Experimental

Materials:

Fresh beech wood chips (harvested in October 2014) were generously provided by Michael Studer (Berner Fahrhochschule, Bern Switzerland). After being air-dried, the chips were ground into 3-10 mm particles using a Wiley mill. Chemical composition of the wood particles is given in Table S1. Commercial enzymes, Cellic®CTec2 (~80 mg protein/mL, 150 FPU/mL) and Cellic®HTec2 were kindly provided by Novozymes and used as received. All the chemical reagents used in this study were purchased from Sigma-Aldrich or Fisher and used as received.

Composition analysis:

Compositional analysis of biomass was performed according to the TAPPI protocol.[1] First, 0.5 g of wood was weighed in a 50 mL beaker followed by the addition of 7.5 mL of 72wt% sulfuric acid. The slurry was stirred every 15 minutes to make sure the material was well dispersed and swollen. After two hours, the slurry was diluted with around 290 mL of water to form a 3 wt% acid solution and transferred into a 500 mL glass bottle. The capped glass bottle was autoclaved at 120 °C for 1 hour to convert polysaccharides into monosugars. After cool down, the solution was filtered and filtrate was used for sugar analysis and acid-soluble lignin determination. The residue was washed with 100 mL of water and then dried at 105 °C to determine Klason lignin.

Pretreatment:

GVL pretreatment:

(1) ***In a 100 mL glass reactor***: 4 g of 3-10 mm wood particles were loaded into a glass reactor followed by the addition of 20 mL solvent (GVL:H_2O 80:20 w/w). Depending on the experiment 50 or 150 mg of 98% sulfuric acid was added to form a 25 or 75 mM sulfuric acid solution. A stir bar was put into the reactor for mixing during pretreatment. The glass reactor was sealed and put into an oil bath at the target temperature for the designated residence time. Details of every reaction condition are shown in Figure 1 and 2. After pretreatment, the reactor was cooled down and the slurry was filtered followed by washing with 500 mL of water. After washing, the pellet was stored in a plastic bag at 4 °C for composition analysis and enzymatic hydrolysis. The filtrate was analysed by HPLC to determine the amount of solubilized sugars.

(2) ***In the 1L Parr reactor***: a certain amount of 3-10 mm wood particles was loaded into 1 L Parr reactor (Parr Instrument Company) followed by the addition of a certain amount of solvent (GVL:H_2O 80:20 w/w) and sulfuric acid. The reactor was heated via an external metal jacket and temperature was ramped to 120°C in 45 min. A custom impeller, which was based on a previously described design,[2] was built into the reactor to allow for high-solids mixing. During pretreatment the impeller rotated at a speed of 70 rpm for mixing. At the conclusion of the reaction, the reactor was cooled by flowing water through the cooling coil imbedded in the heating jacket. Once the pretreated slurry was recovered, 20 g were sampled, filtered and washed with 500 mL of water. After washing, the pellet was placed into a plastic bag and stored at 4°C for composition analysis and enzymatic hydrolysis. The filtrate was analysed by HPLC to determine the amount of solubilized sugars. The leftover slurry was used for GVL recovery experiments.

THF pretreatment: 4 g of 3-10 mm wood particles were loaded into a glass reactor followed by the addition of 20 mL solvent (THF:H_2O 80:20 w/w). 150 mg of 98% sulfuric acid was added to form an acid concentration of 75 mM. A stir bar was put into the reactor for mixing during pretreatment. The glass reactor was sealed and put into an oil bath at 120°C for 1 h. After pretreatment, the reactor was cooled down and the slurry was filtered followed by washing with 500 mL of water. After washing, the pellet was stored in a plastic bag at 4°C for composition analysis and enzymatic hydrolysis.

Ethanol pretreatment: 4 g of 3-10 mm wood particles were loaded into a glass reactor followed by the addition of 20 mL solvent (ethanol:H_2O 50:50 w/w). 150 mg of 98% sulfuric acid was added to form an acid concentration of 75 mM. A stir bar was put into the reactor for mixing during pretreatment. The glass reactor was sealed and put into an oil bath at 120°C for 1 h. After pretreatment, the reactor was cooled down and the slurry was filtered followed by washing with 500 mL water. After washing, the pellet was stored in a plastic bag at 4°C for composition analysis and enzymatic hydrolysis.

Dilute acid pretreatment: 4 g of 3-10 mm wood particles were loaded into a glass reactor followed by the addition of 20 mL water. 150 mg of 98% sulfuric acid was added to form an acid concentration of 75 mM. A stir bar was put into the reactor for mixing during pretreatment. The glass reactor was sealed and put into an oil bath at 120°C for 1 h. After pretreatment, the reactor was cooled down and the slurry was filtered followed by washing with 500 mL water. After washing, the pellet was stored in a plastic bag at 4°C for composition analysis and enzymatic hydrolysis.

Alkali pretreatment: 4 g of 3-10 mm wood particles was loaded into a glass reactor followed by the addition of 20 mL water. 200 mg NaOH was added to form an alkali concentration of 250 mM. A stir bar was put into the reactor for mixing during pretreatment. The glass reactor was sealed and put into an oil bath at 120°C for 1 h. After pretreatment, the reactor was cooled down and the slurry was filtered followed by washing with 500 mL water. After washing, the pellet was stored in a plastic bag at 4°C for composition analysis and enzymatic hydrolysis.

Enzymatic hydrolysis:

(1) Evaluation of the pretreated substrate's digestibility: Enzymatic hydrolysis of the pretreated substrates was conducted as described below. The hydrolysis was carried out at 50 °C on a shaking incubator (New Brunswick Scientific, Model 126) at 250 rev/min. A substrate equivalent to 0.4 g glucan was loaded into a 15 mL glass vial. 1 mL of tetracycline chloride solution (40 mg/mL) was added to prevent the growth of microorganisms and prevent consumption of liberated sugars. Acetate buffer (pH=5, bought from Fisher, catalogue No. 25862-0010) was added to make the final liquid volume of 12 mL (~12 g). In the case of alkali incubation, ~10 mg NaOH (1wt% based on the dry substrate) in 5 mL of water was added into the vial and mixed with the substrate. The slurry wad put into the incubator at 50 °C for 1 h and then was neutralized with acetic acid to form a pH=5. After that, the acetate buffer was added to make the final liquid volume 12 mL (~12 g). Cellulase (15 FPU (Filter Paper Units) of CTech2 per gram glucan) was loaded into the glass vial. In some cases, a stir bar was added and vortexed with the substrate to facilitate the defibrillation and dispersion of cellulose. 200 μL of liquid was sampled at specific intervals for sugar yield determination by HPLC.

(2) High consistency (30 w/v%) hydrolysis of pretreated substrate: Because almost all sugars and lignin were left in the CO_2 extracted substrate, for a better comparison the consistency of 30% was based on the weight of original untreated material (specifically, the pretreated substrate resulting from 4 g of original wood particles was hydrolysed in 12 mL of liquid). During the alkali incubation, 40 mg NaOH (1 wt% based on the dry substrate) in 5 mL of water was added into the vial and mixed with the substrate. The slurry wad put into the incubator at 50 °C for 1 h and then was neutralized with acetic acid to pH=5. The acetate buffer was added to make the final liquid volume of 12 mL (~12 g). Cellulase (15 FPU (Filter Paper Units) of CTech2 per gram glucan) was loaded into the glass vial. In some occasions, a stir bar was added and vortexed with the substrate to facilitate the defibrillation and dispersion of cellulose. During the whole process, a glass rod was used to help the mixing of alkali, acid and enzyme with the substrate as needed. Samples of 200 μL were taken periodically to track sugar yields.

Substrate surface characterization:

125 grams of wood particles were pretreated in the 1L Parr reactor as described above. After pretreatment, the substrate was washed with water until no GVL was detectable by HPLC. The washed substrate was then incubated with 500 mL of a dilute alkali solution (alkali loading: 1.25 gram) at 50 °C for 1 hour. The slurry was filtered and the filtrate was neutralized with concentrated sulfuric acid and then concentrated to 5 mL by evaporation on a hotplate. Any GVL losses were considered to be minimal due to GVL's extremely low vapor pressure. The concentrated solution was analyzed by HPLC to detect GVL.

When GVL-immersed wood particles were used as a control, 125 grams of wood particles were immersed overnight in the same solvent at room temperature instead of the pretreatment procedure. The rest of the treatment was performed as described above.

Analytical methods:

Acid-soluble lignin was determined by UV at 205 nm using an extinction coefficient of 110 L g^{-1} cm^{-1} .[1] Analysis of glucose and xylose was conducted using an Agilent Infinity 1260 HPLC equipped with a Refractive Index Detector and a Bio-Rad Aminex HPX-87P column with water as mobile phase. Furfural, HMF and GVL analysis was conducted using a similar Agilent Infinity 1260 HPLC equipped with UV-Vis Detector and a Bio-Rad Aminex HPX-87H column with a dilute acid solution as the mobile phase.

GVL recovery:

Extraction of GVL from pretreated biomass slurries with liquid CO_2 was performed by placing 700 g of pretreated biomass with glass beads in a separator (2 inch stainless steel tube with a height of 1.2 meters) between two metal sieves (45 mm filtrating disks, VWR) to immobilize the biomass within 2/3 of the separator volume (Figure S**6**). The separator was pressured at 75 bar with CO_2 and left to rest overnight. The extractions were carried out at room temperature with a liquid CO_2 up-flow of 5-8 mL/min and a constant pressure of 75 bar, controlled by a back pressure regulator. The GVL was recovered in a liquid-gas collector and the gas CO_2 was released by a vent. Recovered GVL was weighed and analysed by HPLC to determine the recovery yield.

Table S1. Composition analysis of wood raw material

	glucan	xylan	arabinan	galactan	mannan	Kalson lignin	Acid-soluble lignin
Content (wt%)	38.81±0.4%	16.39±0.26%	1.06±0.07%	1.06±0.07%	0.98±0.13%	24.21±0.07%	1.26±0.08%

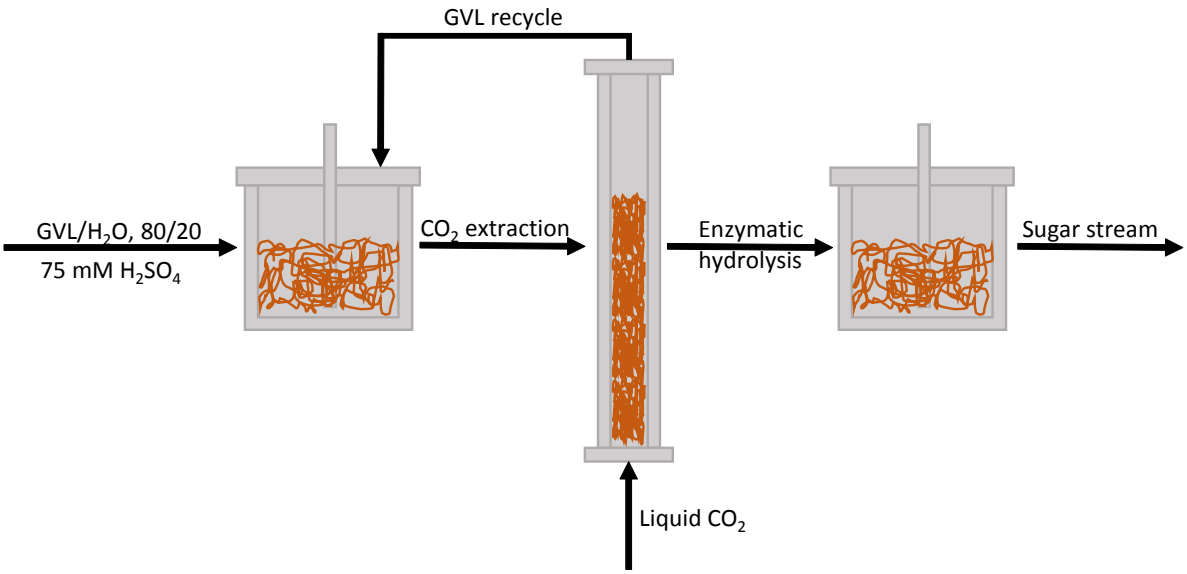

Figure. S1. GVL-based pretreatment process integrated with solvent recycle and enzymatic hydrolysis

Figure. S2. Morphologies of original wood particles and pretreated substrates

Table S2. Mass balance of pretreated substrates and liquors at 120 °C

		50mM acid, 1h	150mM acid, 1h	50mM acid, 2h	150mM acid, 2h
Solid	Glucan	98.9%	96.5%	97.8%	96.6%
	Xylan	51.6%	21.5%	41.8%	19.1%
	Lignin	52.7%	23.0%	41.9%	18.2%
Liquor	Glucose	0.2%	0.6%	0.7%	2.1%
	Gluco-oligosaccharides	1.1%	1.8%	1.8%	2.2%
	Xylose	11.5%	28.2%	17.3%	29.2%
	Xylo-oligosaccharides	33.7%	53.3%	37.4%	48.5%

Table S3. Elemental analysis of separated lignin

C	H	O	N
62.13%	5.73%	31.94%	0.20%

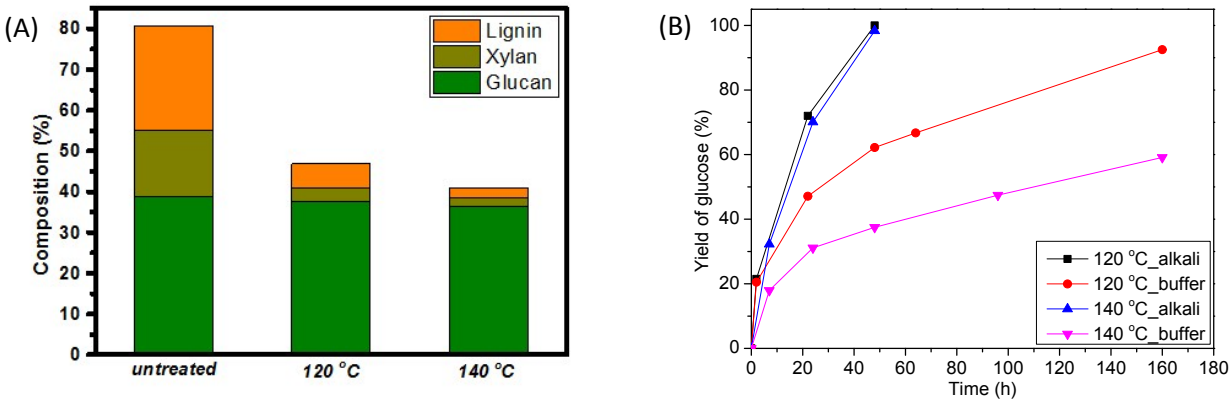

Figure. S3. Comparison of GVL pretreatment at different temperatures (acid concentration: 75 mM H_2SO_4). (a) Composition analyses of pretreated substrates at 120 °C and 140 °C, (b) Enzymatic digestibilities of pretreated substrates at 120 °C and 140 °C

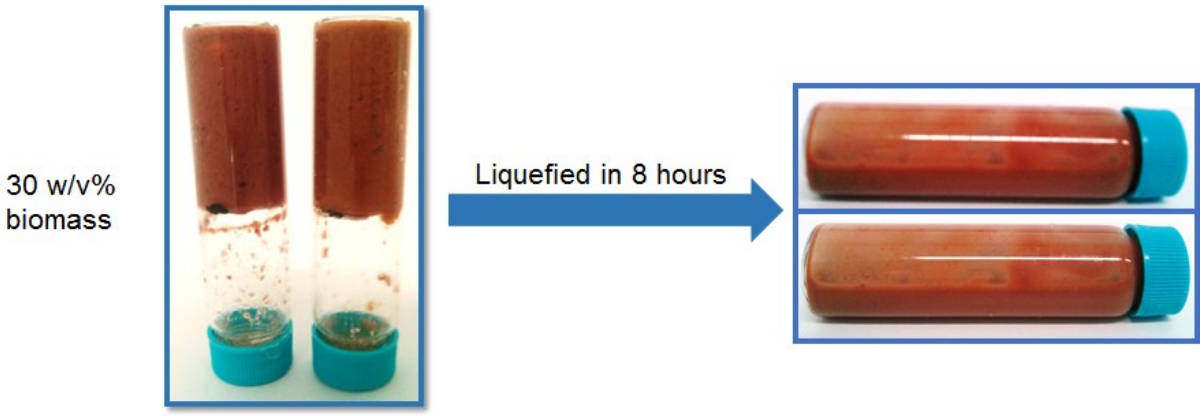

Figure. S4. Liquefaction process of high consistency (30% w/v) enzymatic hydrolysis.

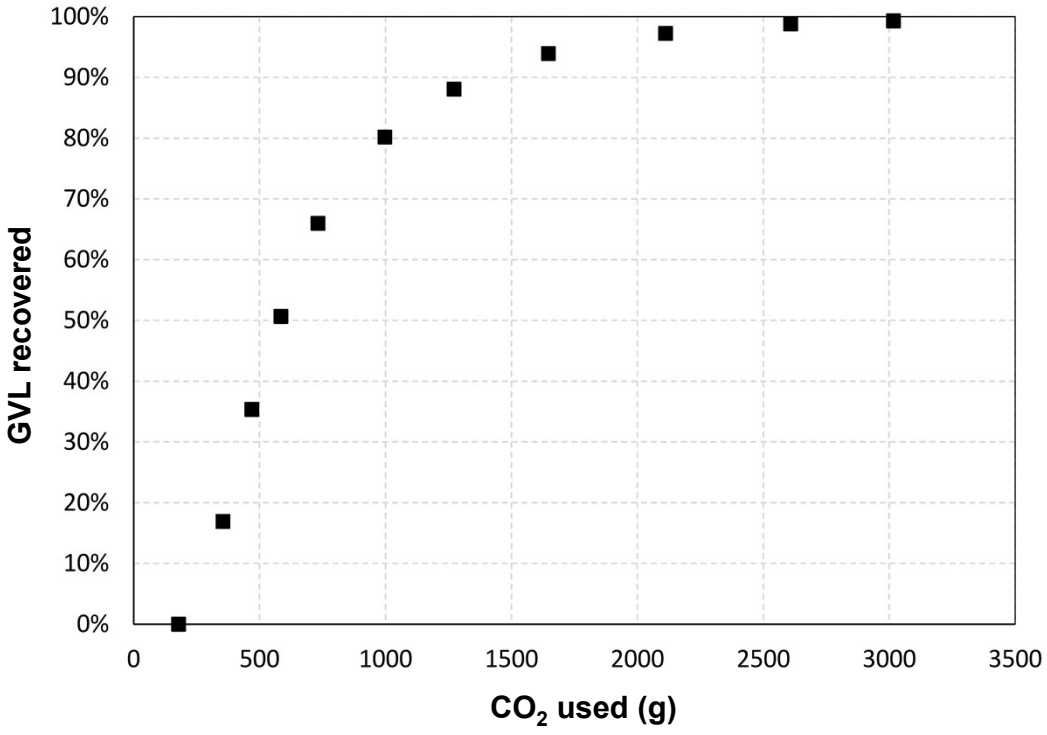

Figure. S5. Consumption of liquid CO_2 versus the recovery yield of GVL. In this case, 100% GVL recovery corresponded to 460 g of GVL.

Table S3. Mass balance of solubilized sugars and GVL in liquid upon CO_2 extraction (16.7 wt% biomass equivalent to liquid/biomass ratio of 5:1 (w/w), 20 wt% biomass equivalent to liquid/biomass ratio of 4:1 (w/w))

Component	Extraction 16.7wt% biomass			Extraction 20wt% biomass		
	Monomer [%]	Oligomer [%]	Total [%]	Monomer [%]	Oligomer [%]	Total [%]
Glucose Extracted	0.0%	0.3%	0.3%	0.0%	0.7%	0.7%
Glucose Retained	16.7%	82.9%	99.7%	65.3%	34.0%	99.3%
Xylose Extracted	0.1%	0.8%	0.9%	0.4%	0.9%	1.3%
Xylose Retained	22.1%	77.0%	99.1%	81.7%	17.0%	98.7%
GVL Extracted	99.5%		99.5%	99.3%		99.3%
GVL Retained	0.5%		0.5%	0.7%		0.7%

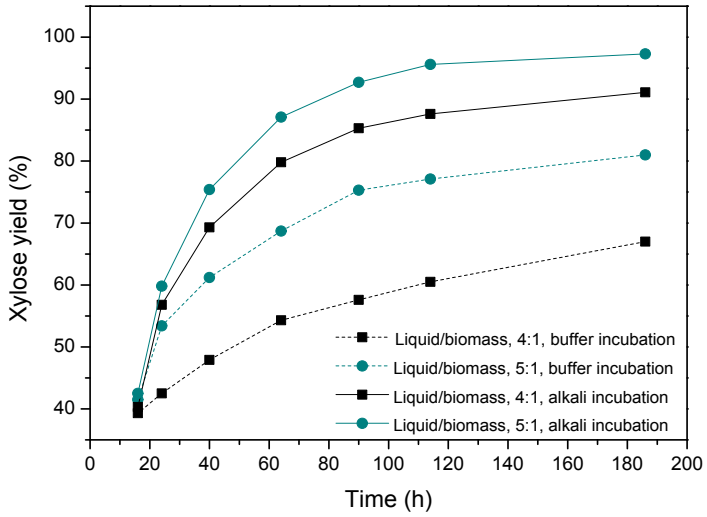

Figure S5. Xylose yields of the CO_2 extracted substrates in high consistency enzymatic hydrolysis.

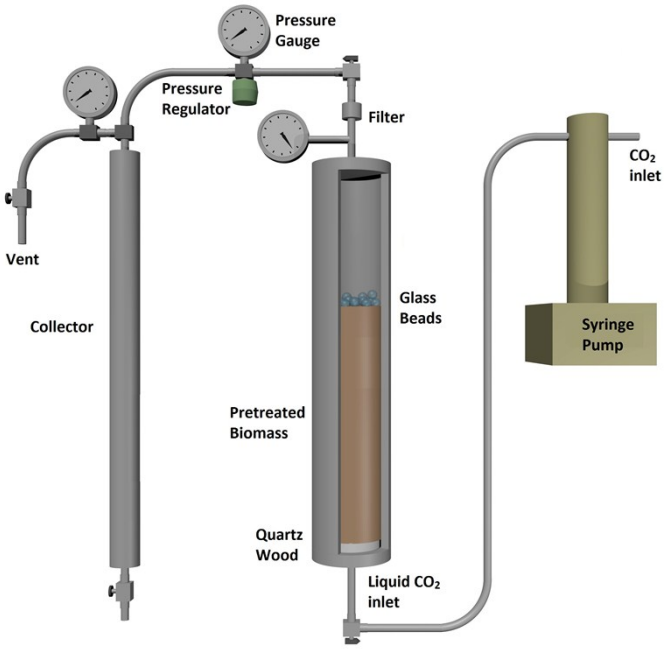

Figure S6. Diagram of the liquid CO_2 extraction unit.

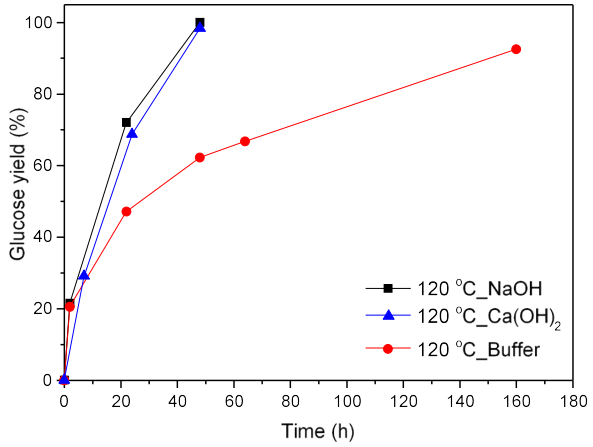

Figure S7. Comparison of the effect of alkali incubations with NaOH and $Ca(OH)_2$ on enzymatic hydrolysis efficiency.

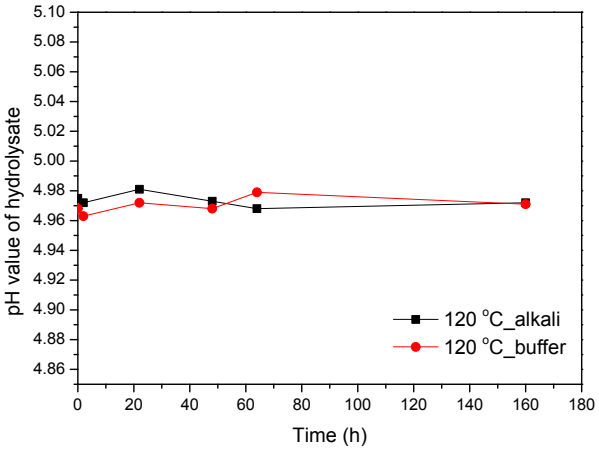

Figure S8. Hydrolysate pH during enzymatic hydrolysis.

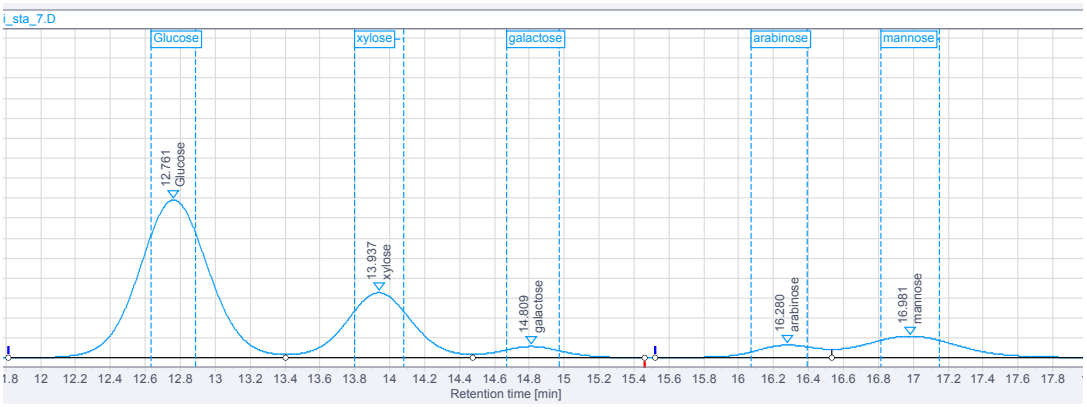

Figure S9. HPLC chromatogram of the hydrolysate

References

1 TAPPI method, available from www.tappi.org/content/SARG/T222.pdf

2 D. J. Klingenberg, Root, Thatcher W., C. Houtman, K. J. Bourne, and V. Subramanian, “High temperature rheometry of lignocellulosic biomass,” presented at the The Society of Rheology 86th Annual Meeting, Philadelphia, US, 07-Oct-2014.