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Optimized Organosolv Pretreatment of Biomass Residues Using 2-Methyltetrahydrofuran and n-Butanol

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Abstract: Wheat straw and eucalyptus residues were pretreated in a biphasic system, constituted of butanol (n-butanol) or 2-methyltetrahydrofuran (2M-THF) and aqueous oxalic acid solutions. The pretreatments were carried out in a 300 mL Parr reactor (Autoclave Buchi Limbo-li®) with a solid load of 5 wt.%, the temperature in the range 140–180 °C, oxalic acid load from 0 to 10 wt.% and a duration of 30–90 min. The obtained slurry was then fractionated in three streams: the aqueous phase which contained solubilized hemicellulose, the organic phase which contained the solvated lignin, and the solid residue which contained cellulose. The solid was hydrolyzed using a commercial mix of enzymes to assess cellulose digestibility and glucose production. The pretreatment was optimized to maximize the purity of the cellulose and hemicellulose fractions and the glucose recovery as free sugar. The optimization was done by using an experimental design and response surface methodology. The mass flow details of the four optimized processes were obtained. In terms of biomass fractionation, butanol demonstrated significant advantages over 2M-THF in the same range of process conditions as shown by the recovery yield of free glucose which reached 98% of the theoretical value with butanol but was 67% with 2M-THF. Tests at low temperature and low enzyme loading highlighted the importance of the solvent choice over the operating conditions. 2M-THF showed interesting performances only in the delignification step, with 90% efficiency for the straw. Regarding the use of different feedstock, fractionation and recovery were generally higher for wheat straw than for eucalyptus wood residues.

Keywords: straw; eucalyptus; organosolv; hydrolysis; biobased economy

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1. Introduction

To efficiently exploit biomass resources, the separation of its macro-constituents has paramount importance. Though the chemical and physical properties of cellulose, hemicellulose, and lignin are very different, their separation is hindered by their co-penetration at the microscopic level and by the chemical bonds among them. Several pretreatments have been proposed to help disrupt such a complex structure making the action of solvents (aqueous or organic) and enzymes more efficient [1–3]. However, the recovery yield and the purity of the resulting streams are common issues to all these processes. Another important property of pretreated biomass that has to be taken into consideration, is the improved digestibility by enzymes that can convert the residual cellulose and hemicellulose into free sugars, both monomers and oligomers, which are the major source of carbon for fermentation processes [4,5]. Among the large variety of tested processes, the pretreatment with organic solvents, called organosolv pretreatment, is economically interesting because of its efficiency in solvating the lignin at relatively low temperatures (below 180 °C), thereby preserving hemicellulose from thermal degradation [6,7]. Moreover, in this process, the enzymatic digestibility of the cellulosic residue reaches quantitative yields [8–10]. More recently, the use of so-called green solvents has raised a lot of interest

because of their potential for sustainability and low environmental impact. These include the biosolvents which are obtained from biomass feedstock and belong to the chemical families of alcohols, furans, esters, terpenes. However, the production of other chemicals, the effect on the environment, as well as human and safety profiles of biosolvents need to be considered [11]. For example, the acid-catalyzed digestion of hemicellulose produces furfural (furan-2-carbaldehyde) which is toxic and carcinogenic. To obtain a greener solvent from hemicellulose, furfural has to be hydrogenated producing 2-methyltetrahydrofuran (2M-THF). 2M-THF has been promoted as an ecologically friendly alternative to furfural and other furyl compounds (furfuryl alcohol, methylfuran, tetrahydrofufuryl alcohol). It has a low tendency to polymerize, is stable in acid and base solutions and has low volatility, which improves safety and recovery efficiency [12]. 2M-THF is considered a valuable alternative to low polar and aprotic solvent in various extractions and is also considered as a liquid medium to pretreat biomass. Data in the literature showed that milled bamboo pretreated in microwave-assisted fractionation with 2M-THF and oxalic acid, resulted in a high cellulose enzymatic hydrolysis rate of 92.89% [13]. Biphasic systems 2M-THF/H_2O were tested with the addition of Lewis acid $AlCl_3$ but showed lower efficiency of cellulose conversion, namely 77% [14]. An integrated approach for its use in biorefinery was proposed at mild temperatures and a long reaction time (140 °C, 3 h) considering solvent recycling and hemicellulose recovery [15]. The synergic action of 2,5-furandicarboxylic acid was recently investigated on 10 types of lignocellulosic biomasses and a large range of efficiency was observed for the hydrolysis of the pulp (from 28% obtained with beech wood to 65.1% for hemp) and delignification (from 0.4% obtained with eucalyptus to 19.4% for hemp) [16]. In a recent paper, the performances in delignification, cellulose accessibility and removal of hemicellulose are reported together with the most investigated solvents used in organosolv pretreatment [17], however, a direct and quantitative comparison among the performances of 2M-THF and those observed with other solvents, is generally lacking in the literature. Alcohols have been used for biomass pretreatment and fractionation for over a century. Ethanol and methanol are most commonly used because of their low cost and low boiling point that allow an easy recovery; while alcohols with high boiling points, like glycerol or butanol, can be used at lower pressure and temperature [18]. All these alcohols can be produced from biomass by first- or second-generation processes [19]. In the case of lignocellulosic feedstock, this results in a smart cycle where the solvent used in the pretreatment is a product of the process itself. In this work, butanol and 2M-THF were investigated to assess their efficiency when both pretreatment and streams separation was carried out in the same conditions. Moreover, two biomasses which are representative of herbaceous crops and woody feedstock were used. Among the herbaceous feedstock, wheat straw (WS) is available as residue in several countries and is used for biorefinery applications [20–23]. Eucalyptus is a fast-growing tree and has become one of the most important hardwood resources for pulp paper production worldwide, a process that generates large amounts of residues like branches, barks, leaves, which can be used for biofuels and biochemicals production [24–27]. Butanol was chosen instead of other alcohols because, like 2M-THF, it leads to the formation of a biphasic system with water. Moreover, the production of n-butanol in the IBE (isopropanol-butanol-ethanol) fermentation has been the subject of a recent economic analysis for the Brazilian market reporting a selling price of 15 USD/GJ similar to 2G ethanol when produced from sugar bagasse [28]. After the pretreatment, three separate fractions were obtained: lignin in the organic phase, hemicellulose in the aqueous phase, and cellulose as a solid residue. Oxalic acid was used to catalyze the depolymerization of hemicellulose as reported by Stein at al. [29]. Different temperatures (140–180 °C), duration (30–90 min), and catalyst load (oxalic acid 1–10 wt.% of the starting dry biomass) were explored and analyzed through the Design of Experiment (DOE). The results were reported in terms of fractions purity and recovery yields of glucose and xylose from the corresponding carbohydrates in raw biomass. The analysis of the experimental outputs will be useful to assess which one of the two solvents is best performing and at which process conditions. The information will be used for techno-

economic analysis and to investigate the underpinning pattern of chemical reactions in future theoretical works.

2. Materials and Methods

2.1. Biomasses

The wheat straw was kindly provided by the ECN part on TNO; the particle size ranged between 0.1–5 cm and it was used without further grinding (Figure 1). The eucalyptus residues (ER) were kindly provided by The Navigator Company from their paper mill in Cacia, Portugal; the particle size ranged between 0.1–2 cm and it was used without further grinding (Figure 1).

Figure 1. Pictures of the utilized biomasses.

2.2. OrganoSolv (OS) Pretreatment and Fractionating

The organosolv pretreatment was carried out in a 300 mL Parr® reactor (Figure 2) loaded with 5 g of raw material, 50 mL of organic solvent, and 50 mL of aqueous solution where oxalic acid was solubilized. The slurry was stirred at 400 rpm. Temperature, time, and amount of acid were set following an experimental design as reported below. At the end of the pretreatment time, the reactor was cooled down, depressurized and opened. The resulting slurry was poured into a beaker and filtered. The solid cellulosic fraction (CF) was washed first with water and then with pure ethanol. The liquid was poured into a funnel to separate the aqueous phase containing the solubilized hemicellulose fraction (HF) from the organic phase containing mostly the organic soluble lignin fraction (LF). The dry matter of CF, HF, and LF was determined after keeping the samples in the oven at 60 °C overnight. The HF was hydrolyzed with sulfuric acid 2 wt.% at 121 °C for 1 h, to convert the oligomers into monomers (i.e., pentosanes in solution, PS). Free monomeric sugars were determined with HPIC (High-Performance Ionic Chromatography). The CF was submitted to enzymatic hydrolysis, then filtered to separate the glucose solution from the unconverted solid that is constituted mostly by the residual lignin embedded in recalcitrant fibers. The procedure is shown in Figure 3.

2.3. Design of Experiments (DOE)

In the case of WS pretreated with butanol, the experiments were planned according to an experimental design where three parameters were varied:

1. Temperature, in the range 140–180 °C;
2. Time, in the range 30–90 min;
3. Catalyst (oxalic acid), in the range of 0–10 as wt.% respect the dry biomass.

The analysis of the experimental design was performed with the software Design-Expert® 10, using the following setting:

Study Type:	Response Surface;
Subtype:	Randomized;
Design Type:	Box-Behnken;
Runs:	15 (3 center points);
Design Model:	Quadratic.

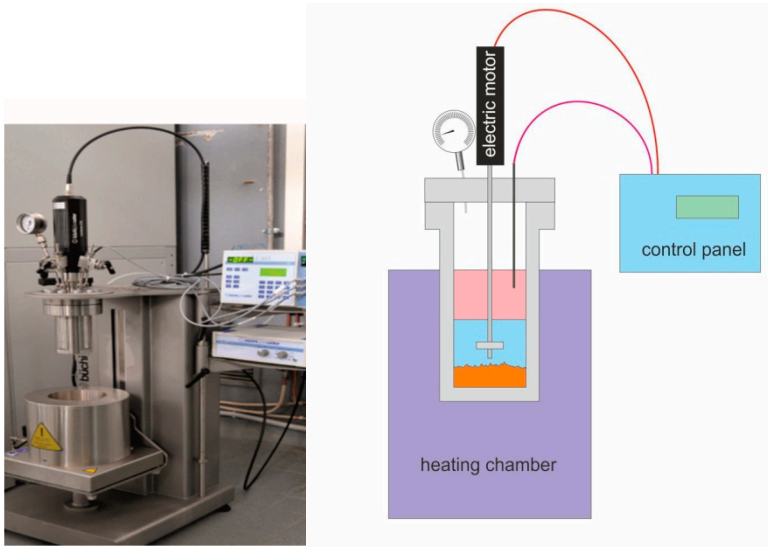

Figure 2. Picture and sketch of the batch reactor used to perform the experiments (PARR, mod. Autoclave Buchi Limbo-li®).

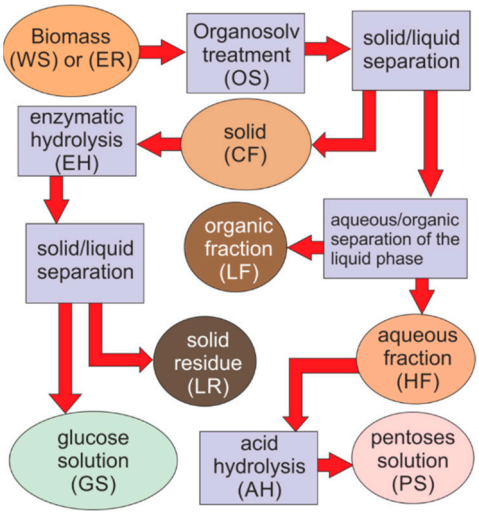

Figure 3. Process scheme of biomass pretreatment, fractionation and hydrolysis.

In the case of ER pretreated with butanol, the experimental design was simplified. Treatment time was fixed at 1 h and only 2 parameters were varied: the temperature in the range 140 °C to 180 °C and Catalyst (oxalic acid), in the range of 3–9 wt.%. In this case, the DOE setting was:

Study Type:	Response Surface;
Subtype:	Randomized;
Design Type:	Central Composite;
Runs:	10 (2 center points);
Design Model:	Quadratic.

In the case of WS pretreated with 2M-THF, the treatment time was fixed at 1 h, the temperature ranged from 140 °C to 180 °C and the catalyst was in the range of 1–7 wt.%. The DOE setting was the same as for pretreating ER.

The analyzed responses included:

- CF purity (i.e., the mass percentage of glucan in the CF)
- HF purity (i.e., the mass percentage of carbohydrates in HF)
- Glucose yield (the produced glucose compared to the glucan content in raw feedstock, as a percentage on the theoretical quantitative hydrolysis).

All the models were analyzed with ANOVA.

2.4. Enzymatic Hydrolysis

The enzymatic hydrolysis (EH) was performed on slurry with 0.5 wt.% of dry CF and 0.05% of enzyme Cellic CTec2® at 50 °C for 72 h, at pH 4.8 and stirring at 180 rpm. Glucose was analyzed in the filtered broth, without diluting it.

2.5. Analytical Methods

The WS and CF were characterized with the NREL (National Renewable Energy Laboratory) procedure to determine the content of carbohydrates, lignin, extractives, and ashes [29]. Sugars content in the HF, as well as the content of glucose after the enzymatic hydrolysis, were determined by HPIC (DIONEX model DX300) with Carbopac PA1 column, using 2 mM NaOH as eluent (flow rate 1.0 mL/min, at 28 °C) and a PED (pulsed electrochemical detector). The most abundant molecules in the HF derived from the degradation of sugar and lignin, like formic acid, furfural, and hydroxymethylfurfural were determined by HPLC (hp1100 series) equipped with diode array UV detector, column Phenomenex Synergi Fusion-RP 80, and using as eluent a mix of acetonitrile and water; the used solvent gradient was 3–50%. Acetic acid was determined by HPLC with a Nucleogel Ion 300 OA column coupled with a refractive index ED50 as the detector, while 0.01 N H_2SO_4 was used as eluent (40 °C, flow 0.4 mL/min). All analyses were conducted in triplicate.

3. Results and Discussion

The compositions of WS and ER are reported in Table 1.

Table 1. Compositions of the starting biomasses: wheat straw (WS) and eucalyptus residue (ER), reported on the dry matter basis.

	WS, wt.%	ER, wt.%
Extractives in water	5.6	2.2
Extractives in ethanol	2.2	1.0
Glucan	29.9	40.4
Xylan	18.9	14.3
Arabinan	2.4	0.7
Galactan	0.9	1.1
Mannan	0.0	1.0
Acetyl groups	2.6	3.9
Lignin (acid insoluble)	13.5	22.5
Lignin (acid soluble)	1.8	3.7
Ash	13.8	1.0
nd	8.4	8.1
Moisture	13.0	10.5

3.1. DOE Analysis of WS Pretreated with Butanol

After OS pretreatment, fraction separations and EH of the CF, the products were analyzed in terms of sugars content. The responses R1 (Cellulosic Fraction purity), R2 (Hemicellulose Fraction purity), R3 (glucose recovery) obtained from the 15 tests are reported in Table 2. The analysis of the glucan content in CF (response R1), produced the interpolating 3D surfaces, or response surface, is shown in Figure 4. Generally, the time of the treatment slightly affected the results; the data corresponding to the variation of reaction time are not reported. The model was significant, with an $R^2 = 0.92$ for the experimental data. A higher purity is predicted at catalyst loading higher than 6 wt.%, with the highest value of 64% predicted at T = 180 °C and oxalic acid = 7.5 wt.%. The analysis of R2 (purity of hemicellulose, i.e., the percentage ratio sugars/HF) produced the response surface shown in Figure 5. The model was significant, but the $R^2 = 0.55$ was indicative of uncertainty regarding the predicted values. The analysis of the overall glucose recovery yield from the starting biomass (R3, the sum of what was determined in the aqueous phase and what

was obtained in the EH) produced the response surface shown in Figure 6. The model was significant, with an R^2 = 0.93. Higher yields were predicted at a catalyst loading higher than 6 wt.%. The highest value of 98% was predicted at T = 180 °C and oxalic acid = 5.6 wt.% (at 60 min. of reaction time). Coefficients of the surface equations and *p* values are reported as Supplementary Data (Tables S1 and S2).

Table 2. Experimental design plan and experimental data obtained for WS pretreatment with and butanol.

Run	A: T	B: t	C: Oxalic Acid	R1 CF Purity	R2 HF Purity	R3 Glucose Recovery
	°C	min	%	%	%	%
1	180	90	5	62.1	43.2	84.1
2	160	90	10	64.5	61.4	83.7
3	160	30	0	38.0	26	33.2
4	160	60	5	57.5	53.4	89.3
5	160	90	0	38.5	57.4	45.9
6	140	60	10	59.0	60.4	80.3
7	180	60	0	46.0	89.9	84.1
8	140	30	5	49.1	58.8	64.5
9	180	60	10	58.0	39.6	84.8
10	160	60	5	52.9	69.8	83.9
11	160	30	10	58.3	80.9	82.3
12	180	30	5	61.3	65.1	90.5
13	140	60	0	35.6	28.1	23.7
14	140	90	5	55.0	62.1	76.2
15	160	60	5	61.0	60.5	84.3

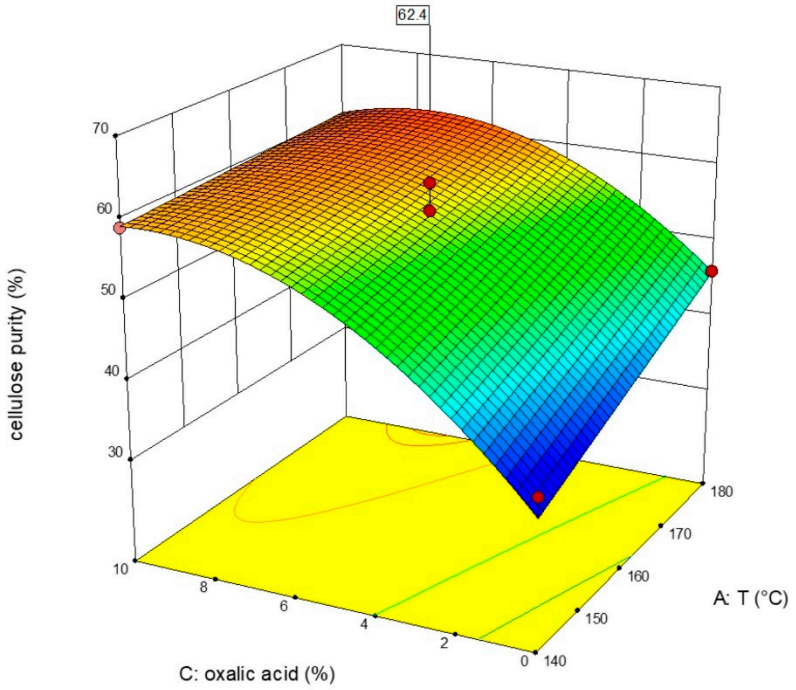

Figure 4. Purity of the CF as a function of temperature and oxalic acid obtained from WS pretreated with butanol, at 60 min. Here and in the following, the surface is the 2-order polynomial best interpolating the experimental data.

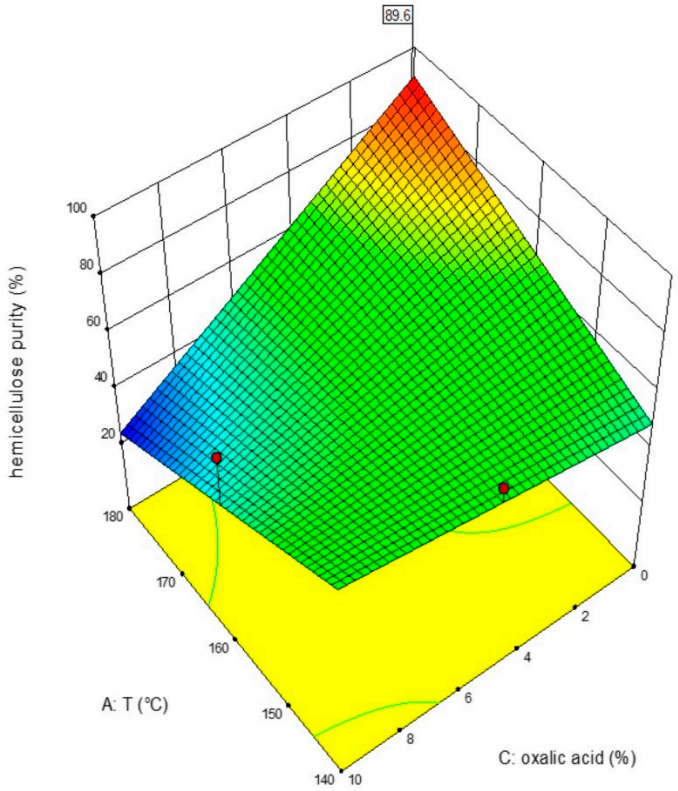

Figure 5. Purity of HF from WS pretreated with butanol as a function of temperature and oxalic acid (time = 90 min).

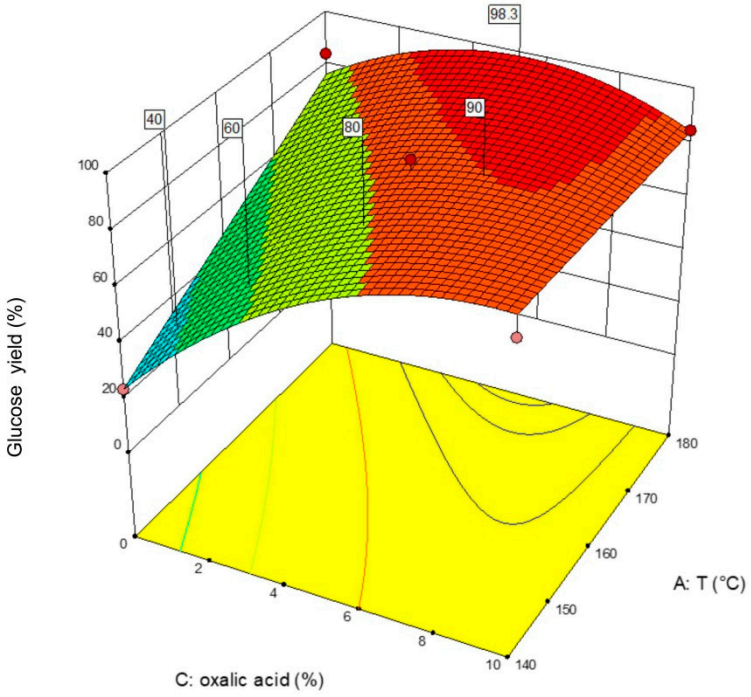

Figure 6. Recovery yield of glucose from WS pretreated with butanol as a function of temperature and oxalic acid (time = 90 min).

3.2. DOE Analysis for ER Pretreated with Butanol

Since the time factor was negligible in the WS case, only the temperature and oxalic acid factors were analyzed in the case of the ER, reducing the experimental design to

10 runs. So, the time was fixed at 60 min and the experimental responses, obtained in 10 different conditions, are reported for ER in Table 3. The analysis of the R1 (glucan content in CF) produced a response surface with a good correlation degree R^2 = 0.90. The highest CF purity of 78% was predicted at T = 168 °C and oxalic acid = 7.1 wt.%. The analysis of R2 (purity of HF) produced a response surface with a correlation degree R^2 = 0.78. The highest HF purity of 60% was predicted at T = 180 °C and oxalic acid = 3 wt.%. The analysis of R3 (overall glucose recovery yield) produced a response surface with a very good correlation degree R^2 = 0.98. The highest value of 86.4% was predicted at T = 172 °C and oxalic acid = 7 wt.%. The surfaces are shown in the supplementary data (Figure S1 for R1, Figure S2 for R2 and Figure S3 for R3). Coefficients of the surface equations are reported in Table S5 with *p* values.

Table 3. Experimental design plan and experimental data obtained for the pretreatment and fractionation of ER with butanol OS.

Run	A: T	B: Oxalic Acid	R1 CF Purity	R2 HF Purity	R3 Glucose Recovery Yield
	°C	%	%	%	%
1	140	9	59.4	50.7	53
2	180	9	72	50.3	78.3
3	180	3	70.1	58.7	70.4
4	140	3	56.6	42	41.4
5	140	6	63.7	40.9	50.5
6	160	3	74.4	51.5	65
7	180	6	77.4	55.5	86.9
8	160	9	79.4	53	80.7
9	160	6	74.3	55.7	80.2
10	160	6	73	54.2	81.6

3.3. DOE Analysis of WS Pretreated with 2M-THF OS

Similar to the previous cases of ER, the responses (R1, R2, R3) obtained in the 10 different conditions (at a fixed reaction time of 60 min) are reported for WS (treated with 2M-THF) in Table 4. In this case, the range of oxalic acid was moved to 1–7% to approach the best values previously found and limit the catalyst consumption. The analysis of the CF purity produced a response surface with an R^2 = 0.82. The highest CF purity of 57% was predicted at T = 180 °C and oxalic acid = 1 wt.%. The analysis of HF purity produced a response surface with a low R^2 (0.58). The highest HF purity of 42% was predicted at oxalic acid = 3.5 wt.% while the temperature did not significantly affect the purity. The analysis of the glucose yield produced a response surface with a high correlation degree R^2 = 0.99. The highest value of 66% was predicted at T = 180 °C and oxalic acid = 4.7 wt.% The surfaces are shown in the supplementary data (Figure S4 for R1, Figure S5 for R2 and Figure S6 for R3). Coefficients of the surface equations are reported in Table S6 with *p* values.

3.4. Optimization

The pretreatment was optimized to maximize the values of the responses R1, R2, R3 and at the same time to minimize the reaction temperature and the load of oxalic acid. The software Design-Expert gave different solutions starting from the experimental data of Tables 2–4. The resulting process conditions are reported in Table 5. In addition to the yields and purity, the production of inhibitors was also considered with the goal of minimizing their production (details are reported in Tables S3 and S4 of Supplementary Data).

Table 4. Design of experiment for WS pretreatment with 2M-THF and obtained experimental responses.

Run	A: T	B: Oxalic Acid	R1 CF Purity	R2 HF Purity	R3 Glucose Recovery Yield
	°C	%	%	%	%
1	160	4	52.4	45.6	57.4
2	140	4	53.4	49.4	48.3
3	180	4	55.7	30.9	64.8
4	160	1	47.0	37.6	44.6
5	180	1	56.6	36.8	56.6
6	160	4	53.3	42.5	55.8
7	140	1	35.1	28.6	27.8
8	140	7	52.9	27.8	51.4
9	160	7	54.3	30.9	59.1
10	180	7	53.4	24.1	62.1

Table 5. Pretreatment conditions and responses predicted by the optimizing model for WS and ER pretreated with butanol and 2M-THF.

	T, °C	Oxalic Acid %	t, min	CF Purity, %	HF Purity, %	Glucose Yield, %	Inhibitors * %
WS, butanol	175	4.6	55	59.0	58.1	94.0	3.3
WS, 2M-THF	180	3.2	60	55.9	42.2	64.8	8.3
ER, butanol	170	4.2	60	75.9	55.1	79.5	9.4
ER, 2M-THF **	170	4.2	60				

* (weight of inhibitors as by HPLC/weight of loaded dry biomass) × 100. ** Conditions not optimized by DOE: the same conditions already optimized for ER with butanol were used (no predicted responses).

3.5. Tests at Optimized Conditions and Mass Balances

Confirmation tests were performed using the optimized conditions reported in Table 5 and the mass flow and the recovery of the main constituents in the different steps of the process were determined from experimental data. The results are summarized in Figure 7 assuming a basis of 100 g of dry biomass. The results were in the confidence range predicted by the model. In Figure 7, we reported the results obtained treating the ER with 2M-THF at the same condition of ER treated with butanol, to observe the only effect of substituting the organic solvent.

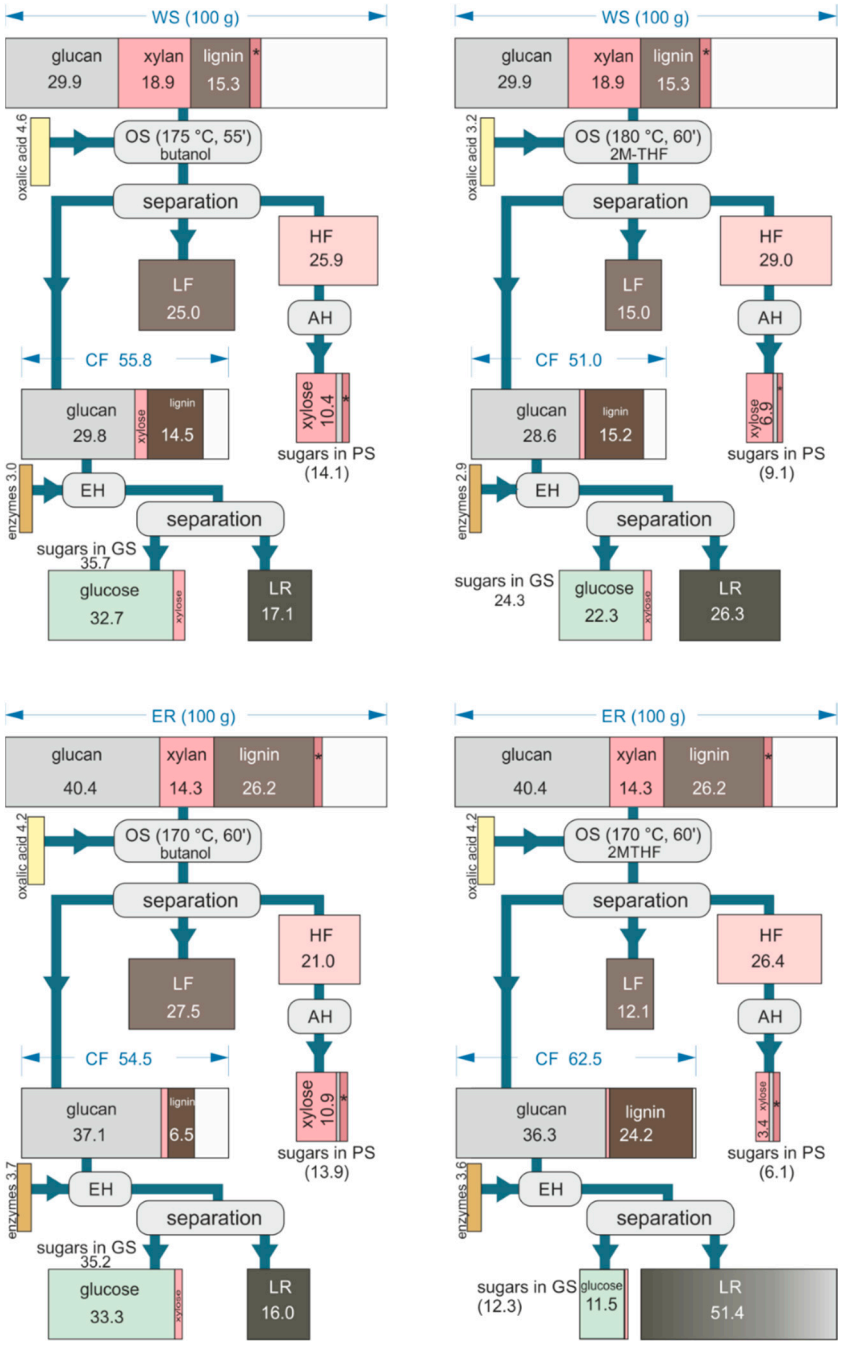

Figure 7. Fractionation at optimized pretreatment conditions of Table 5 and monomers production by EH (glucose) and AH (xylose). From top left, clockwise: WS/butanol; WS/2M-THF; ER/2M-THF, ER/butanol.

In the pretreatment, the presence of the organic acid and the possibility that the organic solvent reacted with the lignocellulosic in condensation reactions could lead to a recovery higher than 100% of the starting material in liquid and solid streams. Indeed, after the pretreatment and fractionation, the sum of the masses of the dried fractions (CF + HF + LF) slightly exceeded the feedstock starting mass. The glucan of the WS was quantitatively recovered in the CF (yield of 99% with butanol and 96% with 2-MTHF), while slightly lower yields were obtained with ER (92% and 90% with butanol and 2-HTMF respectively). After EH, the glucose reached the overall recovery yield of 98% in the case of WS and butanol, while the lowest value was observed for ER pretreated with 2M-THF (26%). For xylose, the highest yield of 69% was achieved from ER pretreated with butanol

while the lowest was observed for ER pretreated with 2M THF. The LF was higher with butanol pretreatment; this fraction contained the organosolv lignin, but also the extractives that accounted for 7.8% of WS and 3.2% of ER. Using TGA and UV analysis the purity of lignin was calculated in the range 50–70% (see Supplementary Data, Figures S7 and S8). The not extracted lignin remained in CF and was separated after the EH as lignin residue (LR). In the case of 2M-THF, LR was higher, because it contained also unconverted fibers. The HF contained mainly xylan and its recovery was higher in the case of the pretreatment with butanol. By adding together all the carbohydrates, the purity of the HF resulted higher in the case of butanol. According to the balance reported in Figure 7, treatment of 100 of dry biomass with butanol resulted in a solution containing 14.1 g of monomeric sugar from WS and 13.9 g from ER after the AH of the HF. By using 2M-THF for the pretreatment, the recovery of sugars in the aqueous phase dropped to 9 g and 6 g from WS and ER respectively. Very high glucan recovery (99%) in CF was achieved with butanol from WS, and also 2M-THF gave a high value of 96%. A lower glucan recovery was obtained treating ER (92% with butanol, 90 with 2M-THF). This finding is in agreement with literature data reporting higher recalcitrance of woody biomass, compared to the herbaceous crops [2,16]. Significant differences were also observed in the saccharification yield after the EH of the CF and a higher yield of glucose was obtained when the biomass was pretreated with butanol. The obtained results are in agreement with the literature, in particular the dependence of hydrolysis during the pretreatment with 2M-THF on the acidic load [9,10,14,15]. The production of inhibitors was higher in the 2M-THF treatment (Table 6). Moreover, also, in this case, the pretreatment of straw with butanol offered significant advantages. These molecules can strongly inhibit bioconversion of the sugar streams and their production has to be duly considered in process design and mechanistic studies [30].

Table 6. Inhibitors produced at the optimized pretreatment conditions (as 100 × weight of inhibitors/weight of loaded dry biomass).

	WS/Butanol	WS/2M-THF	ER/Butanol	ER/2M-THF
acetic acid, %	0.24	1.5	1.02	3.08
formic acid, %	2.4	1.2	6.25	2.62
furfural, %	0.51	5.8	0.47	3.28
5HMF, %	0.06	0.5	0.06	0.51
Total, %	3.21	9.0	7.8	9.49

The comparison between the main results obtained from the different pretreatments is summarized in Figures 8 and 9. Butanol resulted in a higher efficiency to fractionate and produce monomeric sugars after hydrolysis, both acidic and enzymatic. On the other hand, the use of 2M-THF produces a higher quantity of inhibitors. Although the mass flows for the four optimized processes were obtained and demonstrate promising results to promote further techno-economic analysis, this is beyond the object of this work.

3.6. Trials at Reduced Temperature and Enzyme Loading

The OS process with butanol was also tested at a relatively low temperature of 140 °C and halved the enzyme loading from 10% to 5% of the CF weight, in order to validate the efficiency of the process while saving thermal energy and enzyme. The results were compared with those obtained at optimized conditions in Figure 10 (see also Supplementary Data, Figures S9 and S10). As expected, we observed a lower yield of glucose at a lower temperature, however, the loss of fractionation efficiency is more marked in the case of ER than in WS. Further, the processes with butanol at 140 °C were still better than those performed with 2M-THF at higher temperatures (175 °C for WS and 170 °C for ER), highlighting the greater importance of solvent choice than the operating conditions.

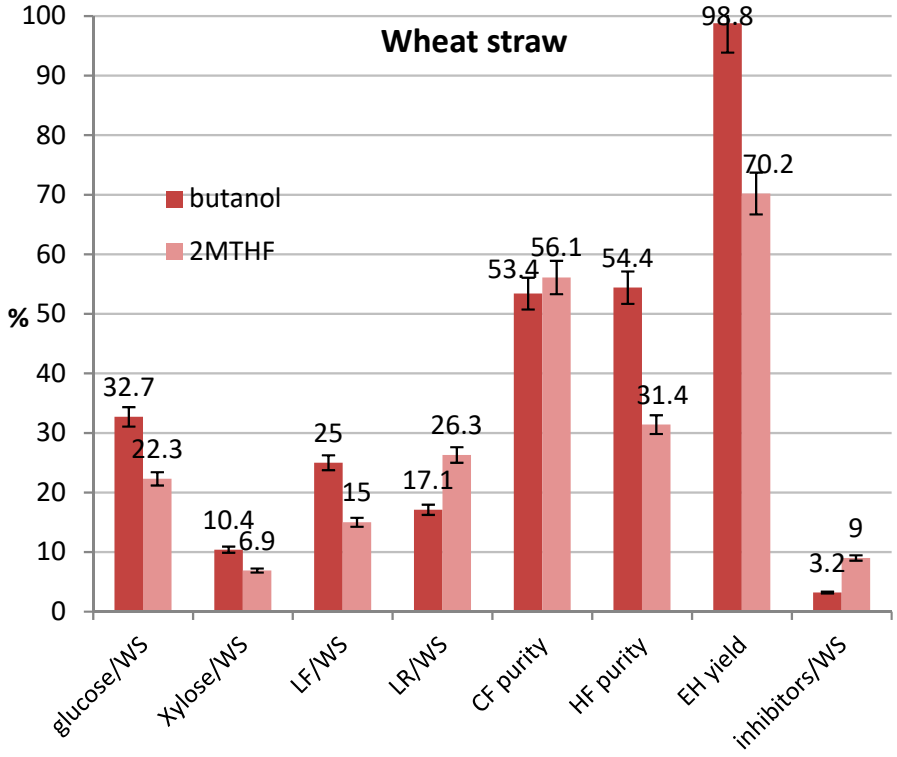

Figure 8. Fractionation and hydrolysis of WS by pretreatment with butanol and 2M-THF.

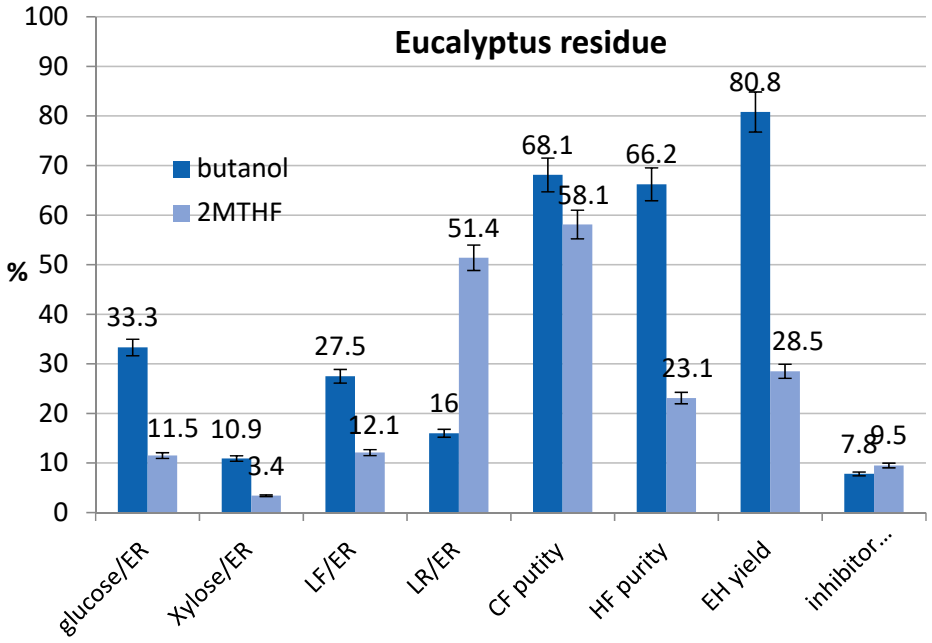

Figure 9. Fractionation and hydrolysis of ER by pretreatment with butanol or 2MTHF.

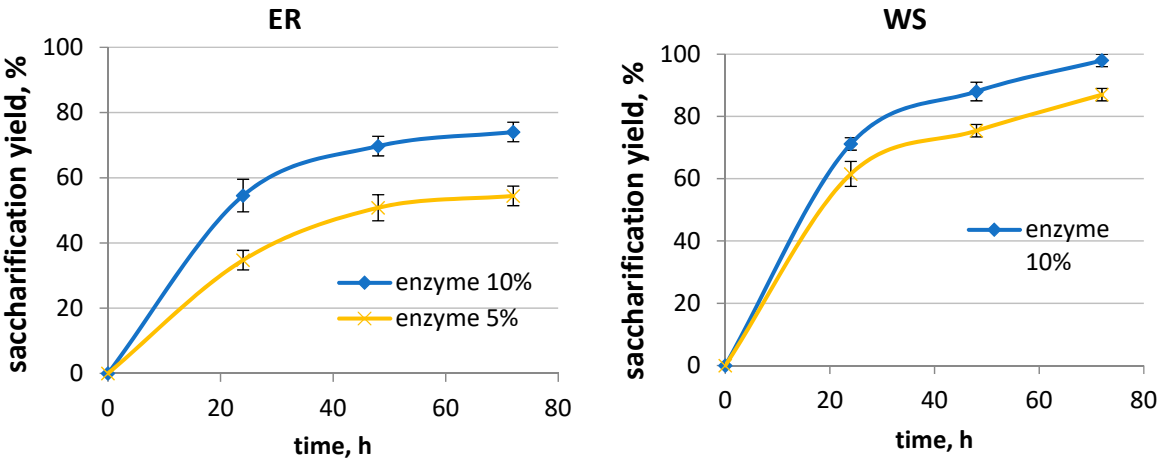

Figure 10. Enzymatic hydrolysis yields of the CF from ER (**left**) and WS (**right**) at different enzyme dosages.

4. Conclusions

Butanol demonstrated significant advantages over 2M-THF in terms of biomass fractionation, recovery of monomeric sugars, and stream purity. Our results showed that optimal pretreatment conditions can be realized at temperatures lower than 180 °C and using less than 5 wt.% of oxalic acid.

At optimized pretreatment conditions, from 100.0 g of WS, we obtained 32.7 g glucose using butanol and 22.3 g using 2M-THF. In the case of ER with butanol, 33.3 g were recoverable, while only 11.5 g were obtained with 2M-THF. Furthermore, with butanol about 14 g of pentoses can be separated in the aqueous phase compared to only 6–9 g with 2M-THF. The extracted organosolv lignin was higher using butanol compared to 2M-THF and, generally, each fraction was significantly purer in the butanol process. The system WS and butanol represented the best combination also regarding the production of inhibitors (acetic acid, formic acid, furfural, 5HMF) and in optimized conditions, accounted for 3.21 g, versus the larger production of 9.49 g measured in the case of ER/2M-THF.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/pr9112051/s1. Figure S1. Purity of CF from ER pretreated with butanol as function of temperature and oxalic acid (time = 60 min). Figure S2. Purity of HF from ER pretreated with butanol as function of temperature and oxalic acid (time = 60 min). Figure S3. Recovery yield of glucose from ER pretreated with butanol as function of temperature and oxalic acid (time = 60 min). Figure S4. Purity of CF obtained from WS pretreated with 2M-THF as function of temperature and oxalic acid (time = 60 min). Figure S6. Glucose yield as function of temperature and oxalic acid (WS, 2M-THF OS) (time = 60 min). Figure S7. UV spectra of the organic solutions obtained from pretreatment of WS with butanol. The legend number are referred to the experimental design reported in Table 2. Figure S8. TGA profile of the dried lignin fraction from WS pretreatment with butanol as reported in Table 5. Figure S9. Recovery yields, purity and byproduct (inhibitors) at 140 °C compared to 170 °C for WS treated with butanol. Figure S10. Recovery yields, purity and byproduct (inhibitors) at 140 °C compared to 170 °C for ER treated with butanol. Table S1. DOE analysis treating straw with butanol: coefficient table with the *p*-value. A = temperature (°C), B = time (min), C = oxalic acid (%). Table S2. DOE analysis treating eucalyptus with butanol: coefficient table with the *p*-value. A = temperature (°C), B = oxalic acid (%). Table S3. Supplementary data on WS treated with butanol (inhibitors details). Table S4. Supplementary data on ER treated with butanol. Table S5. Supplementary data on WS treated with butanol. Table S6. DOE analysis treating straw with 2MTHF: coefficient table with the *p*-value. A = temperature (°C), B = oxalic acid (%).

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Abbreviations

2M-THF	2-methyltetrahydrofuran
AH	Acid Hydrolysis
CF	Cellulosic Fraction
DOE	Design of Experiments
EH	Enzymatic Hydrolysis
ER	Eucalyptus Residues
GS	Glucose Solution
HF	Hemicellulose Fraction
LF	Lignin Fraction
LR	Lignin Residue
OS	Organic Solvent
PS	Pentose Solution
WS	Wheat Straw

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