Electronic supplementary information

Double bonus: surfactant-assisted biomass pelleting benefits both the pelleting process and subsequent enzymatic saccharification of the pretreated pellets.

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Experimental Section

Cellulose accessibility and crystallinity

Cellulose accessibility was studied by Simons' staining according to literature.[1, 2] Briefly, a set of 100 mg pretreated sample was mixed with phosphate-buffered saline solution and growing concentrations of Direct Orange (DO) dye in polypropylene centrifuge tubes. The tubes were then placed into a shaking incubator at 60 °C at a speed of 180 rpm overnight.[3] The samples were subsequently centrifuged, and the absorbance of the supernatant solution was measured at 450 nm by a spectrophotometer. The maximum amount of DO dye adsorbed by the sample was calculated using the Langmuir adsorption isotherm equation. Cellulose crystallinity was studied by X-ray diffraction (XRD). XRD measurements were performed using a D8 Advance (Bruker, Germany) equipped with a Cu tube and a Lynx detector, and operated in reflection geometry. Measurements were performed on forward packed samples prepared in the range 5 to 60 $^{o}2\theta$. The crystallinity index (CrI) was determined using the expression: $CrI = 100 \times (I_{002} - I_{AM})/I_{002}$;[4, 5] I_{002} is the intensity of the crystalline part of the sample at approximately $^{o}2\theta$ = 22.5; and I_{AM} is the amorphous part at approximately $^{o}2\theta$ = 16.6.

Lignin characterization: lignin molecular weight, 2D ^{1}H-^{13}C HSQC NMR, and ^{31}P NMR

The weight average molecular weight (Mw) and number average molecular weight (Mn) of lignin were measured with three replicates by gel permeation chromatography (GPC) based on a previous study.[6] Approximately 5 mg lignin sample was acetylated by 1 mL of pyridine/acetic anhydride (1:1, v/v) in dark with magnetic stirring at room temperature for 24 h. The solvent was evaporated by rotary evaporation at 45 °C with adding ethanol several times until dry. The acetylated lignin was dissolved in tetrahydrofuran (THF), and the solution was filtered through 0.45 μm membrane filter before GPC analysis. The molecular weight distributions were analyzed by a GPC SECurity 1200 system (Agilent Technologies, Inc., Santa Clara, CA) using THF as the mobile phase with a flow rate of 1.0 mL/min. Polystyrene narrow standards were used for establishing the calibration curve.

2D ^{1}H-^{13}C HSQC NMR experiments were performed on a Bruker Ascend™ 500 MHz spectrometer and spectral processing was carried out using software Bruker Topspin 3.6. A standard Bruker heteronuclear single quantum coherence pulse sequence (hsqcetgpspsi2.2) was used on a 5-mm N_2 cryogenically cooled Broadband Observe (BBO) H&F probe with the following acquisition parameters: spectra width 12 ppm in F2 (^{1}H) dimension with 1024 time of domain (acquisition time 85.2 ms), 220 ppm in F1 (^{13}C) dimension with 256 time of domain (acquisition time 4.6 ms), a 1.5 s pulse delay, a $^{1}J_{C\text{-}H}$ of 145 Hz, and 32 scans. About 30 mg lignin sample was dissolved in 0.5 mL deuterated dimethyl sulfoxide (DMSO-*d6*). Chemical shifts calibration was carried out by the central DMSO solvent peak (δC/δH at 39.5/2.49). The assignments

for cross-signals in side-chain and aromatic regions were performed according to previous studies.[7-11] The quantification of each lignin linkage was according to the volume-integration of cross-peak contours in HSQC spectra and the internal standards were selected according to a previous study.[12] Briefly, G_2 and $S_{2,6}/2+G_2+H_{2,6}/2$ signals were set as standards for pine (softwood lignin) and wheat straw (grass lignin).[12] However, in pine samples, the G_2 signal shifts if G_6 or G_5 sites undergo chemical modification during pretreatment, forming a $G_{2,cond}$ peak, hence the sum of G_2 and $G_{2,cond}$ is used as a reference integral.[9]

To measure the content of lignin hydroxyl groups, a lignin sample (~25 mg) was dissolved in a pyridine/$CDCl_3$ (1.5/1.0, v/v) solution and derivatized with TMDP (75 μL) for acquiring ^{31}P NMR spectrum. Chromium acetylacetonate and endo-N-hydroxy-5-norbornene-2,3-dicarboximide (NHND) were also added into the solution as relaxation agent and internal standard, respectively. Quantitative ^{31}P NMR spectra were acquired by an inverse-gated decoupling (Waltz-16) pulse sequence with a 25 second pulse delay and 128 scans. ^{31}P NMR measurements were performed without replicates in agreement with common practice.

Figures and tables

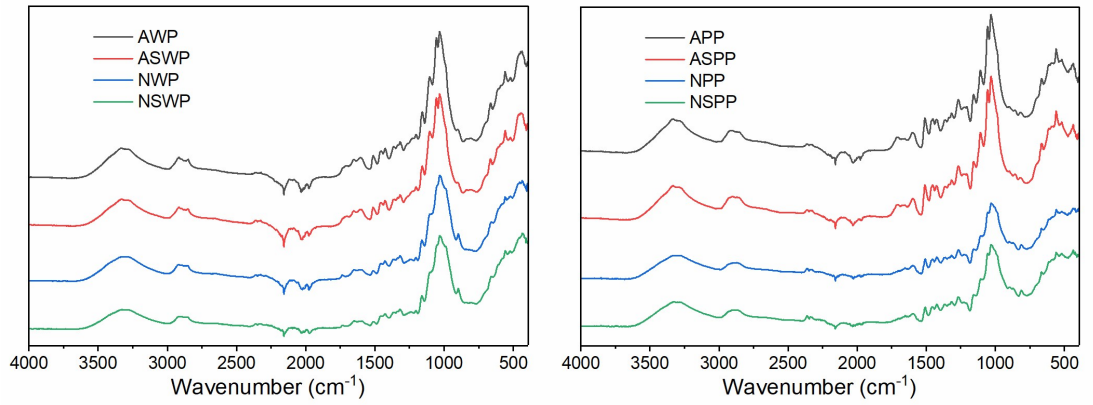

Figure S1. ATR-FTIR spectra of pretreated samples.
Pretreated samples are labeled: AWP and NWP for acid pretreated and alkaline (NaOH) pretreated wheat straw pellets without surfactant, respectively; ASWP and NSWP for acid pretreated and alkaline pretreated wheat straw pellets with 2% surfactant PEG 6000, respectively. APP and NPP for acid pretreated and alkaline pretreated pine pellets without surfactant, respectively; ASPP and NSPP for acid pretreated and alkaline pretreated for pine pellets with 2% surfactant PEG 6000, respectively.

Attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) measurements were done on three replicates using a Nicolet 6700 FT-IR, Pike Technologies GladiATR diamond spectrometer (Termo Scientific, Waltham, MA, USA). Spectra in the range from 4000 to 480 cm^{-1} were obtained using 64 scans for samples and 128 scans for the background at a resolution of 4.0 cm^{-1}.

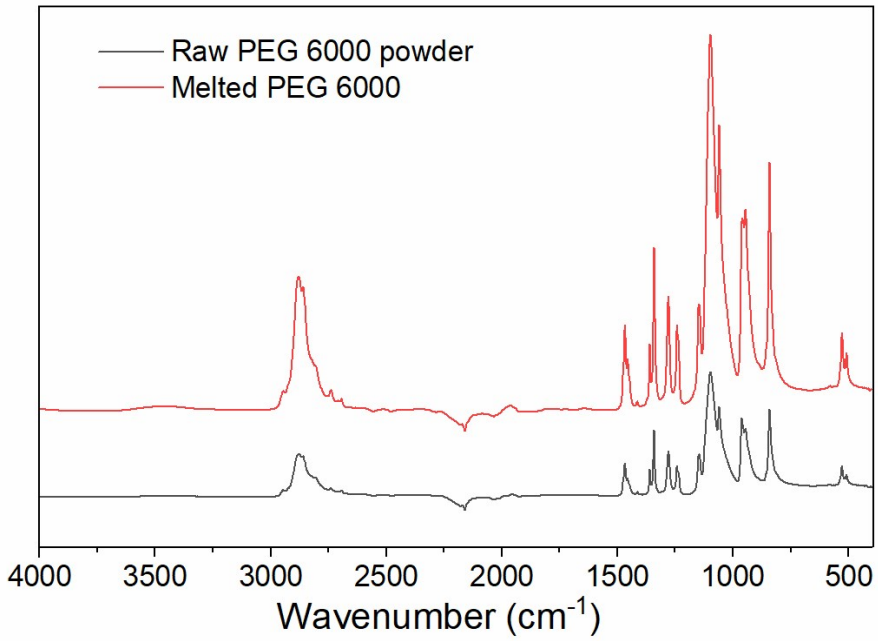

Figure S2. ATR-FTIR spectra of raw PEG 6000 powder and melted PEG 6000.

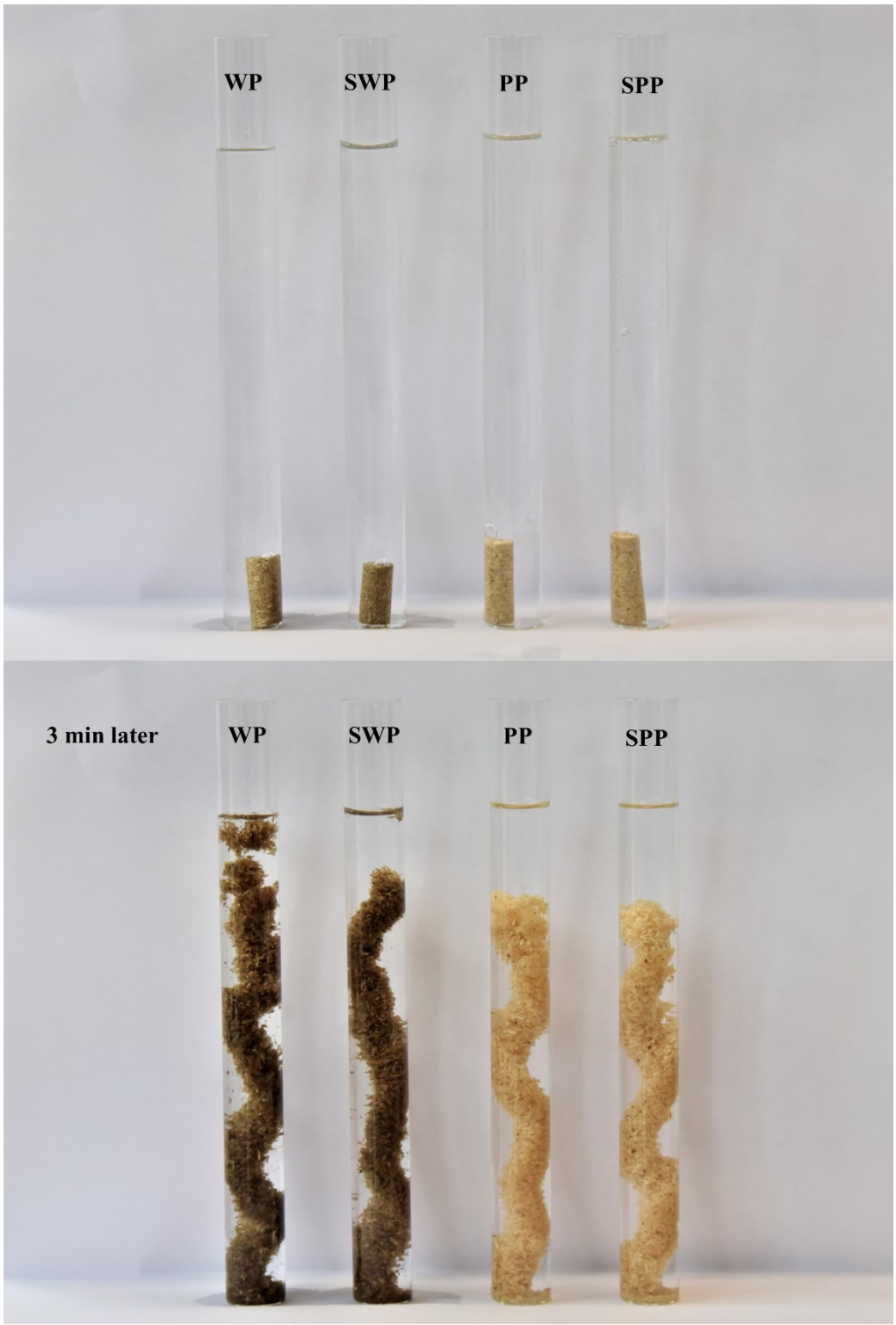

Figure S3. Comparison of water disintegration of pellets (tests performed in triplicates).

Samples are labeled: WP for wheat straw pellets without surfactant; SWP for wheat straw pellets with 2% surfactant PEG 6000; PP for pine pellets without surfactant; SPP for pine pellets with 2% surfactant PEG 6000.

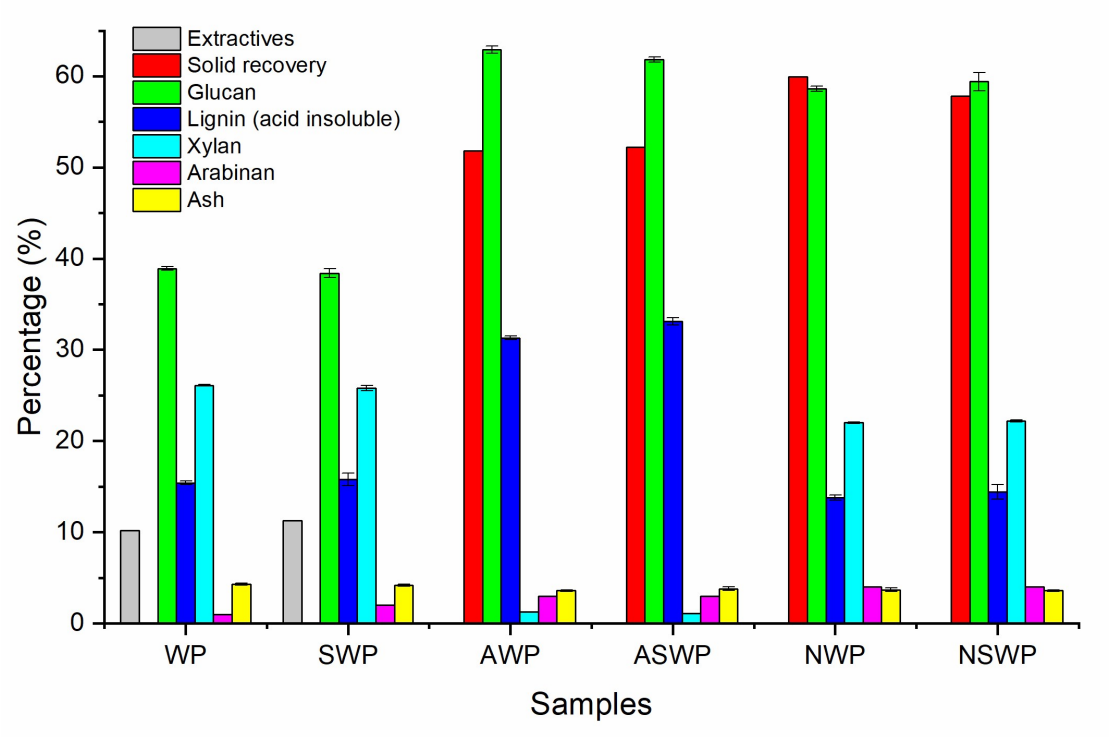

Figure S4. Chemical composition of wheat straw samples.

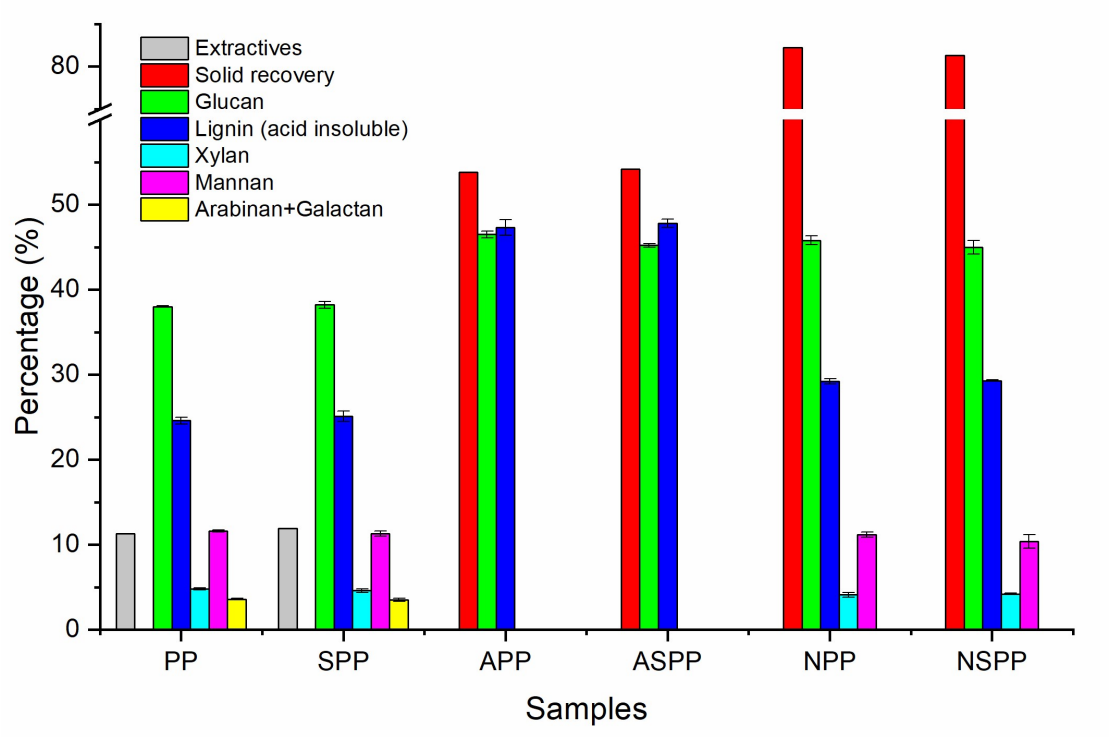

Figure S5. Chemical composition of pine samples.
Ash contents of pine samples are not shown (less than 0.4%).

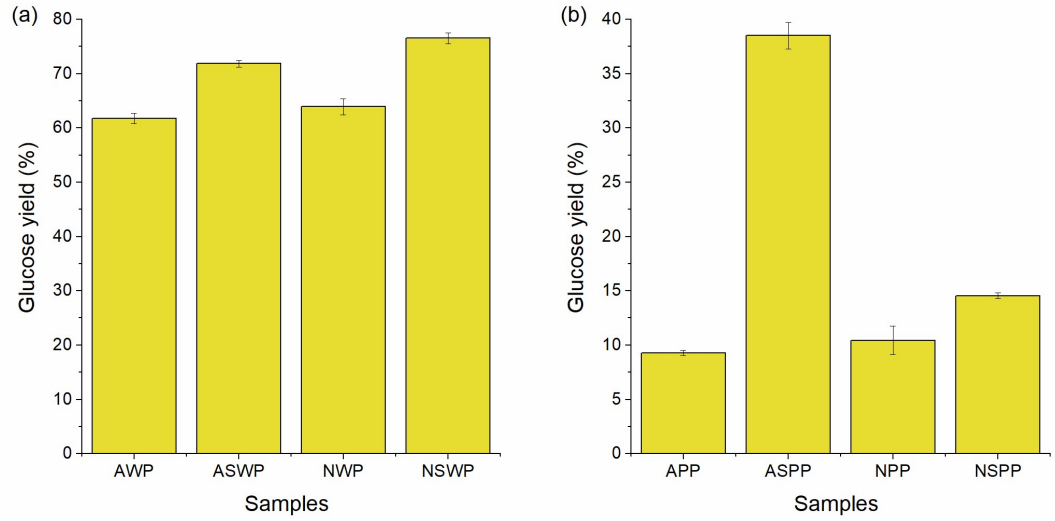

Figure S6. Glucose yields of pretreated wheat straw (a) and pine (b) samples at 25% solid loading saccharification.

Pretreated samples hydrolyzed at 25% solid loading exhibited a similar trend as the 5% solid loading saccharification for wheat straw and pine samples. Pretreated wheat straw samples at 25% solid loading got lower sugar yields than samples at 5% solid loading as expected. Product inhibition and mass transfer limitation are the main factors decreasing the enzymatic sugar yield at high solid loadings.[13] However, higher sugar yield was obtained at 25% solid loading of pretreated pine samples than at 5% solid loading. A previous study reported that a low shaking speed might cause poor mixing of the system, whereas a high shaking speed may lead to deactivation of enzymes because of the shearing force.[14] For saccharification in this study, experiments of both dry matter contents were performed by the same tumbling incubator rotating system. The 25% solid loading samples occupied most of the plastic reactor bottle, while the 5% solid loading samples only used a small part of the volume. Therefore, milder shearing conditions may have prevailed in the 25% solids loading experiments. This result indicated that higher shaking speed was achieved in the 5% set-up, which again may have caused more deactivation of enzymes due to shearing forces.[15] The results of pine pellets in this study are contrary to the general trend of higher dry matter contents causing lower sugar yields reported earlier.[16, 17] However, the findings regarding the effects of solid loading from Jørgensen *et al*. and Kristensen *et al.* were identified using older generations of enzyme preparations,[16, 17] and are known to differ from those found using Cellic Ctec2,[18] i.e., the enzyme preparation used in the current study. The effects of solid loading on enzymatic saccharification deserve deeper studies, but it is out of the scope of current study.

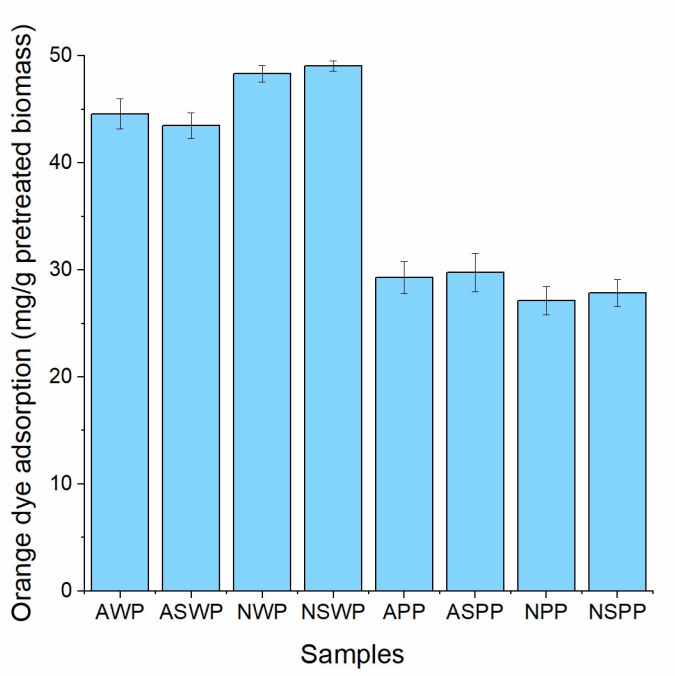

Figure S7. Orange dye adsorption of pretreated samples (error bars showing standard deviations of triplicates).

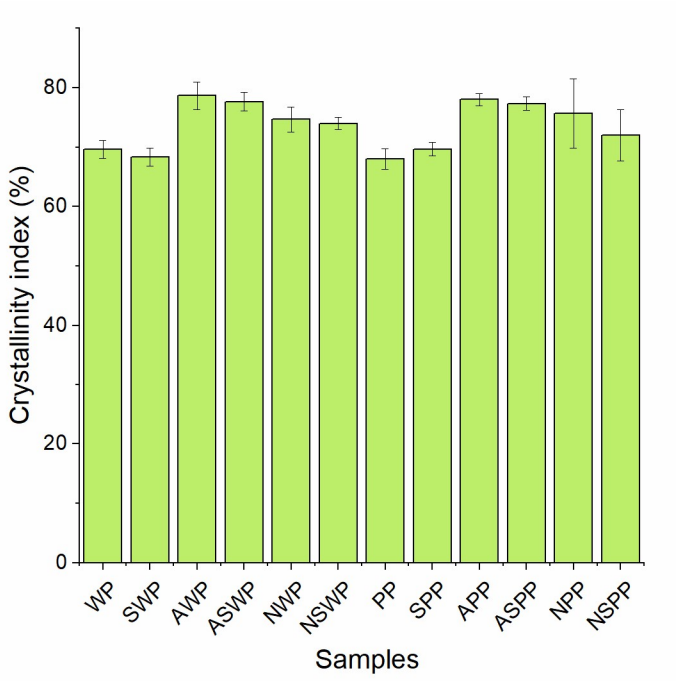

Figure S8. Cellulose crystallinity index (CrI) of un-pretreated and pretreated samples (error bars showing standard deviations of triplicates).

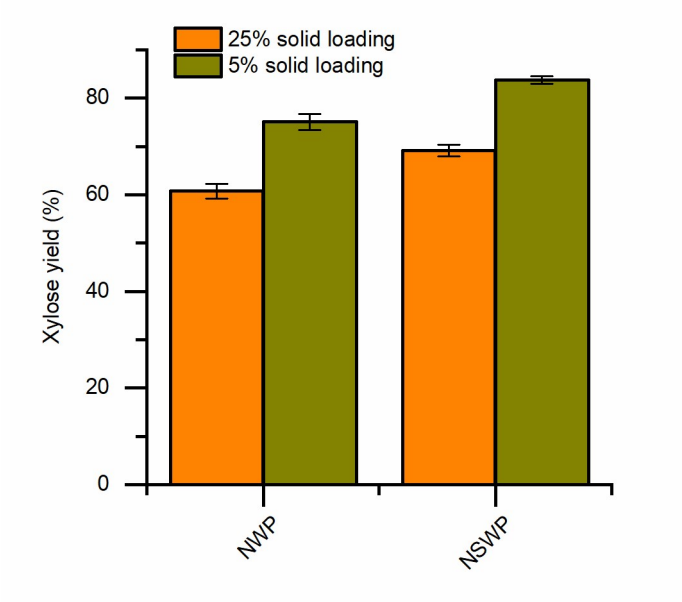

Figure S9. Xylose yields of pretreated wheat straw samples.

Table S1. Glucose and xylose contents in liquid phase after pretreatments of samples

Samples	Glucose (g/L)	Xylose (g/L)
AWP	4.6 (0.0) [a]	12.4 (0.3)
ASWP	5.1 (0.1)	12.0 (0.1)
NWP	0.2 (0.1)	2.0 (0.1)
NSWP	0.5 (0.1)	2.7 (0.0)
APP	11.6 (1.1)	1.3 (0.2)
ASPP	12.7 (0.5)	1.5 (0.0)
NPP	0.1 (0.0)	1.2 (0.0)
NSPP	0.1 (0.0)	1.1 (0.0)

Data is average of three measurements; [a] standard deviation. Glucose and xylose contents were measure according to a standard method.[19]

Table S2. Lignin units and interunit linkages of samples

Samples	S	G	H	β-O-4’	β-β’	β-5’	PCA	FA
WP	39.0%	58.5%	2.5%	54.3%	0.9%	3.1%	7.0%	5.2%
SWP	39.2%	58.1%	2.7%	53.0%	1.0%	3.7%	7.3%	5.5%
AWP	35.3%	64.3%	0.4%	4.1%	na	2.8%	4.6%	na
ASWP	33.4%	64.9%	1.8%	5.1%	na	3.0%	5.2%	na
NWP	45.6%	52.1%	2.4%	19.2%	0.5%	na	na	na
NSWP	45.9%	51.8%	2.3%	22.1%	0.7%	0.3%	na	na
PP	na	98.7%	1.3%	44.5%	12.4%	1.2%	na	na
SPP	na	98.9%	1.1%	45.6%	12.4%	0.9%	na	na
APP	na	100.0%	na	na	0.7%	na	na	na
ASPP	na	100.0%	na	na	0.7%	na	na	na
NPP	na	100.0%	na	16.3%	4.8%	0.4%	na	na
NSPP	na	100.0%	na	19.5%	5.4%	0.4%	na	na

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