

Supplementary data for

Easily measurable pH as an indicator of aqueous cholinium
ionic liquid pretreatment effectiveness of lignocellulose

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X-ray diffraction (XRD) analysis

The samples were scanned on a D8 ADVANCE diffractometer (Brüker, German) from $2\theta = 5-60^\circ$ with a scan speed of $0.07^\circ \text{ min}^{-1}$ and a step size of 0.04° at 40 kV, 40 mA. The peak height method was also used: $\text{CrI} = (I_{002} - I_{\text{am}}) / I_{002} * 100$, in which I_{002} is the intensity of the crystalline portion of biomass (cellulose) at $2\theta \approx 22.5^\circ$, and I_{am} is the peak intensity of the amorphous portion at $2\theta \approx 18.2^\circ$.

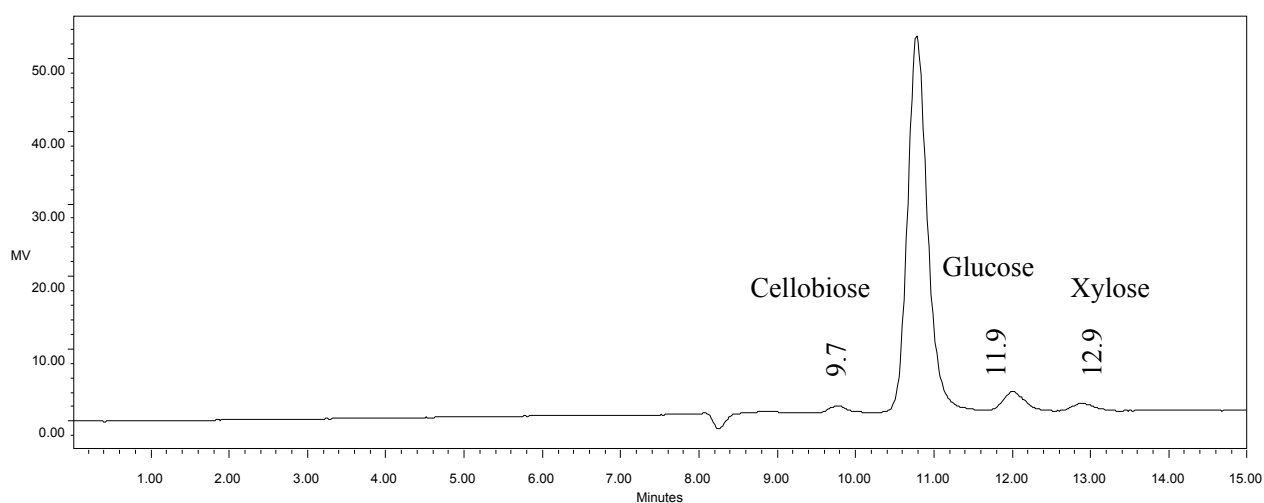


Figure S1 HPLC spectrum of the reaction mixture in the enzymatic hydrolysis of rice straw

Table S1 Effect of 50% IL aqueous solution pretreatment on the composition and enzymatic hydrolysis of rice straw

ILs	Pretreatment ^a			Composition of residues (%) ^b				Enzymatic hydrolysis of residues ^c	
	pH	Lignin extracted (%)	Residues recovery (%)	Cellulose	Xylan	AIL	ASL	Sugar yield (%)	
								Glucose	Xylose
Untreated	–	0	100	36.9	18.4	16.7	2.1	20.0	11.5
[Ch][For]	7.71	20.5	77.7	43.0	19.6	16.9	1.6	38.2	18.2
[Ch][AcO]	8.43	33.4	71.8	45.4	20.6	15.2	1.7	37.6	17.6
[Ch][But]	8.62	33.5	68.0	47.4	21.4	15.3	1.5	36.3	17.8
[Ch][Piv]	8.65	45.4	66.0	51.1	22.3	12.4	1.4	49.0	19.2
[Ch][Hex]	8.56	40.0	67.5	47.4	20.1	12.7	1.2	41.8	16.1
[Ch][iOct]	8.59	49.8	66.8	48.8	22.7	12.2	1.1	52.3	40.4
[Ch][TFA]	7.58	31.8	74.2	43.8	19.6	15.1	1.4	32.6	9.6
[Ch][Nic]	7.85	29.5	77.4	39.9	19.9	13.0	1.4	30.9	15.4
[Ch][Bz]	7.57	24.4	78.5	39.8	18.5	14.6	1.4	27.8	13.3
[Ch][Glc]	7.45	10.6	78.0	40.4	21.4	16.5	1.7	29.8	16.5
[Ch][Lac]	6.10	13.3	77.6	39.8	21.7	16.6	1.4	22.8	12.6
[Ch][Oxa]	3.02	11.4	80.7	39.3	18.6	16.0	1.4	26.0	14.1
[Ch] ₂ [Oxa]	6.35	20.0	79.3	40.1	19.1	14.1	1.5	35.3	20.4
[Ch][Suc]	5.03	10.9	80.9	39.1	19.3	15.5	1.7	29.4	14.3
[Ch] ₂ [Suc]	7.72	21.2	76.7	41.5	19.4	14.3	1.7	34.2	18.6
[Ch][Mal]	4.50	11.0	79.3	38.8	18.4	16.5	1.4	22.9	11.8
[Ch] ₂ [Mal]	8.63	43.4	65.0	40.9	17.8	12.3	1.4	45.6	20.0
[Ch][Gly]	11.56	59.4	57.1	56.5	18.9	9.7	1.40	81.8	48.6
[Ch][Boc-Gly]	8.86	40.7	67.3	41.4	19.0	13.3	1.2	35.1	22.3
[Ch][Cbz-Gly]	10.03	50.0	63.2	44.1	21.5	12.8	1.2	47.0	33.2
[Ch][Ala]	11.70	60.1	57.3	56.1	21.9	10.1	1.22	81.0	43.8
[Ch][Ser]	10.76	52.6	61.6	54.9	18.1	13.6	1.22	83.8	49.0
[Ch][Thr]	10.98	53.9	63.9	51.3	20.5	12.1	1.22	80.9	48.5
[Ch][Val]	11.19	60.9	58.4	55.6	19.9	7.4	1.34	79.0	42.5
[Ch][Leu]	11.75	58.9	60.1	53.2	20.6	9.9	1.18	81.4	48.4
[Ch][Ile]	11.36	38.4	61.7	49.6	17.3	15.4	1.16	77.2	46.7
[Ch][Met]	11.33	61.2	62.4	57.1	16.2	8.5	1.34	83.5	44.3
[Ch][Pro]	11.60	62.2	56.6	56.3	21.0	11.5	1.27	79.5	43.6
[Ch][Phe]	11.08	56.6	70.2	48.6	19.8	11.9	1.06	81.7	47.5
[Ch][Gln]	11.18	53.8	63.4	45.9	20.1	12.0	1.31	68.8	39.1
[Ch][Asp]	7.43	12.2	95.4	40.3	23.0	16.0	1.41	33.9	10.4

[Ch] ₂ [Asp]	11.06	68.9	52.9	48.4	19.8	8.8	0.8	56.4	37.1
[Ch][Glu]	7.80	13.2	91.7	39.5	20.1	15.7	1.35	31.9	9.6
[Ch][Arg]	11.46	76.5	42.7	69.0	17.4	5.4	1.15	75.1	27.8
[Ch][Lys]	11.88	58.9	56.8	61.8	21.6	11.3	1.21	86.2	47.8
[Ch][His]	11.30	52.8	57.7	55.4	18.6	11.7	1.44	70.3	37.9
[Ch][AcO]-[Ch][Gly] (1:1, w/w)	10.95	66.1	52.6	50.8	20.0	8.9	0.7	63.9	44.4
[Ch][iOct]-[Ch][Gly] (1:1, w/w)	10.90	73.8	50.3	54.4	20.7	7.9	0.7	69.5	47.4
[Ch][Bz]-[Ch][Gly] (1:1, w/w)	10.84	73.1	51.6	52.9	20.3	8.7	0.7	69.0	49.8

^a 300 mg Samples of rice straw were incubated in 6 g of aqueous IL solution under N₂ with stirring at 90°C for 6 h.

^b Determined via the NREL protocol (LAP version 2008). Results are expressed as a percentage of the residues. AIL, acid-insoluble lignin; ASL, acid-soluble lignin.

^c Reaction conditions: 20 mg recovered rice straw, 7 mL citrate buffer (50 mM, pH 4.8), 35 U mL⁻¹ cellulase from *Trichoderma reesei*, 50°C, 200 rpm.

Abbreviations: formate (For); acetate (AcO); butyrate (But); pivalate (Piv); hexanoate (Hex); i-octanoate (iOct); trifluoroacetic acid (TFA); nicotinic acid (Nic); benzoate (Bz); glycolate (Glc); lactate (Lac); oxalate (Oxa); succinate (Suc); malate (Mal); glycinate (Gly); N-Boc-glycinate (Boc-Gly); N-Cbz-glycinate (Cbz-Gly); alaninate (Ala); serine (Ser); threoninate (Thr); valinate (Val); leucinate (Leu); isoleucinate (Ile); methioninate (Met); proline (Pro); phenylalaninate (Phe); glutaminate (Gln); aspartate (Asp); glutamate (Glu); arginate (Arg); lysinate (Lys); histidinate (His)

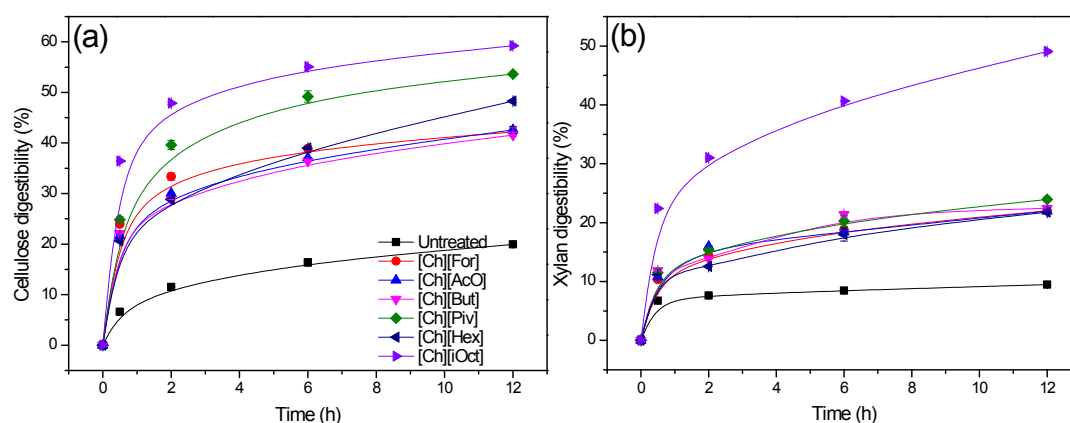


Figure S2 Time courses of enzymatic hydrolysis of rice straw before and after 50% IL aqueous solution pretreatment: (a) cellulose; (b) xylan

Reaction conditions: 20 mg recovered rice straw, 7 mL citrate buffer (50 mM, pH 4.8), 35 U mL⁻¹ cellulase from *Trichoderma reesei*, 50°C, 200 rpm.

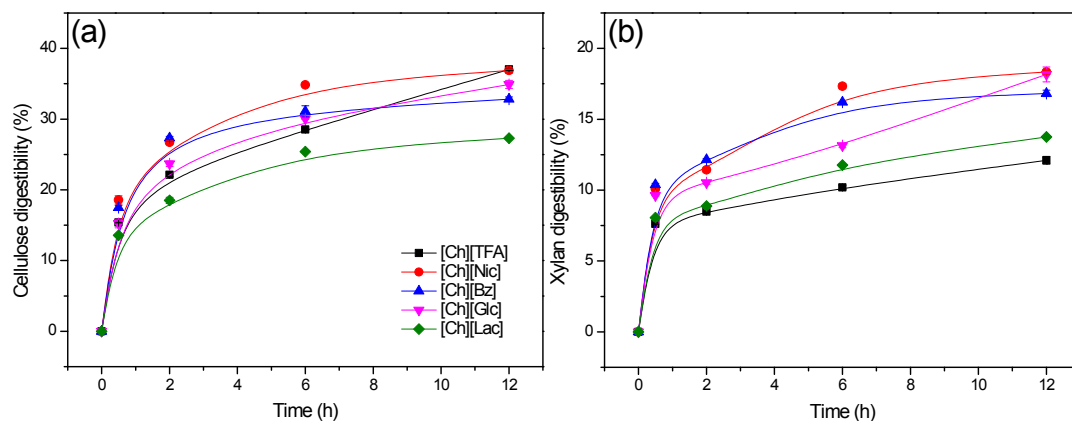


Figure S3 Time courses of enzymatic hydrolysis of rice straw after 50% IL aqueous solution pretreatment: (a) cellulose; (b) xylan

Reaction conditions: 20 mg recovered rice straw, 7 mL citrate buffer (50 mM, pH 4.8), 35 U mL⁻¹ cellulase from *Trichoderma reesei*, 50°C, 200 rpm.

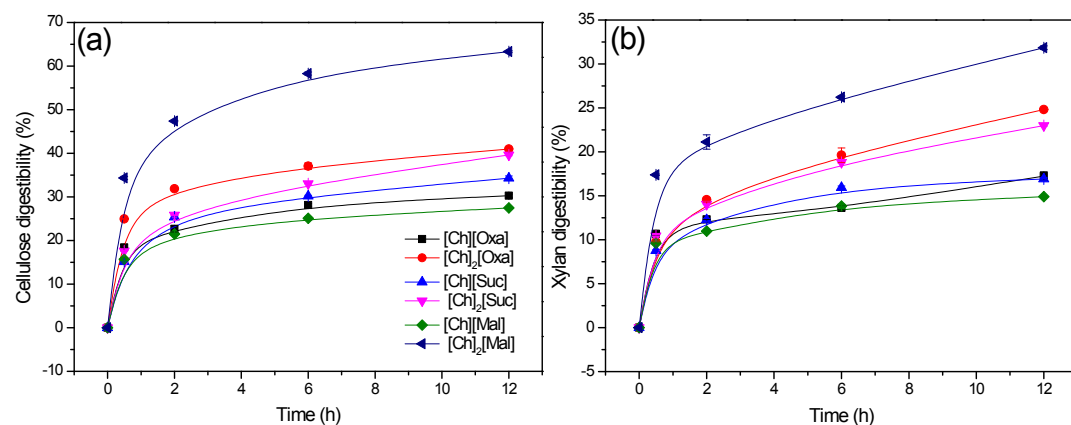


Figure S4 Time courses of enzymatic hydrolysis of rice straw after 50% IL aqueous solution pretreatment: (a) cellulose; (b) xylan

Reaction conditions: 20 mg recovered rice straw, 7 mL citrate buffer (50 mM, pH 4.8), 35 U mL⁻¹ cellulase from *Trichoderma reesei*, 50°C, 200 rpm.

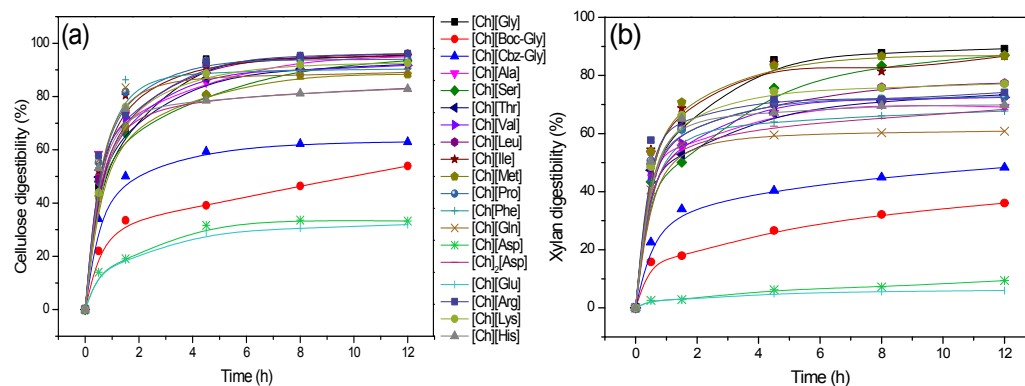


Figure S5 Time courses of enzymatic hydrolysis of rice straw after 50% IL aqueous solution pretreatment: (a) cellulose; (b) xylan

Reaction conditions: 20 mg recovered rice straw, 7 mL citrate buffer (50 mM, pH 4.8), 35 U mL⁻¹

cellulase from *Trichoderma reesei*, 50°C, 200 rpm.

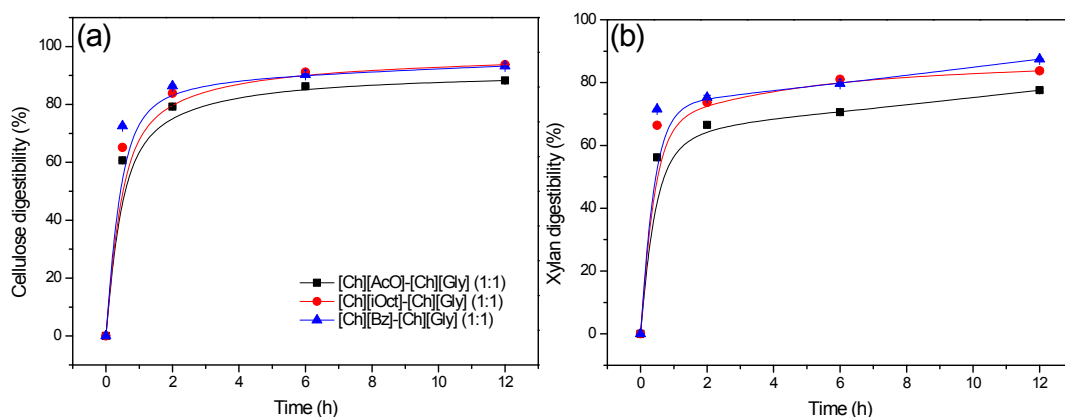


Figure S6 Time courses of enzymatic hydrolysis of rice straw after 50% IL aqueous solution pretreatment: (a) cellulose; (b) xylan

Reaction conditions: 20 mg recovered rice straw, 7 mL citrate buffer (50 mM, pH 4.8), 35 U mL⁻¹ cellulase from *Trichoderma reesei*, 50°C, 200 rpm.

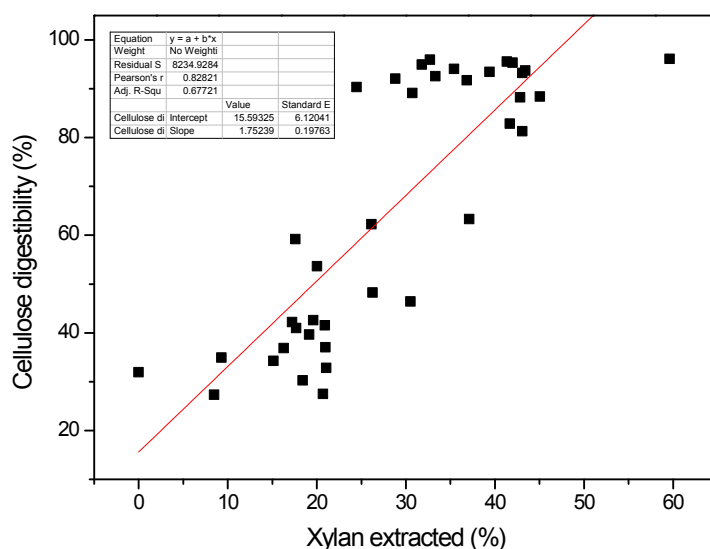


Figure S7 Correlation between xylan extracted and cellulose digestibility

The xylan extracted was calculated on the base of the changes in xylan content before and after

$$\text{pretreatment: Xylan extracted (\%)} = \left(1 - \frac{\text{Residues recovery} \times \text{xylan content in residues}}{\text{Native xylan content}}\right) \times 100$$

Table S2 Cellulose CrI of untreated and neat IL-treated rice straw

ILs	Cellulose CrI (%) ^a
Untreated	59.6
[Ch][For]	66.4
[Ch][AcO]	72.3
[Ch][But]	76.6
[Ch][Piv]	80.0
[Ch][Hex]	72.6
[Ch][iOct]	69.3

[Ch][TFA]	65.9
[Ch][Nic]	65.0
[Ch][Bz]	71.6
[Ch][Glc]	71.5
[Ch][Lac]	64.7
[Ch][Oxa]	70.5
[Ch] ₂ [Oxa]	66.5
[Ch][Mal]	65.7
[Ch] ₂ [Mal]	65.2
[Ch][suc]	70.6
[Ch] ₂ [suc]	66.4
[Ch][Gly]	80.7
[Ch][Boc-Gly]	68.5
[Ch][Cbz-Gly]	69.1
[Ch][Ala]	77.2
[Ch][Asp]	62.1
[Ch] ₂ [Asp]	73.1

^a Crystallinity index (CrI) was calculated by the peak height method: $CrI = [(I_{002} - I_{am}) / I_{002}] * 100$