Cellulosic Ethanol: Improving Cost Efficiency by Coupling Semi-Continuous Fermentation and Simultaneous Saccharification Strategies

Patricia Portero Barahona [1,2], Bernardo Bastidas Mayorga [1], Jesús Martín-Gil [2], Pablo Martín-Ramos [3,*], and Enrique Javier Carvajal Barriga [1]

[1] CLQCA, Neotropical Center for the Biomass Research, School of Biological Sciences, Pontifical Catholic University of Ecuador, Avda. 12 de Octubre 1076 y Roca, Quito, Ecuador; pportero@puce.edu.ec (P.P.-B.), bdbastidas@puce.edu.ec (B.B.-M.), ejcarvajal@puce.edu.ec (E.J. C.-B.)
[2] Agriculture and Forestry Engineering Department, ETSIIAA, Universidad de Valladolid, Avenida de 10 Madrid 44, 34004 Palencia, Spain; mgil@iaf.uva.es.
[3] Instituto Universitario de Investigación en Ciencias Ambientales (IUCA), EPS, Universidad de Zaragoza, Carretera de Cuarte, s/n, 22071 Huesca, Spain; pmr@unizar.es
* Correspondence: pmr@unizar.es; Tel.: +34 9742922668 (P.M.-R.)

SUPPORTING INFORMATION

Vibrational analysis of the solid fraction recovered after pretreatment of SCB

The hemicelluloses- and lignin-related band at *ca*. 1245 cm^{-1}, ascribed to the stretching of C–O bonds, was observed to be notably decreased after pretreatment with sodium hydroxide, sulfuric acid and oxalic acid. The absorption band at 1729 cm^{-1}, attributed to the acetyl and uronic ester groups in hemicellulose or to phenyl ester linkage between hemicellulose and lignin, almost disappeared after alkali pretreatment and was noticeably attenuated by sulfuric and oxalic acid pretreatments, indicating fully cleavage of the ester band from the hemicelluloses and lignin after alkali pretreatment and partial removal of hemicelluloses after acid pretreatments. These results evidenced that the acid pretreatments removed most of the hemicellulose in biomass by cleaving acetyl linkages and that the alkali pretreatment was more conducive to remove lignin compounds, in good agreement with the findings of Q. Qing et al (2017).

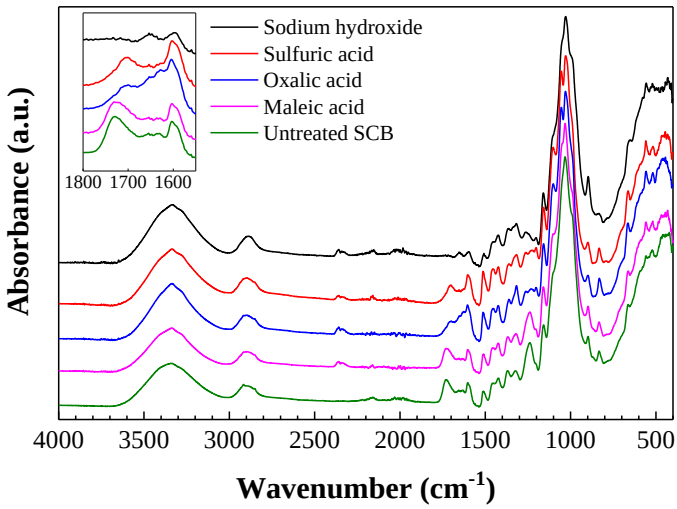

Figure S1. ATR-FTIR spectra of the solid fraction recovered after alkali and acid pretreatments at 160 ºC for 20 min.

Table S1. Solid fraction recovery of pretreated SCB as a function of pretreatment conditions.

Sodium hydroxide															
Temperature (ºC)	130			140			150			160			170		
Time (min)	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
Solid fraction recovery (% w/w)	63.67±0.58	61.33±2.89	61.67±1.15	59.67±3.21	61.67±1.53	60.00±1.00	59.00±3.61	57.67±7.51	56.33±5.51	55.00±1.00	57.33±0.58	58.00±2.65	56.33±0.58	54.33±0.58	54.33±1.53

Sulfuric acid															
Temperature (ºC)	130			140			150			160			170		
Time (min)	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
Solid fraction recovery (% w/w)	57.00±1.00	57.00±1.00	55.67±0.58	56.00±1.00	55.67±1.15	55.67±0.58	54.67±0.58	54.33±0.58	53.67±0.58	53.67±0.58	52.67±0.58	52.33±0.58	51.33±0.58	51.33±1.53	51.33±0.58

Oxalic acid															
Temperature (ºC)	130			140			150			160			170		
Time (min)	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
Solid fraction recovery (% w/w)	60.67±0.58	60.33±0.58	59.67±0.58	59.00±0.00	58.67±0.58	59.33±0.58	58.33±0.58	58.00±0.00	57.33±1.15	57.67±0.58	56.33±0.58	55.67±0.58	55.33±1.15	54.00±1.00	54.00±1.00

Maleic acid															
Temperature (ºC)	130			140			150			160			170		
Time (min)	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
Solid fraction recovery (% w/w)	59.00±1.00	59.33±0.58	58.00±1.00	57.67±0.58	57.67±1.15	57.67±0.58	56.67±0.58	56.33±0.58	55.67±0.58	55.67±0.58	54.00±1.00	52.33±0.58	51.67±0.58	51.67±1.15	52.33±0.58

Table S2. Saccharides composition of SCB hydrolysate as a function of pretreatment conditions.

Chemical pretreatment	Temperature (°C)	Time (min)	Glucose ($g \cdot L^{-1}$)	Xylose ($g \cdot L^{-1}$)	Cellotriose ($g \cdot L^{-1}$)	Cellobiose ($g \cdot L^{-1}$)	Saccharides yield (%)
Sodium hydroxide	130	10	64.26±0.20	29.50±0.14	2.64±0.04	5.72±0.65	91.14±0.00
		20	70.12±0.10	35.70±0.10	2.95±0.09	6.68±4.67	99.68±0.00
		30	70.78±0.10	34.20±0.10	2.65±0.10	6.31±1.82	99.14±0.00
	140	10	78.05±0.20	32.00±0.08	3.25±0.17	9.66±5.47	99.73±0.00
		20	73.52±0.20	32.50±0.08	2.33±0.21	5.48±2.31	99.64±0.00
		30	75.43±0.10	33.40±0.11	2.66±0.14	8.88±1.57	99.34±0.00
	150	10	78.33±0.10	33.20±0.18	2.84±0.22	9.25±6.13	99.85±0.00
		20	80.47±0.60	33.10±0.07	3.15±0.20	10.41±13.56	99.52±0.00
		30	79.79±0.20	35.90±0.18	4.43±0.07	8.16±9.75	99.31±0.00
	160	10	84.66±0.30	34.10±0.10	3.19±0.10	6.68±2.50	99.50±0.00
		20	83.56±2.10	29.40±1.88	4.04±0.17	6.37±1.41	99.77±0.00
		30	83.07±1.20	29.80±0.24	3.03±0.21	6.22±2.88	98.78±0.00
	170	10	82.66±1.10	30.90±0.07	2.97±0.09	6.79±2.39	98.33±0.00
		20	86.93±1.20	35.40±0.39	2.94±0.25	7.13±1.26	99.92±0.00
		30	87.68±1.50	33.60±0.43	2.76±0.54	7.23±6.47	99.18±0.00
Sulfuric acid	130	10	22.56±0.20	4.20±0.15	0.84±0.42	2.22±0.33	22.29±0.00
		20	31.30±0.20	8.50±0.20	1.88±0.17	2.90±0.28	33.77±0.00
		30	32.89±0.20	6.00±0.23	1.69±0.26	2.79±0.43	31.64±0.00
	140	10	38.41±0.20	7.30±0.18	1.70±0.27	2.76±0.54	37.64±0.00
		20	38.01±0.20	5.50±0.25	1.66±0.12	3.11±0.59	35.35±0.00
		30	39.63±0.30	5.00±0.23	1.59±0.19	3.03±0.65	35.89±0.00
	150	10	38.71±0.20	4.10±0.16	1.58±0.25	2.81±0.60	33.73±0.00
		20	38.56±0.20	4.70±0.20	1.32±0.14	3.06±0.33	34.08±0.00
		30	40.37±0.20	3.20±0.14	2.08±0.09	3.21±0.14	33.52±0.00
	160	10	60.75±0.30	1.00±0.03	1.05±0.11	4.33±0.79	46.37±0.00
		20	59.56±0.30	1.40±0.10	0.97±0.16	3.68±1.35	46.19±0.00
		30	60.29±0.20	1.30±0.79	0.87±2.27	2.48±1.24	46.20±0.00
	170	10	56.79±0.20	1.20±0.56	0.89±0.42	3.95±0.39	41.98±0.00
		20	57.98±0.10	1.70±0.15	1.62±0.29	4.02±3.81	45.75±0.00
		30	60.46±0.50	1.60±0.25	1.31±0.72	4.10±3.37	47.38±0.00
Oxalic acid	130	10	33.72±0.10	6.80±0.14	2.48±0.07	3.96±0.35	35.98±0.00
		20	29.58±0.20	5.40±0.10	1.82±0.21	3.44±0.50	30.94±0.00
		30	36.26±0.10	5.80±0.20	2.03±0.16	3.73±0.43	36.55±0.00
	140	10	42.02±0.00	6.60±0.13	1.96±0.12	3.32±0.12	41.86±0.00
		20	42.89±0.20	6.50±0.13	1.96±0.19	3.91±0.43	42.09±0.00
		30	43.58±0.20	6.50±0.23	2.01±0.12	3.79±0.33	43.26±0.00
	150	10	42.12±0.10	3.90±0.07	1.98±0.19	3.33±0.41	38.59±0.00
		20	42.69±0.10	5.00±0.00	0.00±0.13	3.61±0.17	39.93±0.00
		30	42.78±0.20	4.30±0.13	1.52±0.25	3.63±0.53	38.88±0.00
	160	10	52.76±0.10	1.00±0.67	1.76±0.26	4.25±2.72	41.82±0.00
		20	47.85±0.10	0.80±0.31	0.81±0.58	3.48±1.67	38.31±0.00
		30	47.99±0.10	0.60±0.11	1.01±0.28	4.00±1.51	39.97±0.00
	170	10	55.98±0.10	0.70±0.08	1.07±0.56	4.24±1.63	42.86±0.00
		20	58.14±0.10	0.50±0.08	0.94±0.01	4.23±0.57	44.33±0.00
		30	57.11±0.10	0.60±0.36	0.95±0.60	4.07±1.88	43.41±0.00

Chemical pretreatment	Temperature (°C)	Time (min)	Glucose ($g \cdot L^{-1}$)	Xylose ($g \cdot L^{-1}$)	Cellotriose ($g \cdot L^{-1}$)	Cellobiose ($g \cdot L^{-1}$)	Saccharides yield (%)
Maleic acid	130	10	25.70±0.10	6.80±0.06	3.17±0.03	2.95±0.47	28.51±0.00
		20	26.23±0.10	8.20±0.02	3.02±0.20	3.24±0.56	30.58±0.00
		30	27.65±0.10	9.00±0.17	2.87±0.15	3.03±0.60	31.91±0.00
	140	10	28.88±0.10	9.30±0.10	2.68±0.07	2.96±0.31	32.95±0.00
		20	27.81±0.10	9.90±0.19	2.55±0.17	2.78±0.79	32.78±0.00
		30	30.60±0.10	11.10±0.08	2.61±0.11	2.81±0.34	36.26±0.00
	150	10	30.68±0.10	10.90±0.10	2.85±0.08	3.03±0.50	35.39±0.00
		20	31.89±0.10	12.80±0.05	2.94±0.23	3.09±0.32	38.16±0.00
		30	30.90±0.10	12.90±0.20	2.66±0.13	2.90±0.28	37.03±0.00
	160	10	45.65±1.70	18.80±0.89	1.02±0.07	3.19±2.01	55.01±0.00
		20	58.70±0.70	23.30±0.00	0.00±0.15	3.76±2.47	66.11±0.00
		30	55.05±1.30	21.70±0.88	0.51±0.12	3.69±2.74	62.57±0.00
	170	10	54.12±0.70	20.50±0.00	0.00±0.17	3.30±2.14	58.84±0.00
		20	62.51±0.80	18.80±0.86	0.50±0.20	3.72±0.33	62.60±0.00
		30	68.75±0.70	17.10±0.00	0.00±0.20	3.74±3.13	67.02±0.00

Table S3. Ethanol produced by fermentation of pretreated SCB hydrolysate as a function of pretreatment conditions.

Chemical pretreatment	Temperature (°C)	Time (min)	Ethanol ($g \cdot L^{-1}$)	Ethanol yield (%)
Sodium hydroxide	130	10	32.76±0.10	99.85±0.38
		20	34.79±0.15	97.18±0.48
		30	34.94±0.08	96.72±0.28
	140	10	39.41±0.18	98.82±0.28
		20	29.53±0.11	78.63±0.16
		30	37.56±0.07	97.79±0.31
	150	10	39.37±0.07	98.63±0.22
		20	40.72±0.08	99.09±0.08
		30	40.63±0.06	99.84±0.10
	160	10	42.98±0.77	98.76±0.57
		20	43.25±1.27	98.07±1.13
		30	42.22±1.40	99.31±0.29
	170	10	43.02±1.34	99.23±0.80
		20	43.04±1.02	98.26±2.00
		30	43.91±1.86	98.81±1.07
Sulfuric acid	130	10	9.50±1.22	82.64±10.92
		20	15.68±0.10	97.96±0.13
		30	16.51±0.18	98.67±0.87
	140	10	19.48±0.08	99.09±0.15
		20	18.94±0.08	97.16±0.39
		30	19.98±0.10	99.06±0.60
	150	10	19.37±0.13	97.92±0.74
		20	19.60±0.12	99.22±0.54
		30	20.50±0.09	99.42±0.21
	160	10	30.11±0.25	97.78±0.93
		20	29.96±0.52	96.74±1.43
		30	30.72±0.45	98.40±1.64
	170	10	24.88±0.52	85.85±1.46
		20	30.27±1.23	96.87±2.01
		30	32.17±1.81	98.88±1.09
Oxalic acid	130	10	16.84±0.11	98.16±0.71
		20	11.42±0.10	75.47±0.63
		30	17.58±0.10	95.03±0.52
	140	10	21.08±0.13	98.13±0.84
		20	21.75±0.10	99.55±0.43
		30	21.62±0.14	97.16±0.77
	150	10	21.53±0.08	100.00±0.12
		20	21.18±0.04	97.11±0.17
		30	21.28±0.12	97.42±0.42
	160	10	24.84±2.41	96.36±2.86
		20	23.29±0.56	96.18±1.72
		30	24.84±0.87	96.72±1.84
	170	10	21.36±1.54	77.00±4.82
		20	22.49±0.68	76.20±1.54
		30	24.76±0.64	85.89±4.08

Chemical pretreatment	Temperature (°C)	Time (min)	Ethanol ($g \cdot L^{-1}$)	Ethanol yield (%)
Maleic acid	130	10	12.50±0.10	95.29±0.61
		20	13.12±0.12	97.92±1.43
		30	13.57±0.08	95.97±0.76
	140	10	14.40±0.12	97.77±0.71
		20	13.62±0.08	95.75±0.33
		30	14.99±0.11	95.91±0.36
	150	10	14.83±0.13	94.93±0.73
		20	15.22±0.07	93.37±0.67
		30	14.99±0.07	95.11±0.51
	160	10	22.44±0.43	94.92±1.29
		20	27.89±0.72	95.28±4.03
		30	27.78±1.41	94.68±0.79
	170	10	27.25±0.63	97.16±0.56
		20	30.71±0.43	96.57±0.89
		30	34.03±1.52	96.15±2.56