.30 | 0.33 | 0.33 | 0.00 | 23.02 | 53.58 |
| 23 | 20.40 | 0.00 | 0.37 | 0.27 | 0.00 | 21.04 | 51.81 |
| 24 | 15.36 | 0.00 | 0.37 | 0.05 | 0.04 | 15.82 | 46.85 |
| 25 | 19.10 | 0.87 | 0.33 | 0.23 | 0.00 | 20.54 | 49.58 |
| 26 | 13.80 | 0.00 | 0.00 | 0.00 | 0.00 | 13.80 | 44.01 |
| 27 | 9.40 | 0.00 | 0.00 | 0.00 | 0.00 | 9.40 | 41.15 |
| 28 | 4.13 | 0.00 | 0.00 | 0.00 | 0.00 | 4.13 | 36.99 |
| 29 | 2.38 | 0.00 | 0.00 | 0.00 | 0.00 | 2.38 | 36.44 |
| 30 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 34.70 |

* Acid concentration and reaction time in treatments are the same described in the previous tables (Tables S1, S2, and S3)
* Classic method used as the reference