Supporting Information

Studer et al. 10.1073/pnas.1009252108

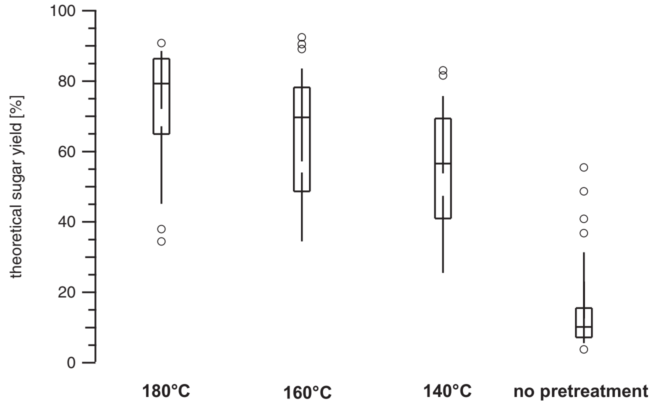

Fig. S1. Box plot representing the variability in monosaccharide yield for the analyzed 47 natural *Populus trichocarpa* variants pretreated at 180 °C, 160 °C, and 140 °C as well as no pretreatment. The sugar yield is calculated based on the composition of the BioEnergy Science Center (BESC) standard poplar. The bottom and top whiskers represent the 10 and 90 percentiles, respectively (○ represents outliers).

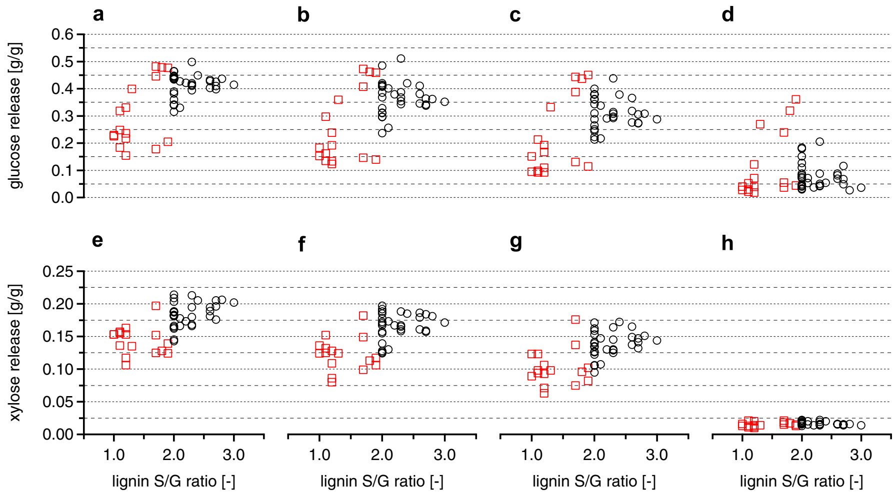

Fig. S2. Relationship of glucose and xylose release to syringyl and guaiacyl units (S/G ratio) from pretreatment of poplar at different temperatures followed by enzymatic hydrolysis using cellulase and xylanase. Samples were pretreated in just water at 180 °C for 18 min (*A* and *E*), 160 °C for 68 min (*B* and *F*), or 140 °C for 464 min (*C* and *G*), or they were directly subjected to enzymatic hydrolysis without pretreatment (*D* and *H*). Each marker represents the mean value of three replicates. The sugar yields are displayed in grams sugars per grams raw biomass. The influence of lignin content is distinguished between samples featuring an S/G ratio < 2 (□) and ≥ 2 (○). Glucose and xylose release follow the trend of the total sugar yield described in Fig. 3.

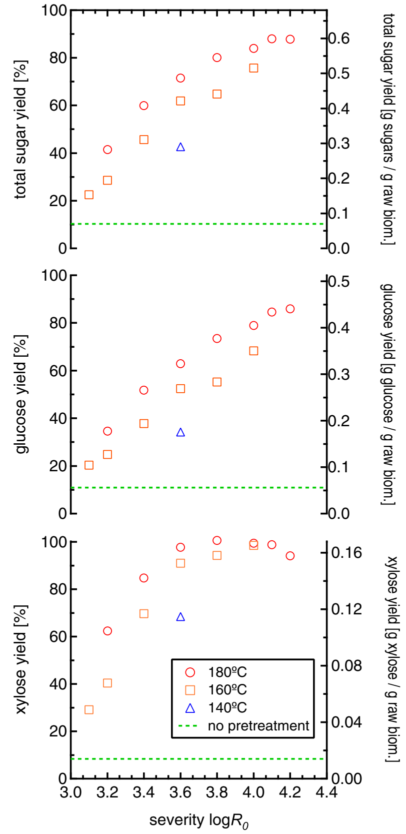

Fig. S3. Sugar yields from pretreatment and enzymatic hydrolysis for different pretreatment severities and temperatures using a BESC internal standard poplar. The standard poplar has a composition of 46.2% glucan, 14.8% xylan, and 27.0% lignin and a lignin S/G ratio of 1.7. The pretreatment and enzymatic hydrolysis conditions were identical to the method described in the text, except that larger volumes were used. Pretreatments were carried out in 10 mL tube reactors (each containing 8.333 g of biomass slurry) heated with condensing steam (1). For enzymatic hydrolysis, the content of three tube reactors was poured into 125-mL Erlenmeyer flasks to yield 25 mL aliquots of pretreated slurry. The total sugar yield for a pretreatment temperature of 180 °C peaks at a severity of $\log R_0$ 4.1, corresponding to a pretreatment time of 55.5 min. However, xylose yields peak at a severity of 3.8 (27.8 min) because of xylose degradation at higher severities, whereas glucose release increases over the entire severity range tested. Based on these experiments, we selected a severity of 3.6 to be the starting point for the screening experiment of the 47 *Populus* samples to minimize degradation of sugars and make them available for enzymatic release.

1. Studer MH, DeMartini JD, Brethauer S, McKenzie HL, Wyman CE (2010) Engineering of a high-throughput screening system to identify cellulosic biomass, pretreatments, and enzyme formulations that enhance sugar release. *Biotechnol Bioeng* 105:231–238.

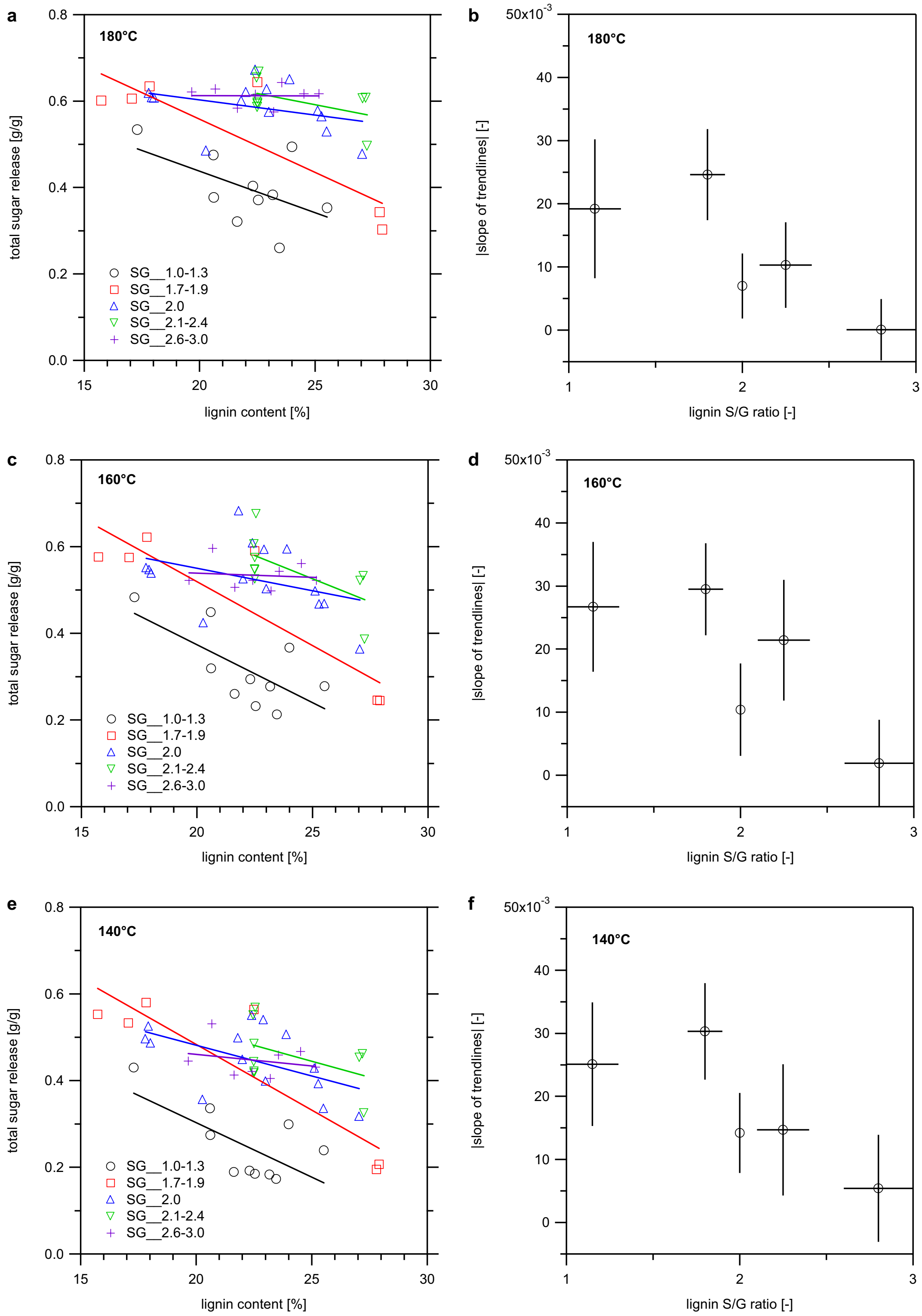

Fig. S4. Trend lines for sugar release vs. lignin content for individual S/G groups for all tested pretreatment temperatures (*A*, *C*, and *E*). The absolute values of the slopes of these trend lines are plotted against the S/G ratio (*B*, *D*, and *F*). The vertical error bars represent SDs; the horizontal error bars represent band widths of the S/G group. In addition to the previously described trend that the slope is smaller for S/G ≥ 2.0 than for S/G < 2.0, there is a general trend that higher S/G ratios reduce the dependency of sugar release on lignin content after pretreatment and enzymatic hydrolysis.

Table S1. Statistical analysis based on Eq. 2 showed that the slopes of the regressions lines for total sugar release vs. lignin content for the data subsets S/G < 2 and S/G ≥ 2 are not identical at the 0.05 level for each pretreatment temperature applied (indicated by asterisk), whereas total sugar and glucose release vs. lignin content for the data subsets S/G < 2 and S/G ≥ 2 are identical at the 0.05 level for the different pretreatment temperatures

Comparing slopes of regressions lines for sugar yields	95% confidence interval for b_2	Statistically identical slopes?
Total sugar		
180 °C S/G < 2.0 vs. S/G ≥ 2.0	(0.005, 0.032)	No
160 °C S/G < 2.0 vs. S/G ≥ 2.0	(0.005, 0.037)	No
140 °C S/G < 2.0 vs. S/G ≥ 2.0	(0.003, 0.036)	No
180 °C vs. 160 °C S/G ≥ 2	(−0.015, 0.007)	Yes
180 °C vs. 160 °C S/G < 2	(−0.014, 0.026)	Yes
160 °C vs. 140 °C S/G ≥ 2	(−0.011, 0.014)	Yes
160 °C vs. 140 °C S/G < 2	(−0.022, 0.023)	Yes
180 °C vs. 140 °C S/G ≥ 2	(−0.005, 0.016)	Yes
180 °C vs. 140 °C S/G < 2	(−0.015, 0.028)	Yes
Glucose		
180 °C vs. 160 °C SG ≥ 2	(−0.002, 0.007)	Yes
180 °C vs. 160 °C SG < 2	(−0.005, 0.012)	Yes
160 °C vs. 140 °C SG ≥ 2	(−0.004, 0.006)	Yes
160 °C vs. 140 °C SG < 2	(−0.009, 0.010)	Yes
180 °C vs. 140 °C SG ≥ 2	(−0.001, 0.008)	Yes
180 °C vs. 140 °C SG < 2	(−0.005, 0.012)	Yes
Xylose		
180 °C vs. 160 °C SG ≥ 2	(−0.001, 0.003)	Yes
180 °C vs. 160 °C SG < 2	(−0.00005, 0.005)	Yes
160 °C vs. 140 °C SG ≥ 2	(−0.001, 0.003)	Yes
160 °C vs. 140 °C SG < 2	(−0.002,0.003)	Yes
180 °C vs. 140 °C SG ≥ 2	(0.00008, 0.004)	No
180 °C vs. 140 °C SG < 2	(0.0003, 0.005)	No

Xylose releases at 180 °C and 140 °C follow statistically different trends.

Table S2. List of the nine highest- and lowest-performing biomasses (sample identifications) for all pretreatment conditions as well as no pretreatment

	Pretreatment conditions			
Rank	180 °C	160 °C	140 °C	No pretreatment
1	273*	833	819*†	876
2	909*†	909*†	909*†	081
3	349*	819*†	152*	869
4	332	273*	876	819†
5	152*	349*	349*	909†
6	351	462*	273*	338
7	819*†	332	314*	818
8	462*	314*	081	833
9	314*	152*	462*	296
47	006*	006*	006*	163*†
46	121*	163*†	102*	290*†
45	829*†	121*	163*†	829*†
44	103*	103*	829*†	102*
43	077*	829*†	290*†	351*
42	163*†	102*	103*	258*
41	080*	077*	121*	319*
40	102*	290*†	077*	816*
39	290*†	080*	080*	332*
				006 (23.); 077 (10.); 80 (37.); 102 (44.); 103 (36.); 121 (27.)‡

The average lignin content and S/G ratio with SEM (standard error of the mean) of the seven highest- and nine lowest-performing biomasses using any hot-water pretreatment: lignin, 21.6 ± 0.7% (highest) and 23.9 ± 0.3% (lowest); S/G ratio, 2.1 ± 0.1 (highest) and 1.3 ± 0.1 (lowest).

*Seven of the top nine sugar-releasing biomasses and nine of nine worst sugar-releasing biomasses were the same, respectively, independent of the hot-water pretreatment condition applied.

†The top two and bottom three biomass variants that exhibited high or low monosaccharide yields, respectively, for any condition tested are indicated.

‡These are the six lowest-performing biomasses for any pretreatment condition applied with the respective rank for the no pretreatment case.

Table S3. Composition and characteristics of the highest- and lowest-performing nine biomass variants

Sample ID	Lignin content (% wt/wt)	S/G ratio (–)	dbh (cm)
006	23.46	1.2	70
014	20.28	2.0	25
077	25.53	1.2	31
080	20.62	1.0	82
081	17.06	1.8	16
102	23.17	1.0	50
103	27.8	1.9	12
121	27.91	1.7	20
152	22.5	1.7	38
163	22.54	1.2	60
258	22.01	2.0	24
273	22.42	2.0	23
290	22.31	1.1	109
296	17.94	2.0	15
314	22.92	2.0	32
319	25.17	3.0	8
332	23.91	2.0	12
338	17.81	2.0	4
349	22.48	2.4	14
351	23.56	2.8	9
462	20.68	2.6	6
816	22.52	2.2	16
818	18.03	2.0	30
819	17.83	1.7	49
829	21.63	1.1	83
833	21.81	2.0	26
869	17.3	1.3	92
876	15.74	1.9	8
909	22.57	2.3	24

dbh, diameter on breast height (i.e., the diameter of the sampled tree); ID, identification.

Table S4. Endogenous glucose and starch contents in select biomass samples along with their lignin contents and S/G ratios

Sample ID	Endogenous glucose and/or starch content (% wt/wt)	Lignin content (% wt/wt)	S/G ratio (–)
125	0.15	18.29	1.8
291	0.16	18.75	1.7
294	0.49	18.25	2.0
443	0.39	18.63	2.2
807	0.22	18.83	1.7
818*	0.28	18.03	2.0
833*	0.18	21.81	2.0
897	0.57	18.46	2.1
909*	0.20	22.57	2.3

The starch content was determined using a downscaled (5 mg biomass), enzyme-based (α-amylase and amyloglucosidase) analysis kit from Megazyme (catalog number K-TSTA; http://www.megazyme.com/booklets/KTSTA.pdf). The starch content was determined in duplicates. Endogenous glucose and/or starch were only present in very small amounts [<1% (wt/wt) of total raw biomass].

*Three samples were from the analyzed 47 samples subset; 6 samples featuring very low lignin contents were from the complete 1,100 sample set.

Table S5. To test amylase activity in the cellulase/xylanase mixture used, starch from potato (product number S2630; SigmaAldrich) was subjected to a 72-h enzymatic hydrolysis using the same equipment and reaction conditions as for the real samples

Starch loading (% wt/wt)	Corresponding starch in biomass (% wt/wt)	Glucose yield (%)
1.0	100	7.4 ± 0.1
0.1	10	7.2 ± 1.2

Two loadings were tested along with enzyme and starch blanks. The uncertainties are calculated from four replicates and are reported as SEM.