SUPPLEMENTARY DATA

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

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DOE analysis of pretreated biomass with OS, purity of the streams.

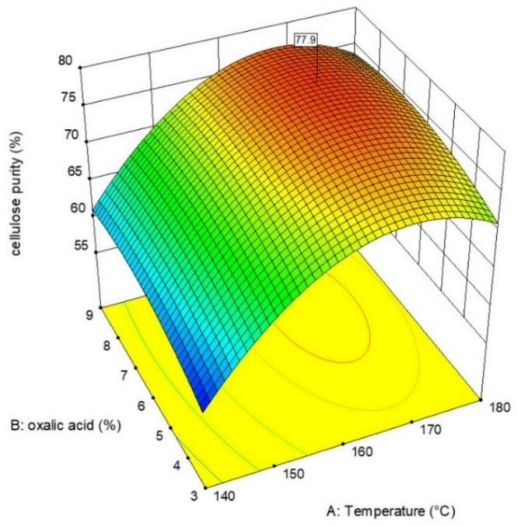

Figure S1. Purity of CF from ER pretreated with butanol as function of temperature and oxalic acid (time = 60 min.).

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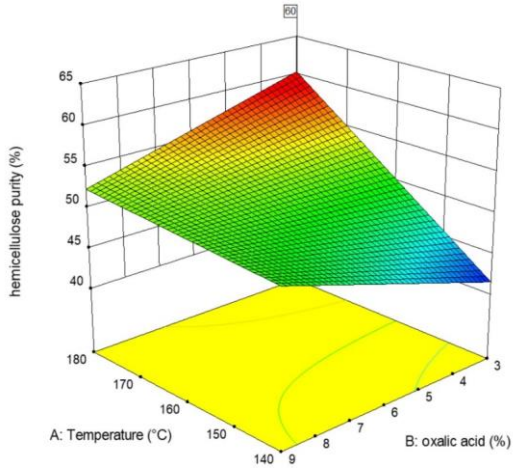

Figure S2. Purity of HF from ER pretreated with butanol as function of temperature and oxalic acid (time = 60 min.).

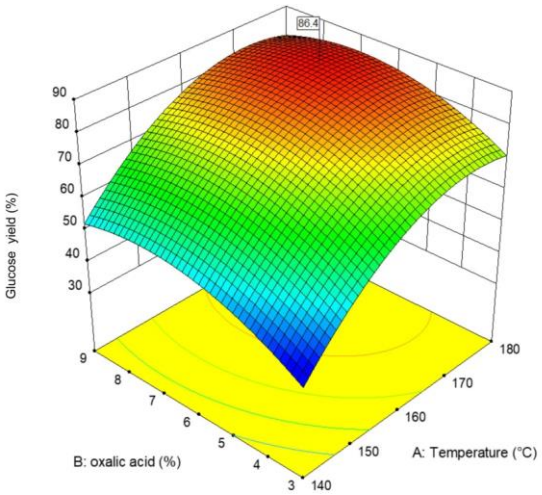

Figure S3. Recovery yield of glucose from ER pretreated with butanol as function of temperature and oxalic acid (time = 60 min.).

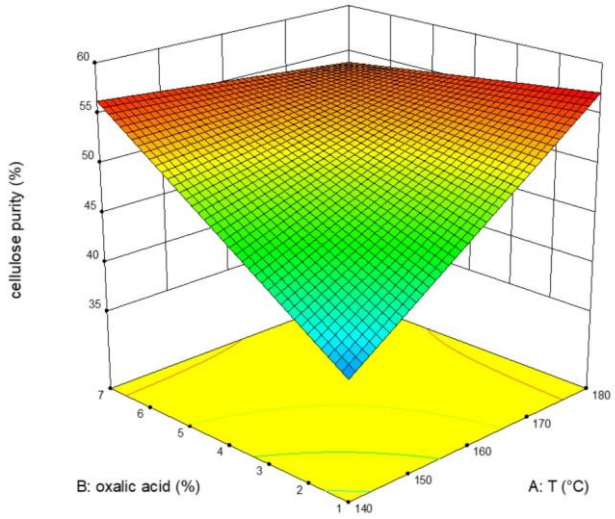

Figure S4. Purity of CF obtained from WS pretreated with 2M-THF as function of temperature and oxalic acid (time = 60 min).

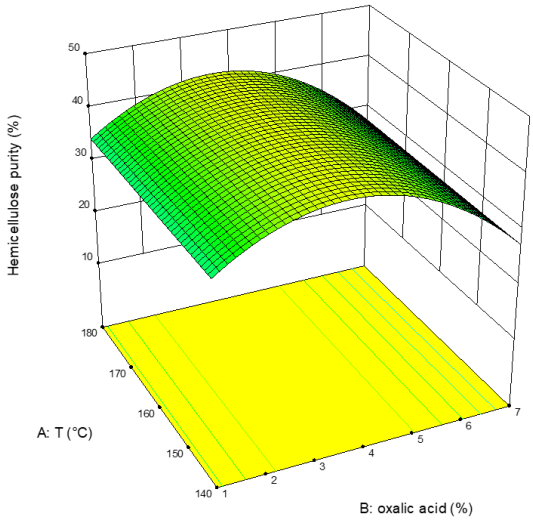

Figure S5. Fraction purity of HF as function of temperature and oxalic acid (WS, 2M-THF OS) (time = 60 min.).

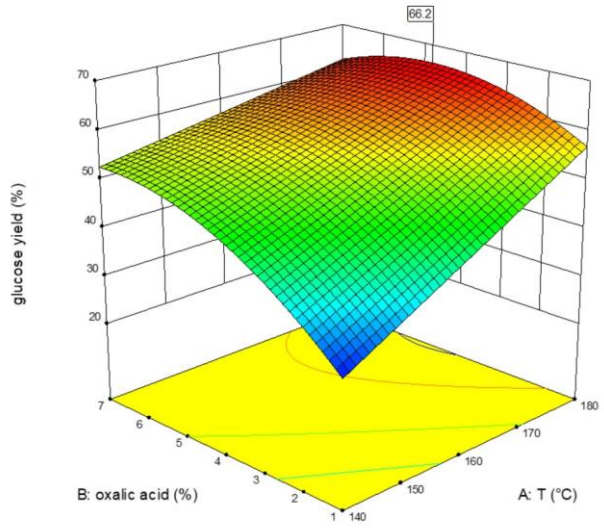

Figure S6. Glucose yield as function of temperature and oxalic acid (WS, 2M-THF OS) (time = 60 min.).

Determination of the lignin purity by UV. This measurement is based on the UV spectra of the organic solutions obtained after the pretreatments (total organic solution was 200 ml from 5 g of started material; it was diluted 1:250 bevore UV analysis).
In the Figure 1 the UV spectra of the organic solutions after the pretreatments (Organosolv with butanol on wheat straw).

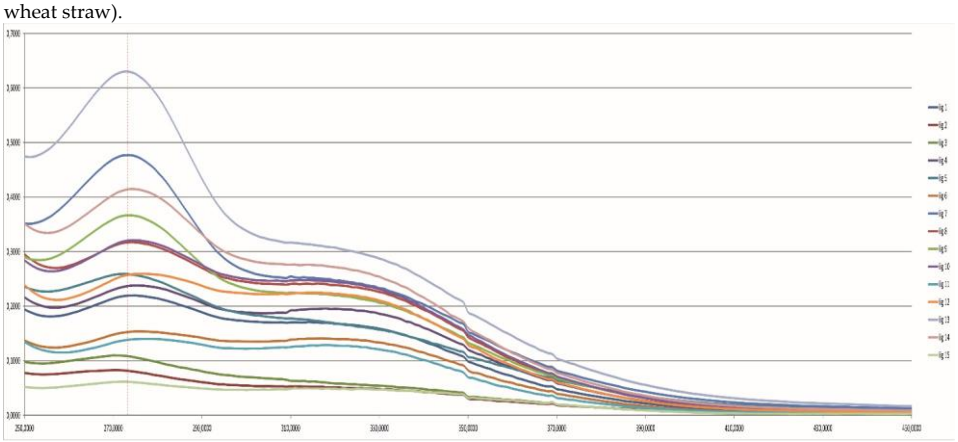

Figure S7. UV spectra of the organic solutions obtained from pretreatment of WS with butanol. The legend number are referred to the experimental design reported in Table2.

Data in the literature reported that the absorbance at 273 nm is characteristic of lignin [1]. The absorbance value per g of the obtained lignin fraction (abs/g) gives an indication of the lignin purity, with higher values indicating higher purity.

Determination of the lignin purity by TGA. This is based on the TGA profile of the dried solute in the organic phase. A TGA profile of a single sample is reported in Figure S2. In the TGA profile we can observe two flexes: the first could be assigned to the combustion of low molecular weight substances (extractives), the second could be assigned to the combustion of macromolecules (lignin). Higher values of the % area of the second inflection indicate higher abundance of macromolecules (lignin), and higher lignin purity [2].

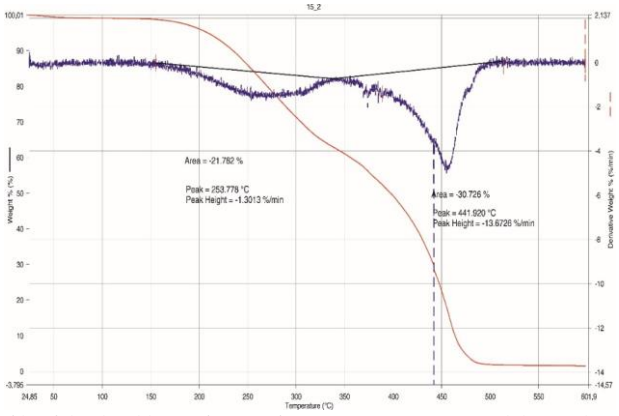

Figure S8. TGA profile of the dried lignin fraction from WS pretreatment with butanol as reported in Table 5.

Trials at reduced temperature and enzyme loading. The purpose of those tests was to verify the efficiency of the process while saving thermal energy and enzyme. The results were compared in the following figures S9- S10, with those obtained at optimized conditions at 175°C.

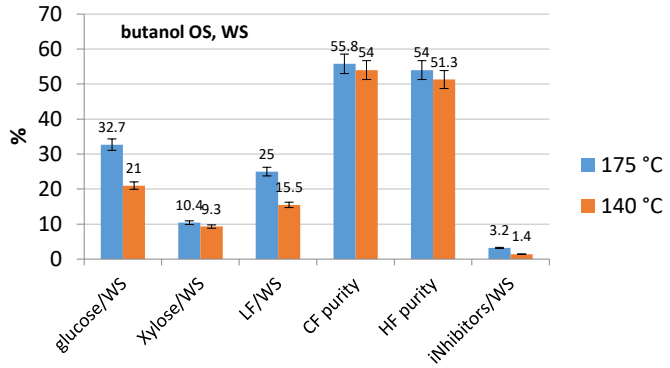

Figure S9. Recovery yields, purity and byproduct (inhibitors) at 140°C compared to 170°C for WS treated with butanol.

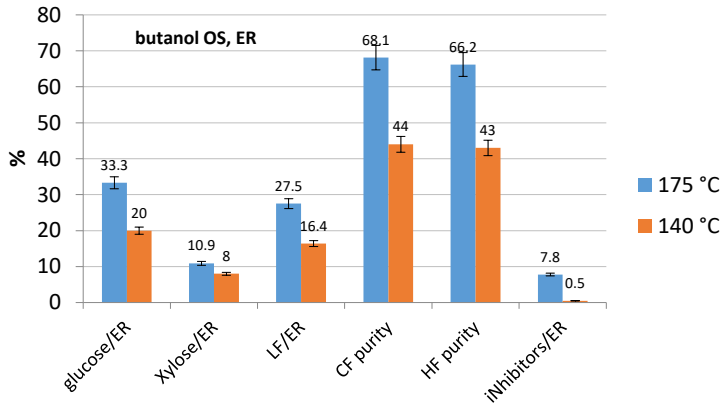

Figure S10. Recovery yields, purity and byproduct (inhibitors) at 140°C compared to 170°C for ER treated with butanol.

Table S1. DOE analysis treating straw with butanol: coefficient table with the p-value. A=temperature (°C), B=time (min), C=oxalic acid (%).

Response	Intercept	A	B	C	AC	BC	A^2	B^2	C^2
cellulose purity	56.9857	3.5875		10.21	-2.85				-7.248
p=		0.0087		< 0.0001	0.0977				0.0012
hemicellulose purity	57.1036				-20.6492	-12.7			
p=					0.0066	0.0658			
Glucose yield	85.8034	12.342		18.0196	-13.9832			-6.986	-17.55
p=		0.0006		< 0.0001	0.0026			0.079	0.0008
inhibitors	2.25446	1.29422	0.231056	0.959	0.810696		0.499		0.5575
p=		< 0.0001	0.0741	< 0.0001	0.0009		0.0165		0.0097

Legend p <.01 .01<= p <.05 .05<= p <.10 p >=.10

Table S2. DOE analysis treating eucalyptus with butanol: coefficient table with the p-value. A=temperature (°C), B=oxalic acid (%).

RESPONSE	INTERCEPT	A	B	AB	A^2	B^2
CELLULOSE PURITY	76.2971	6.62	1.62333	-0.24	-8.38429	-2.05429
P=		0.0110	0.3333	0.9008	0.0240	0.4345
HEMICELLULOSE PURITY	51.2371	5.15347	0.276769	-4.25552		
P=		0.0091	0.8455	0.0432		
GLUCOSE YIELD	80.8489	15.1261	5.85849	-0.9283	-12.1269	-7.98238
P=		0.0003	0.0110	0.5934	0.0044	0.0190
INHIBITORS	9.30071	4.66833	1.42333	0.6225	-3.14143	-1.40643
P=		0.0003	0.0230	0.2691	0.0078	0.0914

Legend p <.01 .01<= p <.05 .05<= p <.10 p >=.10

Table S3. DOE analysis treating straw with 2MTHF: coefficient table with the p-value. A=temperature (°C), B=oxalic acid (%).

Response	Intercept	A	B	AB	B^2
Cellulose purity	51.412	4.05667	3.65333	-5.28	
p=		0.0236	0.0349	0.0185	
Hemicellulose purity	42.0843		-3.3625		-11.1233
p=			0.2222		0.0264
glucose yield	56.5719	9.3431	7.27907	-4.53119	-6.31399
p=		< 0.0001	< 0.0001	0.0022	0.0016
Inhibitors	4.999	3.85833	2.01		
p=		0.0018	0.0393		

Legend p <.01 .01<= p <.05 .05<= p <.10 p >=.10

Table S4. Supplementary data on WS treated with butanol (inhibitors details).

	Formic acid, %	Acetic acid, %	5HMF, %	Furfural, %	Total, %
1	2.2	1.4	0.4	0.1	4.02
2	1.9	1.0	0.2	0.7	3.66
3	0.8	0.8	0.0	0.0	1.53
4	1.2	1.0	0.1	0.3	2.59
5	1.1	1.1	0.0	0.1	2.20
6	0.9	0.9	0.1	0.4	2.19
7	1.5	1.3	0.0	0.2	3.03
8	0.7	0.6	0.0	0.0	1.37
9	2.7	1.5	0.4	2.2	6.81
10	1.18	0.86	0.08	0.33	2.45
11	1.9	1.1	0.1	0.3	3.42
12	1.5	1.0	0.2	0.8	3.45
13	1.0	0.7	0.0	0.0	1.65
14	0.8	0.8	0.0	0.1	1.74
15	1.3	0.8	0.1	0.0	2.16

Table S5. Supplementary data on ER treated with butanol.

RUN	**T**	**OXALIC ACID**	**LIGNIN PURITY [1]**	**LIGNIN PURITY [2]**	**INHIBITORS**
	°C	%	abs/g	%	%
1	140	9	0.26	56	0.76
2	180	9	0.498	68	11.1
3	180	3	0.413	61	7.89
4	140	3	0.335	60	0.02
5	140	6	0.294	67	0.88
6	160	3	0.31	59	5.22
7	180	6	0.473	69	10.7
8	160	9	0.365	56	9.79
9	160	6	0.351	63	10.1
10	160	6	0.31	56	9.28

[1] Determinated by UV.
[2] Determinated by TGA.

Table S6. Supplementary data on WS treated with butanol.

RUN	T	T	OXALIC ACID	LIGNIN PURITY [1]	LIGNIN PURITY [2]
	°C	min	%	abs/g	%
1	180	90	5	0.61	54.8
2	160	90	10	0.343	41.9
3	160	30	0	0.43	40.7
4	160	60	5	0.21	41.2
5	160	90	0	0.218	40.8
6	140	60	10	0.127	36.5
7	180	60	0	0.373	53.7
8	140	30	5	0.233	36.4
9	180	60	10	0.404	46.6
10	160	60	5	0.324	39.4
11	160	30	10	0.206	37.8
12	180	30	5	0.323	44.9
13	140	60	0	0.349	30.7
14	140	90	5	0.297	40.2
15	160	60	5	0.295	37.8

[1] Determinated by UV.
[2] Determinated by TGA.

REFERENCES

1. Sun R.C.; Lu Q.; Sun X.F. Physico-chemical and thermal characterization of lignins from Caligonum monogoliacum and Tamarix spp.,Polymer Degradation and Stability, 2001, 72, 229-238.
2. Freda, C., Zimbardi, F., Nanna, F., & Viola, E., Mathematical tool from corn stover TGA to determine its composition, Applied biochemistry and biotechnology, 2012, 167(8), 2283-2294.