Electronic Supplementary Material (ESI) for Green Chemistry.

Cellulose and Lignin Colocalization at the Plant Cell Wall Surface Limits Microbial Hydrolysis of Populus Biomass

Alexandru Dumitrache[1,2], Allison Tolbert[1,2,3], Jace Natzke[1,2], Steven D. Brown[1,2], Brian H. Davison[1,2], Arthur J. Ragauskas[1,2,4]

[1] Bioenergy Science Center (BESC), Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA

[2] Biosciences Division, Energy and Environment Directorate, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA

[3] School of Chemistry and Biochemistry & Renewable Bioproducts Institute, Georgia Institute of Technology, Atlanta, Georgia, USA

[4] Department of Chemical and Biomolecular Engineering, Center for Renewable Carbon, Department of Forestry, Wildlife, and Fisheries, University of Tennessee, Knoxville, Tennessee, USA

Corresponding author: Arthur J. Ragauskas, aragausk@utk.edu

Supplemental figures and tables

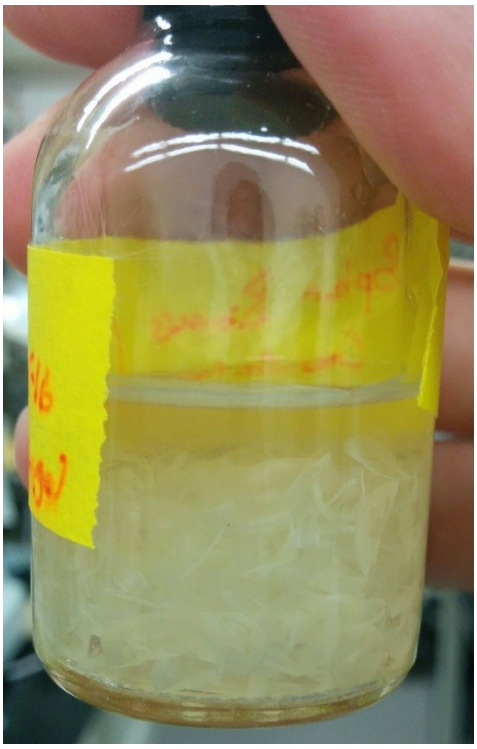

Figure S.1. Typical preparation of anaerobic batch culture bottle of cryotome transverse-sectioned poplar stem in MTC media loaded at 10g biomass/L.

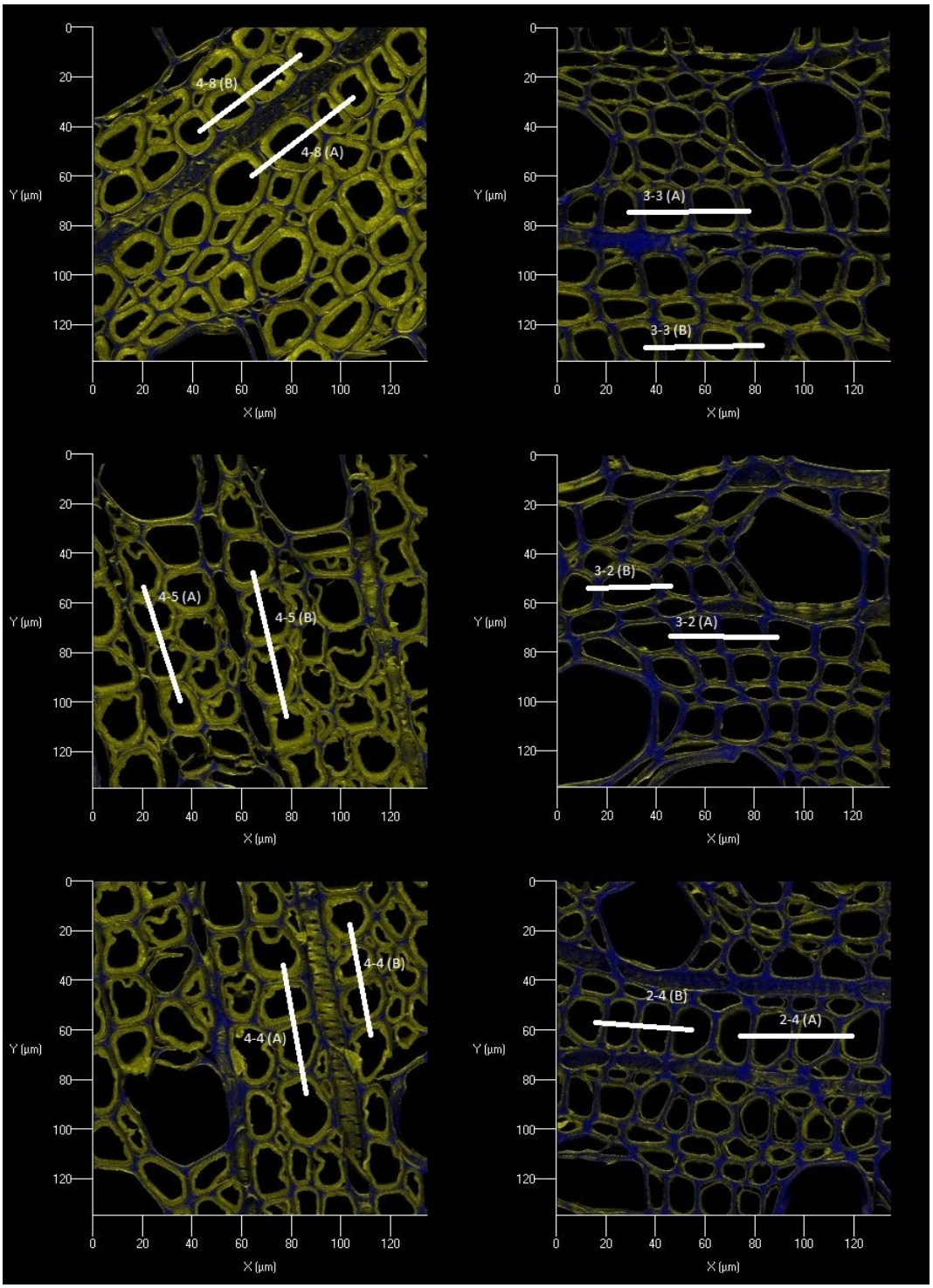

Figure S.2. Microtome sections of poplar analyzed for co-localization of cellulose and lignin across cell walls (white lines). Control biomass (left side images) and fermented biomass (right side images)

Raw data from Figure 2.

Time (h)	Fermentation ethanol (mg/g biomass)	Fermentation acetate (mg/g biomass)	Biomass-acetate in control (mg/g biomass)
0	1.7 ± 0.1	3.2 ± 0.2	0
24	5.3 ± 0.6	20.8 ± 2.4	1.1
48	19.2 ± 4.3	52.8 ± 1.7	2.1
72	22.4 ± 2.0	55.9 ± 5.9	2.6
92	24.2 ± 3.0	60.0 ± 3.0	3.8

Raw data from Figure 4.

	Fluorescence intensity (8-bit scale)				Localization across cell walls (μm)			
Rep	**Control Cellulose**	**Control Lignin**	**Ferm. Cellulose**	**Ferm. Lignin**	**Control Cellulose**	**Control Lignin**	**Ferm. Cellulose**	**Ferm. Lignin**
1	46.61	53.31	43.67	56.67	10.67	6.06	3.43	3.29
2	37.66	49.79	40.08	54.99	6.46	3.95	4.74	4.09
3	44.17	49.73	65.27	67.5	10.41	3.82	5.54	4.88
4	43.17	52.74	48.46	69.65	8.3	3.43	3.29	2.9
5	50.3	52.67	56.57	79.26	6.72	3.03	4.61	3.95
6	50.33	56.45	49.02	59.1	11.2	6.33	3.56	3.03
7	-	-	-	-	6.33	2.24	3.69	3.82
8	-	-	-	-	4.61	1.71	3.16	3.03
9	-	-	-	-	7.12	2.24	3.03	3.16
10	-	-	-	-	8.17	2.5	4.48	4.48
11	-	-	-	-	7.38	2.24	5.4	4.74
12	-	-	-	-	6.46	3.69	2.24	2.5
13		-	-	-	7.91	1.98	4.88	4.48
14	-	-	-	-	7.91	2.37	4.74	4.22
15	-	-	-	-	7.91	1.45	4.74	4.22
16	-	-	-	-	6.85	1.32	5.01	4.22
17	-	-	-	-	8.43	1.98	6.33	5.14
18	-	-	-	-	9.75	1.71	5.93	5.14

Raw data from Figure 6.

	Average normalized ion intensities			Average fraction of normalized ion counts	
	Cellulose	**S-lignin**	**G-lignin**	**Sugars**	**Lignin**
Control	1.66 ± 0.17	1.84 ± 0.10	1.78 ± 0.10	0.56 ± .05	0.44 ± 0.05
Fermented	0.84 ± 0.09	2.38 ± 0.09	1.98 ± 0.14	0.35 ± 0.02	0.65 ± 0.02

Raw data from Figure 7.

	Glucose equivalent (mg/g biomass	Xylose equivalent (mg/g biomass)

Control	95.53 ± 2.02	29.42 ± 1.17
Fermented	23.42 ± 1.1	8.59 ± 0.62