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Rapid mechanoenzymatic saccharification of lignocellulosic biomass without bulk water or chemical pre-treatment

Supplementary Information

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1) Experimental section

Material and general methods

Wheat straw (WS) and sugarcane bagasse (SB) were kindly provided by Iogen Corporation (Ottawa, Canada), and corn stover (CS) was from POET (Sioux Falls, SD, USA). Cellulases from *Trichoderma longibrachiatum* (G4423), xylanase from *Thermomyces lanuginosus*, and the CTec2 preparation (SAE0020) were purchased from Sigma-Aldrich. 3,5-Dinitrosalicylic acid, potassium sodium tartrate tetrahydrate, and sodium azide were purchased from Millipore Sigma (Oakville, ON, Canada). Microcrystalline cellulose (MCC) was obtained from Sigma Aldrich (Saint-Louis). Water was from a MilliQ system with a specific resistance of 18.2 MΩcm at 25˚C. Mechanochemical reactions were performed in SmartSnap stainless steel jars (15 or 30 mL) from FormTech Scientific, containing stainless steel balls (7 or 15 mm in diameter) set up on a FTS1000 shaker mill from FormTech Scientific. Aging was performed in an IsoTemp vented oven from Fischer Scientific set at 55°C.
All experiments were performed at least in triplicates, and presented as the average with the standard deviation.

Biomass preparation and handling

As measured using the standard NREL protocol,[1] WS and SB had water contents of 6.1% and 6.4% w/w respectively and were used as is. CS was noticeably more humid and partially mouldy, with a water content of roughly 30%. To increase reproducibility, the CS sample was dried at 75°C in a vented oven for several days to a water content of 1.8% w/w, and stored at r.t. None of the substrates were washed before use.

Reaction analysis and yield determination

Reaction yields were first estimated using the classical dinitrosalicylic acid (DNS) method.[2] DNS reacts with the reducing end of sugars and does not discriminate between them. Since both enzyme cocktails possess xylanase activity, unless otherwise noted, the yields presented in this study are for hydrolysis of holocellulose i.e. cellulose and hemicellulose combined. Detailed sugar analysis was performed on key samples to determine specific glucose concentrations and yields.
The DNS reagent solution was prepared by mixing 3,5-dinitrosalicylic acid (1 g) in deionized water (50 mL), before addition of sodium potassium tartrate tetrahydrate in small portions (total 30 g). An aqueous solution of sodium hydroxide (20 mL of 2 M) was next added. The mixture turned from transparent to an intense yellow color. The volume was adjusted to 100 mL with water and the solution was filtered through cotton. This reagent solution was stored at 4°C in an inactinic container for up to one month.
The frozen reaction mixture aliquots to be analyzed were thawed on ice. Ice cold water was added to obtain a 10 mg/mL suspension of initial biomass weight. The samples were incubated for 30 min at 100°C to inactivate the enzymes. The samples were returned to room temperature and aggregates were broken down using a spatula. The suspensions were then centrifuged for 5 min at 21,100 × *g* and the supernantant was kept for analysis.
The standard procedure for the DNS assay[2] was followed with a few modifications. In a 1.5 mL microtube, the supernatant of the reaction mixture (50 µL) was diluted in deionized water (150 µL) and the DNS reagent solution (100 µL) was added, before vortexing for 2 seconds, and incubating for 5 minutes at 100°C. After cooling down to room temperature, a portion of the sample (200 µL) was introduced in the well of a 96-well microtiter plate. Absorption at 540 nm was measured using a SpectraMax i3x from Molecular Device with PathCheck enabled. For each new DNS batches, a calibration curve was plotted from analysis of freshly prepared glucose solutions of known concentrations. The reagent was found to be stable for several weeks. The amount of carbohydrates present in the commercial enzymatic mixture was measured and subtracted from the results.
Due to the polydispersity of cellulose, the heteropolymeric nature of hemicellulose, and the presence of both cellulases and hemicellulases in the enzyme mixtures used, yields were calculated on a dry mass basis by approximating cellulose and hemicellulose to linear infinite chains of linked glucose or xylose, respectively.

Small scale (15 mL jars) pre-milling of biomass

The biomass substrate (400 mg) was introduced in a 15 mL stainless steel jar containing two 7 mm stainless steel balls and milled at 30 Hz for the desired duration. The resulting powder was either used directly or stored in a closed glass vial under ambient conditions.

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[1] A. Sluiter, B. Hames, D. Hyman, C. Payne, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, J. Wolfe *NREL Technical report* NREL/TP-510-42621 "Biomass and Total Dissolved Solids in Liquid Process Samples", **2008**.
[2] T. K. Ghose, *Pure Appl. Chem.* **1987**, *59*, 257–268.

Large scale (30 mL jars) pre-milling of biomass

The biomass substrate (1.5 g) was introduced in a 30 mL stainless steel jar containing one 15 mm stainless steel ball and milled for 1 min at 30 Hz. Additional substrate was added if needed to reach the desired final mass (up to 3 g) and with further milling for the desired time (5 to 90 min).
The resulting powder was recovered and stored at room temperature in a closed glass vial.

Enzymatic reactions under milling and aging conditions

Unless otherwise noted, all milling reactions were performed at a 400 mg (small) scale in a 15 mL stainless steel jar containing two 7 mm stainless steel balls. The raw or pre-milled biomass was combined with the *T. longibrachiatum* solid enzyme preparation and the desired amount of water, or with a solution of CTec2 diluted in the appropriate volume of water so that no additional water needs to be added, with or without sodium azide (0.04% w/v). The reaction mixtures were milled at 30 Hz for the desired duration (5-60 min). Aliquots (10-20 mg) were collected, weighed precisely, and frozen until analysis. Aging was accomplished at 55°C for the desired duration (1 h to 3 days) in a closed container inside a vented oven. Aliquots (10-20 mg) were collected, weighed precisely, and frozen until analysis.

Enzymatic reactions under RAging conditions

All RAging reactions were performed at a 400 mg (small) scale in a 14 mL Teflon jar containing two 7 mm stainless steel balls. The biomass substrate (pre-milled or not) was combined with the *T. longibrachiatum* solid enzyme preparation and the desired amount of water, or with a solution of CTec2 diluted in the appropriate volume of water so that no additional water needs to be added, with or without sodium azide (0.04% w/v). The mixture was milled at 30 Hz for 5 min and transferred to a vented oven set at 55°C for 55 min with the jar kept closed. After this incubation period, the jar was taken out, the mixture milled again for 5 min and aged for 55 min. The jar was submitted to a total of 12 cycles. Aliquots (10-20 mg) were collected after 4, 8 and 12 h, weighed precisely, and frozen until analysis. In some cases, aging was continued uninterrupted for another 1 h to 3 days. Aliquots (10-20 mg) were collected, weighed precisely, and frozen until analysis.

Reactions with wet CS biomass for comparison

Corn stover (400 mg dried or 570 mg wet to account for 30% humidity) was introduced in a 15 mL stainless steel jar containing two 7 mm stainless steel balls and milled for 30 min at 30 Hz. The *T. longibrachiatum* enzyme preparation (final enzyme loading of 45 mg/g cellulose) was added followed by water (600 or 430 µL for dried or wet biomass respectively; no sodium azide was used for this experiment). The mixture was milled for 30 min at 30 Hz and aged for 3 days at 55°C. The samples were collected, treated, and analyzed as described above.

Xylanase addition

Corn stover (1.5 g) was pre-milled for 60 min following the protocol described above. The resulting powder (400 mg) was introduced in a 15 mL stainless steel jar containing two 7 mm stainless steel balls and combined with a xylanase preparation from *Thermomyces lanuginosus* (50 mg, 1.5 mg/g cellulose) and a freshly prepared CTec2 solution in water (final enzyme loading 45 mg/g cellulose). The mixture was milled for 30 min at 30 Hz and aged for 3 days at 55°C. The samples were collected, treated, and analyzed as described above.

Larger scale enzymatic reactions

The pre-milled biomass substrate (1.5 g) was introduced in a 30 mL stainless steel jars containing one 15 mm stainless steel ball. A freshly prepared CTec2 solution (2.25 mL) in aqueous sodium azide 0.04% w/v was added. The mixture was milled for 5 min at 30 Hz. An aliquot (10-20 mg) was collected, weighed precisely, and frozen at -20°C. The rest of the mixture was transferred to a glass vial and aged for 3 days at 55°C. An aliquot (10-20 mg) was collected, weighed precisely, and frozen until analysis. The remaining hydrolysate was stored at -20°C to be later used for bacterial growth and fermentation experiments.

Analysis of corn stover (CS) crystallinity after pre-milling

X-Ray diffraction (PXRD) patterns of pre-milled CS (1.5 or 3 g in a 30 mL stainless steel jar for 5-90 min) were collected at room temperature on a Bruker D8 Discovery instrument equipped with a LYNXEYE XE-T detector (1D mode), using nickel-filtered CuK_α (λ = 0.154056 Å) radiation.

Sugar analysis

The monosaccharides were analyzed in a sugar analyzer (YSI 2900, Yellow Spring Instruments). Crude reaction mixtures were vortexed for 10 seconds, and an aliquot (400 µL) was centrifuged for 5 min at 9400 × *g* before analysis. For the RAging reaction mixtures, a sample was collected (ca. 100 mg), extracted in distilled water (10 mL) by vortexing for 30 seconds. Next, an aliquot (1 mL) was centrifuged for 5 min at 9400 × *g*, and the supernatant was separated and analyzed. This analyzer uses glucose and xylose specific membranes, and calibrates itself automatically with standard glucose (2.5 g/L) and xylose (20 g/L) solutions. The pH is kept at 7.0 by using a mono and dibasic sodium phosphate buffer (YSI 2357, 0.1 M, pH 7.0)

Agar plates from biomass hydrolysates for the culture of *E. coli* and *S.* Typhimurium

Growth media were prepared according to a protocol by Causey *et al.*[3] and contained: 3.5 g of KH_2PO_4, 5.0 g of K_2HPO_4, 3.5 g of $(NH_4)_2HPO_4$, 0.25 g of $MgSO_4.7H_2O$, 15 mg of $CaCl_2.2H_2O$, 0.5 mg of thiamine chloride, and 1 mL of trace elements solution per liter. The trace elements solution was prepared in 0.1 M HCl and contained: 1.6 g of $FeCl_3$, 0.2 g of $CoCl_2.6H_2O$, 0.1 g of $CuCl_2$, 0.2 g of $ZnCl_2.4H_2O$, 0.2 g of $NaMoO_4$, and 0.05 g of H_3BO_3 per liter.

Hydrolysates from aging reactions (21 g containing roughly 4.3 g monosaccharides) from all three biomass substrates (CS, WS, SB) were thawed, suspended in growth medium (20 mL), and transferred to 40 mL centrifuge tubes. The suspensions were centrifuged at 20,000 × *g* for 5 min. The supernatant was collected. The pellet was further extracted by re-suspension in growth medium (20 mL), and breaking down the aggregates with a spatula. After centrifugation (5 min at 20,000 × *g*), both supernatants were combined. The total volume was adjusted to 140 mL with growth medium to afford ca. 3% w/v monosaccharides. These concentrated sugar solutions were partitioned into 10 mL tubes and stored at -20°C until needed.

When needed, a sugar solution (10 mL) was thawed and combined with 90 mL of growth medium for a final concentration of 0.3% w/v monosaccharide. Agar (1.5 g) was added and the liquid was autoclaved, then cooled to approximately 50-60°C, before being poured into petri dishes, and allowed to cool to r.t.

Frozen stocks of bacterial strains (*E. coli* ATCC 25922 or *Salmonella enterica* Typhimurium ATCC 14028) were thawed on ice, streaked on the agar plates, and the plates were incubated overnight at 37°C.

Biomass hydrolysates for liquid cultures of *Paraburkholderia sacchari*

Paraburkholderia sacchari IPT 101 (DSM 17165) was purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ, Germany). The strain was resuscitated in Reasoner's 2A (R2A)[4] medium (0.50 g/L yeast extract, 0.50 g/L proteose peptone (Difco no. 3), 0.50 g/L casamino acids, 0.50 g/L glucose, 0.50 g/L soluble starch, 0.30 g/L Na-pyruvate, 0.30 g/L K_2HPO_4, 0.50 g/L $MgSO_4.7H_2O$, final pH 7.2) as recommended by DSMZ and stored at –70°C.

The growth medium composition was adapted from a previous study.[5] In brief, the nitrogen-limited medium (before carbon source addition) was composed of 1 g/L $(NH_4)_2SO_4$, 6.78 g/L $Na_2HPO_4.7H_2O$, 1.5 g/L KH_2PO_4, 1 g/L yeast extract, 0.2 g/L $MgSO_4$, and 1 mL/L of trace elements solution (see agar plate section above).[6] The pH was adjusted to 6.8. The $MgSO_4$ stock solution (X100) was autoclaved and the trace elements stock solution (X1,000) was sterilized by filtration (0.22 µm). The remaining components were sterilized by autoclaving.

Carbon source stocks were prepared either from commercial glucose or diluted biomass hydrolysate. The glucose solution (80 g/L in growth medium) was prepared from pure glucose, purchased from Sigma Aldrich and sterilized by filtration (0.22 µm). The hydrolysate was suspended in culture medium to obtain 46.2 g/L glucose and 22.6 g/L xylose, and homogenized by vortexing. The supernatant was recovered after centrifugation (5 min, 4,700 x *g*), and sterilized by filtration (0.22 µm).

The bacterium from frozen stocks was first streaked on agar plates prepared from R2A medium before incubation at 30 C for 18 hours. One colony was used to inoculate the seeding medium (100 mL) in 250 mL shake flasks. The seeding medium had the same composition as the culture medium, except for a lower sugar concentration (10 g/L glucose and 5 g/L xylose). Following inoculation, the seeding medium was incubated at 30°C and 170 rpm for 18 hours to approximately 0.5 g/L cell dry mass (CDM). An aliquot of the seeding culture (1 mL) was transferred to the growth medium (15 mL) with the carbon source stocks (5 mL) or growth medium (5 mL, blank) and incubated at 30°C and 170 rpm for 24 hours.

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[3] T. B. Causey, S. Zhou, K. T. Shanmugam, L. O. Ingram, *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 825–32.

[4] https://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium830.pdf

[5] M. T. Cesário, R. S. Raposo, M. C. M. D.de Almeida, F. van Keulen, B. S. Ferreira, M. M. R. da Fonseca, *New Biotechnol.* **2014**, *31*, 104-113.

[6] B.S. Kim, S.C. Lee, S.Y. Lee, H.N. Chang, Y.K. Chang, S.I. Woo, *Biotechnol. Bioeng.* **1994**, *43*, 892-898.

Bacterial growth medium aliquots were taken and centrifuged for 5 min at 4,700 x *g*. The supernatant was filtered on 0.22 µm filter, and the filtrate was injected in the sugar analyzer.

Cell dry mass determination

The cell dry mass was determined gravimetrically. In brief, 1.5 mL of culture broth was centrifuged (15,900 x *g* rpm for 5 min). The pellet was washed twice with distilled water (2 × 1 mL) and frozen-dried until constant weight (ca. 24 hours).

2) Supplementary figures

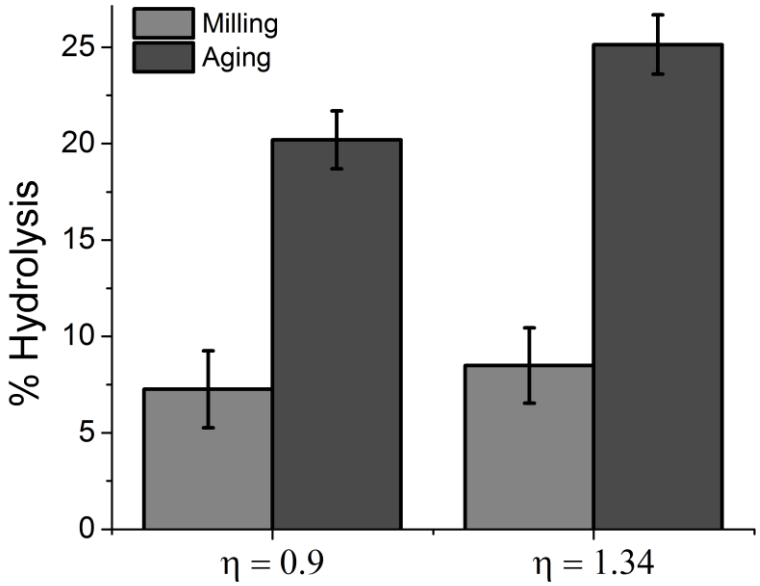

Figure S1: Effect of water loading on milling and aging reactions of WS (*η* in mg/mL. Pre-milled substrate (400 mg, 15 min at 30 Hz in a 15 mL s.s. jar with two 7 mm s.s. balls) was combined with *T. longibrachiatum* enzyme preparation (45.4 mg, 86 mg protein/g cellulose) and water (400 or 600 µL). The mixtures were milled at 30 Hz for 30 min and aged at 55°C for 3 days. Yield is DNS-based; error bars are standard deviation with n = 3.

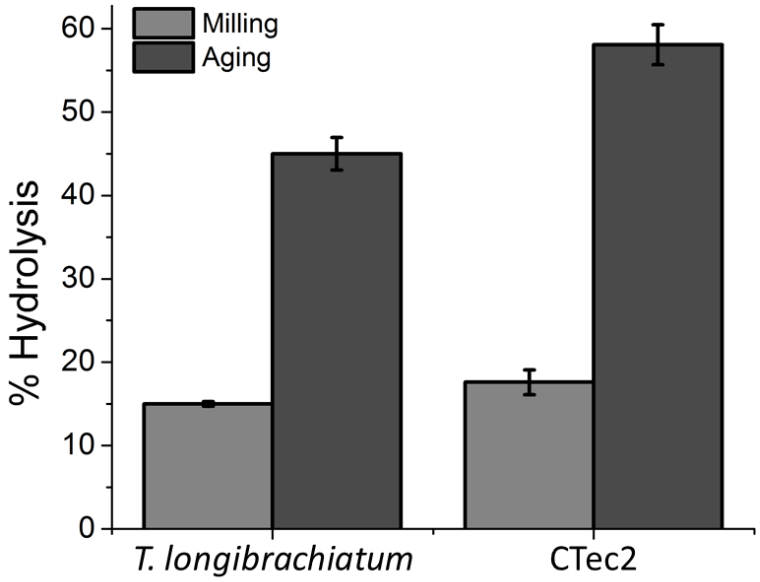

Figure S2: Mechano-enzymatic digestion of MCC (200 mg) at *η* = 1.0 µg/mL with *T. longibrachiatum* enzyme preparation (100 mg, 68 mg protein/g cellulose) or *CTec2* (57 µL, 45 mg protein/g cellulose). The mixtures were milled at 30 Hz for 30 min in a 15 mL s.s. jar with two 7 mm s.s. balls and aged at 55°C for 3 days. Yield is DNS-based; error bars are standard deviation with n = 3.

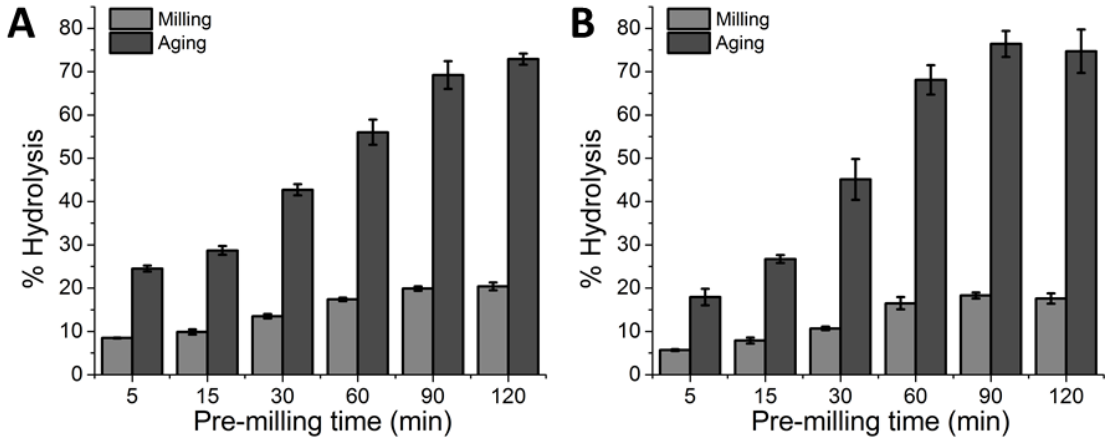

Figure S3: Influence of pre-milling time (3 g in 30 mL s.s. jar, 30 Hz) on the outcome of milling and aging reactions of WS (A) and SB (B). Substrate was combined with *CTec2* enzymes (45 mg/g cellulose) as a solution in water (600 µL, η = 1.5 µL/mg), milled 30 min at 30 Hz, aged 3 d at 55°C). Yield is DNS-based; error bars are standard deviation with n = 3.

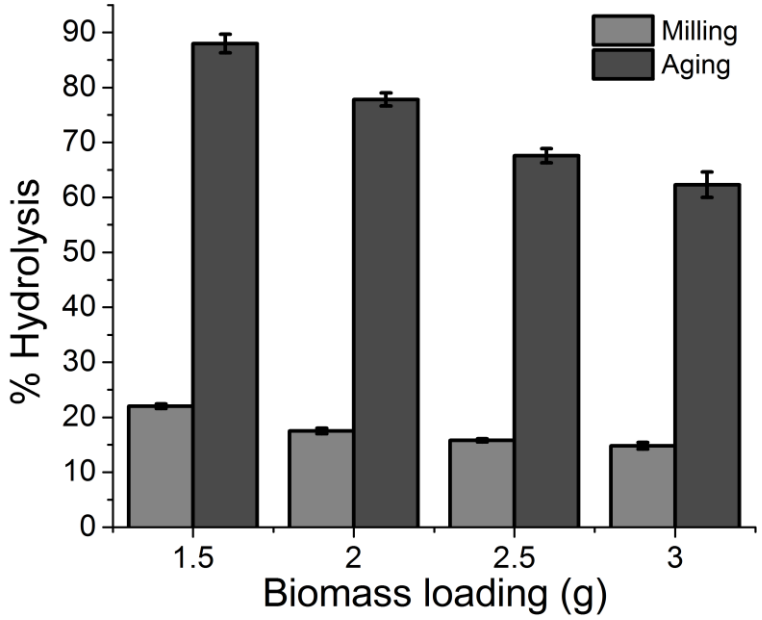

Figure S4: Influence of biomass loading on pre-milling efficacy of CS (60 min, 30 Hz) followed by milling and aging reactions with *CTec2* enzymes (45 mg/g cellulose) as a solution in water (600 µL, η = 1.5 µL/mg), milled 30 min at 30 Hz, aged 3 d at 55°C). Yield is DNS-based; error bars are standard deviation with n = 3.

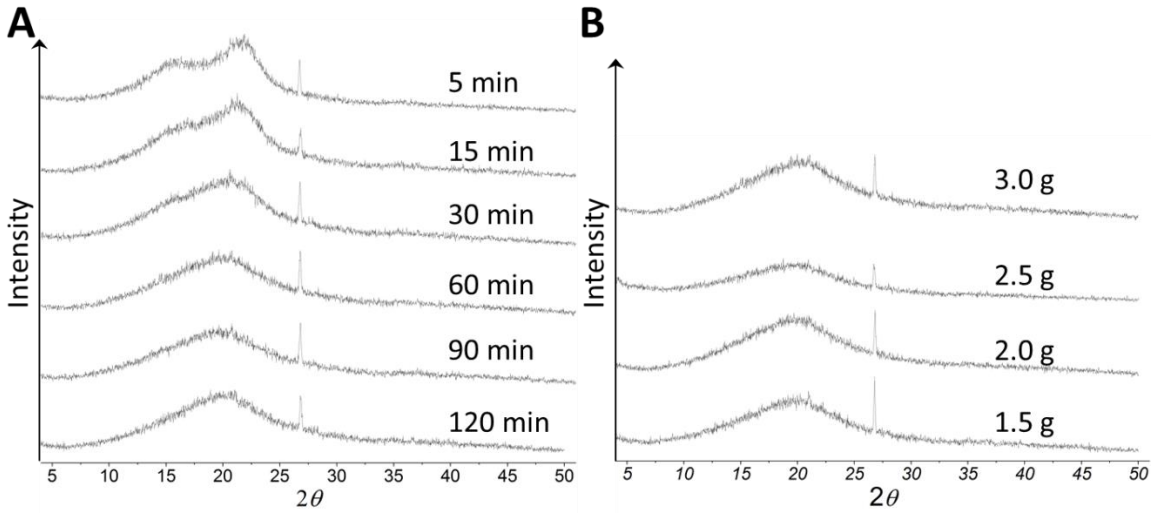

Figure S5: Comparison of PXRD patterns for CS after pre-milling. A) CS (3 g) pre-milled for various durations. B) CS pre-milled for 60 min at various loadings. Milling took place at 30 Hz in a 30 mL s.s. with one 15 mm s.s. ball. The crystalline cellulose peak at 22° disappears as ball-milling becomes longer and harsher. Peak at 27° is probably steel contamination.

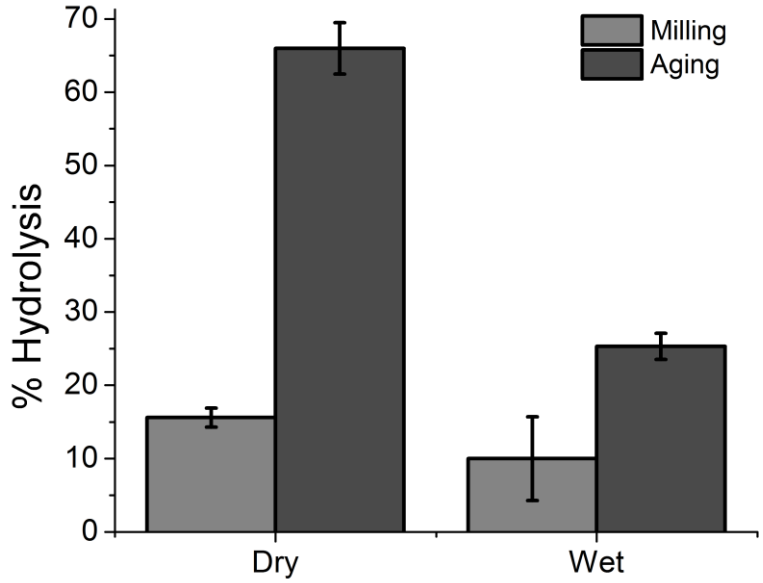

Figure S6: Influence of substrate humidity during pre-milling on the outcome of milling and aging reactions of CS. Substrate (400 mg dry i.e. 1.1% w/w water or 570 mg wet i.e. 30% w/w) was pre-milled for 30 min at 30 Hz in a 15 mL s.s. jar with two 7 mm s.s. balls. The resulting powder was then combined with *CTec2* enzymes (45 mg/g cellulose) as a solution in water (600 µL if dry or 430 µL if wet, η = 1.5 µL/mg), milled 30 min at 30 Hz, and aged 3 d at 55°C). Yield is DNS-based; error bars are standard deviation with n = 3.

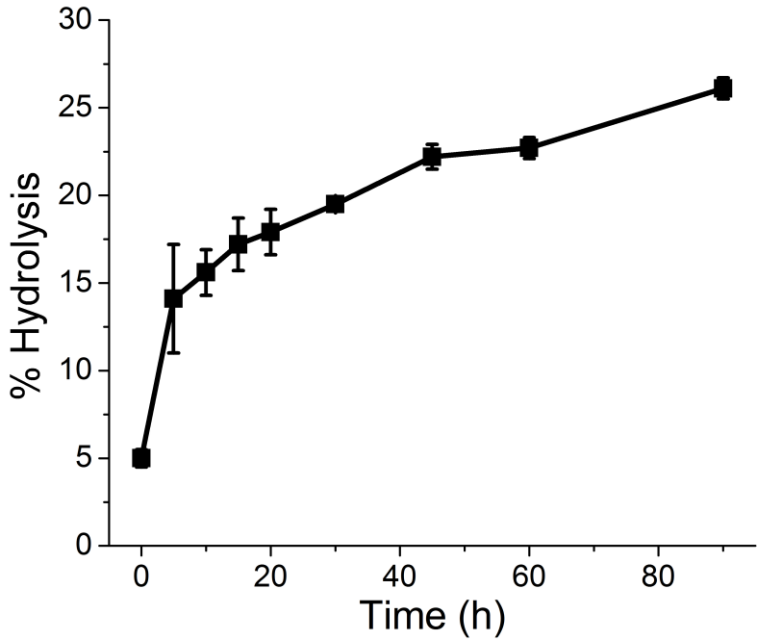

Figure S7: Kinetics of the hydrolysis reaction during the milling phase. CS (400 mg) was pre-milled (1.5 g, 60 min at 30 Hz in a 30 mL s.s. jar with one 15 mm s.s. ball) was combined with *CTec2* enzymes (45 mg/g cellulose) as a solution in water (600 µL, η = 1.5 µL/mg) and milled at 30 Hz for 90 min. Yield is DNS-based; error bars are standard deviation with n = 3.

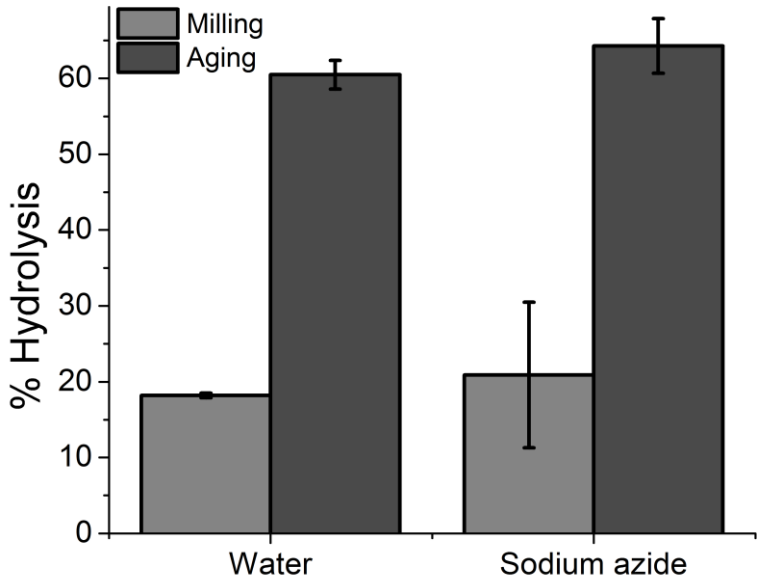

Figure S8: Influence of sodium azide on the outcome of milling and aging reactions. CS (400 mg) pre-milled (1.5 g, 60 min at 30 Hz in a 30 mL s.s. jar with one 15 mm s.s. ball) was combined with *CTec2* enzymes (45 mg/g cellulose) as a solution in water or NaN_3 0.04% w/v (600 µL, η = 1.5 µL/mg), milled 30 min at 30 Hz, and aged 3 d at 55°C). Yield is DNS-based; error bars are standard deviation with n = 3.

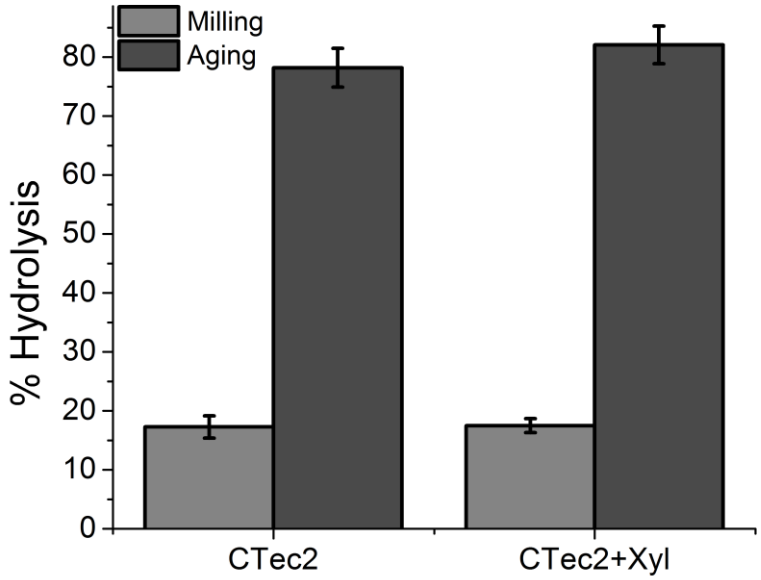

Figure S9: Influence of xylanase addition on the outcome of milling and aging reactions. CS (400 mg) pre-milled (1.5 g, 60 min at 30 Hz in a 30 mL s.s. jar with one 15 mm s.s. ball) was combined with *CTec2* enzymes (45 mg/g cellulose) as a solution in NaN_3 0.04% w/v (600 µL, η = 1.5 µL/mg) and 50 mg xylanase preparation from *Thermomyces lanuginosus* (1.5 mg/g cellulose), milled for 30 min at 30 Hz, and aged 3 d at 55°C. Yield is DNS-based; error bars are standard deviation with n = 3.

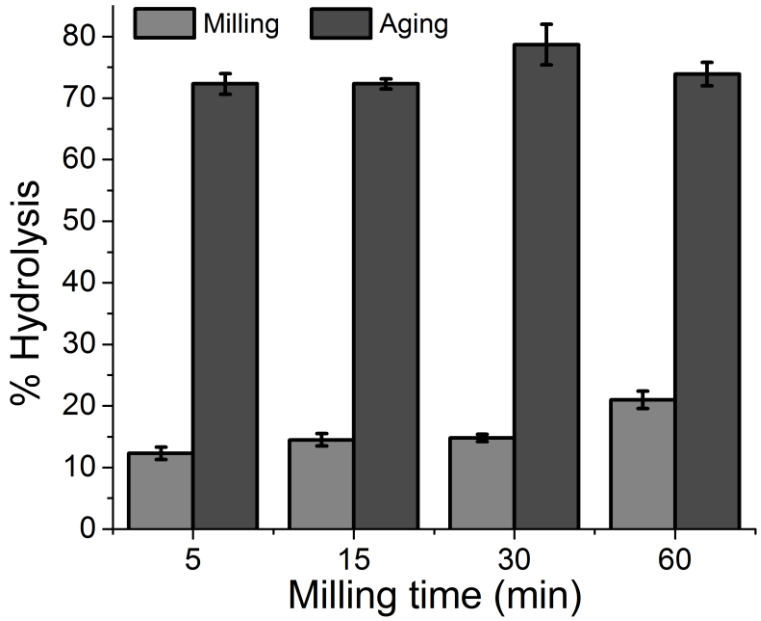

Figure S10: Influence of milling time on the outcome of milling and aging reactions. CS (400 mg) pre-milled (1.5 g, 60 min at 30 Hz in a 30 mL s.s. jar with one 15 mm s.s. ball) was combined with *CTec2* enzymes (45 mg/g cellulose) as a solution in NaN_3 0.04% w/v (600 µL, η = 1.5 µL/mg), milled at 30 Hz, and aged 3 d at 55°C). Yield is DNS-based; error bars are standard deviation with n = 3.

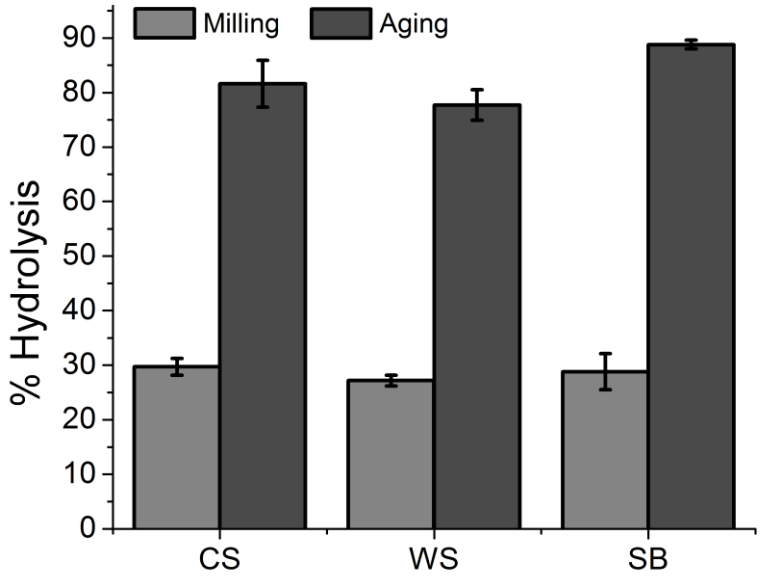

Figure S11: Scaling up of milling and aging reactions. Substrate (1.5 g) was pre-milled (60 min at 30 Hz in a 30 mL s.s. jar with one 15 mm s.s. ball) and combined with *CTec2* enzymes (45 mg/g cellulose) as a solution in NaN_3 0.04% w/v (2.25 mL, η = 1.5 µL/mg), milled for 5 min at 30 Hz, and aged 3 d at 55°C. Yield is DNS-based; error bars are standard deviation with n = 3.

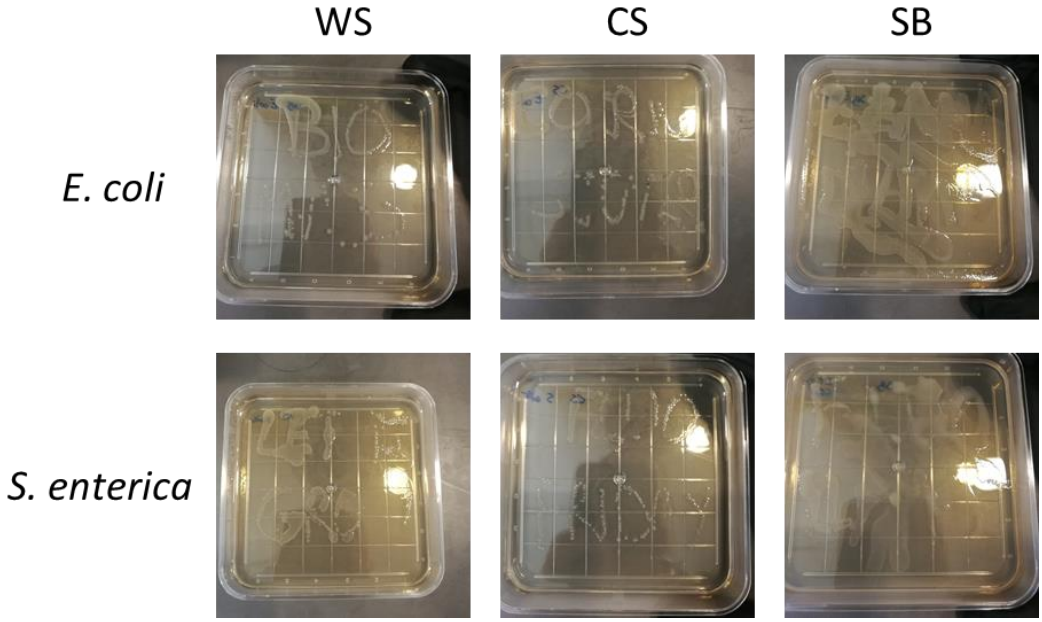

Figure S12: Bacterial (*E. coli* ATCC 25922 and *S. enterica* ATCC 14028) culture on media made from hydrolysates from CS, WS, and SB.

3) Supplementary tables

Table S1. Comparison of published processes for the digestion of cellulose in lignocellulosic biomass.

Ref.	Substrate	Pre-treatment	Cellulases	Solid loading (g/L)	Enzyme loading[a] (FPU/g glucan)	Time (h)	Cellulose Conversion (%)	[Glucose][b] (g/L)	Productivity[c] (mg glucose/FPU)	Efficacy[d] (mg glucose/FPU/h)	Space-time yield[e] (g glucose/L/h)
	Corn stover[f]	Shaker milling (60 min)	CTec1	667	35[h]	72	58	143	18.4	0.26	2.0
	Corn stover[g]	Shaker milling (60 min)	CTec2	667	35[h]	12	77	189	24.4	2.04	15.8
	Sugarcane Bagasse[g]	Shaker milling (60 min)	CTec2	667	35[h]	12	66	184	21.0	1.75	15.3
	Wheat Straw[g]	Shaker milling (60 min)	CTec2	667	35[h]	12	65	154	20.6	1.72	12.8
32	Corn crop residues	Bioextrusion	ACCELERASE DUET	400	58	48	11	22	2.1	0.04	0.5
32	Corn crop residues	Bioextrusion	ACCELERASE DUET	300	58	48	15	22	2.9	0.06	0.5
32	Corn crop residues	Dilute base, bioextrusion	ACCELERASE DUET	300	58	48	33	91	6.3	0.13	1.9
29	Wheat straw	Hydrothermal (195°C, 19 min)	CTec2	300	14	72	60	94	47.6	0.66	1.3
55	Corn stover	Steam explosion	Accelerase 1000	300	92	12	74	80	8.9	0.74	6.7
56	Agave bagasse	Blade milling, hydrothermal (194°C, 30 min)	CTec2	250	15	72	98	196	72.6	1.01	2.7
57	Rice straw	Dilute base, 24h	*T. reesei* + *A. niger* BGi	250	18	48	56	155	34.6	0.72	3.2
58	Corncob	Alkali liquor (12h, 70°C)	Cellulase A1 + Xylanase B1	200	40	120	41	57	11.4	0.09	0.5
59	Rice straw	Dilute sulfuric acid (162°C, 10 min)	SaccharISEB-6	200	9	24	64	125	79.0	3.29	5.2
60	Sugarcane Bagasse	Atmospheric glycerol organosolv (240°C, 30 min)	CTec2 + BGi + Endoxylanase + LPMOj AA9	200	3	72	83	105	307.4	4.27	1.5
61	Brewers spent grains	Dilute HCl (121°C, 30 min)	CTec2	150	10k	72	44	45	48.9	0.68	0.6
62	Sugarcane Bagasse	Dilute alkaline (120°C, 60 min)	*C cubensis*, *P. pinophilum*	120	34	120	51	40	16.7	0.14	0.3
63	Wheat Straw	Oxygen delignification (10% NaOH, 150°C, 60 min)	Celluclast 1.5L + Novozyme 188	50	40	24	81	25	22.5	0.94	1.0

a) reported as Filter Paper Unit (FPU) per gram of cellulose. b) Final glucose concentration measured *in situ*. c) defined as the mass of glucose produced per enzymatic activity unit. d) defined as the mass of glucose produced per enzymatic activity per hour. e) defined as the mass of glucose produced per volume unit per hour. f) Result from aging reactions. g) Result from RAging reactions. h) extrapolated from Cannella et al. who used the same commercial enzyme cocktail and reported both protein titer and FPU activity. i) BG: β-glucosidase. j) LPMO: Lipid polysaccharide monooxygenase k) reported as FPU per gram of substrate.

Table S2. Comparison of published processes for the digestion of holocellulose (cellulose and hemicellulose) in lignocellulosic biomass.

Ref.	Substrate	Pre-treatment	Cellulases	Solid loading (g/L)	Enzyme loading[a] (FPU/g glucan)	Time (h)	Holocellulose Conversion (%)	[RS][b] (g/L)	Productivity[c] (mg RS/FPU)	Efficacy[d] (mg RS/FPU/h)	Space-time yield[e] (g RS/L/h)
	Corn Stover[f]	Ball-milling (shaker, 60 min)	CTec2	667	16[h]	24	69	306	47.9	2.00	16.6
	Corn stover[g]	Ball-milling (shaker, 60 min)	CTec2	667	35[h]	12	83	377	26.3	2.20	31.4
	Sugarcane Bagasse[g]	Ball-milling (shaker, 60 min)	CTec2	667	35[h]	12	76	353	24.1	2.01	29.4
	Wheat Straw[g]	Ball-milling (shaker, 60 min)	CTec2	667	35[h]	12	70	290	22.2	1.85	24.2
32	Corn crop residues	Bioextrusion	ACCELERASE DUET	400	58	48	20	62	3.8	0.08	1.3
32	Corn crop residues	Bioextrusion	ACCELERASE DUET	300	58	48	25	58	4.8	0.10	1.2
32	Corn crop residues	Dilute base, bioextrusion	ACCELERASE DUET	300	58	48	42	97	8.0	0.17	2.0
57	Rice straw	Dilute base, 24h	*T. reesei* + *A. niger* BG[i]	250	18	48	73	203	45.1	0.94	4.2
58	Corncob	Alkali liquor (12h, 70°C)	Cellulase A1 + Xylanase B1	200	40	120	43	103	11.9	0.10	0.9
59	Rice straw	Dilute sulfuric acid (162°C, 10 min)	SaccharISEB-6	200	9	24	68	133	83.9	3.50	5.5
60	Sugarcane Bagasse	Ambient glycerol organosolv (240°C, 30 min)	CTec2 + BG[i] + Endoxylanase + LPMO[j] AA9	200	3	72	94	155	348.1	4.83	2.2
62	Sugarcane Bagasse	Dilute alkaline (120°C, 60 min)	*C. cubensis*, *P. pinophilum*	120	34	120	58	63	19.0	0.16	0.5
63	Wheat Straw	Oxygen delignification (10% NaOH, 150°C, 60 min)	Celluclast 1.5L + Novozyme 188	50	40	48	84	37	23.3	0.49	0.8

a) reported as Filter Paper Unit (FPU) per gram of cellulose. b) Final reducing sugar (RS) concentration measured *in situ*. c) defined as the mass of reducing sugar produced per enzymatic activity unit. d) defined as the mass of reducing sugar produced per enzymatic activity per hour. e) defined as the mass of reducing sugar produced per volume unit per hour. f) Result from aging reactions. g) Result from RAging reactions. h) extrapolated from Cannella et al. who used the same commercial enzyme cocktail and reported both protein titer and FPU activity. i) BG: β-glucosidase. j) LPMO: Lipid polysaccharide monooxygenase