Electronic Supplementary Information for:

Quantification of acidic compounds in complex biomass-derived streams

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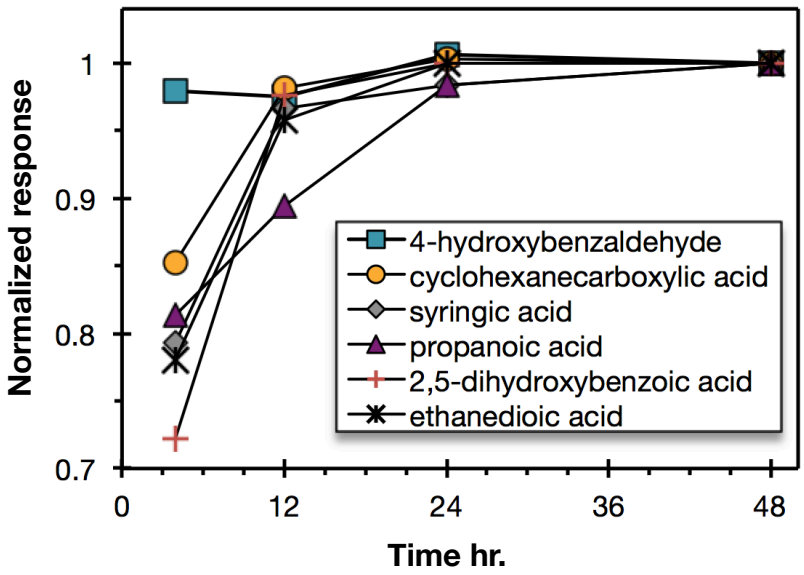

Figure S1: Normalized peak area observed on the GCxGC-TOFMS instrument over a period of 48 hours for a solution of 6 compounds (4-hydroxybenzaldehyde, cyclohexanecarboxylic acid, syringic acid, propanoic acid, 2,5-dihydroxybenzoic acid, and ethandioic acid) dissolved in pyridine at 3 mg/ml and derivatized with BSTFA. These compounds were chosen to mimic functionalities of compounds present in APL. Results of this experiment show that 24 hours of derivatization time at room temperature is sufficient to achieve complete derivatization of the samples.

Table S1: GCxGC-TOFMS settings used for sample analysis.

Columns			
Primary	10m x 180µm; 0.18µm RTX-5		
Secondary	0.75m x 100µm; 0.10µm DB-1701		
Injector			
Split	Helium; 30:1 split ratio, 300°C		
Column flow	1 mL/min, constant flow		
Injection volume	2 µL		
Oven			
Primary	40°C – 1 minute hold Ramp 7.5°C/min to 115 °C 15°C/min to 225°C, no hold Total time 20 minutes		
Secondary	+10°C offset from primary		
Modulator	+15°C offset from secondary		
Modulator cycle timing	Modulator per second	Hot time (sec)	Cold time (sec)
Start – 596 sec	4	0.7	1.3
596 – end	4	0.85	1.15
Transfer line	250°C		
TOF			
TOF mass range	29 – 350 amu		
TOF acquisition rate	200 spectra / sec		
Delay			
Solvent delay	54 sec		

Table S2: Standard compounds that were derivatized and analyzed with the GCxGC-TOFMS instrument. Masses used for quantification are listed with retention times and information on the supplier/lot number of each compound. In total, 61 compounds were tested and 19 were identified in APL with an additional 4 compounds quantified that were produced from microorganisms.

IUPAC name	Common name	Present in APL?	R.T. (s)	Quant masses of TMS derivative	Supplier	Lot#
acetic acid	SAME	**Yes**	90 , 1.080	73+75+117	Fisher scientific	106218
2-hydroxypropanoic acid	lactic acid	**Yes**	410 , 1.815	219+147+117	Sigma-Aldrich	MKBT5753V
2-hydroxyethanoic acid	glycolic acid	**Yes**	426 , 1.950	73+147+205	TCI America	N7QDG-RM
2-hydroxy-butanoic acid	SAME	**Yes**	486, 1.820	233+205+190+147+131+73	Sigma-Aldrich	CD5000492
ethanedioic acid	oxalic acid	Yes at trace levels and is also a biological product	498, 2.195	219+147+73	Sigma-Aldrich	SHBC5374V
3-hydroxypropanoic acid	SAME	**Yes**	510,1.820	219,177,147,133,116,103,101,75,73,66,45	TCI America	FH7RE-TA
propane-1,2,3-triol	glycerol	**Yes**	654.1.625	218+147+73	Sigma-Aldrich	011M01132V
butanedioic acid	succinic acid	**Yes**	694,1.785	262,218,172,157,131,129,117,77,75,71,47	TCI America	ELQGA
2,3-Dihydroxypropanoic acid	glyceric acid	**Yes**	710,1.525	307,292,205,189,147,133,117,103,73	TCI America	N5RLG-MK
(2E,4E)-hexa-2,4-dienedioic acid	muconic acid	Biological product	850 , 1.525	73+147+169+196	Sigma-Aldrich	MKBL8281V
4-hydroxy benzoic acid	SAME	Yes at trace levels and is also a biological product	882 , 1.550	73+193+223+267+282	Sigma-Aldrich	574444V
3,4 dihydroxy benzoic acid	protocatechuic acid	Biological product	974 , 1.435	223+267+281+311+73	Sigma Aldrich	02608MR
4-hydroxy-3-methoxybenzoic acid	vanillic acid	**yes**	946 , 1.565	223+253+267+297+312	Sigma-Aldrich	BCBH4868V
4-hydroxycinnamic acid	p-coumaric acid	**yes**	1018 , 1.695	73+219+249+293+308	MP biomedicals Inc.	4886kA
(E)-3-(4-hydroxy-3-methoxy-phenyl)prop-2-enoic acid	ferulic acid	**yes**	1074 , 1.730	73+219+249+308+323+338	Fluka	1105704
2-hydroxy-4-methylvaleric acid	2-hydroxyisocapronic acid	**Yes (trace)**	610, 1.750	261,203,177,159,147,103,75,73,45	Sigma-Aldrich	0221HPV

4-(2-hydroxyethyl)phenol	tyrosol	**Yes (trace)**	862, 1.325	282,267,193,179,149,117,103,73,45	Sigma-Aldrich	MKBK6451V
maleic acid	SAME	**Yes (trace)**	686 , 1.920	73+75+147+245	Sigma-Aldrich	1307MK
4-hydroxy benzaldehyde	SAME	**Yes (trace)**	746 , 1.935	194+179+151	Sigma-Aldrich	02425JM
D-gluconic acid	gluconic acid	**Yes (trace)**	1050, 1.285	333+305+292+147+73	TCI America	RYL7N-SM
4-hydroxy-3,5-dimethoxybenzoic acid	syringic acid	**Yes (trace)**	1002 , 1.600	223+253+297+312+342	Sigma-Aldrich	BCBB6440V
1-(4-Hydroxy-3-methoxyphenyl)ethanone	Acetovanillone*	**Yes***	915,1.632	N/A*	Sigma-Aldrich	
cyclohexane carboxylic acid	SAME	INTERNAL STANDARD	588 , 1.960	73+75+185+200	Sigma-Aldrich	MKBD6586V
propanoic acid	SAME	LOST IN SOLVENT	N/A		Sigma-Aldrich	SHBC7498V
1,2-ethanediol	glycol	LOST IN SOLVENT	N/A		Fluka	BCBM6462V
2-oxopropanoic acid	pyruvic acid	LOST IN SOLVENT	N/A		TCI America	48DJE-BD
formic acid	SAME	LOST IN SOLVENT	N/A		Fluka	BCBB9543V
Hexanoic acid	SAME	no	426, 1.725	173,131,117,75,73,55,47,45,43,41	Sigma-Aldrch	MKBR6972V
4-oxopentanoic acid	levulinic acid	no	490, 2.725	73+145+173	Sigma-Aldrich	JI19403EI
3-hydroxybutanoic acid	SAME	no	526, 1.775	233,294,191,147,133,117,88,75,73,45	Sigma-Aldrich	MKBQ6538V
2-hydroxy-3-methylbutyric acid	2-hydroxyisovaleric acid	no	530, 1.755	247,219,147,145,133,75,73,45	Sigma-Aldrich	BGBC119V
propanedioic acid	malonic acid	no	574, 2.22	73+75+147+233	Sigma-Aldrich	MKBH6537V
benzoic acid	SAME	no	606, 2.34	77+105+135+179+194	AlfaAesar	F02W053
2-methylbutanedioic acid	methylsuccinic acid	no	702, 1.725	261,217,147,143,133,129,75,73,69,55,45	TCI America	DEIGE-0Q
3-hydroxybenzaldehyde	SAME	no	702, 2.005		Sigma-Aldrich	MKBK4423V
(E)-butenedioic acid	fumaric acid	no	722, 1.635	245,55,147,143,133,115,83,75,73,45	Sigma-Aldrich	16F-0465
1,3-dimethoxy-2-hydroxybenzene	syringol	no	758 , 1.705	226+211+196+181	Sigma-Aldrich	13729ER
2,5 dihydroxybenzoic acid	gentisic acid	no	806 , 1.220	267+233+149+73	Sigma-Aldrich	N/A
2-hydroxybenzoic acid	salicylic acid	no	822 , 1.540	267+135+73	Sigma-Aldrich	MKBF0928V

3-hexene 1,6-dioic acid	trans-β-hydromuconic acid	no	830 , 1.525	73+82+147+273	Sigma-Aldrich	STBD2139V
3-hydroxy benzoic acid	SAME	no	854, 1.525	282+267+223+193+73	TCI America	FHM01-NPDO
3-hydroxy-4-methoxybenzaldehyde	isovanillin	no	866, 1.680	209,194,193,163,83,73,45	Sigma-Aldrich	PW013126W
3,4-dihydroxybenzaldehyde	protocatechuic aldehyde	no	882 ,1.670	73+193+267+282	Sigma-Aldrich	MKBH5707V
4-hydroxy-3-methoxybenzaldehyde	vanillin	no	898, 1.405	73+194+209+224	Sigma-Aldrich	04808PH
2-oxopentanedioic acid	α-ketoglutaric acid	no	902, 1.265	292,219,189,147,133,102,73,45	Sigma-Aldrich	18723MA
methyl-4-hydroxy-3-methoxybenzoate	methyl vanillate	no	906, 1.530	254,239,224,193,165,149,137,104,89,73,45	Sigma-Aldrich	CX001810EV
1,2-benzene carboxylic acid	phthalic acid	no	922, 1.565	295,221,163,147,135,119,105,91,73,45	TCI America	VNXEI-T0
4-(2-hydroxyethyl)-2-methylphenol	homovanillic alcohol	no	930, 1.350	312,297,282,209,192,179,149,103,91,73	Sigma-Aldrich	13023HS
4,-hydroxy-3,5-methoxybenzaldehyde	syringaldehyde	no	930, 1.715	254,239,224,209,193,153,137,104,73,59,45	Sigma-Aldrich	KR051310R
prop-1-ene-1,2,3-tricarboxylic acid	*cis*-aconitic acid	no	946, 1.410	285,257,229,221,147,133,97,75,73,67,45	AlfaAesar	I4708A
2-(4-hydroxy-3-methyoxyphenol)acetic acid	homovanillic acid	no	958, 1.470	326,311,267,209,179,163,149,135,104,73	Sigma-Aldrich	13023HS
4-hydroxy-3,5-dimethoxyaceteophenone	acetosyringone	no	962, 1.685	268,253,238,223,73,59	Sigma-Aldrich	01531EG
(Z)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enal	coniferyl aldehyde	no	994 , 1.845	250+220+219+192	Sigma-Aldrich	07513MZ
2-hydroxy-3-(4-hydroxyphenyl)propanoic acid	DL-*p*-Hydroxyphenyllactic acid	no	1010,1.395	308,179,147,133,119,73,45	Sigma-Aldrich	SLBD9757V
3,5-dimethoxy-4-hydroxyphenlactic acid	homosyringic acid	no	1014,1.525	341,326,267,239,209,193,179,163,151,89,71	Sigma-Aldrich	MKBQ9362V
4-(3-hydroxy-1-propenyl)-2-methoxyphenol	coniferyl alcohol	no	1022 ,1.500	73+204+235+293+324	Sigma-Aldrich	N/A
hexadecanoic acid	palmitic acid	no	1062, 1.270	145,132,129,117,75,73,55,43,41	Sigma-Aldrich	105F-00232
3,4-dihydroxyphenyllactic acid	dopacetic acid	no	1062, 1.340	287,221,147,73,45	Sigma-Aldrich	2405DK
3-(3,4-dihydroxyphenyl)-2-propenoic acid	caffeic acid	no	1090, 1.7	73+191+219+249	TCI America	W7WS6-EL
3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoic acid	sinapinic acid	no	1130, 1.7580	338+323+279+249+73	Sigma-Aldrich	SLBH4664V

* Analysis software had difficulty routinely quantifying this peak despite its relatively large size.

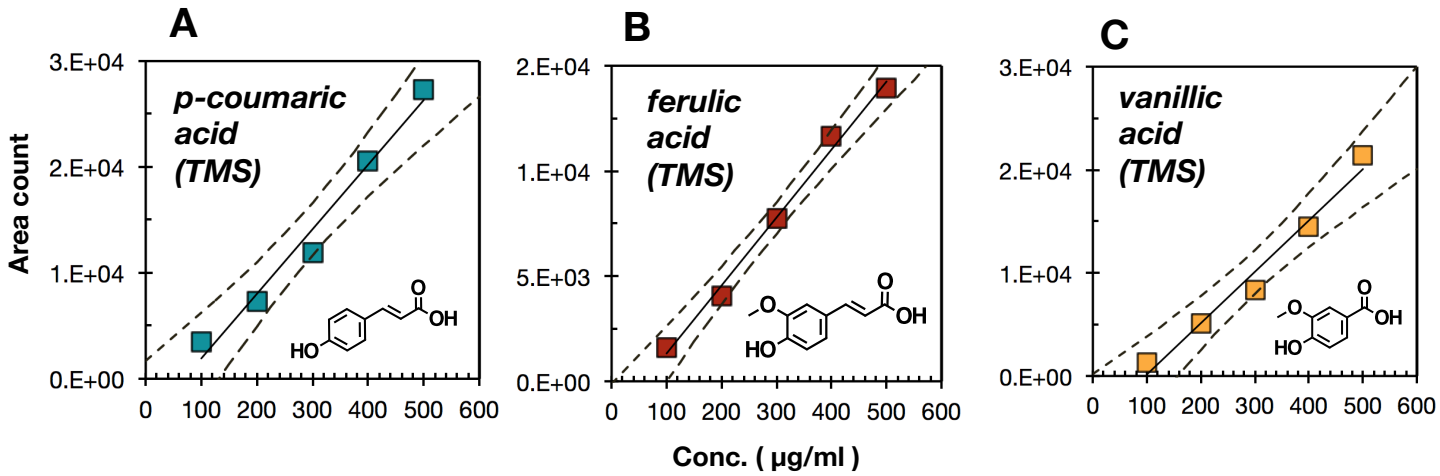

Figure S2: calibration curves for lignin derived monomers.

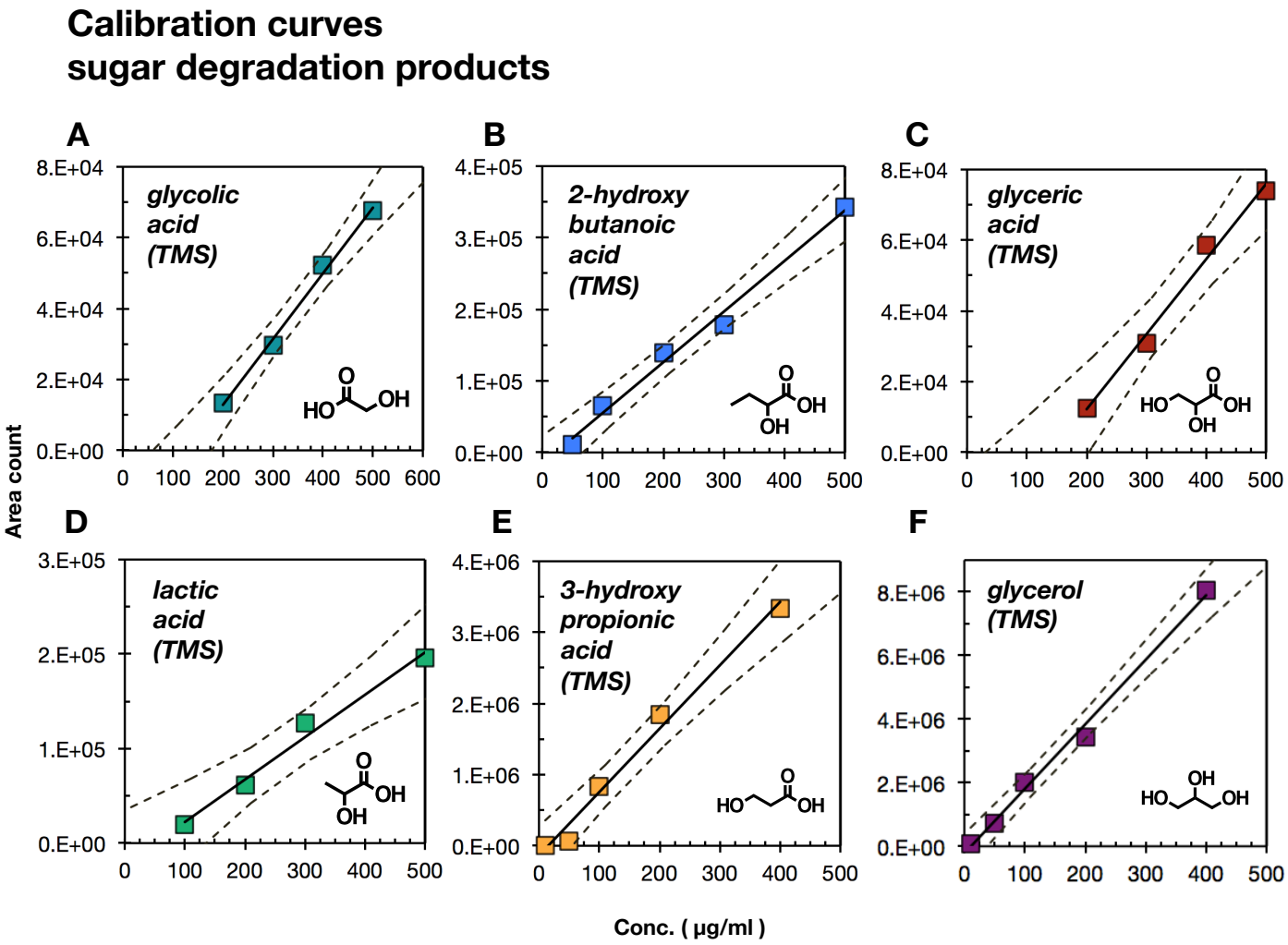

Figure S3: Calibration curves for sugar degradation products.

Calibration curves biological products

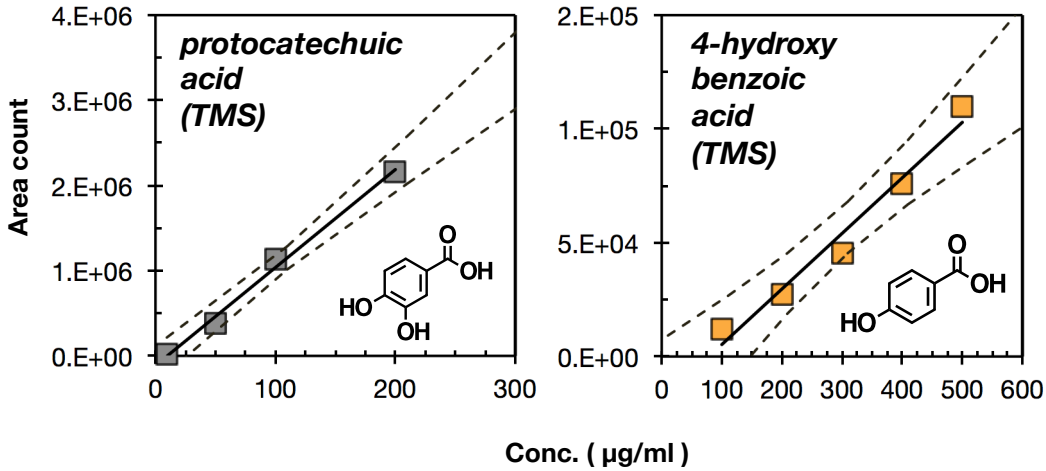

Figure S4: Calibration curves for the TMS derivatives of protocatechuic acid and 4-hydroxy benzoic acid. These compounds are produced biologically during fermentation of APL samples.

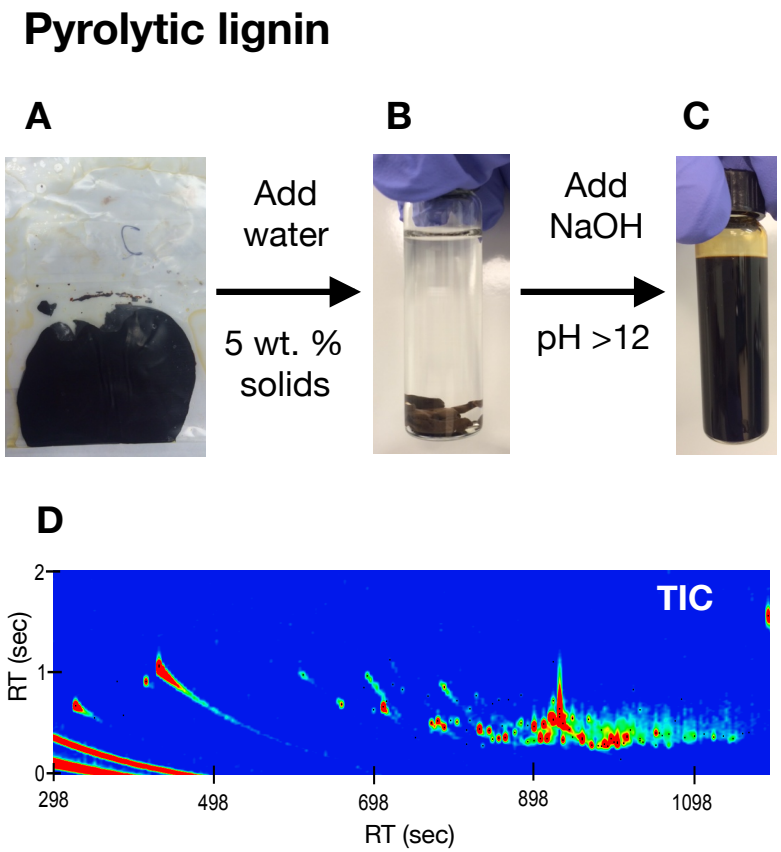

Figure S5: (A) Pyrolytic lignin sample at ~20-30 wt.% exhibits a tar like consistency. (B) The sample is diluted in water to ~5 wt.% solids. Here the pyrolytic lignin is completely insoluble. (C) Solid sodium hydroxide is added to the solution until the pH is > 12 solubilizing the material. Then the sample is derivatized following the same workup protocol as that used for the APL samples. (D) TIC chromatogram from the GCxGC-TOFMS analysis of the derivatized sample.

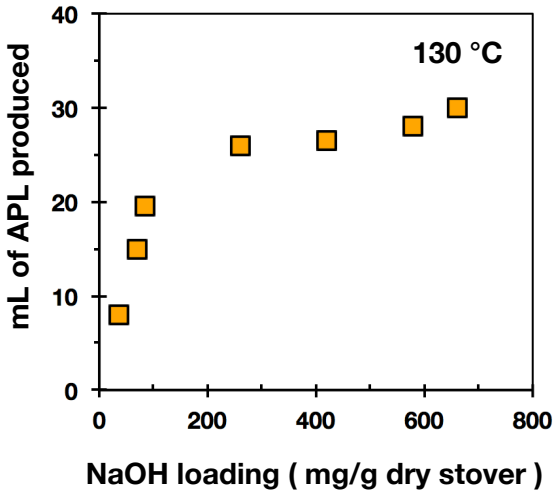

Figure S6: Volume of APL produced (in mL) as a function of NaOH loading for corn stover pretreatments at 130 °C in accordance with our previous work presented in Ref. **1**

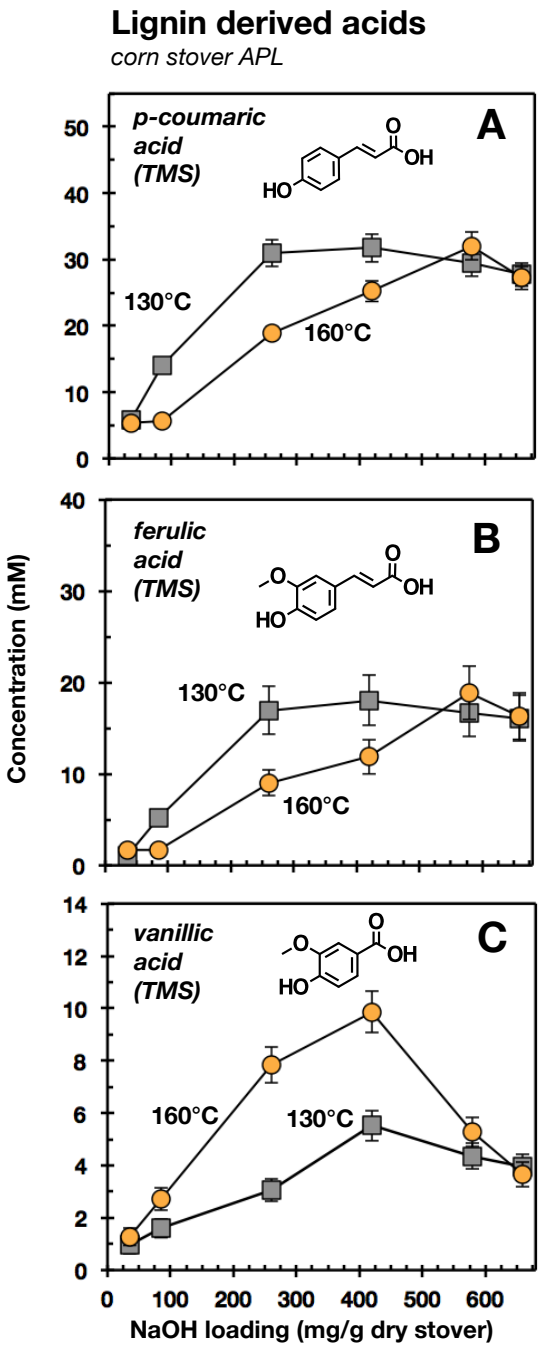

Figure S7: Replicate of Figure 2 within the manuscript but reporting mM units instead of the mg/mL units used in the text.

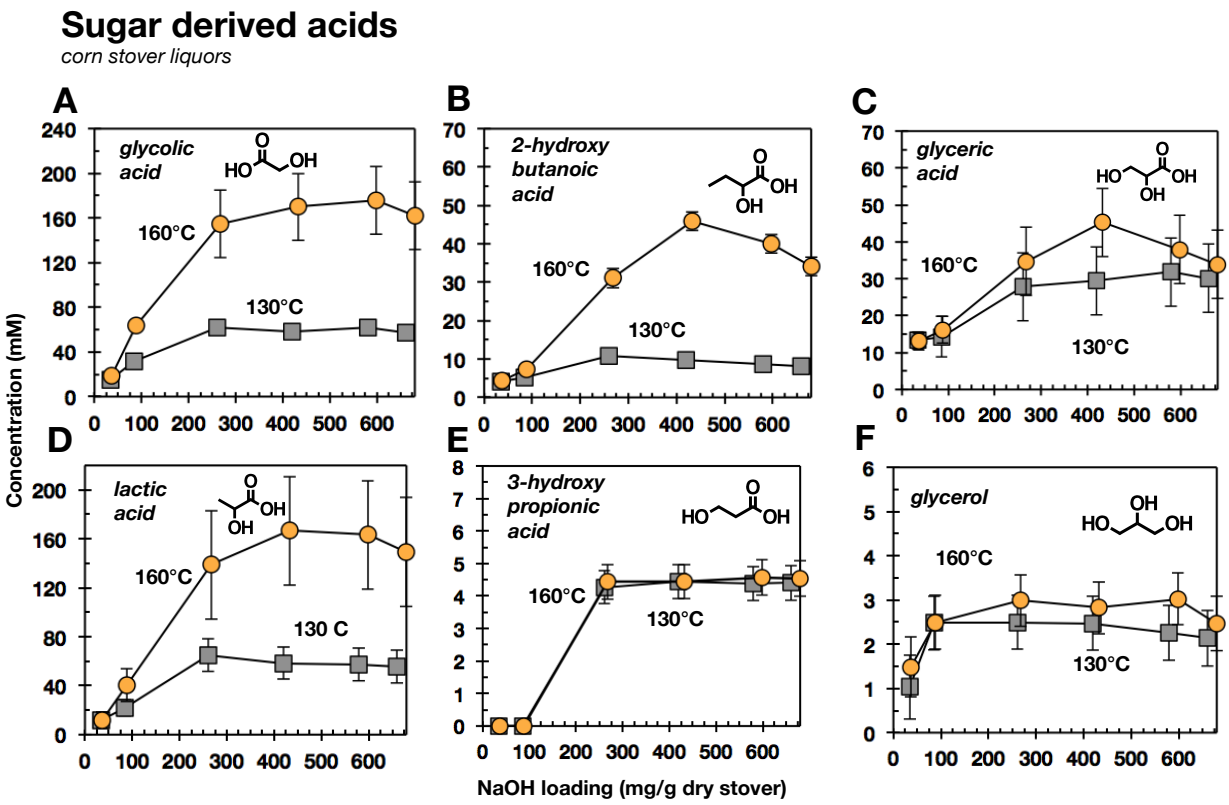

Figure S8: Replicate of Figure 3 within the manuscript but reporting mM units instead of the mg/mL units used in the text.

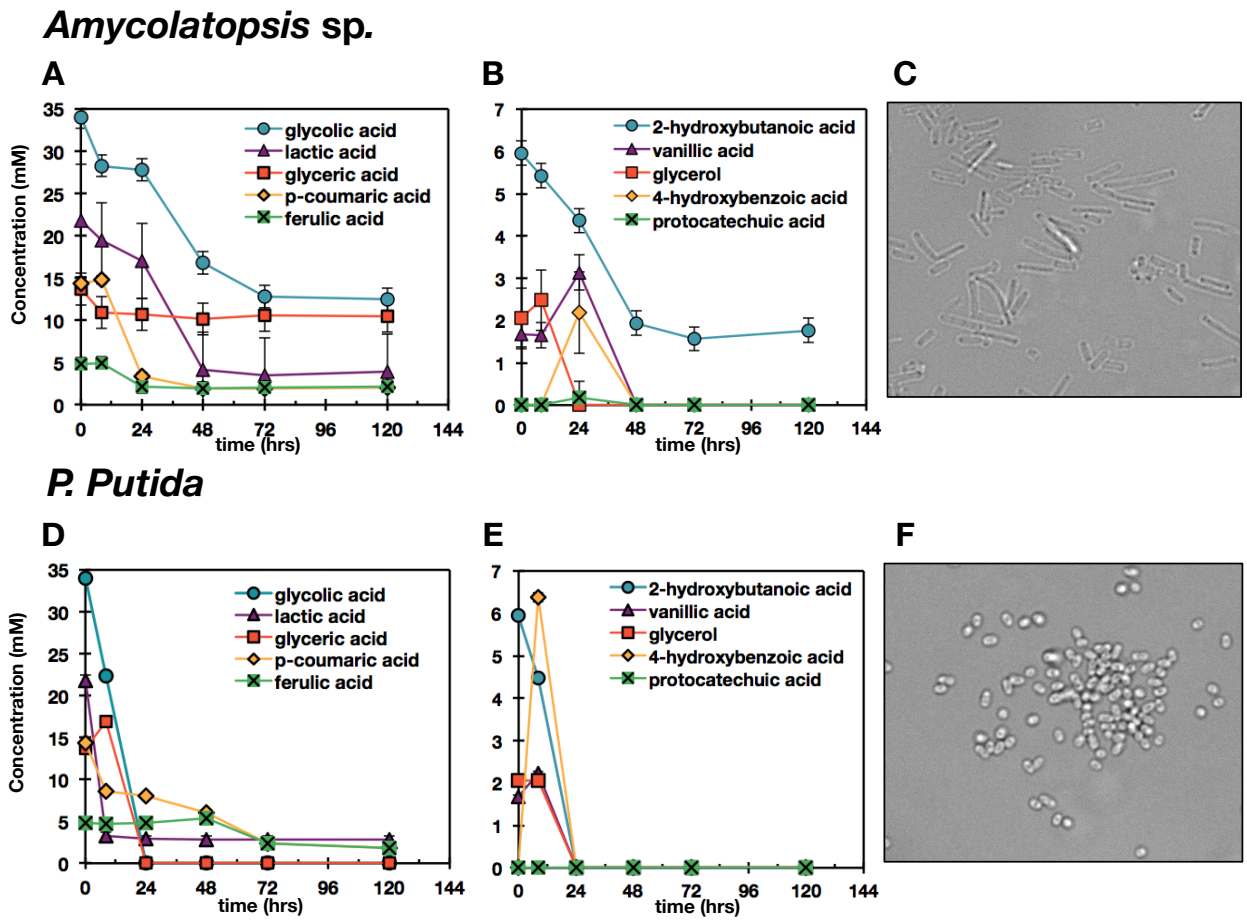

Figure S9: Replicate of Figure 4 within the manuscript but reporting mM units instead of the mg/mL units used in the text.

SUPPORTING REFERENCES

1. E. M. Karp, B. S. Donohoe, M. H. O'Brien, P. N. Ciesielski, A. Mittal, M. J. Biddy and G. T. Beckham, *Acs Sustain. Chem. Eng.*, 2014, **2**, 1481–1491.