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Steam Explosion Pretreatment of Beechwood. Part 2: Quantification of Cellulase Inhibitors and Their Effect on Avicel Hydrolysis

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The supplementary information contains 2 tables and 2 figures.

1. Supplementary Tables

Table S1. Selected literature data on inhibitors generation in different kinds of pretreatment and their influence on enzymatic hydrolysis.

Biomass; Pretreatment	Composition of Prehydrolysate [mg $g^{-1}_{biomass}$; g L^{-1}]	Substrate for Enzymatic Hydrolysis	Reduction of Glucose Yield in Enzymatic Hydrolysis [%]	Reference
Barley straw; Steam explosion, 210 °C, 5 min, log R_0=3.94	Xylose: 71; 17.4 Glucose: 19; 4.6 Arabinose: 8; 1.9 Galactose: 5; 1.3 Acetic acid: 9; 2.1 Formic acid: 3.3; 0.8 Furfural 2.8; 0.7 HMF 0.8;0.2 Phenolics*: 0.9; 0.2	washed pretreated barley straw	25 (original prehydrolysate) 40 (2-fold concentrated prehydrolysate) 10 (2-fold diluted prehdyrolysate)	[1]
Maple; Liquid hot water, 200 °C, 5 min, log R_0=4.25, 23% w/w solids	Xylose:40; 9.2 Xylo-oligomers: 49; 11.2 Glucose: 3; 0.6 Gluco-oligomers: 7; 1.5 Acetic acid: 57, 13.1 Furfural, HMF: 18; 4.1 Phenolics 6; 1.3	Solka Floc	57	[2]
Poplar; Steam explosion, 214 °C, 6 min log R_0= 4.13	Acetic acid: 28; n/a Formic acid 11; n/a Levulinic acid 0.8; n/a Furfural 6; n/a	Washed steam exploded poplar	n/a (only single compound model	[3]

	HMF 3; n/a Phenolics**:0.9; n/a		solutions were tested)	
Poplar; SO_2-catalyzed steam explosion, 3% SO_2, 200 °C, 5 min	Xylose: n/a; 52.1 Xylo-oligomers: n/a; 11.3 Glucose: n/a; 22.9 Gluco-oligomers: n/a; 5.8 Other monomeric sugars: n/a; 7.9 Other oligomeric sugars: n/a; 3.6 Acetic acid: n/a; 12.4 Furfural: n/a; 1.1 HMF: n/a; 3.0 Phenolics: n/a; 9.8	dissolving pulp	70 (low Celluclast loading) 30 (high Celluclast loading) 37 (low CTec3 loading) 25 (high CTec3 loading)	[4]
Lodgepole pine; SO_2-catalyzed steam explosion, 4% SO_2, 200 °C, 5 min	Xylose: n/a; 15.4 Xylo-oligomers: n/a; 2.4 Glucose: n/a; 19.8 Gluco-oligomers: n/a; 6.5 Mannose: n/a; 27.2 Manno-oligomers: n/a; 7.9 Other monomeric sugars: n/a; 13.2 Other oligomeric sugars: n/a; 2.4 Acetic acid: n/a; 4.4 Furfural: n/a; 2.1 HMF: n/a; 0.8 Phenolics: n/a; 4.5	dissolving pulp	60 (low Celluclast loading) 30 (high loading of Celluclast) 33 (low CTec3 loading) 19 (high CTec3 loading)	[4]
Spruce; SO_2 catalyzed steam explosion, 2.4% SO_2, 215 °C, 3 min	Xylose: n/a; 9.2 Glucose: n/a; 13.8 Mannose: n/a; 15.5 Other sugars: n/a; 4.4 Acetic acid: n/a; 5.7 Furfural: n/a; 0.6 HMF: n/a; 1.0	Washed, pretreated spruce	36	[5]

*: Sum of 4-hydroxybenzaldehyde, 4-hydroxybenzoic acid; catechol, syringaldehyde, syringic acid, vanillin, vanillic acid, ferulic acid, coumaric acid; ** Sum of vanillin, syringaldehyde; 4-hydroxybenzaldehyde.

Table S2. Summary of inhibitor concentrations in the enzymatic hydrolysis mixture and their effect on enzymatic hydrolysis of Avicel. Shown are the actual concentrations of the inhibitors during enzymatic hydrolysis of 3.5% Avicel and the corresponding glucose yield reductions after 24 h and 168 h. The last column shows the calculated contribution of the phenolics to the final glucose yield reduction according to equation 2 in the main manuscript. The enzymatic hydrolysis of Avicel in a buffer solution without inhibitors resulted in a final glucose concentration of 34.5 g/L, which corresponds to a yield of 88.8%.

log R_0 (T [°C])	Acetic Acid [g/L]	Formic Acid [g/L]	Furfural [g/L]	HMF [g/L]	Phenolics [g/L]	Total Xylose Equivalents [g/L]	Yield Reduction 168 h [%]	Yield Reduction 24 h [%]	Ratio of Yield Reduction (24 h/168 h)	Phenolics Contribution to Final Yield Reduction [%]
3.00 (160)	0.04	0.01	0.00	0.00	0.15	0.28	4.65	0.00	0.00	88.8
3.50 (160)	0.32	0.08	0.00	0.00	0.34	3.54	7.20	13.33	1.85	59.2
3.25 (170)	0.07	0.02	0.00	0.00	0.25	1.10	6.89	12.53	1.82	77.8
3.50 (170)	0.24	0.07	0.00	0.01	0.35	4.58	9.41	23.55	2.50	53.9
3.75 (170)	0.48	0.12	0.02	0.01	0.48	8.21	13.19	34.11	2.59	47.2
3.50 (180)	0.42	0.10	0.00	0.00	0.35	2.88	11.47	33.97	2.96	64.9
4.00 (180)	1.05	0.20	0.00	0.00	0.70	12.87	18.25	41.39	2.27	45.6
4.00 (190)	1.12	0.17	0.15	0.05	0.73	12.32	20.85	47.39	2.27	47.4
3.75 (200)	0.77	0.15	0.07	0.03	0.53	8.58	19.82	46.53	2.35	48.8
4.00 (200)	1.21	0.27	0.12	0.04	0.70	9.57	20.75	43.75	2.11	52.9
4.25 (200)	1.86	0.35	0.33	0.11	0.96	11.33	22.49	40.41	1.80	56.4
4.50 (200)	2.60	0.48	0.58	0.14	1.08	8.39	21.92	30.70	1.40	66.3
4.00 (210)	1.61	0.53	0.21	0.06	0.76	9.10	17.54	33.87	1.93	56.1
4.25 (210)	2.23	0.61	0.35	0.12	0.96	9.36	15.91	21.97	1.38	61.1
4.50 (210)	2.99	0.78	0.46	0.23	1.11	7.28	16.77	17.90	1.07	69.9
4.75 (210)	3.17	0.74	0.60	0.33	1.37	4.88	20.22	20.31	1.00	81.1
4.50 (220)	2.49	0.63	0.42	0.19	1.20	1.65	16.89	20.72	1.23	91.7
4.75 (220)	3.04	0.74	0.50	0.31	1.36	1.26	18.74	21.21	1.13	94.3
5.00 (220)	3.58	0.84	0.55	0.48	1.52	1.23	22.73	23.18	1.02	95.0
4.50 (230)	2.88	0.75	0.33	0.23	1.18	1.58	17.06	17.28	1.01	92.0
4.75 (230)	3.20	0.80	0.32	0.33	1.33	1.45	17.78	16.88	0.95	93.4
5.00 (230)	3.49	0.91	0.35	0.48	1.55	0.75	21.53	20.16	0.94	96.9
5.25 (230)	3.75	0.94	0.44	0.71	1.85	0.09	26.47	23.36	0.88	99.7

2. Supplementary Figures

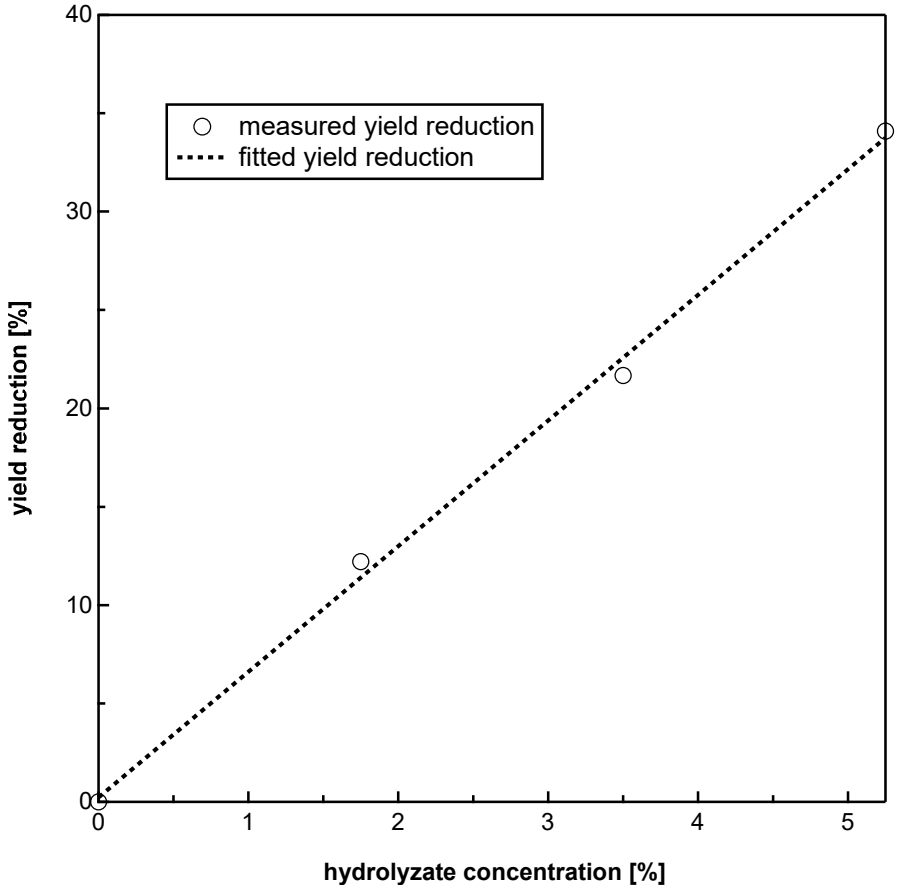

Figure S1. Influence of the amount of prehydrolyzate (230°C, log R0 = 5.0) on the yield reduction in the enzymatic hydrolysis of Avicel at 3.5% solids concentration. The prehydolyzate concentration is expressed as the hypothetic glucan concentration in a whole slurry hydrolysis. The linear regression model had an adjusted r^2 of 0.9985 and a significant regression equation was found ($p = 0.0005$).

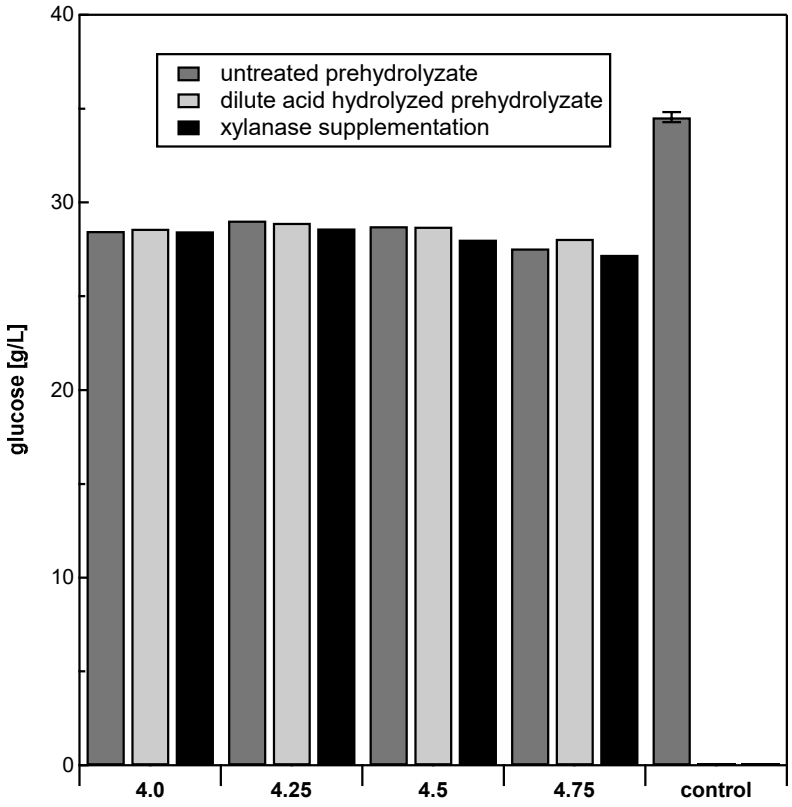

Figure S2. Final glucose concentrations (168 h) in the enzymatic hydrolysis of Avicel in the presence of 210°C °C steam explosion prehydrolyzates: effect of xylanase supplementation and acid hydrolysis of xylooligomers. The dark grey bar on the right site of the graph denotes the glucose concentration in the control without prehydrolyzate and without supplementation of xylanase. One group of prehydrolyzates was subjected to a dilute acid hydrolysis to convert xylooligomers to xylose (light grey bars). Alternatively, xylanase was added to the enzymatic hydrolysis mixtures (black bars). The addition of xylanase did not improve the final glucose yields.

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