SUPPLEMENTARY MATERIAL

Liquid hot water pretreatment of lignocellulosic biomass at lab and pilot scale

Jose M. (Chema) Jimenez-Gutierrez[a], Rob Verlinden[b], Peter van der Meer[b], Luuk A. M. van der Wielen[a,c], Adrie J. J. Straathof[a]

[a] Delft University of Technology, Department of Biotechnology, Delft, The Netherlands

[b] Bioprocess Pilot Facility, Delft, The Netherlands

[c] Bernal Institute, University of Limerick, Castletroy, Limerick, Ireland

Conditions and pH values

Table S1 – Conditions and pH values for LHW pretreatment of poplar biomass at lab and pilot scales.

Sample	Biomass fraction	Temperature (°C)				Pressure (MPa)				Time (min)			pH final
		T.set	T.init	T.pret	T.final	P.set	P.init	P.pret	P.final	t.heat	t.pret	t.cool	
Lab													
P19N2	10%	180	22.2	180	20.8	0.1	0.1	0.98	0.15	80	120	52	3.64
P33N	10%	180	22.0	180	24.0	0.1	0.1	0.96	0.13	70	6	30	4.10
P34N	10%	180	21.7	180	21.7	0.1	0.1	0.98	0.16	62	240	418	3.60
P35N	10%	180	21.8	180	20.1	0.1	0.1	0.98	0.16	55	180	40	3.60
P36N	10%	180	21.7	180	21.8	0.1	0.1	0.97	0.13	56	30	177	4.30
P37N	10%	188	21.5	188	13.0	0.1	0.1	1.17	0.15	57	120	93	3.66
P38N	15%	180	21.6	180	22.4	0.1	0.1	1.00	0.14	60	120	135	3.60
P39N	11%	180	20.3	180	21.7	0.1	0.1	0.98	0.15	55	120	150	3.75
P40N	10%	180	18.1	180	13.3	0.1	0.1	0.98	0.14	56	120	54	3.67
P34N2	10%	180	25.5	180	20.0	0.1	0.1	0.97	0.16	94	240	26	3.61
Pilot													
71	10%	180	18.9	180	20.1	0.1	0.1	0.98	n.a.	105	33	95	3.92
73	10%	180	17.0	180	24.0	0.1	0.1	0.88	n.a.	80	30	93	4.12
75	10%	180	16.9	180	28.2	0.1	0.1	1.02	n.a.	80	180	71	3.71
77	10%	180	20.3	180	28.3	0.1	0.1	0.90	n.a.	105	243	78	3.68
79	10%	180	20.9	180	19.8	0.1	0.1	1.03	n.a.	74	122	127	3.74
82	10%	180	20.0	180	29.0	0.1	0.1	0.96	n.a.	81	33	76	4.01
83	10%	188	16.4	187	24.7	0.1	0.1	1.20	n.a.	125	120	95	3.68
85	10%	180	19.1	180	27.4	0.1	0.1	0.96	n.a.	79	245	78	3.69
87	16%	180	17.3	180	24.1	0.1	0.1	1.01	n.a.	70	121	69	3.68
89	10%	180	15.3	180	21.1	0.1	0.1	1.01	n.a.	81	123	128	3.75

Temperature profiles at pilot plant

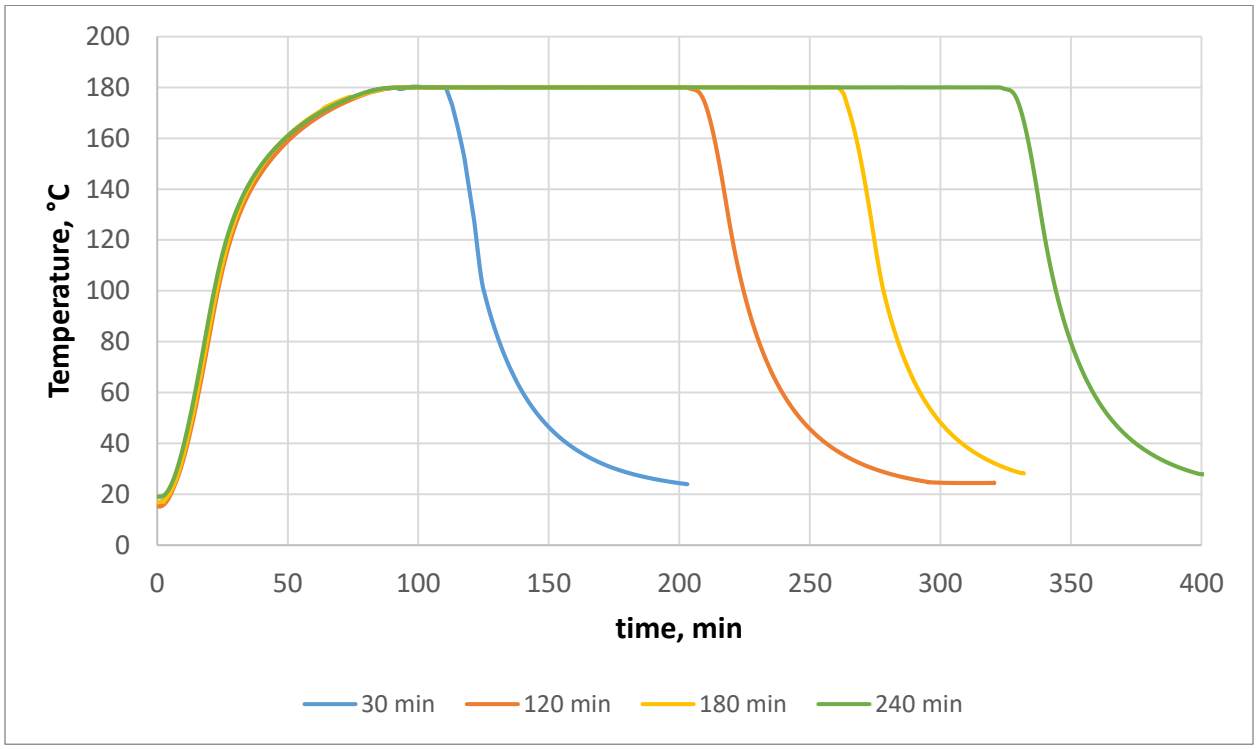

Figure S1 – Temperature profiles during LHW pretreatment of poplar biomass at pilot plant at different pretreatment times.

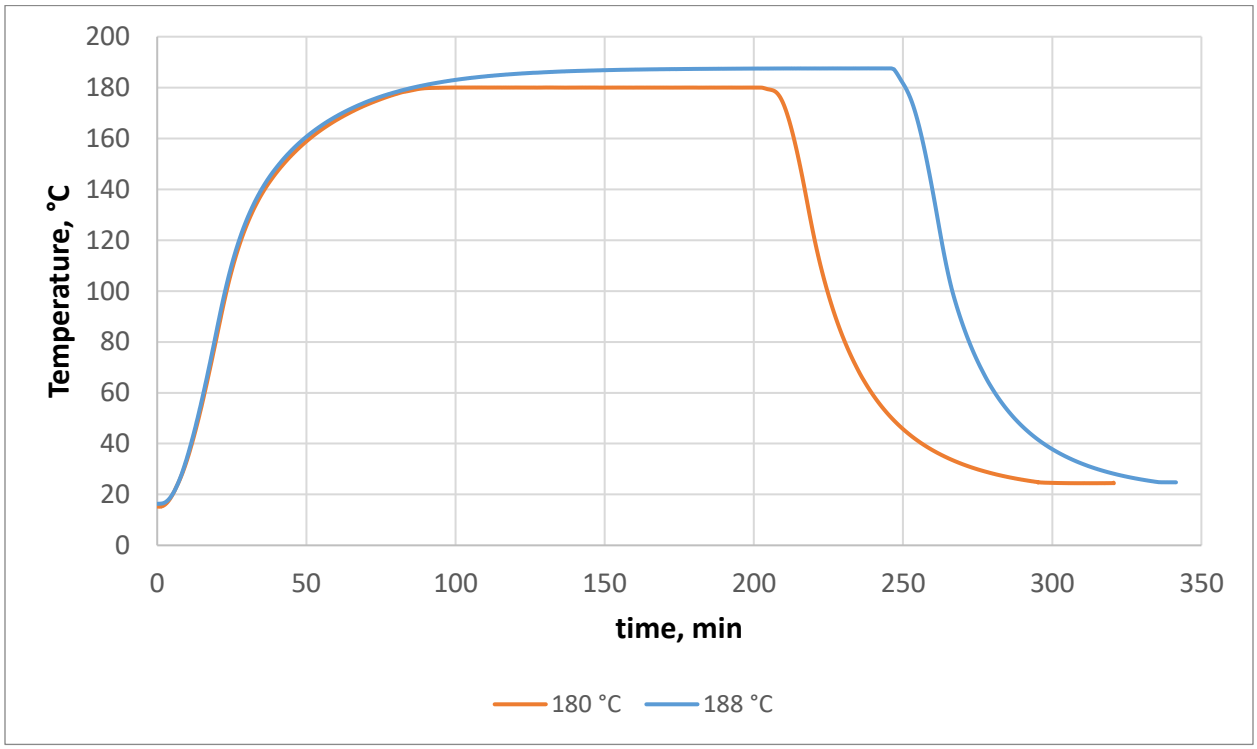

Figure S2 – Temperature profiles during LHW pretreatment of poplar biomass at pilot plant at two different pretreatment temperatures, for 120 min pretreatment time and 10% initial biomass fraction.

Calculations for analysis of solid fractions

The composition of the solid fraction was expressed in grams of component "A" per gram of dry biomass, calculated from the concentration in mmol/L obtained in the analysis after the LAP for solid compositions, by the following expression:

$$\frac{C_A * V * M_A * \alpha_A}{m_{dry}} \qquad \left[\frac{g_A}{g\ dry\ biomass}\right] \tag{S1}$$

C_A is the measured dissolved molar concentration of component "A" (being glucose, xylose or acetic acid) after the two-step hydrolysis, V the final liquid volume of the mixture after the two-steps acid hydrolysis (0.435 L), M_A is the molar mass of the component A, α_A is a correction factor for the anhydrous form of component A (0.9 for glucose to anhydroglucose unit, 0.88 for xylose to anhydroxylose unit, 0.72 for acetic acid to acetyl group), and m_{dry} is the initial mass of the raw biomass on dry basis.

When expressing the composition of solid fraction in grams of component A per gram of dry biomass (equation S1), the m_{dry} is not the mass of dry biomass used in the experiment but the proportional dry biomass that was assumed to been used, as calculated from the actual pretreated biomass used for the analysis m_{pret}. The reason to do this is because the same amount of pretreated biomass was used for the LAP analysis of all experiments: 1.5 g. However, not all the experiments gave the same dry solid fraction, as every experiment converted a different amount from the initial biomass used. Thus, in order to have comparable results, from the 1.5 grams of pretreated biomass analysed ($m_{solids.LAP}$), a proportional amount of dry biomass was calculated from a simple cross multiplication:

$$\frac{m_{biomass(dry)}}{m_{pret}} = \frac{m_{dry}}{m_{solids.LAP}} = \beta \tag{S2}$$

This ratio was called β and is expressed in dry basis. Therefore, the initial dry biomass used $m_{biomass(dry)}$ should be calculated from the raw biomass using the moisture content θ:

$$m_{biomass(dry)} = m_{raw} * (1 - \theta) \tag{S3}$$

Additionally, there is a loss of mass due to handling and it differs also from one experiment to another. Therefore, a proportional amount of this initial dry biomass used needs to be subtracted in order to use the "actual" dry biomass used:

$$m_{biomass(dry)} = m_{raw} * (1 - \theta) * (1 - \%_{loss}) \tag{S4}$$

Therefore, from equations S2 and S4 we obtained the following expression, which was applied in the calculations:

$$\beta = \frac{m_{raw} * (1 - \%m_{loss}) * (1 - \theta)}{m_{pret}} \quad \text{(S5)}$$

In case of the pilot plant trials, it was not feasible to wash and dry all the pretreated biomass obtained (7-10 kg) to get the value of m_{pret}. Instead, only a sample of the washed pretreated solids was used. Assuming that the pretreated washed solids have similar fraction of liquid adsorbed after filtration and washing at both scales, values of raw biomass/washed solids ratio *R* were calculated from each lab experiments. These were then applied for their coupled pilot plant trial (experiment at the pilot plant with similar conditions than at the lab) to obtain the proportional raw biomass used for each sample of washed pilot-pretreated solids. Thus, in case of the pilot plant analysis, the value of β was calculated as follow:

$$\beta = \frac{(m_{pret.sample.washed} * R) * (1 - \%m_{loss}) * (1 - \theta)}{m_{pret.sample.dry}} \quad \text{(S6)}$$

With $m_{pret.sample.washed}$ as the amount of washed pilot-pretreated solids sample taken and used for this analysis, and $m_{pret.sample.dry}$ is this amount of solids after a drying step.

Visual analysis of pretreated biomass

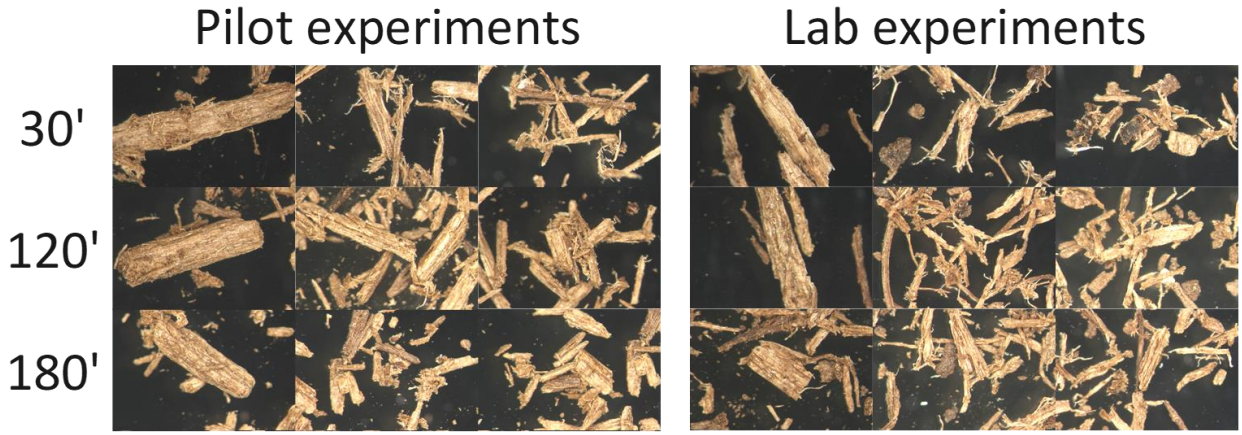

Figure S3 – Visual analysis at the microscope of the surface of the LHW pretreated material at different pretreatment times at lab and pilot scales.

Results from higher biomass loading fraction, in g/L

Table S2 – Concentration of different compounds for LHW experiments for 120 min pretreatment time at 180 °C.

Scale	Initial biomass fraction	Glucose g/L	Xylose g/L	Formic acid g/L	Acetic acid g/L	HMF g/L	Furfural g/L
Lab	10 %	0.28	2.05	0.88	4.20	0.30	2.02
	15 %	0.49	1.69	1.56	6.44	0.51	2.85
Pilot	10 %	0.28	1.62	0.96	4.22	0.31	2.67
	16 %	0.568	1.888	1.849	7.168	0.625	3.210

In the pilot plant trials, increasing 50% the dry mass content loading provided proportional increments in the concentrations in g/L of most of the compounds analysed, with the exception of xylose and furfural (see Table S2). Whereas glucose, formic acid and HMF experienced an increase in double concentration, acetic acid was 70% higher and a 20% growth was observed for furfural. At the lab trials, except for xylose and furfural, all the species show an average increment of approximately 70% in concentrations in g/L. On the other hand, xylose showed only a decrease of 18% at the lab experiments. By increasing the biomass/water ratio for LHW pretreatment, the concentration of glucose, carboxylic acids and furans increased mostly proportionally due to the lower liquid fraction used.

Overall mass balance

The overall mass balance would state:

$$m_{biomass} + m_{water} = m_{solids} + m_{liquid} + m_{loss} \quad \text{(S7)}$$

Combining to initial biomass and water as total mass, then the mass loss is converted to a percentage of loss:

$$m_{TOTAL} = m_{solids} + m_{liquid} + m_{loss} \quad \text{(S8)}$$

$$m_{TOTAL} - m_{solids} - m_{liquid} = m_{loss} \quad \text{(S9)}$$

$$\frac{m_{loss}}{m_{TOTAL}} = \frac{m_{TOTAL} - m_{solids} - m_{liquid}}{m_{TOTAL}} = \%_{loss} \quad \text{(S10)}$$

Table S3 – Overall mass balance for all LHW pretreatment experiments.

Sample	**Biomass**	**Water**	**Total mass**	**Liquid frac.**	**Solid frac.**	**% mass loss**	**% water adsorbed**	**Feature**
Lab	**g**	**g**	**g**	**g**	**g**			
P34N	5	45	50	27.50	18.40	8.2 %	29.8 %	240 min
P35N	5	45	50	25.23	20.26	9.0 %	33.9 %	180 min
P36N	5	45	50	23.50	23.15	6.7 %	40.3 %	30 min
P37N	5	45	50	24.36	17.83	15.6 %	28.5 %	188 °C
P38N	5.25	29.75	35	16.24	13.24	15.8 %	26.9 %	15 %DM
P39N	5	40	45	23.33	14.49	16.0 %	23.7 %	11 %DM
P40N	5	45	50	25.18	20.08	9.5 %	33.5 %	120 min
P34N2	5	45	50	27.58	12.58	19.7 %	16.9 %	240 min
Pilot	**kg**	**kg**	**kg**	**kg**	**kg**			
71	2.5	22.5	25	14.88	9.54	2.3 %	31.3 %	30 min
73	2.5	22.5	25	13.56	10.18	5.0 %	34.1 %	30 min
75	2.5	22.5	25	15.40	7.14	9.8 %	20.6 %	180 min
77	2.5	22.5	25	17.26	7.30	1.8 %	21.3 %	240 min
79	2.5	22.5	25	15.72	8.00	5.1 %	24.4 %	120 min
82	2.5	22.5	25	15.30	9.28	1.7 %	30.1 %	30 min
83	2.5	22.5	25	16.06	7.02	7.7 %	20.1 %	188 °C
85	2.5	22.5	25	16.46	6.82	6.9 %	19.2 %	240 min
87	2.0	10.5	12.5	4.90	6.30	10.4 %	41.0 %	16 %DM
89	2.5	22.5	25	16.70	7.40	3.6 %	21.8 %	120 min

Acetic acid in washing steps

Table S4 – Amount of acetic acid in the recovered liquid fraction and in the washing water, compared with the total acetic acid per amount of biomass, estimated from the initial liquid added (including mass loss).

Sample	in liq. fraction†, mg	in W1*, mg	in W2*, mg	Total recovered, mg/g biomass	Total estimated‡, mg/g biomass	Feature
Lab						
P35N	108.7	46.4	7.7	32.53	38.76	180 min
P36N	44.4	37.9	8.1	18.08	16.99	30 min
P37N	107.1	73.9	9.6	38.13	39.57	188 °C
P38N	104.5	64.9	14.2	36.72	38.29	15 %DM
P39N	101.2	70.4	26.1	37.67	33.06	11 %DM
P40N	107.1	55.1	6.0	33.65	38.27	120 min
P34N2	146.5	37.4	16.7	40.12	47.81	240 min
Pilot						
72	171.2	86.2	24.6	22.54	20.21	30 min
74	91.9	52.5	10.5	14.97	14.00	30 min
76	449.3	163.1	53.1	44.23	39.33	180 min
78	423.5	138.8	38.5	46.75	42.20	240 min
80	372.4	137.6	38.8	36.71	33.83	120 min
81	179.8	74.7	19.3	20.14	19.13	30 min
84	555.4	185.1	52.7	45.61	41.30	188 °C
86	609.6	178.7	61.4	48.93	44.68	240 min
88	286.8	272.4	84.6	37.40	31.98	16 %DM
90	367.3	130.6	35.5	41.05	36.71	120 min

*W1 regards the first washing steps, and w2 the second washing steps, both cases with 100ml demi-water.
†Liquid fraction at pilot plant estimated from wet solids sampled and the proportion liquid/solid fractions obtained (Table S3).
‡Values estimated by multiplying the concentration of the liquid fraction by the total initial water added and divided by the initial biomass (both initial liquid and biomass minus the proportional mass loss (Table S3)). In case of pilot plant values, the initial water added was estimated by the proportion liquid fraction†/initial water added. Similarly, the initial biomass was also estimated by multiplying the wet solids sampled by the average raw/wet biomass ratio obtained at the lab (0.335 $g._{raw.biomass}/g._{pretreated.biomass}$).