

Supplemental Online Materials

Supplemental Methods: HSQC NMR

Heteronuclear Single Quantum Coherence (HSQC) NMR spectra were acquired for ball-milled whole biomass samples (30-40 mg), dissolved in a DMSO-d₆:Pyridine-d₅ 4:1 mixture (500 µL). Spectra were acquired at 40 °C on a Bruker Avance Nanobay 400 MHz spectrometer at 9.4 T using a BBO room temperature probe. Spectra were acquired and processed using parameters set out in Mansfield et al.⁽¹⁾ and integrations were performed using TopSpin 3.5.

(1) S. D. Mansfield, H. Kim, F. Lu and J. Ralph, *Nat. Protoc.*, 2012, **7**, 1579.

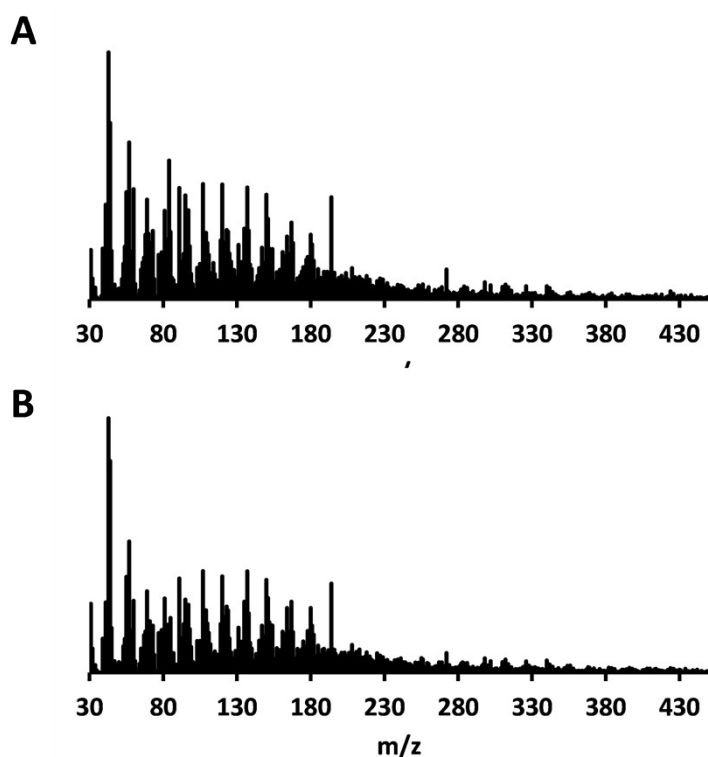


Figure S1. PyMBMS spectra of (A) Residue remaining after *C. thermocellum* fermentation with cotreatment. (B) Residue remaining after *C. thermocellum* fermentation with cotreatment after removal of metal particles by sonication after suspension in the EDTA solution.

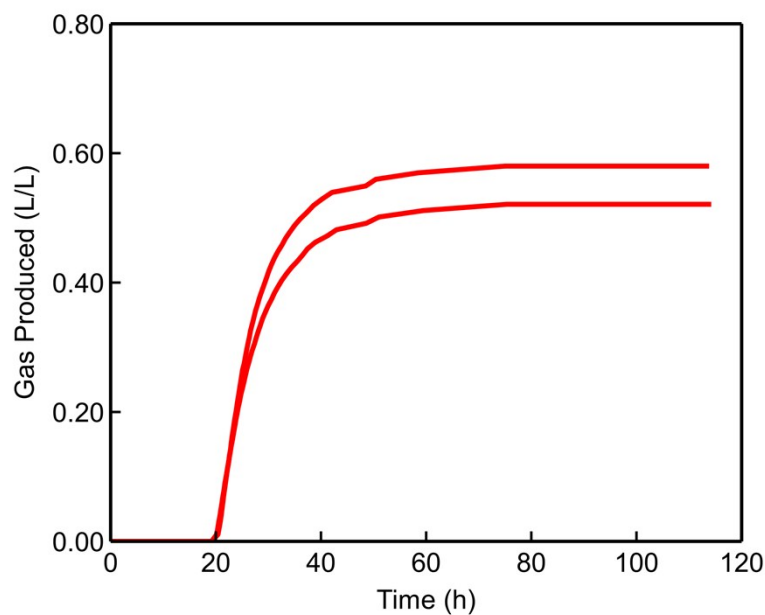


Figure S2. Volumetric gas production for duplicate fermentation of switchgrass following hydrothermal pretreatment (further results reported in Table 1).

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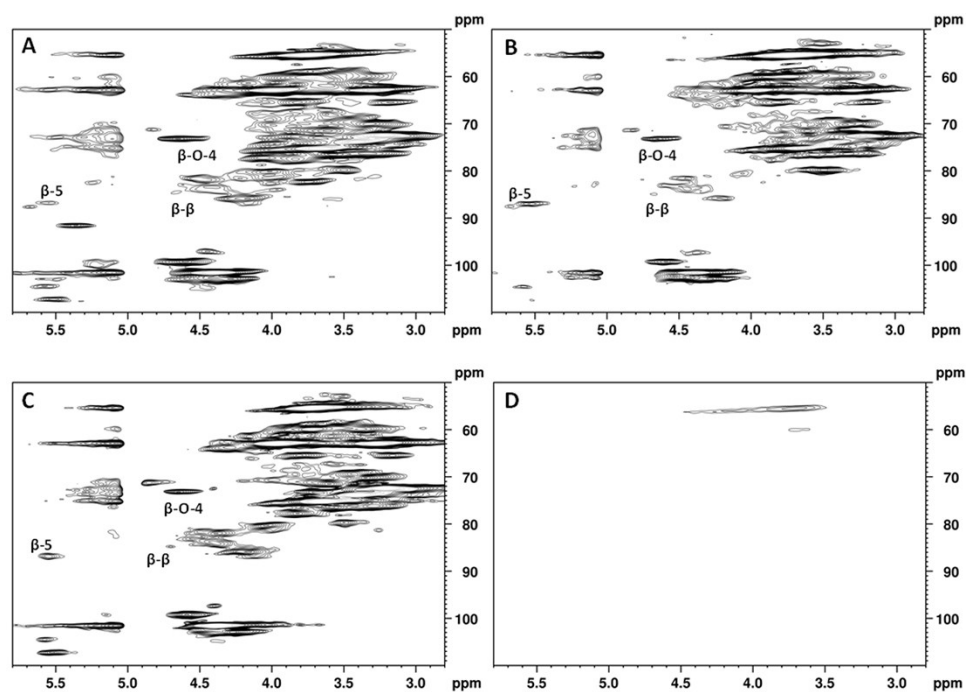


Figure S3. Aliphatic region of ^1H - ^{13}C HSQC spectra of switchgrass feedstock before and after conversion. (A) Switchgrass feedstock before conversion. (B) Feedstock after hydrothermal pretreatment. (C) Residue remaining after *C. thermocellum* fermentation.

25 (D) Residue remaining after *C. thermocellum* cotreatment following attempted removal of metal particles (lines were too broad from residue metal to get useful information for this sample). The following C-O-C bonds are labeled on the spectra (^1H , ^{13}C in ppm): β -O-4 (4.63, 73.0), phenylcoumaran (β -5) (5.54, 86.75), and resinol (β - β) (4.68, 84.78).

30 **Table S1.** Integrations of ^1H - ^{13}C HSQC peaks assigned to β -O-4 (α C-H correlation), phenylcoumaran (β -5), and resinol (β - β) linkages.

Sample	% β -O-4	% (β -5)	% (β - β)
Unpretreated Feedstock	85	8	7
Pretreated Feedstock	57	37	6
<i>C. thermocellum</i> fermentation residue	68	24	8
Cotreatment residue	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>

n.d. = not determined