Supplementary Information

AqSO biorefinery: a green and parameter-controlled process for the production of lignin-carbohydrate hybrid materials

Dmitry Tarasov[a], Philipp Schlee[a], Andrey Pranovich[b], Adrian Moreno[c], Luyao Wang[b], Davide Rigo[a], Mika Sipponen[c], Chunlin Xu[b], Mikhail Balakshin[a]*

[a]Department of Bioproducts and Biosystems, Aalto University, Vuorimiehentie 1, Espoo, Finland, 02150

[b]Laboratory of Natural Materials Technology, Åbo Akademi University, Henrikinkatu 2, Turku, Finland, 20500.

[c]Department of Materials and Environmental Chemistry, Stockholm University, Svante Arrhenius väg 16C, Stockholm, Sweden, 10691.

*Corresponding author: mikhail.balakshin@aalto.fi

Experimental procedure for birch wood sawdust preparation and details on calculations

Birch wood chips (1 kg) were grinded with Wiley Mill M02 grinder (passed 0.5 mm) and then screened in the range 0.15-0.5 mm with Retsch AS 300 Control Vibratory Sieve Shaker – RAMI. The moisture content (MC) of the sawdust was measured by drying a sample to a constant weight at 100-105°C. According to the obtained MC, the amount of dry matter (4 g) and water (4 g) required for the process were calculated as follows:

$$Msd = Msd^{'} \times \left(L/S + \frac{MC,\%}{100}\right) = 4 \times \left(1 + \frac{MC,\%}{100}\right) \qquad (1)$$

Where **Msd** is the mass of wet sawdust needed for the process, **Msd´** is the mass of dry sawdust needed (in this case it is 4 g), **MC** is the moisture content of the sawdust used, and **L/S** is the liquid to solid ratio between dry matter and water. The amount of water needed (**Mw**) was calculated as follows:

$$Mw = (Ml' + Msd') - Msd \qquad (2)$$

Where **Ml´** is the mass of liquid (H_2O) theoretically needed. For instance, taking into consideration that liquid to solid ratio is 1 (L/S=1), it means that **Ml´** = **Msd´** = 4g.

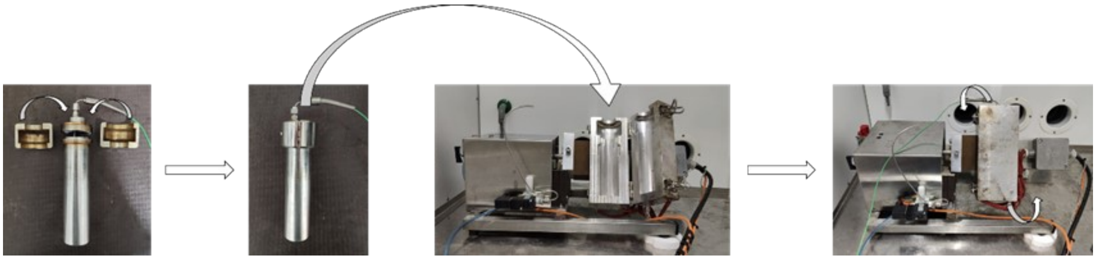

Fig. SI1. Experimental apparatus (swing reactor) used for HTT experiments.

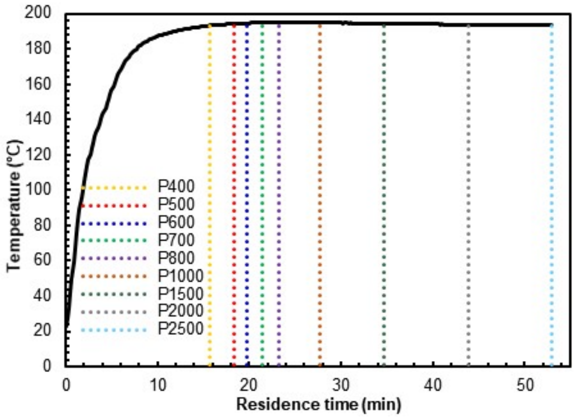

Fig. SI2. Temperature profile in the HTT experiments.

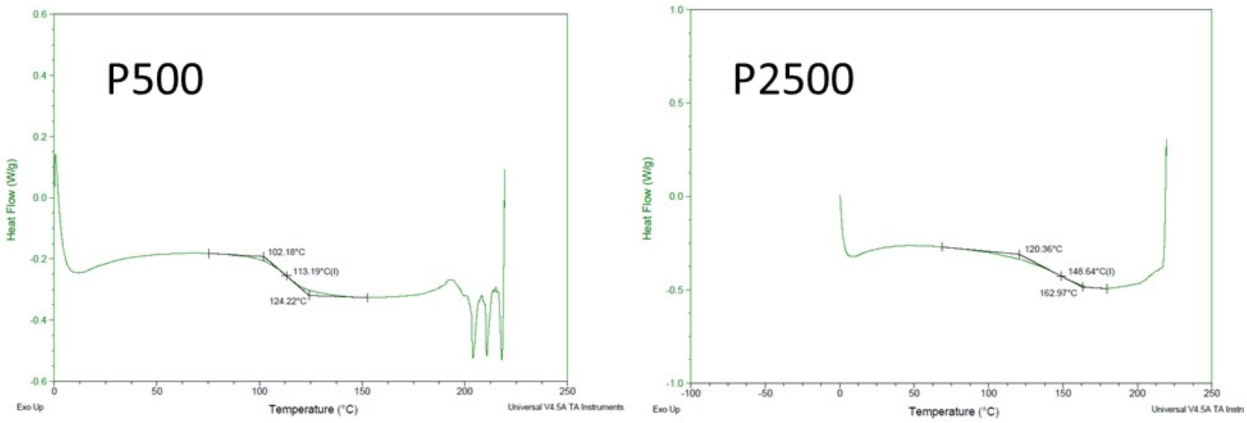

Figure SI3. DSC curve of lignin samples obtained under different process severity (left: P500; right: P2500).

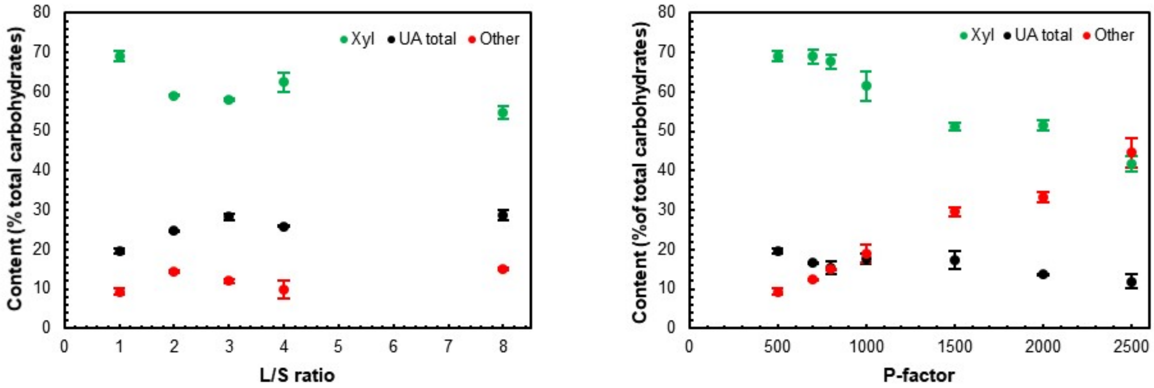

Fig. SI4. The effect of L/S ratio (P-factor 500) on proportion of main carbohydrates in AELs.

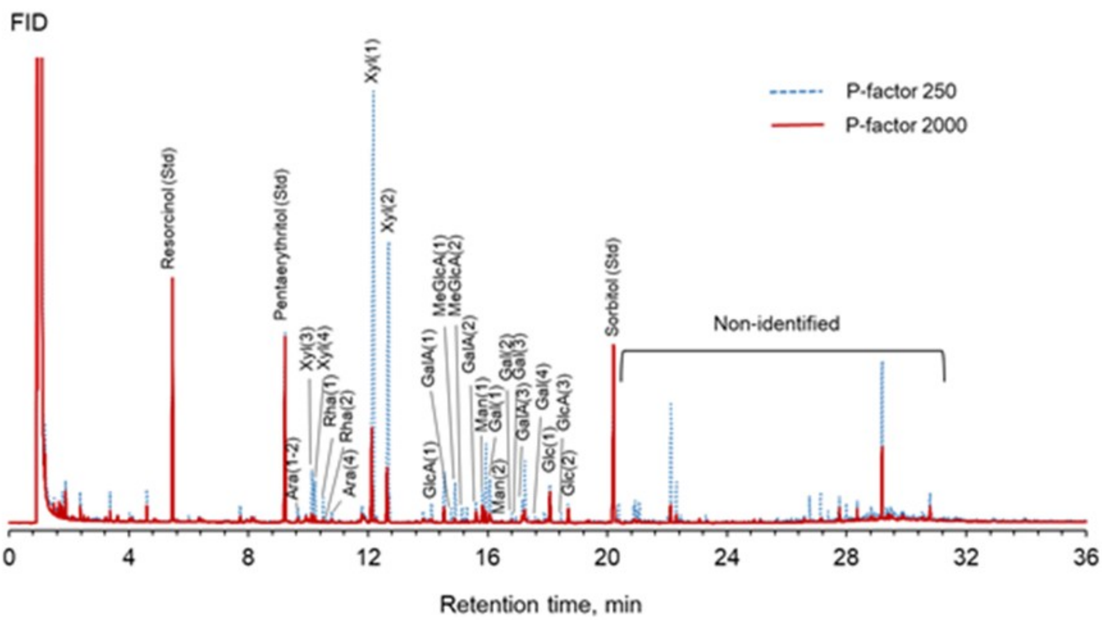

Fig. SI5. GC-chromatograms of acid methanolysed AEL-250 and AEL-2000.

Table SI1. Carbohydrate composition (mg/g sample) of AELs by the methanolysis method.

P factor	L/S ratio	Ara	Xyl	Man	Gal	Glc	GlcA	GalA	MeGlcA	Total
500	1	2.53±0.19	69.7±15.5	2.01±0.46	1.75±0.50	2.93±0.50	1.61±0.24	3.52±0.51	14.7±3.79	101±21.8
700	1	2.75±0.03	53.0±2.04	2.82±0.21	1.21±0.05	2.83±0.05	1.08±0.38	2.36±0.21	9.47±0.67	76.8±0.97
800	1	3.21±0.14	51.1±8.46	3.28±0.66	1.30±0.19	3.57±0.19	1.26±0.05	2.10±0.20	8.15±0.15	75.2±10.5
1000	1	1.66±0.51	25.3±3.51	3.50±0.14	0.77±0.03	1.79±0.03	0.57±0.03	1.27±0.04	5.37±0.02	41.0±3.18
1500	1	2.32±0.06	9.83±0.24	1.51±0.12	0.38±0.05	1.48±0.05	0.34±0.15	0.51±0.03	2.49±0.22	19.2±0.12
2000	1	2.19±0.03	8.26±0.75	1.21±0.05	0.34±0.04	1.58±0.04	0.20±0.18	0.26±0.02	1.73±0.03	16.0±1.02
2500	1	1.66±0.17	3.28±0.06	0.60±0.03	0.14±0.02	1.10±0.18	0.03±0.02	0.09±0.02	0.82±0.07	7.85±0.23
500	2	2.97±0.36	38.2±0.61	2.34±0.07	1.52±0.16	2.51±0.13	1.80±0.50	2.99±0.13	11.2±0.64	64.7±0.81
500	3	1.82±0.86	25.5±3.60	1.27±0.03	0.90±0.09	1.35±0.02	1.35±0.11	2.42±0.25	8.68±0.97	44.0±5.98
500	4	2.58±0.66	42.1±8.01	1.22±0.07	1.29±0.11	1.23±0.04	2.71±0.53	2.92±0.41	11.7±1.56	67.0±10.2
500	8	1.70±0.34	16.7±0.87	0.95±0.03	0.64±0.04	1.36±0.37	1.06±0.00	1.68±0.06	6.11±0.16	30.8±0.71
BCEL		3.71	21.1	1.95	4.84	9.36	3.59	8.39	8.71	65.1
PLCC		18.3	30.4	29.8	15.7	49.3	1.92	15.1	21.7	189

Table SI2. Quantification of LCC moieties (per 100 Ar) in AELs by the HSQC method.

Moieties	P-factor (L/S=1)									L/S ratio (P-factor=500)		
	400	500	600	700	800	1000	1500	2000	2500	2	3	8
BE total	3.8	3.5±0.0	3.8	3.7±0.0	3.7	3.7	3.4	3.5±0.1	3.4±0.0	3.7±0.2	3.4	3.4±0.2
Acetyl/3	10.1	7.0±0.5	7.9	5.0±0.5	4.9	4.0	2.4	2.4±0.0	2.5±0.3	2.9±0.7	2.4	1.4±0.0
γ−esters	0.7	0.4±0.0	0.3	0.6±0.0	0.6	0.5	0.5	0.6±0.0	0.4±0.0	0.4±0.0	0.4	0.3±0.1
GlcU Acid	1.2	0.8±0.2	0.9	0.6±0.0	0.5	0.3	0.1	0.1±0.0	0.0±0.0	0.4±0.1	0.3	0.3±0.0
GlcU Esters	1.2	0.8±0.1	0.9	0.6±0.1	0.5	0.4	0.1	0.1±0.0	0.0±0.0	0.3±0.1	0.3	0.2±0.0
PhGly	0.8	0.6±0.0	0.7	0.8±0.1	0.9	0.7	0.4	0.3±0.0	0.2±0.0	0.5±0.1	0.3	0.2±0.0
Term carb.	7.2	3.3±0.0	7.4	5.3±0.9	6.9	3.8	1.8	1.0±0.0	0.5±0.0	2.6±1.1	1.4	0.9±0.0
Internal carb.	20.4	13.9±1.3	17	9.7±1.1	9.3	6.6	2.4	1.7±0.0	0.9±0.1	6.3±1.0	5.6	4.0±0.1
Total carb.	27.6	17.3±1.3	24	15±2.9	16	11	4.2	2.7±0.0	1.4±0.0	8.9±2.2	7.0	4.9±0.0
carb. DP	3.8	5.2±0.4	3.3	2.8±0.1	2.4	2.7	2.3	2.7±0.0	2.9±0.2	3.8±0.8	4.9	5.5±0.2

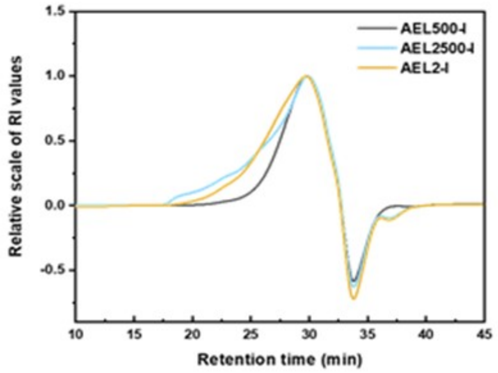

Fig. SI6. SEC chromatograms of AEL500, AEL2500, and AEL2 using RI values.

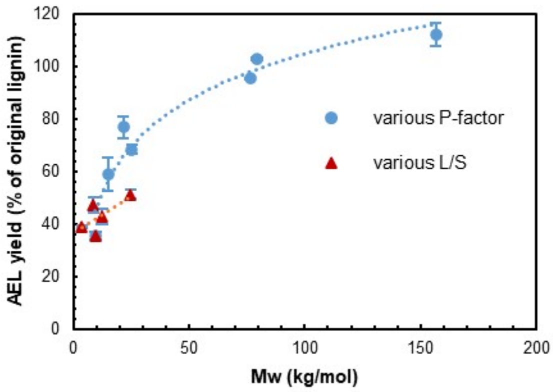

Fig. SI7. Correlation between AEL yield and Mw.

Table SI3. The Molecular Mass Distribution of AELs and reference lignins and LCCs.

P-factor	L/S ratio	Mw (kg/mol)	Mn (kg/mol)	D	Mp (kg/mol)	Mz (kg/mol)
500	1	9.50±2.02	2.66±1.00	3.99±1.17	1.59±0.17	2.26±0.75
700	1	14.9±0.27	2.77±0.50	5.54±1.39	2.85±0.59	2.94±1.13
800	1	25.3±7.95	2.51±0.98	13.4±8.38	2.42±0.81	1.45±0.42
1000	1	21.7±0.00	5.78±0.01	3.75±0.03	5.96±0.00	1.56±0.0
1500	1	76.3±0.20	2.44±0.02	31.3±0.00	6.32±0.00	2.37±0.00
2000	1	79.3±6.94	3.48±0.09	22.8±1.44	3.07±0.12	1.21±0.15
2500	1	157±17.4	4.8±0.20	33.0±12.0	3.92±0.10	1.98±0.12
500	2	24.65±4.74	2.26±0.39	11.6±4.10	4.74±0.28	0.39±1.66
500	3	12.35±1.63	1.86±0.04	6.68±1.04	1.63±0.03	0.04±0.72
500	4	8.64±3.11	1.33±0.20	6.31±1.42	3.11±0.20	0.20±1.88
500	8	3.65±0.53	1.39±0.27	2.65±0.13	0.53±0.23	0.27±0.47
Reference lignins						
Alcell		4.84	1.71	2.83	2.11	3.34
Indulin		13.9	2.91	4.78	3.83	1.84
Native LCCs						
BCEL		35.0	14.3	2.45	1.43	9.36
PLCC		2.02	0.98	2.06	1.17	5.17

Table SI4. Tg of AELs and reference lignins determined by DSC.

P-factor	L/S	Tg (°C)
500	1	113.0
700	1	118.0
1000	1	125.0±4.3
1500	1	132.0
2000	1	140.0
2500	1	150.0±4.5
500	2	105.0±1.1
500	3	116.5±1.1
500	4	122.5±5.2
500	8	106.0±0.2
Alcell		91.0
Indulin		143.0

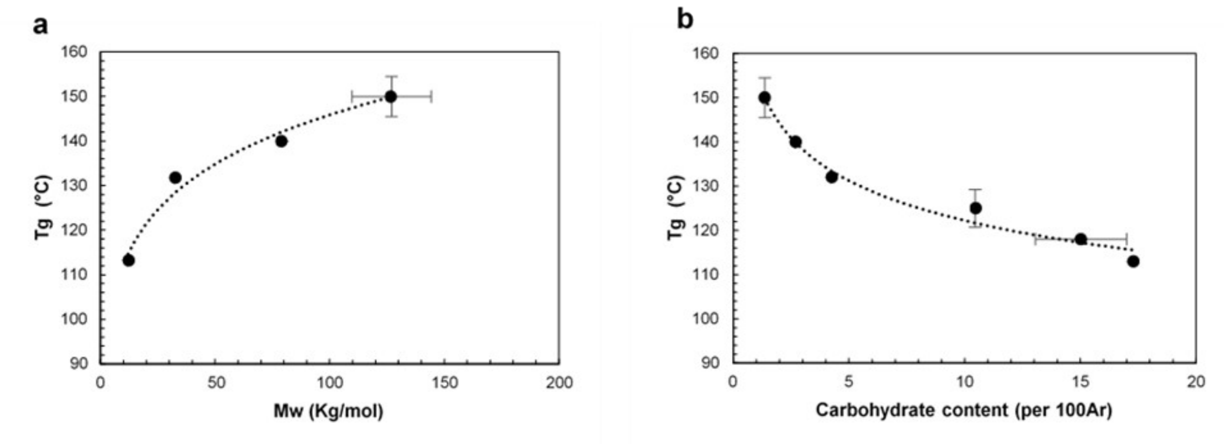

Fig. SI8. Correlation between Tg and Mw (a), carbohydrate content (b).

Table SI5. Surface tension of AEL and reference lignin as well as native LCC in ultrapure water and pH 11.5 alkaline solution.

Sample	**Surface tension in ultrapure water[a] (mN/m)**	**Surface tension in pH 11.5 solution[b] (mN/m)**
AEL-500 (L/S=1)	60.69±3.15	60.65±1.09
AEL-8 (P=500)	67.13±2.65	59.22±3.11
AEL-2000	67.94±0.05	69.03±0.05
BCEL	70.96±0.01	59.68±0.07
PLCC	64.74±0.03	69.44±0.02
Alcell	68.85±0.02	54.84±0.09
Indulin AT	69.81±0.10	67.85±0.04

The color of green indicated the state of lignin was totally soluble; the color of yellow indicated the state of lignin was partially soluble/dispersed; the color of red indicated the state of lignin was totally insoluble.

Table SI6. Hydrolysate main products (% per original wood).

Sample	Carbohydrates				Lignin	Degradation products			
	Xylan		Total			HMF	Furfural	HCOOH	AcOH
	Mono	Bonded	Mono	Bonded*					
H500	2.4	12.1	3.0	14.1 (1.7)	3.3	0.02	0.26	0.28	0.97
H2500	1.5	0.0	3.3	0.3 (0.2)	2.7	0.58	4.91	1.14	5.74

* the values in the parentheses correspond to the direct analysis (without acid hydrolysis) of oligosaccharides

Table SI7. Composition of free carbohydrates in hydrolysates (% of detected).

Sample	mono-	di-	tri-	tetra-	penta-
H500	64.0	18.7	8.7	6.2	2.3
H2500	93.2	4.5	1.9	0.4	0.0

Table SI8. Xylan balance (mol% per original xylan).

Sample	Mono	Bonded	Total Xyl	Furfural	RXyl	Sum	Others
H500	10.1	58.2	68.3	1.7	28.6	98.6	1.4
H2500	6.4	0.2	6.7	32.4	7.2	46.2	53.8

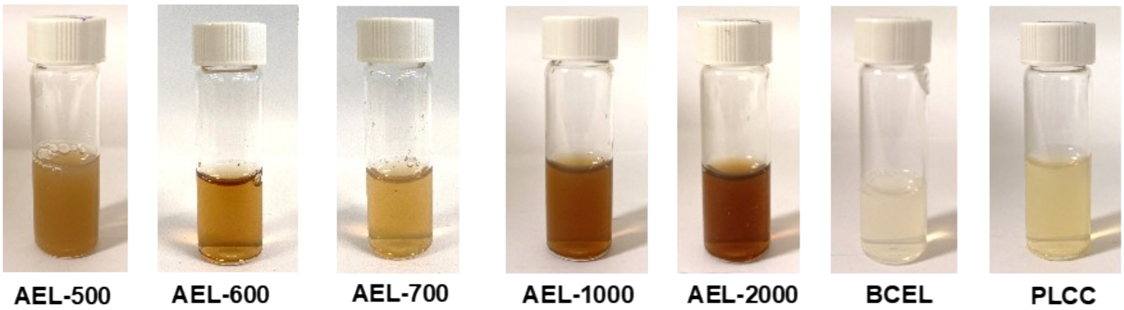

Fig. SI9. Digital images of LCCNPs samples after 1 month of their preparation.

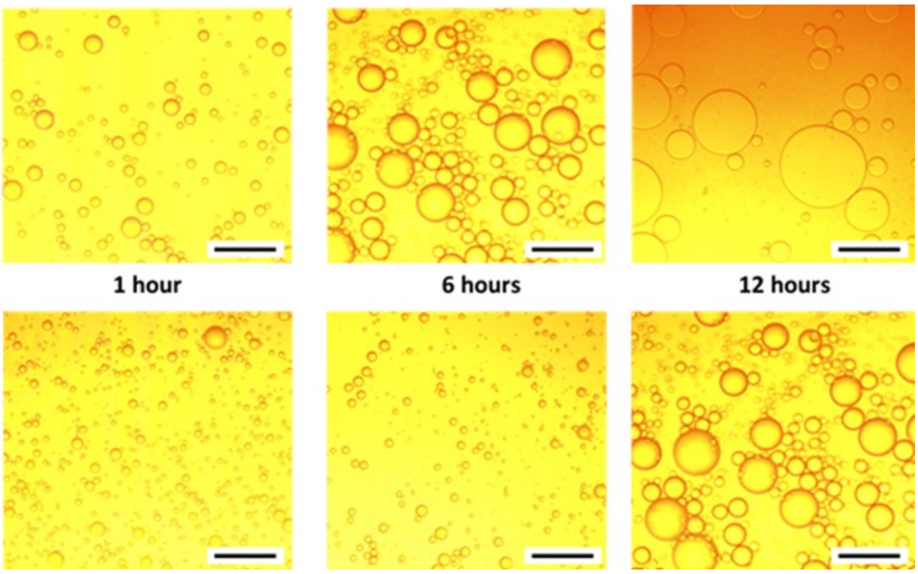

Fig. SI10. Optical microscope images of THFMA-Pickering emulsions stabilized by LCCNPs (PLCC on the top, AEL-500 on the bottom) at different time durations.