Supporting Information

Lignin valorization through integrated biological funneling and chemical catalysis

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SI Methods

Compositional Analysis of Corn Stover Pretreated Solids. Compositional analysis was conducted on the pretreated residual solids to quantify the carbohydrate retention and lignin removal. The mass of the recovered dry solids was measured by drying a subsample of solid fraction for several days in a 40°C vacuum oven until the mass stabilized to a constant value. Compositional analysis of the recovered solids was subsequently performed in accordance with standard NREL Laboratory Analytical Procedures (LAPs) (1, 2). The APL is a heterogeneous mixture of acids, polysaccharides, monosaccharides, aromatic monomers (derived from lignin), high molecular weight lignin, and acetate (3). The complexity of black liquor and its sensitivity to pH changes significantly complicates direct, detailed compositional analysis. Therefore, for the purposes of this work, we report the composition of the APL by difference from the known mass and composition of the dry biomass loaded into the pretreatment vessel and the resulting mass and composition of the retained solids, as shown in Figure S1.

Gel Permeation Chromatography (GPC) Analysis of APL. To determine the molecular weight distribution of the APL, 20 mg of APL obtained from the alkaline pretreatment of corn stover was acetylated in a mixture of pyridine (0.5 mL) and acetic anhydride (0.5 mL) at 40°C for 24 h with stirring. The reaction was terminated by addition of methanol (0.2 mL) to neutralize the acetic anhydride. The acetylation solvents were then evaporated from the samples at 40°C under a stream of nitrogen gas. The samples were further dried in a vacuum oven at 40°C overnight. A final drying was performed under vacuum (1 torr) at room temperature for 1 h. The dried acetylated samples were dissolved in tetrahydrofuran (THF, Baker HPLC grade) and then filtered (0.45-µm nylon membrane syringe filters) before GPC analysis. The acetylated samples appeared to be completely soluble in THF.

GPC analysis was performed using an Agilent HPLC with 3 GPC columns (Polymer Laboratories, 300 x 7.5 mm) packed with polystyrene-divinyl benzene copolymer gel (10-µm beads) having nominal pore diameters of 10^4, 10^3, and 10^2Å. The eluent was THF and the flow rate was 1.0 mL/min. An injection volume of 25 µL was used. The HPLC was attached to a diode array detector measuring absorbance at 260 nm (band width 40 nm). Retention time was converted into molecular weight (MW) by applying a calibration curve established using polystyrene standards.

Liquid Chromatography Component Identification of APL. Liquid chromatography was employed to identify the primary components in APL. Individual chemical standards representing the compounds listed in Table S1 without asterisks were purchased from Sigma-Aldrich, with the exception of acetic acid (Fisher Scientific). HPLC solvents and modifiers consisted of deionized water (DI) (Barnstead Easy Pure[II]), acetonitrile (Fisher HPLC grade), and formic acid (Sigma-Aldrich).

Analysis of samples was performed on an Agilent 1100 LC equipped with a G1315B Diode Array Detector (DAD) and in-line Electrospray Ionization (ESI) 2440A Mass Selective Detector (MSD) Ion Trap SL (Agilent Technologies). Each sample was placed in a cooled auto-sampler (10°C) and injected at a volume of 50 µL into the LC/MS system. Sample compounds were separated using reverse-phase chromatography on an YMC C30 Carotenoid 0.3 µm, 4.6 x 150 mm column (YMC America). The LC/MS method consisted of eluent gradients, flow rates, temperatures, and configurations according to prior methods (4-7). The degassed solvent regime consisted of eluent A) DI modified with 0.03% formic acid, and eluent B) 9:1 acetonitrile and DI water also modified with 0.03% formic acid, which was prepared fresh at least 4 hours prior to analyses.

Flow from the HPLC-DAD was directly routed to the ESI-MSD Ion Trap. Tandem MS of major contributing ions was carried out via direct infusion on an Agilent 2440A MSD Ion Trap SL equipped with ESI source operating in negative mode. Source and ion trap conditions were calibrated and optimized with Agilent ESI-T tuning mix (P/N:G2431A) and using smart parameter setting

(SPS) tuning with target m/z set to 165, compound stability 70%, trap drive 50%, capillary at 3500 V, fragmentation amplitude of 0.8 V with a 30 to 200% ramped voltage implemented for 50 ms, and an isolation width of 2 m/z (He collision gas). The ESI nebulizer gas was set to 60 psi, with dry gas flow of 11 L/min held at 350°C. A MS scan and precursor isolation-fragmentation scans were performed across the range m/z: 40-350.

An internal spectral database consisting of compounds previously identified as degradation products (4, 7-10) was developed based on ESI-MS-MS scans for the precursor $(M-H)^-$ ion and product ion of each compound (Table S1, compounds not marked with an asterisk) through direct infusion experiments, as previously described (7). Both the retention time and database search results for total and extracted ion chromatography for the precursor $(M-H)^-$ ion and at least one product ion were used to confirm the identity of compounds, while deconvolution of mass/charge ion fragmentation patterns was utilized to predict the identity of unknown compounds observed within the samples.

Glucose YSI Measurement of APL. The glucose concentration in neutralized APL was measured using a YSI 7100 MBS (YSI Life Sciences, Yellow Springs, Ohio) instrument. To confirm the accuracy of this method, a calibration curve was produced by adding known amounts of glucose into six samples of neutralized APL. Glucose concentrations of these six samples were measured using the YSI instrument and the results confirmed the known concentration of glucose added into these samples.

Enzymatic Hydrolysis of Residual Solids. Enzymatic hydrolysis of the residual solids was conducted to determine the digestibility. Alkaline pretreated corn stover solids were washed five times with deionized water and stored in 30 mM NaAc pH 5.0 and at 4°C prior to enzymatic hydrolysis. Fungal cellulase enzymes (CTec2, Novozymes) were loaded at 10 or 20 mg of protein per g of glucan in a 1% biomass solids slurry and incubated at 50°C in 20 mM NaAc, pH 5.0 for 120 hours. Digestions were conducted in sealed 1.5-mL vials with continuous mixing by inversion at 10-12/min. Substrates were loaded at 10 mg dry biomass per mL in 1.4-mL reaction volumes. Representative (with respect to both solid and liquid phases of the digestion slurry) 0.1-mL samples were withdrawn from well-mixed digestion slurries at selected time-points during the digestions. The aliquots were then diluted 10-fold with deionized water and immersed in a boiling-water bath for 10 min to inactivate the enzymes and terminate the reaction. The diluted and terminated digestion aliquots were then filtered through 0.2-μm nominal-pore-size nylon syringe-filters (Pall/Gelman Acrodisc-13) to remove residual substrate and, presumably, most of the denatured enzyme. Released cellobiose and glucose and xylose in the diluted samples were then determined by HPLC analysis on an Aminex HPX-87H column (Bio-Rad Laboratories, Inc.) operated at 55°C with 0.01 N H_2SO_4 as mobile phase at 0.6 mL/min in an Agilent 1100 HPLC system with refractive-index detection. The resulting glucose, cellobiose, and xylose concentrations calculated (in mg/mL) for each digestion mixture was converted to *anhydro*-glucose and *anhydro*-cellobiose concentrations, respectively, by subtracting out the proportional weight added to each molecule by the water of hydrolysis. The sum of the concentrations of *anhydro*-glucose and *anhydro*-cellobiose, which sum is equivalent to the weight-concentration of the glucan chain that was hydrolyzed to produce the soluble sugars, was then divided by the initial weight-concentration of glucan and xylan in the digestion mixture and multiplied by 100% to yield activity results as percent conversion.

Fluorescent Microscopy of *P. putida*. *mcl*-PHA accumulation was visually assayed using epifluorescence microscopy. To prepare cells for imaging, 1 mL of culture grown in APL was harvested at t=0, 6, 12, 24, and 48 hours post-inoculation via centrifugation at 5,000 × *g* at room temperature for 5 min. The culture supernatant was removed, and cells were washed twice in PBS, fixed in 3:1 ethanol:acetic acid for 10 min, and washed twice in 1X PBS, followed by resuspension in 1 mL PBS, as described previously (11). Cells were stained with 10 μg/mL Nile Red (Molecular Probes, Invitrogen Corporation) for 5 min, and immobilized on microscope coverslips by mixing with 1% low-melting-temperature agarose (heated to 65°C to solubilize) in a 1:1 ratio. Images were acquired using a Nikon Eclipse 80i microscope. Nile Red fluorescence was detected between 560 and 590 nm using band-pass filtering.

Fluorescence Quantitation of *P. putida*. Fluorescence emission of Nile Red was obtained using a FLUOstar Omega microplate reader (BMG Labtech), equipped with emission and excitation filters of 485/12 and 590/10 nm, respectively. Cells were harvested, diluted to OD_{600} = 0.1 in M9 media, washed in PBS, and stained with Nile Red, as described above. Top optic positioning was utilized with 0.2 s positioning delay, a gain setting of 500, and 10 flashes per well. All measurements were obtained in 96-well, black, round-bottom plates (Corning Costar) at room temperature, in 200-μL reaction volumes.

Flow Cytometry of *P. putida*. To assay PHA accumulation, we used Nile Red staining and detection using a FACS aria (BD Biosciences, San Jose CA). One milliliter of cell culture was centrifuged and washed in phosphate buffered saline (PBS). Cells were then stained using 0.5mg/ml Nile Red dissolved in DMSO for 15 minutes, then washed twice in PBS. Samples were loaded into the FACS aria and screened for Nile Red Fluorescence using a 488 nm wavelength laser coupled with 610/20 nm detection. For each sample 20,000 events were recorded to generate the histograms. For time-course experiments, cells were frozen at -20° C following the suspension in Nile Red/DMSO. Following the conclusion of the time course, all samples were analyzed by flow cytometry in parallel.

Accelerated Solvent Extraction of *P. putida*. *mcl*-PHAs were extracted from *P. putida* with dichloromethane using a Dionex 200 and Dionex 350 Accelerated Solvent Extractor (ASE) (Dionex Instruments). Extractions were performed at 50°C and 10 MPa over 4 cycles with a 100% flush volume. The extract was concentrated to 10% of the initial volume using a rotary evaporator, and the mcl-PHAs were precipitated from the crude extract using ice-cold ethanol (36). Residual solvent was removed under flowing N2 at 40°C, prior to drying under vacuum at 40°C overnight to recover purified mcl-PHAs.

Gas Chromatography (GC) Analysis of *mcl*-PHA Monomer Distribution. The *mcl*-PHA monomer hydroxyacid profile was determined by methanolysis of the purified PHA extract with BF_3 (12). Hydroxyacid methyl esters were identified and the distribution quantified by gas chromatography mass spectroscopy (GC-MS) using an Agilent 7890A GC equipped with a 5975C MSD (Agilent Technologies). The GC was outfitted with an Agilent DB-FFAP column (30 m × 0.25-mm id, 0.25-µm film), and helium (0.8 mL/min column flow) was used as the carrier gas. The injector volume was set to 1 µL using an Agilent auto-sampler. The GC/MS method consisted of a front inlet temperature of 250°C, MS transfer line temperature of 250°C, and scan range from 35 m/z to 550 m/z. A starting temperature of 40°C was held for 5 minutes and then ramped at 5°C/min to a temperature of 250°C and held for 20 minutes. HP MSD Chemstation software (Agilent) equipped with NIST11 database Rev. 2.0G (May 19, 2011 build) was used to identity unknown compounds found within the samples. The mass spectrometer signal response for C_8 and C_{10} hydroxyacid methyl esters (HAME) was determined with HAME standards prepared from known quantities of 3-hydroxyoctanoic and 3-hydroxydecanoic acid obtained from Sigma Aldrich. The remaining HAME response factors were normalized to the nearest HAME standard.

Conversion of ^{13}C-Labeled p-coumarate and xylose to *mcl*-PHAs by *P. putida* KT2440. To definitively conclude that lignin-derived molecules are being metabolized to *mcl*-PHAs in the APL, we supplemented APL with labeled p-coumaric acid-1,2,3-$^{13}C_3$ (Sigma-Aldrich), and monitored for the inclusion of ^{13}C in extracted and derivatized *mcl*-PHAs via Gas Chromatography-Isotope-Ratio Mass Spectrometry (GC-IRMS). As a negative control, we also separately used labeled D-xylose-$^{13}C_5$ (Omicron), a compound present in APL that is unable to be metabolized by *P. putida* KT2440. To ensure sterility, APL was centrifuged (30', 22,000 x g) and the supernatant was sequentially filtered via vacuum filtration using reduced pore sizes (11 µm, 6 µm, 2 µm, to minimize filter clogging) followed by sterile 0.2-µm filtration. As described in the main text, this APL was mixed with 10X M9 salts to make 0.9X APL; 1X M9 (supplemented with 1mM $(NH_4)_2SO_4$). *P. putida* was grown overnight in LB medium, and then diluted 20-fold in LB and allowed to outgrow for 2h. Cells were centrifuged and washed in 0.9X APL; 1X M9 and then inoculated to an OD_{600} of 0.1 in two separate 500-mL baffled flasks in a total volume of 200 mL. These twin cultures were grown for 4 h in the absence of labeled compounds. *mcl*-PHAs accumulation typically is not detectable prior to 6 h in our experiments, and given the small amount of ^{13}C-labeled compounds we were adding to the APL, we wanted to maximize flux to PHAs to ensure detection. Following this 4 h growth, 3 mg of either ^{13}C-labeled p-coumarate or xylose was added (pre-dissolved in 3 mL of M9-medium) to the growing cultures. Cultures were allowed to grow for an additional 44 h (48 h total), then harvested and lyophilized for analysis.

GC Isotope Ratio Mass Spectroscopy (IRMS) Analysis of *mcl*-PHAs

For ^{13}C-analysis by GC-IRMS, lyophilized cell pellets grown on APL supplemented with labeled p-coumaric acid and D-xylose were derivatized directly by methanolysis with BF_3 to produce HAMEs (12). The $^{13}C/^{12}C$ ratios of major derivatized hydroxyacids (3-hydroxyoctanoic acid, 3-hydroxydecanoic acid, 3-hydroxydodecanoic acid) were compared for (1) APL supplemented with ^{13}C-labeled p-coumaric acid, (2) APL supplemented with ^{13}C-labeled D-xylose, and (3) APL without ^{13}C-labeled supplement. HAME $^{13}C/^{12}C$ ratios were identified using a Thermo Trace GC Ultra coupled to a Delta V Advantage IRMS via a GC Isolink Device (Thermo Scientific, Bremen Germany). The temperature in the isolink was set to 100°C. The GC was outfitted with a SGE BPX5 column (60m × 0.25-mm id, 0.25-µm film). Splitless injections were performed with an injection volume of 1 µL or 0.1 µL. All other conditions (e.g., gas carrier, flow rate, injector temperature, oven settings) were identical to those described above for HAME profile analysis by GC-MS. Signal areas from m/z 44, indicative of ^{12}C, and signal areas from m/z 45, indicative of ^{13}C, were used to calculate $\delta^{13}C$ values. Signal areas from m/z 46, indicative of ^{18}O, were not used since oxygen from the combustion tube can be incorporated into in CO_2. To compare levels of ^{13}C-enrichment, $\delta^{13}C$ values were calculated (Eqn S1**)** by comparing the HAME $^{13}C/^{12}C$ ratio to Vienna Pee Dee Belemnite (PDB). Results are

$$\delta^{13}C = 1000\left[\frac{\left({}^{13}C/{}^{12}C\right)_{HAME} - \left({}^{13}C/{}^{12}C\right)_{PDB}}{\left({}^{13}C/{}^{12}C\right)_{PDB}}\right] \quad [S1]$$

Positive $\delta^{13}C$ values are indicative of significant ^{13}C-enrichment due to labeled substrate incorporation into the *mcl*-PHA polymer. The GC-IRMS mass 45 signal intensities, corresponding primarily to $^{13}C^{16}O_2$, were plotted as shown in Figure S8 to compare relative sample HAME monomer distribution ratios.

GPC and Thermal Analysis of *mcl*-PHAs. The mcl-PHA molecular weight distribution was analyzed by GPC with a refractive index detector, as described above, with the exception of a 100-µL injection volume and refractive index detector. The GPC chromatogram and corresponding MW-distribution parameters are shown in Figure S10. Thermal properties of mcl-PHAs were analyzed by differential scanning calorimetry (DSC) and thermal gravimetric analysis (TGA) (Figure S11). The glass transition temperature (Tg) was measured using a TA DSC 2000 (TA Instruments) by heating the sample to 100°C at a rate of 25°C/min under flowing N2 (20 mL/min), followed by a isothermal hold for 1 min, and rapid cooling to -90°C. The melting temperature (Tm) was analyzed using a TA DSC 1000 by heating the sample from 0-100°C at a rate of 10°C/min. Lastly, the thermal decomposition temperature (Td), indicative of 5 wt.% loss, was determined using a Setaram SETSYS Evolution TGA instrument (Setaram) by heating the sample from 50-400°C at a rate of 10°C/min.

GC Analysis of Thermally Depolymerized *mcl*-PHAs. *mcl*-PHA thermal depolymerization products were recovered in dichloromethane, filtered (0.2-µm PTFE), and identified by GC-MS using the method described above for HAME analysis. The total ion chromatogram (TIC) and listing of major identified products with retention times is provided in Figure S12 and Table S2.

GC Analysis of Catalysis Products. Catalytic upgrading products were recovered in dichloromethane, filtered (0.2-µm PTFE), and the distribution quantified by GC-MS using an Agilent 6890N gas chromatograph and 5973N MSD. The GC was outfitted with an Agilent HP-5MS column (30 m × 0.25-mm id, 0.25-µm film), and helium (0.8 mL/min column flow) was used as the carrier gas. The injector volume was set to 1 µL using an Agilent auto-sampler. The GC/MS method consisted of a front inlet temperature of 270°C, MS transfer line temperature of 280°C, and scan range from 35 m/z to 550 m/z. A starting temperature of 35°C was held for 3 minutes and then ramped at 15°C/min to a temperature of 225°C with a final hold time of 1 minute. HP MSD Chemstation software (Agilent) equipped with NIST11 database Rev. 2.0G (May 19, 2011 build) was used to identity unknown compounds found within the samples. Mixed alkane standards (Sigma Aldrich) were used to determine mass spectrometer instrument response factors, and cyclic hydrocarbon response factors were estimated based on the nearest linear hydrocarbon. The total ion chromatogram (TIC) and listing of major identified products with retention times is provided in Figure S13 and Table S3.

SI Figures and Tables

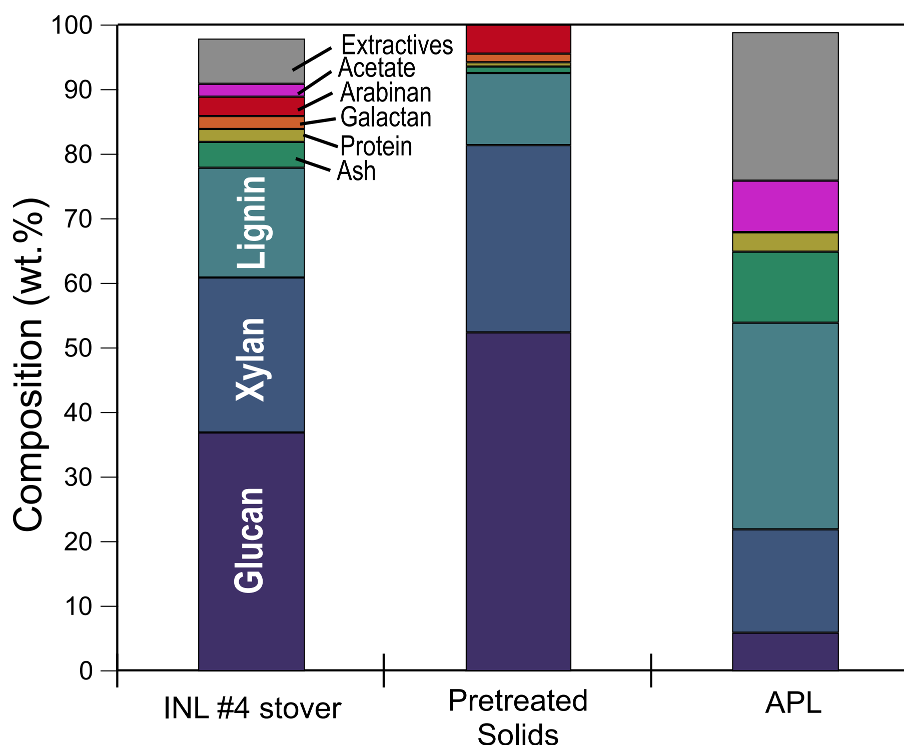

Figure S1. Biomass compositional analysis for the alkaline pretreatment step. The composition of the starting corn stover, resulting pretreated solids, and the composition of the material solubilized into the APL are shown on a w/w basis. The pretreatment conditions for the material shown here are 100°C for 30 minutes with an NaOH loading of 70 mg NaOH/g dry stover at 7 wt% solids and 0.2% AQ charge (w/w on dry corn stover). The initial corn stover (Idaho National Laboratories Lot #4) is shown in the left most bar and has a w/w composition of: 37% glucan, 24% xylan, 17% lignin, 4% ash, 2% protein, 2% galactan, 3% arabinan, 2% acetate, and 7% extractives. The recovered solids are enriched in carbohydrates and retain 67% of the mass of the dry corn stover loaded into the reactor. The measured w/w composition of the pretreated solid, shown in the middle bar, is: 53% glucan, 29% xylan, 11% lignin, 1% ash, 1% protein, 1% galactan, 5 % arabinan, 0% acetate, and 0% extractives. The composition of the material solubilized into the APL is presented in the right most bin labeled "APL" and its w/w composition is: 6% glucan, 16% xylan, 32% lignin, 11% ash, 3% protein, 0% galactan, 0% arabinan, 8% acetate, and 23 % extractives.

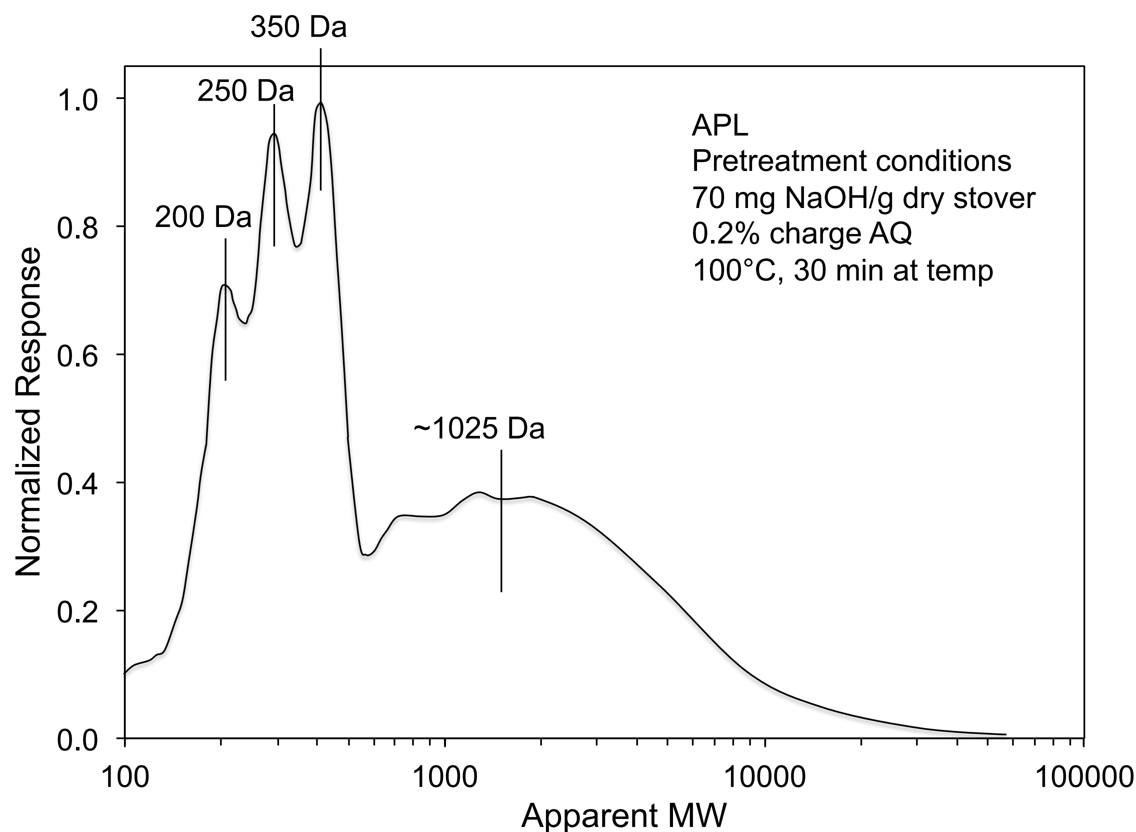

Figure S2. GPC chromatogram of APL. The APL chromatogram shows three large peaks of low molecular weight components at apparent molecular weights of ~200, ~250, and ~350 Da, suggesting the majority of the components in the APL are in the monomer, dimer, and trimer range. A broad peak centered at an apparent molecular weight of ~1,025 Da likely represents lignin fragments that are not fully depolymerized. The overall apparent molecular weight average of the APL is estimated to be 1,100 Da. Here, the larger intensity of the low molecular weight components points to the effectiveness of the anthraquinone additive, which increases the fragmentation of the lignin polymer during pretreatment.

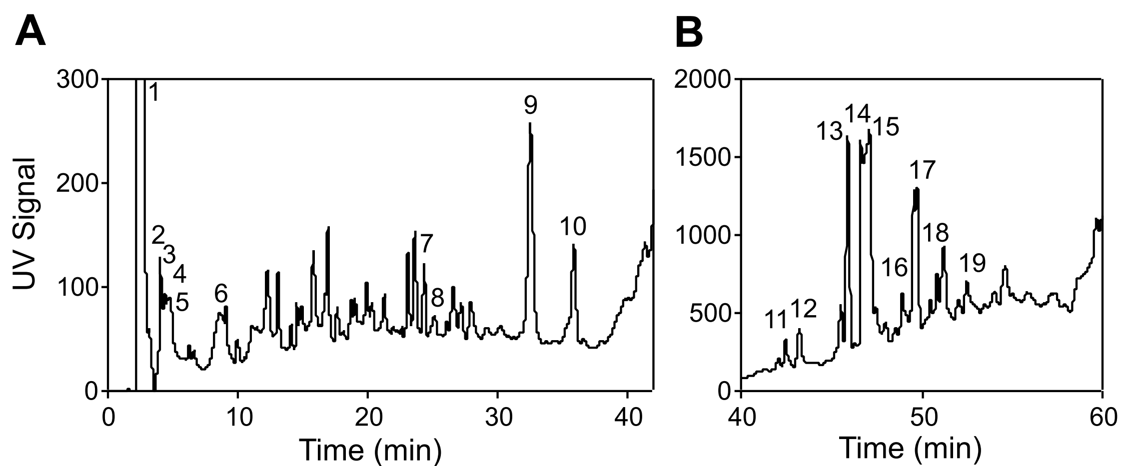

Figure S3. LC chromatogram of APL. (A) Chromatogram obtained from APL using the liquid chromatography method described above, for retention times between 0 and 42 min. (B) Chromatogram from APL for retention times between 40 and 60 min; the y axis scale for these retention times has been expanded from that presented in (A) to capture the full peak heights in this region. Major identified compounds are listed in Table S1.

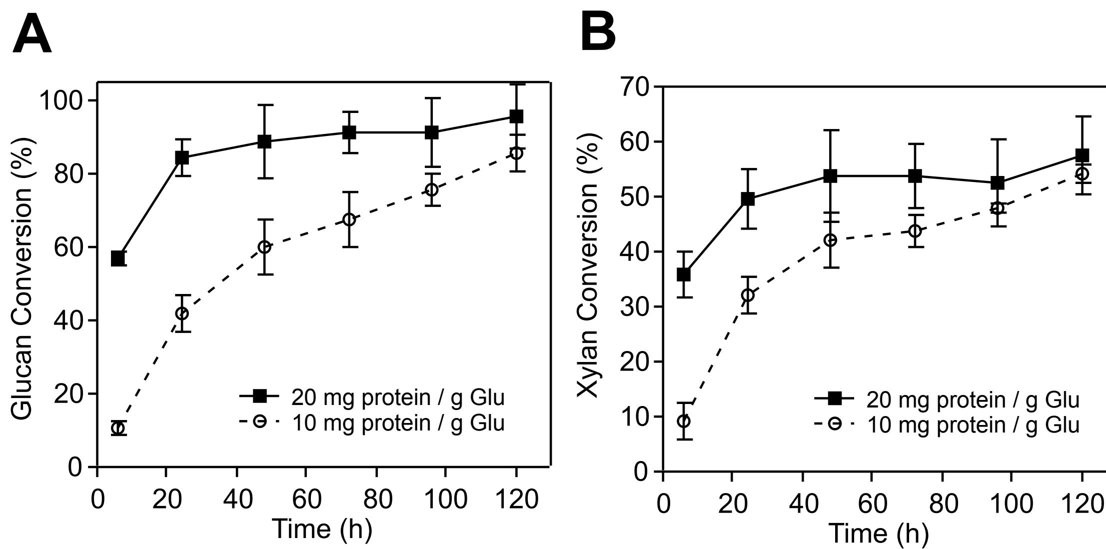

Figure S4. Enzymatic hydrolysis of residual corn stover solids after alkaline pretreatment. (A) Glucan (cellulose) conversion at 20 mg enzyme/g glucan and 10 mg enzyme/g glucan. (B) Xylan conversion at 20 mg enzyme/g glucan and 10 mg enzyme/g glucan. Digestions were conducted at 1 wt% solids loading at 50°C, pH = 5.0.

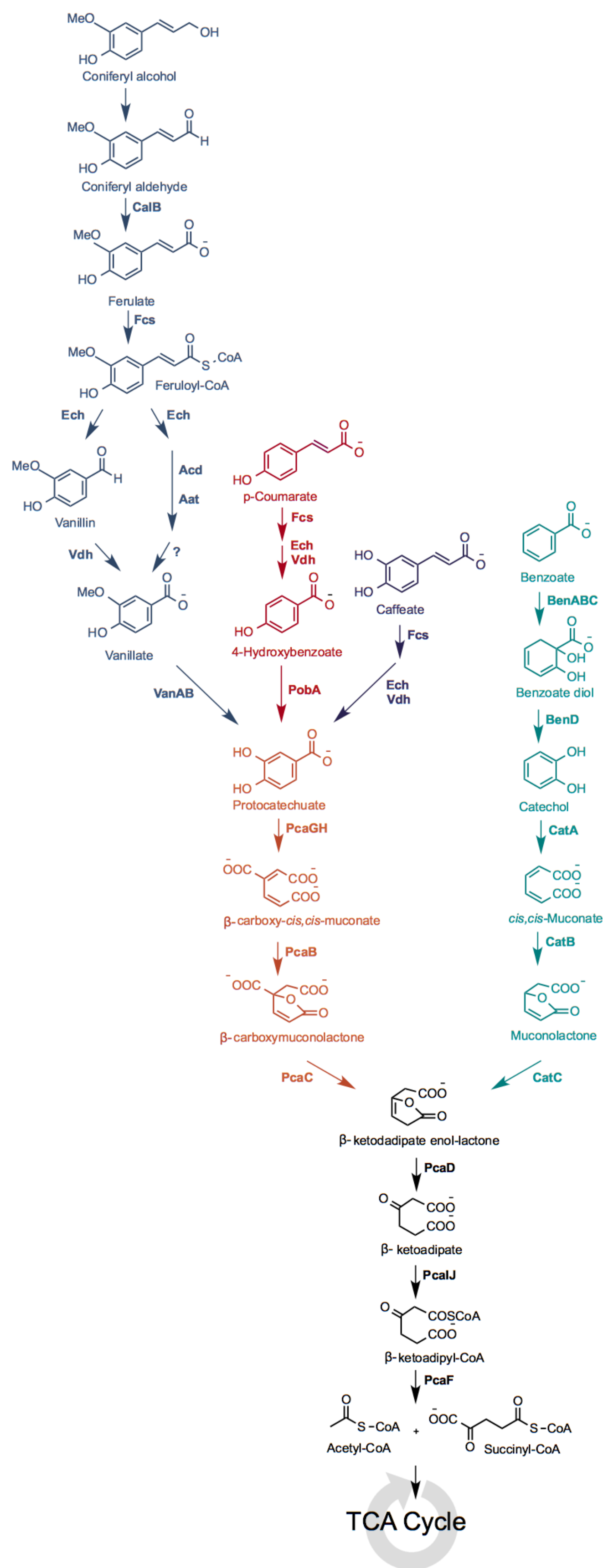

Figure S5. Catabolic pathways for funneling aromatic molecules to central metabolism (TCA cycle) in *P. putida* strain KT2440.

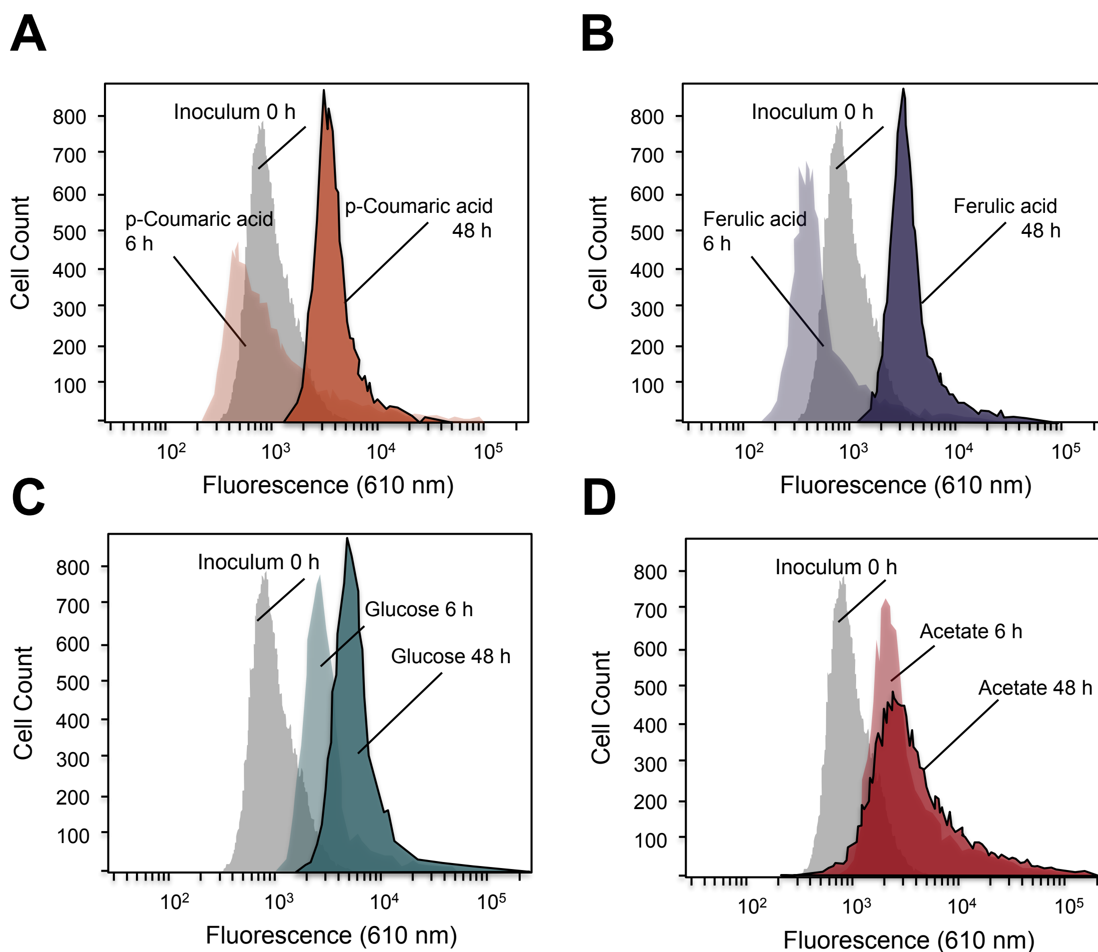

Figure S6. Flow cytometry of model compounds. Shake flask cultures of *P. putida* were grown with single model compounds including (A) p-coumaric acid, (B) ferulic acid, (C) glucose, and (D) acetate, each at a concentration of 2 g/L. Flow cytometry analysis of the relative cell fluorescence (610 nm), indicative of *mcl*-PHA production, was performed on the initial inoculum, 6 h, and 48 h cultivation time points.

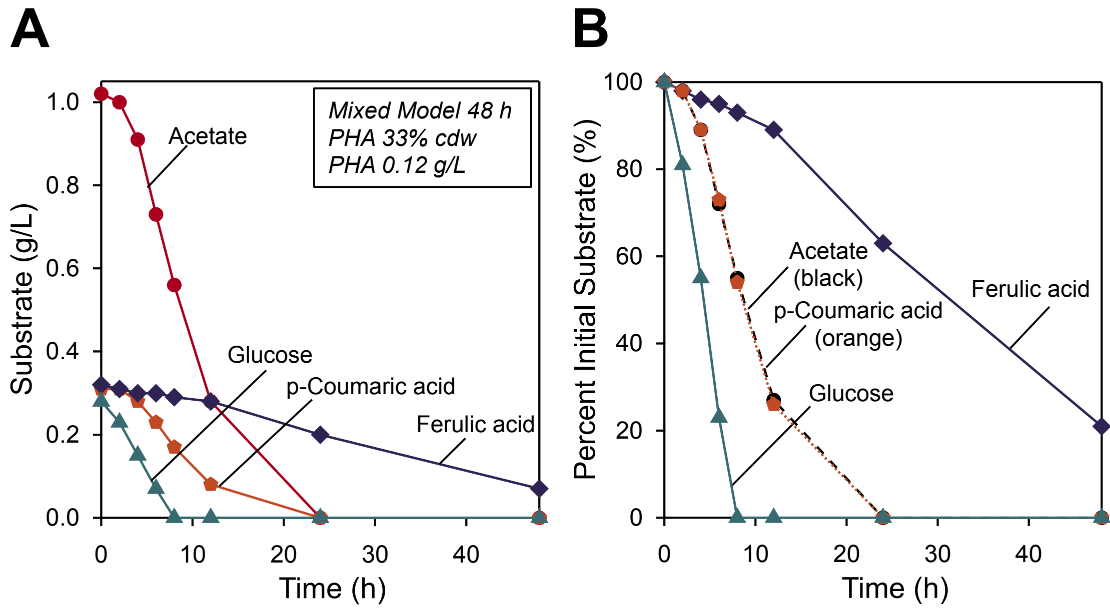

Figure S7. Mixed model compound substrate utilization. Shake flask cultures of *P. putida* were grown with mixed model compounds including p-coumaric acid, ferulic acid, glucose, and acetate, each at a concentration of 0.3 g/L with the exception of acetate at 1.0 g/L. Data are provided in (A) g/L of substrate, and (B) percent substrate concentration normalized to the initial loading, with the corresponding total *mcl*-PHA production shown in the inset.

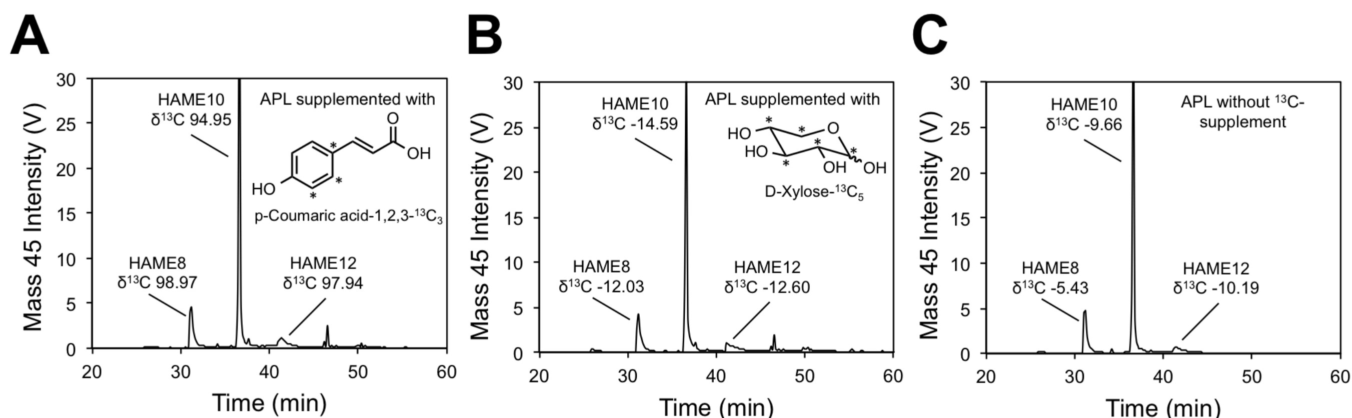

Figure S8. ^{13}C-enrichment levels in *mcl*-PHAs derived from APL. Mass 45 signal intensities and δ^{13}C values for derivatized methyl esters of major hydroxyacids, 3-hydroxyoctanoic acid (HAME8), 3-hydroxydecanoic acid (HAME10), and 3-hydroxydodecanoic acid (HAME12) recovered from cultures grown on complex APL with (A) supplemented ^{13}C-labeled p-coumaric acid, (B) supplemented ^{13}C-labeled D-xylose, and (C) no ^{13}C-labeled supplement. Comparable mass 45 signal profiles indicate similar HAME monomer distribution ratios, while positive δ^{13}C values indicate significant ^{13}C-enrichment due to labeled substrate incorporation into the *mcl*-PHA polymer. The lack of enrichment with ^{13}C-labeled xylose demonstrates that ^{13}C transfer from supplemented carbon sources to *mcl*-PHAs is specific and dependent upon the presence of functional metabolic pathways, as *P. putida* KT2440 is unable to utilize xylose.

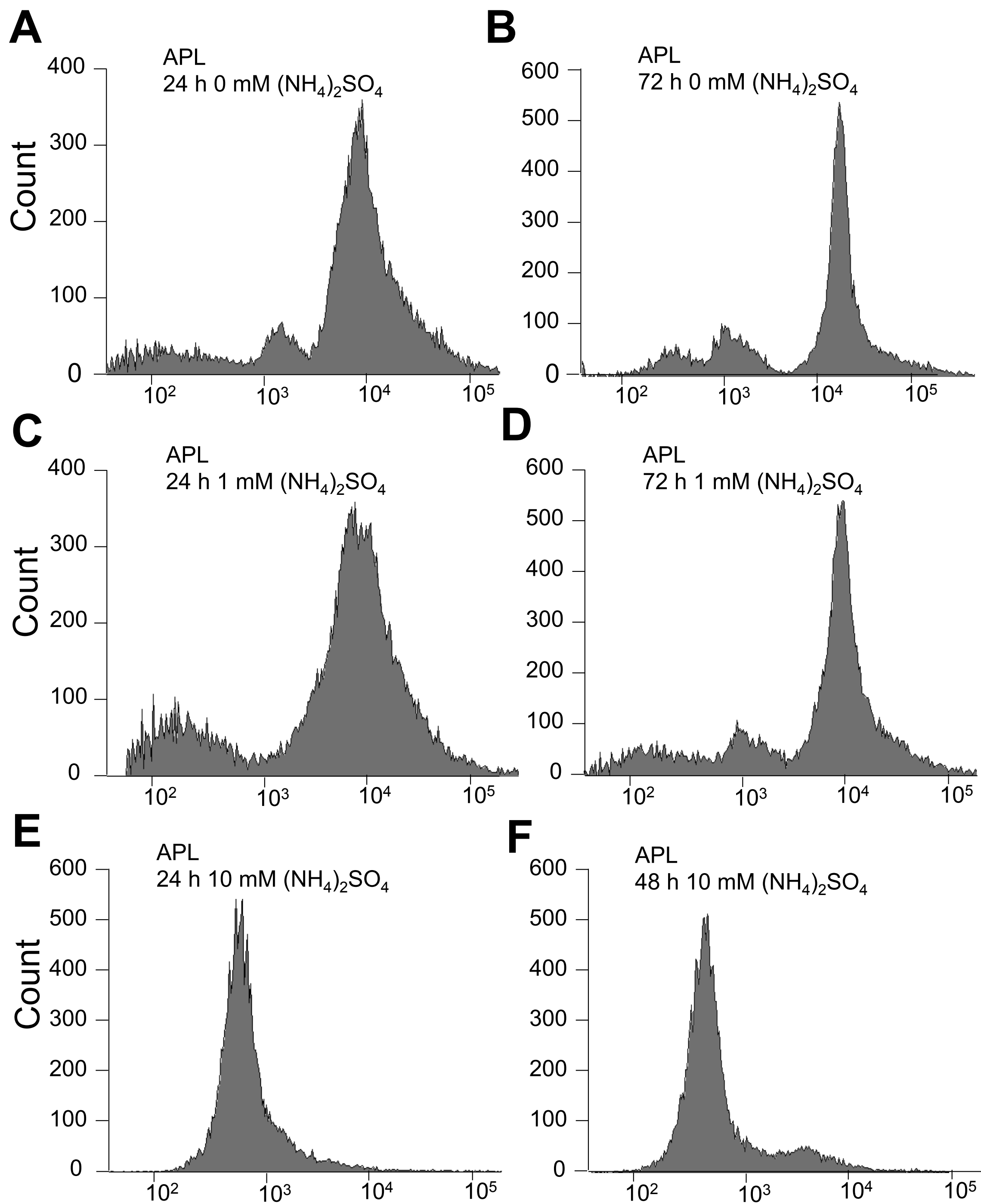

Figure S9. Flow cytometry of APL-derived Nile Red stained cells. *mcl*-PHA production in *P. putida* grown in (A, B) APL or (C, D) APL supplemented with 1 mM $(NH_4)_2SO_4$ during 14-L cultivations. Additionally, a culture grown in APL supplemented with 10 mM $(NH_4)_2SO_4$ (50-mL culture volume in 250-mL baffled flasks) is shown to highlight the nitrogen dependence of *mcl*-PHA production in APL over 48 h (E, F).

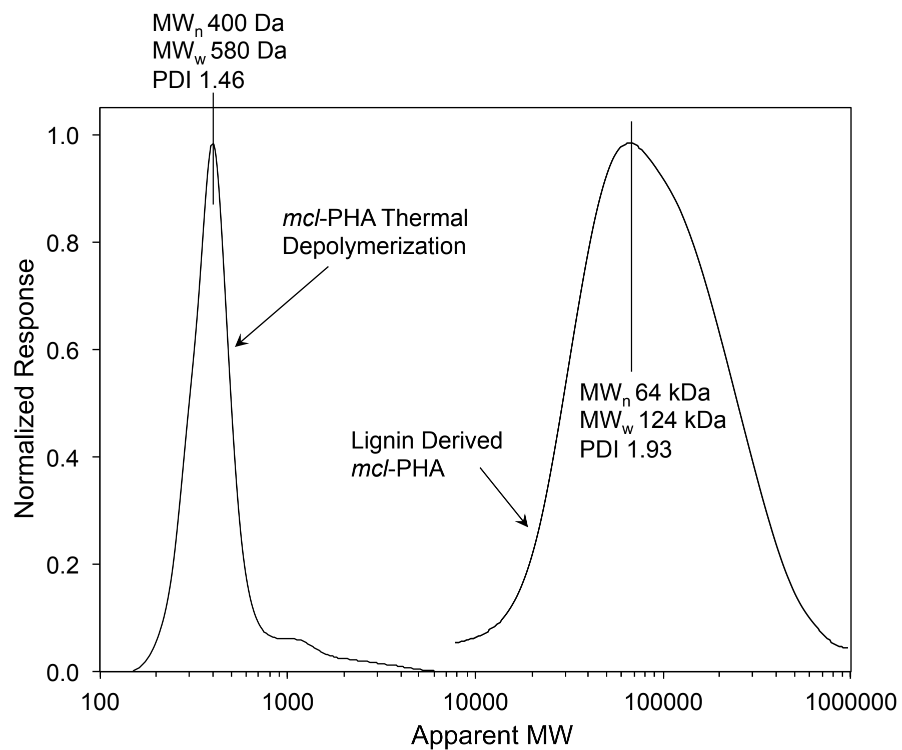

Figure S10. GPC chromatogram of *mcl*-PHAs before (right) and after (left) thermal depolymerization. The molecular weight distribution number average (MW_n), weight average (MW_w), and polydispersity index (PDI) are provided for each sample. Thermal depolymerization of *mcl*-PHAs was performed at 250°C for 30 min at temperature under an inert Ar atmosphere.

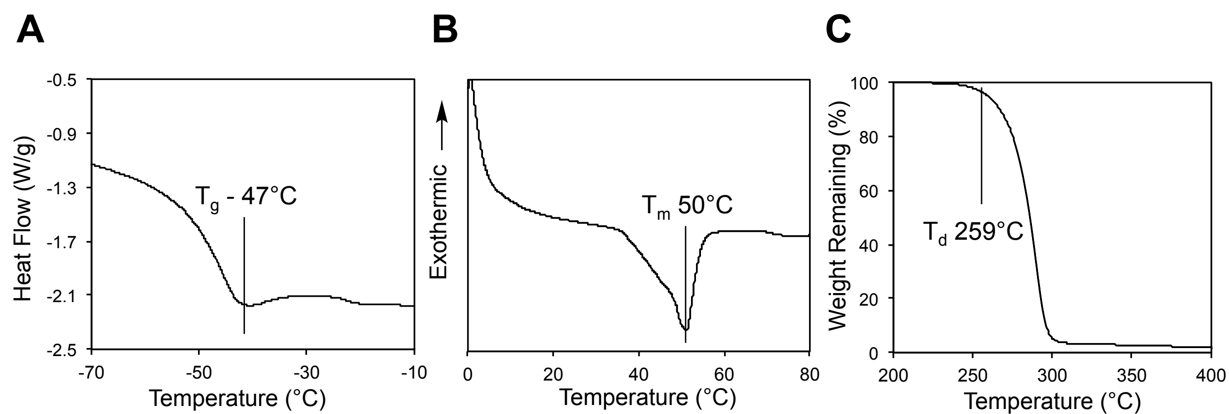

Figure S11. Thermal properties of *mcl*-PHAs determined by DSC and TGA. (A) Glass transition temperature (T_g), and (B) melting temperature (T_m) of *mcl*-PHAs, as measured by DSC. (C) Thermal decomposition temperature of *mcl*-PHAs, indicated by 5 wt% sample loss, as measured by TGA.

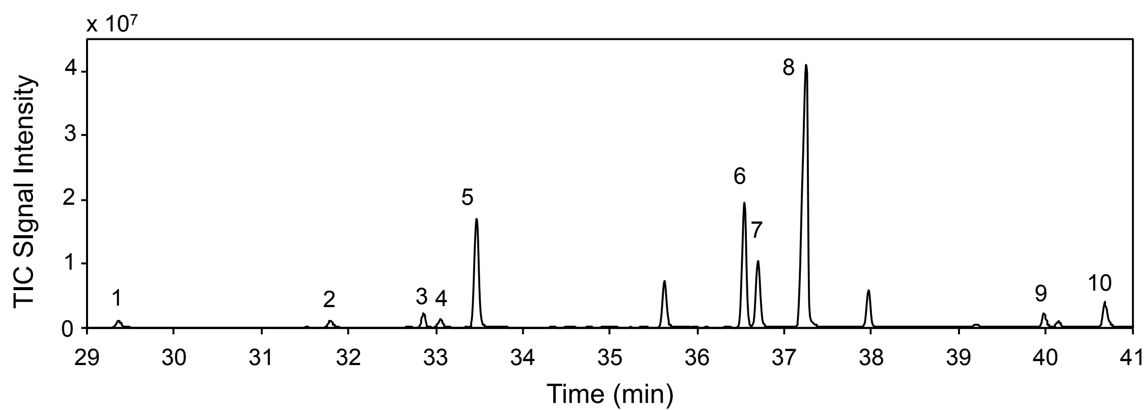

Figure S12. GC-MS TIC of alkenoic acids produced by thermal depolymerization of *mcl*-PHAs. Thermal depolymerization of *mcl*-PHAs was performed at 250°C for 30 min at temperature under an inert Ar atmosphere. Major identified compounds are listed in Table S2.

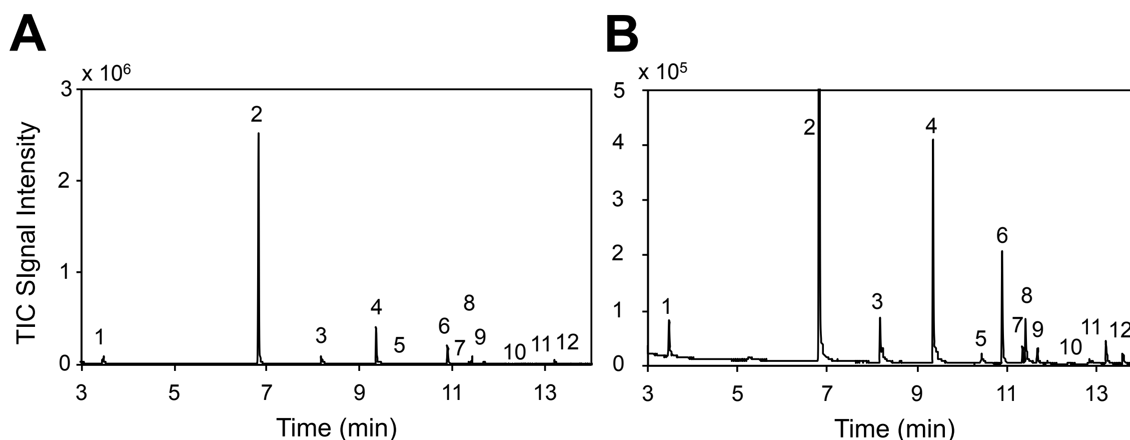

Figure S13. GC-MS TIC of hydrocarbons derived from the thermal depolymerization and catalytic deoxygenation of *mcl*-PHAs. Catalytic deoxygenation of *mcl*-PHAs was performed using with a Pt-Re/C catalyst using water as a solvent at 300°C for 180 min, with an initial reactor headspace pressurized to 2.75 MPa with H_2 at ambient temperature. (A) GC-MS chromatogram and major identified product peaks for retention times between 3 and 14 minutes. (B) Chromatogram y-axis scale has been expanded from that presented in (A) to capture the full peak heights in this region. Major identified compounds are listed in Table S3.

Table S1.

Low molecular weight compounds identified or reported (*) in APL by LC-MS-MS.

Peak No.	RT (min)	Identified or reported compounds in APL
1	2.3	Malonic acid
2	2.7	Malic acid
3	2.8	Acetic acid
4	2.9	Lactic acid
5	4.9	Citric acid
6	9.4	Levulinic acid
7	25.2	Salicylic acid
8	25.3	Vanillyl alcohol*
9	32.5	4-Hydroxybenzaldehyde
10	35.6	Vanillic acid
11	42.5	Syringic acid
12	43.3	Vanillin
13	46.4	Syringaldehyde
14	46.6	Coniferyl alcohol*
15	46.6	p-Coumaric acid
16	47.7	Sinapyl alcohol*
17	49.0	Ferulic acid
18	49.9	Sinapic acid
19	53.4	Coniferyl aldehyde*
ND	-	Eugenol*
ND	-	Guaiacol*
ND	-	Syringol*

* Known from previous literature *(13-23)*

Table S2.

Volatile components identified by GC-MS for the thermal depolymerization of *mcl*-PHAs. Repeated entries are indicative of isomeric forms, which are chromatographically separated.

Peak No.	RT (min)	Catalytic Deoxygenation Species
1	29.4	2-Hexenoic acid
2	32.9	3-Octenoic acid
3	33.1	2-Octenoic acid
4	33.5	2-Octenoic acid
5	36.5	3-Decenoic acid
6	36.7	3-Decenoic acid
7	37.3	2-Decenoic acid
8	40.0	1-Tetradecene
9	40.2	5-Dodecenoic acid
10	40.7	2-Dodecenoic acid

Table S3.

Volatile hydrocarbons identified by GC-MS derived from the thermal depolymerization and catalytic deoxygenation of *mcl*-PHAs. Linear hydrocarbons were matched with known standards and cyclic hydrocarbons were identified using GC-MS NIST library matches.

Peak No.	RT (min)	Compound Name
1	3.4	Heptane
2	6.8	Nonane
3	8.2	Decane
4	9.4	Undecane
5	10.4	Dodecane
6	10.9	Cyclic hydrocarbon
7	11.3	Cyclic hydrocarbon
8	11.4	Tridecane
9	11.7	Cyclic hydrocarbon
10	12.3	Tetradecane
11	12.9	Cyclic hydrocarbon
12	13.6	Branched hydrocarbon

Table S4.

Fluorescence intensity as a function of APL concentration. The fluorescence intensity was normalized at an equivalent optical density.

APL Concentration	Fluorescence Intensity (t=24 hours)
1x	99,600 ± 6,040
5x	105,400 ± 10,842

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