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Optimization of hydrothermal conversion of bamboo (*Phyllostachys aureosulcata*) to levulinic acid via response surface methodology

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Abstract

In this study, the dilute acid hydrolysis of lignocellulosic bamboo (*Phyllostachys aureosulcata*) particles to levulinic acid in a hydrothermal synthesis reactor is reported. The aim of the study was to optimize the reaction conditions for maximum levulinic acid production in terms of reaction time (t), reaction temperature (T) and HCl concentration (c_{HCl}) via Response Surface Methodology (RSM). A maximum levulinic acid yield of 9.46 w% was predicted at the following reaction conditions: t of 3 h, T of 160 °C and c_{HCl} of 0.37 M. A maximal experimental yield of levulinic acid of 10.13 w% was observed, which in respect to the cellulose fraction of the bamboo particles corresponds to 34.60 w% or 48.05 mol%. Furfural, which is formed by the hemicellulose fraction of bamboo, has not been observed within the boundaries of the RSM model, since it is already degraded under the given reaction conditions. The conversion of levulinic acid and furfural occurred more or less simultaneously, however, furfural was more vulnerable to degradation reactions at the given process conditions. Therefore, if both fractions (cellulose + hemicellulose) are required to be valorized, further optimization is required. However, the global results of this study provide insight in the potential of lignocellulosic bamboo as an alternative platform to fossil sources.

Keywords

Dilute acid hydrolysis, Levulinic acid, Response Surface Methodology, Cellulose

1. Introduction

The depletion of non-renewable fossil resources and the environmental pollution associated with the exploitation of these resources have resulted in a growing awareness for a transition towards a bio-based economy. Sugars and starch extracted from agricultural crops are much desired feedstocks for the production of bio-based chemicals and materials. However, competition with the existing food and feed production questions the sustainability of using these feedstocks [1, 2]. Lignocellulosic biomass, being the most abundant and renewable resource on earth, can be used as a raw feedstock for the production of a large variety of chemical building blocks and polymers [3]. Lignocellulosic biomass sources which include corn stover, bagasse, forestry residues, sawdust, municipal waste, etc. are currently considered as low-value waste [7]. When applying lignocellulosic biomass on a large scale, a vast demand of feedstock is required and thus, energy crops dedicated to bio-fuel and bio-based chemicals need to be grown [7, 9]. This demand can be met by growing energy crops on polluted, infertile and non-arable land, where there is no competition with food and feed production. In this study, bamboo (*Phyllostachys aureosulcata*) was used as a lignocellulosic biomass feedstock. Bamboo is ranked within the C4 crops, which can be grown in high biomass yield, even in polluted and infertile land [9, 10]. Plant biomass resources (e.g. bamboo) consists primarily of three polymer constituents: lignin, hemicellulose and cellulose along with smaller amounts of lipids, proteins, extractives and inorganic ash. The composition of these constituents vary from one biomass species to another and even within a single plant species, the composition can vary dependent on the age, geographic location, *etc.* Lignin is a complex, high molecular weight structure containing heterogeneously linked monomeric phenolic compounds.

Levulinic acid has been reported as being a building block [4–7] that can be produced from lignocellulosic biomass. It can be used for the production of wide variety of fine chemicals such as diphenolic acid, valeric acid, methyltetrahydrofuran, etc [8]. The development and optimization of chemical processes involved in levulinic acid production can help reducing the cost to a sufficiently low level so that the production becomes more economically viable. This can help opening the market for a broader range of applications. The thermocatalytic conversion of carbohydrates from lignocellulosic biomass to platform chemicals in the presence of an acid catalyst has been reported extensively in literature [9–14]. A schematic overview of the different reactions taking place during acid hydrolysis of lignocellulosic carbohydrates is presented in Figure 1.

Figure 1: Composition of lignocellulosic bamboo (*Phyllostachys aureosulcata*) particles and the conversion pathway to furfural and levulinic acid.

When applying dilute acid hydrolysis to lignocellulosic biomass, cellulose and hemicellulose are converted into their respective platform chemicals but lignin remains unaffected, due to its high recalcitrant structure. Processes applying extreme conditions (high temperature), such as pyrolysis, are required to convert lignin to hydrocarbon fuels [15, 16]. The hemicellulose fraction primarily consists of C5 sugars such as xylose and arabinose that can be converted to furfural under acidic conditions. The cellulose fraction of lignocellulosic biomass consists solely of C6 carbohydrates that can be hydrolyzed to glucose monomers in the presence of an aqueous medium at high temperature ($> 100\text{ }^{\circ}\text{C}$). Furthermore, this glucose monomers can be converted into fructose through isomerization in an acidic medium. Upon fructose formation, fructose is dehydrated to 5-hydroxymethylfurfural (HMF). When a monophasic system is applied, i.e., solely containing an aqueous medium, HMF is immediately rehydrated to levulinic acid and formic acid. This ring opening reaction, leading to the formation of levulinic acid, does not occur with furfural due to the absence of the hydroxymethyl group. When applying a biphasic system, an organic solvent (e.g. MIBK, THF, *etc*), the formation of HMF from cellulose can be promoted by suppressing the rehydration reaction of HMF to levulinic acid [17, 18]. However, in this study, a monophasic reaction system consisting of acidified (HCl) water is used, which allows the conversion of cellulose and hemicellulose to levulinic and furfural, respectively. From literature, it is known that furfural is formed under milder reaction conditions (lower T and/or lower c_{HCl}) than levulinic acid [19]. Under the more harsh conditions of levulinic acid production, furfural is prone to degradation reactions, which makes a simultaneous production of furfural and levulinic acid difficult [10, 11, 20]. For this reason and the fact that levulinic acid is ranked amongst the top 10 bio-based platforms chemicals for the future [21], the focus of this study is the optimization of levulinic acid production. Furthermore, during the course of the reaction, intermediary products can undergo unwanted polymerization reactions to produce humins (insoluble carbonaceous species), resulting in a lower yield of the desired reaction products. Up to date, the molecular structure as well as the stoichiometry involved in humin formation have not been identified [22].

Generally, two types of acidic catalyst can be applied in this conversion process with both their (dis)advantages. A first group are the liquid homogenous catalysts (i.e. HCl, H_2SO_4 , *etc.*) which are used extensively for the conversion of C6 carbohydrates to levulinic acid. This type of catalysts are relatively low-cost and highly effective [5, 6, 23]. However, the drawback of homogeneous catalysts is that they are difficult to recover. This leads to excessive amounts of wastewater, which is not convenient from a sustainable and environmental point of view [23]. A second type of catalysts are the solid heterogeneous acid catalysts (i.e. zeolites, ion exchange resins, *etc.*). The greatest advantage of this type of catalysts is the ease of separation, and thereby overcoming the recovery difficulties [5, 24, 25]. However, heterogeneous catalysts often suffer from limited activity and selectivity, resulting in lower reaction yields, slower reaction rates and a higher catalyst/substrate ratio compared to homogeneous catalysts [5, 24]. Heterogeneous catalysts are difficult to separate from the solid lignin residue formed in the present study and therefore, the liquid homogeneous HCl was chosen as the acidic catalyst for this study.

The aim of this study is the optimization of T , c_{HCl} and t for maximizing the yield of levulinic acid from bamboo particles. The optimization was performed by means of Response Surface Methodology (RSM), which allows us to predict the optimum reaction conditions for a maximum levulinic acid yield.

2. Materials and methods

2.1. Feedstock and chemicals

2. All experiments were conducted using bamboo leaves (*Phyllostachys aureosulcata*) from the Arboretum in Bokrijk, Belgium. The bamboo was approximately 3 years old. Prior to the experiments, it was mechanically grinded and sieved, resulting in particles $< 1\text{ mm}$. The reactions took place in an aqueous medium (demineralized water) with varying concentrations of hydrochloric acid (Fisher scientific, 5M) as the homogeneous acid catalyst.

2.2. Experimental set-up

All experiments were carried out in a PTFE-lined hydrothermal synthesis reactor. The outer material of this reactor consists of stainless steel (internal diameter 33 mm, wall thickness 5.5 mm). Inside this stainless steel casing fits a PTFE container (internal diameter 22 mm, wall thickness 5 mm) with a volume of 18 mL, preventing contact of the reaction mixture with the outer casing and thereby avoiding possible corrosion. In an experiment, the reactor was filled with 1 g of bamboo particles and 10 mL of HCl solution. The hydrothermal synthesis reactors were placed in a muffle furnace to obtain the desired reaction temperature. When the reaction was completed, the reactor was cooled rapidly by placing it in an ice bath to prevent further reaction. After cooling, the reaction mixture was filtered (Macherey-Nagel glass fiber filters, GF-3, 0.6 μm retention capacity) and the samples were analyzed as described in the next section.

2.3. Analytical techniques

The residual particulate matter was determined and characterized by a modified Van Soest fractionation method [26]. Furfural and levulinic acid concentrations were determined in the filtrate. All experiments were performed in triplicate. Levulinic acid and furfural concentrations were measured by means of GC-FID. Prior to GC analysis, both furfural and levulinic acid were extracted from the aqueous reaction medium with diethyl ether (Sigma Aldrich), and 2-methylvaleric acid (Acros Organics) was used as an internal standard to quantify the recovery. An aliquot (1 μL) was subsequently injected into the GC. The GC was equipped with a HP-FFAP column (Agilent, J&W HP-FFAP length 30 m, internal diameter 0.25 mm, film thickness 0.25 μm) and a flame ionization detector (FID) to determine levulinic acid and furfural concentrations. The mass of residual particulate matter after the reaction was completed, was gravimetrically determined by filtering the reaction mixture through a glass fiber filter (Macherey Nagel, 0.6 μm retention capacity) and drying it 24 h at 105 $^{\circ}\text{C}$. After drying, the particulate matter was gravimetrically characterized by a modified Van Soest fractionation method that is described in [26]. In brief, a first fraction (the so-called “soluble” fraction or extractives) is determined by an extraction step with a neutral detergent at 100 $^{\circ}\text{C}$ using microwave irradiation. The residual particulate matter was subsequently treated with sodium chlorite and acetic acid at 75 $^{\circ}\text{C}$ in a water bath. The loss in particulate matter can be attributed to the lignin fraction. The solid matter which remains is holocellulose (hemicellulose + cellulose). In a final extraction step, hemicellulose is extracted with a mildly acidified detergent at 100 $^{\circ}\text{C}$ using microwave irradiation, which only leaves cellulose as the sole particulate matter. The ash content is determined by heating the bamboo particles for 2h at 550 $^{\circ}\text{C}$ in a muffle furnace. The residual inorganic matter is the ash content.

2.4. Response Surface Methodology

Response Surface Methodology (RSM) is an optimization tool widely used in literature amongst a variety of research topics [10, 12, 17, 27]. In this study, RSM was used to systematically investigate the effects of three independent variables (t , T and c_{HCl}) on the dilute acid hydrolysis of lignocellulosic biomass, and thereby optimizing the levulinic acid yield. The levulinic acid yield is chosen over the furfural yield, since the cellulose fraction (29.28 w%) of bamboo is almost double the hemicellulose fraction (16.62 w%), making a higher yield (in absolute mass) of levulinic acid possible. A full factorial design in triplicate was applied to evaluate the effect (linear, quadratic, 2-way interaction) of these variables, leading to 81 (3×3^3) unique experiments. The levels and ranges of the variables are listed in Table 1.

Table 1: Levels of the independent variables

Independent variables	Unit	Variable ranges=		
		low	medium	high
c_{HCl} : HCl concentration	M	0.3	0.6	0.9
T : Reaction temperature	$^{\circ}\text{C}$	160	180	200
t : Reaction time	h	3	6	9

The yield of levulinic acid was chosen as the response factor and fitted to the following second-order polynomial model equation:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \sum \beta_{ij} x_i x_j \quad (1)$$

With Y the yield of levulinic acid/furfural (w%), β_0 the model intercept, $\beta_i, \beta_{ii}, \beta_{ij}$ the linear, quadratic and interaction coefficients, respectively, and x_i the independent variables.

The yield of levulinic acid was defined by using the weight of the raw bamboo material as a basis and is calculated from Equation 2;

$$Y_{LA} = \frac{m_{LA}}{m_{Bamboo}} \times 100\% \quad (2)$$

With Y_{LA} the yield of levulinic acid (w%), m_{LA} the mass of levulinic acid formed (mg), m_{Bamboo} the mass of bamboo particles used (mg).

3. Results and discussion

3.1. Heat-transfer experiment

At the start of an experiment, the hydrothermal synthesis reactor is placed in a constant-temperature muffle furnace. The heat of the hot air from the muffle furnace is transferred to the stainless steel reactor through convection. Subsequently, the stainless steel reactor will transfer the heat to the PTFE lined container through conduction, which subsequently will transfer it to the reaction mixture. The temperature is monitored as a function of the time during the course of the reaction process to assess the various heat transfer steps and to determine the time that is required to heat the mixture until the desired temperature is reached, as shown in Figure 2. The temperature is measured by a thermocouple placed inside the stainless steel reactor. The temperature profile as a function of time can be modelled by fitting it against the heat balance provided by Equation 3.

$$\frac{d(m C_p T)}{dt} = UA(T_e - T) \quad (3)$$

With T; the actual temperature (°C), t; time (min), m; the mass of the reaction mixture (kg), C_p ; the heat capacity of the reaction mixture ($J\ kg^{-1}\ K^{-1}$), U; the heat transfer coefficient ($W\ m^2\ K^{-1}$) A; total surface of the reactor (m^2); T_e ; the temperature of the muffle furnace (°C).

Since A, U, m and C_p can be considered constant during the reaction process, these terms can be combined in a constant factor h (min^{-1}) and Equation 3 can be rewritten as follows:

$$\frac{dT}{dt} = \frac{UA}{m C_p} (T_e - T) = h (T_e - T) \quad (4)$$

Solving this differential Equation 4 for $t = 0$ and $T = T_i$ (initial temperature of the reaction mixture (°C)), results in the following Equation:

$$T = h (T_e - T_b) e^{-ht} \quad (5)$$

The value of h was determined by fitting Equation 5 iteratively with Excel solver to the experimental data at the different muffle furnace temperatures and was found to be $0.0095\ min^{-1}$, which is rather small compared to literature. A study by Girisuta and coworkers for instance noted an h value of $0.359\ min^{-1}$ when using glass ampoules with a wall thickness of 1.5 mm. this material provides a faster heating profile compared to the reactors used in this study [28]. Thus, a total wall thickness of 15 mm (5.5 stainless steel and 5 mm PTFE) is responsible for the small h factor and the extended reaction times compared to the available literature.

Figure 2: Temperature profile of hydrothermal synthesis reactor.

3.2. Optimization of levulinic acid conversion

The conversion of bamboo to levulinic acid was optimized by conducting a three-level full factorial design leading to 27 unique experiments (each performed in triplicate), as depicted in Table 2. The levulinic acid yield was chosen as the response factor to evaluate the conversion efficiency. The analysis of variance (ANOVA) was applied to determine the significance of the different effects (linear, quadratic, two-way interaction) of the independent variables as well as the lack-of-fit test of the obtained model. The ANOVA with the yield of levulinic acid as a response is shown in Table 3 (left side). From Table 3, it is clear that the quadratic term t^2 and the two-way interaction terms $t * T$ and $t * c_{HCl}$ are not significant (p -values > 0.05). Hence, these insignificant terms are excluded from the model, leading to a new ANOVA depicted on the right in Table 3. By excluding these terms, the significance of the lack-of-fit test increases from 0.056 to 0.063. Therefore, it can be concluded that the resulting model is significant, represented by a coefficient of determination ($adj R^2$) of 94.57%, meaning that the model explains 94.57% of the variability in the responses for the region studied. Equation 6 predicts a maximum levulinic acid yield of 9.46 w% for a t of 3 h, a T of 160 °C and a c_{HCl} of 0.37 M. The obtained model in uncoded factors is:

$$Y_{LA} = 38.42 - 0.1012 t - 0.3268 T + 12.89 c_{HCl} + 0.000894 T^2 - 5.5 c_{HCl}^2 - 0.5543 T * c_{HCl} \quad (6)$$

With Y_{LA} : the yield of levulinic acid (w%); t : the reaction time (h); T : the reaction temperature (°C); and c_{HCl} : the HCl concentration (M).

Since the interaction term $T * c_{HCl}$ and the quadratic terms T^2 and c_{HCl}^2 are significant to the model equation, the independent variables cannot be analyzed independently. Figure 3 depicts 2D contour and 3D surface plots for the levulinic acid yield (w%), generated by plotting levulinic acid yield versus two independent variables. The third independent variable is kept constant at a fixed value, corresponding to the value of this parameter at the predicted maximum levulinic acid yield (i.e. c_{HCl} of 0.37 M, T of 160 °C, t of 3 h for Figure 3A, 3B and 3C, respectively). These plots visualize the interaction of the independent variables at optimum reaction conditions, leading to a region in the 3D plot where a maximum levulinic acid yield of 9.46 w% can be achieved. In Figure 3A, where c_{HCl} is kept constant at 0.37 M, it is clear that a maximum levulinic acid yield is achieved at a temperature of 160 °C and a reaction duration of 3 h. Operating at longer duration and higher temperatures is not favorable since it has a negative effect on the levulinic acid yield. For instance, a reaction duration of 9 h at 200 °C would only result in a 7.8 w% levulinic acid yield. Figure 3B illustrates the effects of varying c_{HCl} and t at a fixed T of 160 °C (predicted optimal T). From the contour plot it is clear that also in this case, long reaction durations are not desired, especially at high c_{HCl} . The surface of the envelope where a maximum levulinic acid yield is noted, reaches from 3 h till a max t of 3.5 h and a c_{HCl} of 0.46 M. In Figure 3C, T is plotted against c_{HCl} , whilst t is fixed at 3 h. At 3 h, the maximum levulinic acid yield is situated within a T range of 160 °C - 183 °C and a c_{HCl} range of 0.3 M - 0.425 M. From these optimization plots it can be concluded that harsh reaction conditions (high T and high c_{HCl}) do not have a positive effect on the levulinic acid yield, especially in combination with long t . The

reason for a decreasing levulinic acid yield can be explained by the reaction pathway depicted in Figure 1: it is depicted that during the course of the reaction, various side reactions occur. One particular side reaction is the (much undesired) formation of humins due to polymerization reactions from a variety of intermediary products. Several studies also indicated that the formation of humins in the conversion of C6 carbohydrates to levulinic acid is more pronounced when applying harsh reaction conditions [10, 22, 29, 30]. In general, optimization of the process parameters involves a trade-off between all three: t , T and c_{HCl} . In this study, the priority was given to T and c_{HCl} : the optimal T of 160 °C and c_{HCl} of 0.37 M are fairly low. For instance, a review study by Rackemann and Doherty show a minimum T of 175 °C and a minimum acid concentration (HCl or H_2SO_4) of 3.5 w% for the conversion of lignocellulosic biomass species to levulinic acid [31]. A lower T and c_{HCl} are interesting from an economic point of view, since T and c_{HCl} are directly related to the cost of materials of construction: milder conditions require less expensive materials of construction. In a dilute acid hydrolysis, a low c_{HCl} is desired because corrosion rates rise exponentially with increasing acid concentration, leading to a higher cost of reactor materials [13, 32]. However, the downside of working at relatively low T and c_{HCl} is the longer reaction durations to reach the same conversion efficiency and thereby promoting the formation of humins and decreasing the yield of levulinic acid.

Table 2: Experimental design and results

Run	Experimental variables			Response factor - Levulinic acid Yield		
	t (min)	T (°C)	c (M)	$Y_{\text{avg. exp}}$ (w%)	Std (w%)	$Y_{\text{LA. model}}$ (w%)
1	3	160	0.3	9.43	0.38	9.43
2	3	160	0.6	9.40	0.26	9.15
3	3	160	0.9	7.62	0.16	7.88
4	6	160	0.3	9.31	0.21	9.12
5	6	160	0.6	7.75	0.22	8.84
6	6	160	0.9	8.73	0.21	7.58
7	9	160	0.3	8.61	0.11	8.82
8	9	160	0.6	8.46	0.21	8.54
9	9	160	0.9	7.37	0.29	7.27
10	3	180	0.3	8.49	0.20	8.64
11	3	180	0.6	7.96	0.28	8.03
12	3	180	0.9	6.38	0.16	6.42
13	6	180	0.3	8.45	0.19	8.33
14	6	180	0.6	7.71	0.22	7.72
15	6	180	0.9	6.02	0.18	6.12
16	9	180	0.3	8.09	0.19	8.03
17	9	180	0.6	7.46	0.16	7.42
18	9	180	0.9	6.09	0.31	5.82
19	3	200	0.3	8.36	0.33	8.56
20	3	200	0.6	7.73	0.40	7.62
21	3	200	0.9	5.82	0.07	5.69
22	6	200	0.3	8.44	0.30	8.26
23	6	200	0.6	7.68	0.03	7.32
24	6	200	0.9	5.39	0.39	5.38
25	9	200	0.3	8.01	0.33	7.96
26	9	200	0.6	6.65	0.11	7.01
27	9	200	0.9	4.93	0.45	5.08

Table 3: ANOVA of the proposed model for levulinic acid yield ($R^2 = 94.99\%$, adj $R^2 = 94.57$, pred $R^2 = 93.93$). Right table represents ANOVA after dropping insignificant terms of the proposed model.

Source	DF	Sum of squares	Mean square	F-value	p-value	Source	DF	Sum of squares	Mean square	F-value	p-value
Model	9	111.7160	12.4128	152.39	0.000	Model	9	111.4580	18.5764	227.56	0.000
Linear	3	99.4470	33.1489	406.97	0.000	Linear	3	99.6900	33.2299	407.06	0.000
T (°C)	1	30.7540	30.7542	377.57	0.000	T (°C)	1	30.8160	30.8160	377.49	0.000
c (M)	1	64.4620	64.4617	791.40	0.000	c (M)	1	64.5810	64.5812	791.11	0.000
t (h)	1	4.7520	4.7523	58.34	0.000	t (h)	1	4.7760	4.7763	58.51	0.000
Square	3	6.9180	2.3059	28.38	0.000	Square	3	6.7010	3.3507	41.05	0.000
T (°C) * T (°C)	1	2.2400	2.2397	27.50	0.000	T (°C) * T (°C)	1	2.2250	2.2249	27.25	0.000
c (M) * c (M)	1	4.2380	4.2378	52.03	0.000	c (M) * c (M)	1	4.2620	4.2622	52.21	0.000
t (h) * t (h)	1	0.2190	0.2192	2.69	0.105						
2-way interaction	3	3.9230	1.3076	16.05	0.000	2-way interaction	3	3.8620	3.8616	47.30	0.000
T (°C)* c (M)	1	3.8600	3.8596	47.38	0.000	T (°C)* c (M)	1	3.8620	3.8616	47.30	0.000
t (h) * T (°C)	1	0.0350	0.0347	0.43	0.516						
t (h) * c (M)	1	0.0010	0.0009	0.01	0.918						
Error	69	5.6200	0.0815			Error	69	5.8780	0.0816		
Lack-of-fit	17	2.0720	0.1219	1.79	0.056	Lack-of-fit	17	2.3290	0.1165	1.71	0.063
Pure error	52	3.5490	0.0682			Pure error	52	3.5490	0.0682		
Total	78	117.3360				Total	78	117.336

Figure 3: 3D Response surface plots and 2D contour plots of the levulinic acid yield (w%) at optimal reaction conditions whilst (A) varying T_r and t ; (B) varying c_{HCl} and t ; (C) varying T and c_{HCl} .

The statistical analysis of the model already confirmed that the model provides a good fit of the data, shown by ANOVA which revealed a significant lack-of-fit test. However, to be able to validate the accuracy of the model and verify that the predicted values of the model lay close to the experimental data, the predicted values are plotted against the experimental values. If the model has a good fit, the data points should be closely centered around a straight line with slope 1. The confidence interval (CI) is defined by Equations 7 and 8. As seen in Figure 4, most of the values are within the 95% confidence interval, implying a good model prediction.

$$RMSE = \sqrt{\frac{\sum_{j=1}^n (P_j - E_i)^2}{n}} \quad (7)$$

$$CI = \bar{x} \pm t_{95} * RMSE \quad (8)$$

With RMSE; the Root Mean Square Error, P_j ; the predicted value of an experiment, E_i ; the experimental value of an experiment, n ; number of experiments, $\bar{x}$; the mean value of an unique experiment, t_{95} ; the t value of the inverse two-sided Student t distribution for a confidence level of 95%.

Figure 4: Predicted vs. experimental levulinic acid yield. Dashed lines represent the RMSE (95%).

As an external validation of the proposed model and to be able to gain insights in the conversion pathway of lignocellulosic bamboo particles, the conversion to levulinic acid is experimentally tested on the optimum predicted conditions (T of 160 °C and c_{HCl} of 0.37 M; experiments performed in triplicate). For these experiments, the furfural yield is also presented. As seen in Figure 5, furfural is only present under a t of 3 h, therefore, furfural has not been taken into account in the previous optimization experiments where reaction times of 3 h - 9 h were applied. From a previous study by the authors, where the conversion of xylose to furfural was investigated, it was already shown that furfural is more vulnerable to polymerization reactions (i.e. humin formation) than levulinic acid, which explains its absence at t longer than 3 h [10]. The RSM model shown in Figure 5 only provides a fit between the boundaries studied in the optimization experiments (3 h - 9 h). Furthermore, a sharp increase in levulinic acid content is observed between 2 h and 3.5 h. This can be explained by the temperature profile of the reactor shown in Figure 2: at 2 h, a temperature of 130 °C inside the reactor is reached. At shorter durations, the temperature is not high enough to initiate the conversion to levulinic acid, and hence the yield remains low. The relatively large standard deviations (max 1.2 w%) observed for levulinic acid can be explained by the fact that the reaction mixture is not stirred, so mixing only occurs through molecular diffusion. A maximum levulinic acid yield of 10.13 w% may appear quite low, however, this should be placed in perspective with respect to the cellulose fraction of the bamboo particles. The bamboo particles have a cellulose fraction of 29.28 w%. This means that a levulinic acid yield of 34.60 w% (10.13 w / 29.28 w%) or 48.05 mol% is achieved.

Figure 5: The external validation of the conversion of bamboo particles to levulinic acid. Conditions: $T = 160\text{ }^{\circ}\text{C}$, $c_{\text{HCl}} = 0.37\text{ M}$, reactor content: 1 g bamboo and 10 mL reaction medium.

Figure 6 depicts the change in lignocellulosic composition over the course of the reaction by performing a modified Van Soest fractionation method on the residual particulate matter that remains after the hydrolysis reaction. It is trivial that the extractive or “soluble” part is instantaneously extracted from the bamboo particles since it is soluble in the aqueous medium and thereby separated from the solid matter. As soon as the reaction mixture reaches a T of $100\text{ }^{\circ}\text{C}$ ($t = 1.5\text{ h}$), the hemicellulose fraction is completely converted. Hemicellulose mainly consists of C5 carbohydrates which will be converted to furfural. This is confirmed by Figure 5, where a maximum furfural yield of 2.75 w% is observed after a t of 2 h. When the reaction mixture reaches $130\text{ }^{\circ}\text{C}$ ($t = 2.5\text{ h}$), the cellulose fraction (C6 carbohydrates) rapidly decreases. This corresponds to the steep increase in levulinic acid yield, shown in Figure 5. After 3 h, both hemicellulose and cellulose fractions are completely converted to furfural and levulinic acid, respectively. This leaves the lignin fraction as the major component (90-95 w%) in the particulate matter which cannot be degraded by dilute acid hydrolysis. The dashed line in Figure 6 represents the w% of the solid particles after reaction. The slight increase at longer duration ($t > 6\text{ h}$) indicates that solid particles are formed during the reaction. These solid particles are the result of polymerization reactions in the form of humins, which is confirmed by the decrease of levulinic acid in Figure 5 at longer duration.

Figure 6: Lignocellulosic composition of bamboo particles during the dilute acid hydrolysis ($T = 160\text{ }^{\circ}\text{C}$, $c_{\text{HCl}} = 0.37\text{ M}$).

4. Conclusion

The dilute acid hydrolysis of bamboo particles to levulinic acid in a hydrothermal synthesis reactor was investigated, using HCl as the homogeneous acid catalyst. The crucial process parameters (c_{HCl} , T , t) were optimized by means of RSM in a three level full factorial design, in such a way that a maximum levulinic acid yield could be predicted. ANOVA revealed a model equation where the parameters T , c_{HCl} , t , T^2 , c_{HCl}^2 and the interaction of $T * c_{\text{HCl}}$ were significant. The model predicted a maximum levulinic acid yield of 9.46 w% at the following optimal reaction conditions: a t of 3 h, a T of 160 °C and a c_{HCl} of 0.37 M. The goodness of fit was tested by an external validation which resulted in a 9.98 w% levulinic acid yield at the optimal reaction conditions. With respect to the cellulose fraction of the bamboo particles (29.28 w%), this corresponds to a levulinic acid yield of 34.60 w% or 48.05 mol%. From the results, it is clear that furfural is far more vulnerable for degradation reactions than levulinic acid, since it is completely degraded after a t of 3.5 h. These degradation reactions can be attributed to the formation of humins, which is confirmed by an increase in solid particles during the reaction. In our opinion, the key in creating an industrially viable process lies within the simultaneous conversion of lignocellulosic hemicellulose and cellulose fractions into their corresponding derivatives. In this respect, a next step in the research should include the optimization of the simultaneous conversion of both lignocellulosic polysaccharides.

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