

Hemicelluloses negatively affect lignocellulose crystallinity for high biomass
digestibility under NaOH and H₂SO₄ pretreatments in *Miscanthus*

Supplementary

Table S1. Hexoses yield (% cellulose) released from enzymatic hydrolysis after pretreatment

Pair	Sample	NaOH						H ₂ SO ₄					
		0.5%		1%		4%		0.25%		1%		4%	
I	Mlu26 (H)	54.9±0.9	1.6 *	72.8±0.4	1.6	98.8±1.7	1.3	29.2±0.2	1.5	40.8±1	1.5	47.0±1.0	1.4
	Msi34 (L)	34.7±0.6		44.4±0.6		75.6±0.9		18.9±0.7		26.5±0.7		34.2±0.9	
II	Mfi03 (H)	37.1±1.3	1.4	48.6±0.8	1.4	80.6±0.6	1.3	22.9±0.6	2	37.9±0.4	2.1	41.9±0.6	1.7
	Mlu01(L)	26.3±1.5		34.8±0.2		60.5±1.6		11.2±0.7		18.2±0.8		22.5±0.8	
III	Mfi40 (H)	33±0.5	1.9	54.4±1.4	1.5	74.6±7.7	1.3	16.2±1.8	1.4	32.4±0.4	2.6	40.8±0.4	1.8
	Msa02 (L)	17.4±1.7		36.5±1.5		58.5±5.7		11.9±0.4		12.7±0.4		22.6±0.2	
IV	Msi56 (H)	48.8±3.1	1.4	57.9±0.7	1.1	84.1±6	1.1	27.6±1.1	1.5	43.1±0.9	1.5	51.9±0.3	1.4
	Mfi04 (L)	33.8±2.4		50.8±0.8		77.1±1.1		18.2±0.9		29.1±1.5		38.4±0.6	
V	Msa20 (H)	47.3±2.9	2.0	64±1.2	1.8	90.9±1.4	1.2	27.2±1.2	2.7	35.1±1.4	1.2	51.0±0.5	1.6
	Mfi08 (L)	24.2±2.1		35.3±0.4		76.3±1.5		10.3±0.5		28.4±0.9		31.9±1.9	
VI	Mfi27 (H)	46.3±1.5	2.1	65.6±3.4	2.2	96.6±3.4	1.8	20.8±1.0	1.8	42.1±0.9	2.0	49.2±1.2	2.1
	Mlu12 (L)	21.8±0.8		30.1±1.5		54.0±1.3		11.3±0.4		21.2±0.8		23.7±0.6	

* Ratio of two sample values at pair

Table S2. Total sugar yield (% dry matter) released from both enzymatic hydrolysis and pretreatment

Pair	Sample	NaOH						H ₂ SO ₄					
		0.5%		1%		4%		0.25%		1%		4%	
I	Mlu26 (H)	30.9±0.2	1.8*	43.7±0.4	1.7	59.0±0.9	1.3	29.3±0.4	1.3	40.2±0.4	1.4	44.1±0.7	1.1
	Msi34 (L)	17.1±0.5		26.5±0.6		46.6±0.6		23.3±0.1		29.7±0.4		38.7±0.6	
II	Mfl03 (H)	19.3±1.4	1.2	28.6±0.9	1.2	49.9±0.1	1.2	26.9±0.2	1.4	37.5±1.2	1.4	40.1±0.4	1.2
	Mlu01 (L)	16.6±0.1		23.9±0.2		42.3±0.8		18.6±0.3		27.0±0.3		34.8±0	
III	Mfl40 (H)	21.8±0.3	2	38.1±0.2	1.6	50.6±2.4	1.3	26.9±0.8	1.5	36.6±0.4	1.5	39.3±0.8	1.2
	Msa02 (L)	11.1±1		24±0.7		39.2±2.2		17.4±0.1		24.7±0.3		33.9±1.3	
IV	Msi56 (H)	36.0±2.5	2.3	47.5±0.5	1.8	63.7±2.5	1.4	33.5±0.8	1.5	46.1±0.7	1.4	52.7±0.7	1.3
	Mfl04 (L)	15.5±0.6		26.8±0.2		45.1±0.9		22±0.2		32.5±0.9		39.2±0.4	
V	Msa20 (H)	24.4±1.1	1.4	37.7±0.9	1.3	52.8±0.5	1.1	28.2±0.5	1.2	37.3±0.4	1.3	44.2±1.6	1.2
	Mfl08 (L)	17.5±0.5		28.5±0.5		49.9±0.3		23±0.8		28.1±0.6		38.4±1.3	
VI	Mfl27 (H)	23.9±1.0	1.5	36.5±1.1	1.4	54.4±1.0	1.2	31.6±0.4	1.5	42.3±1.3	1.5	45.2±0.6	1.1
	Mlu12 (L)	16.5±0.5		26.3±1.1		44.0±0.7		21.0±0.1		29.2±0.6		39.6±0.4	

* Ratio of two sample values at pair

Table S3. Monosaccharide composition of hemicelluloses (% of total)

Pair	Sample	Rha	Fuc	Ara	Xyl	Man	Glu	Gal
IV	Msi56 (H)	0.29% *	ND	13.08%	83.45%	0.20%	1.10%	1.86%
	Mfl04 (L)	0.28%	ND	12.06%	82.34%	0.13%	3.83%	1.36%

* Percentage of total monosaccharides; ND, non-detectable.

Table S4. Monosaccharide composition of hemicelluloses (% of total)

Pair	Sample	Rha	Fuc	Ara	Xyl	Man	Glu	Gal
V	Msa20 (H)	0.24% *	ND	12.06%	83.68%	0.59%	1.69%	1.74%
	Mfi08 (L)	0.19%	ND	10.88%	85.17%	0.14%	2.66%	0.95%

* Percentage of total monosaccharides; ND, non-detectable.

Table S5. Monosaccharide composition of hemicelluloses (% of total)

Pair	Sample	Rha	Fuc	Ara	Xyl	Man	Glu	Gal
VI	Mfl27 (H)	0.26% *	ND	12.68%	80.53%	0.14%	4.95%	1.43%
	Mlu12 (L)	0.17%	0.01%	9.88%	87.45%	0.20%	0.99%	1.31%

* Percentage of total monosaccharides; ND, non-detectable.

Table S6. Monomer composition of lignin (% of total)

Pair	Sample	H	G	S	S/G	H/G	S/H
VI	Mfi27 (H)	27.79%*	49.02%	23.19%	0.47	0.57	0.83
	Mlu12 (L)	21.41%	45.11%	33.48%	0.74	0.47	1.56

* Percentage of total monomers

Table S7. Linked Phenols of Mfl27 and Mlu12 ($\mu\text{mol/g}$ dry matter)

Linkage	Sample	H-	G-	S-	AV-	AS-	PCA-	FA-	Total
Ester-	Mfl27 (H)	0.13 (2.59%)*	0.26 (5.12%)	0.09 (1.76%)	ND	ND (0%)	1 (19.31%)	3.69 (71.22%)	5.17
	Mlu12 (L)	0.5 (6.77%)	0.99 (13.34%)	0.42 (5.63%)	ND	0.03 (0.36%)	1.48 (20.1%)	3.97 (53.81%)	7.38
Ether-	Mfl27 (H)	2.86 (3.75%)	11.29 (14.78%)	9.21 (12.05%)	6 (0.78%)	13.77 (18.02%)	7.56 (9.89%)	25.73 (33.67%)	76.41
	Mlu12 (L)	3.35 (3.48%)	19.46 (20.16%)	29.75 (30.83%)	6.0 (0.63%)	12.59 (13.05%)	5.91 (6.13%)	19.39 (20.1%)	96.49

H-: *p*-Hydroxybenzaldehyde, G-: Vanillin, S-: Syringaldehyde, AV-: Acetovanillone, AS-: Acetosyringone, PCA-: *p*-Coumaric acid, FA-: Ferulic acid, SA-: Sinapic acid;

* percentage of total linked-phenols.

Table S8. Correlation coefficient between cellulose CrI and total sugar yield released from both enzymatic hydrolysis and pretreatment

	NaOH			H ₂ SO ₄		
	0.50%	1%	4%	0.25%	1%	4%
Raw material CrI	-.750**	-.748**	-.838**	-.875**	-.839**	-.812**

** significant differences at $p < 0.01$.

Table S9. Correlation coefficient between cellulose CrI and hexoses yield released from enzymatic hydrolysis after pretreatment.

	NaOH			H ₂ SO ₄		
	0.50%	1%	4%	0.25%	1%	4%
Raw material CrI	-0.735**	-0.685*	-0.763**	-0.580*	-0.891**	-0.777**

* and ** significant differences at $p < 0.05$ & 0.01 , respectively.