Flow-through solvolysis enables production of native-like lignin from biomass

David G. Brandner,[a] Jacob S. Kruger,[a] Nicholas E. Thornburg,[a] Gregory G. Facas,[a] Jacob K. Kenny,[a,b] Reagan J. Dreiling,[a] Ana Rita C. Morais,[a] Tom Renders,[a,c] Nicholas S. Cleveland,[a] Renee M. Happs,[a] Rui Katahira,[a] Todd B. Vinzant,[a] Daniel G. Wilcox,[a] Yuriy Román-Leshkov,[d,*] Gregg T. Beckham[a,b,*]

a. Renewable Resources and Enabling Sciences Center, National Renewable Energy Laboratory, 15013 Denver West Parkway, Golden, CO 80401, US
b. Department of Chemical and Biological Engineering, University of Colorado–Boulder, Jennie Smoly Caruthers Biotechnology Building, 596 UCB, Boulder, CO 80309, US
c. Department of Chemical Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, US

Materials and Methods

Biomass substrate

Clean chips of hybrid poplar clone OP-367 (*P. deltoides x P. nigra*) harvested by Greenwood Resources in 2013 from Morrow county, OR. The chips were dried in a bale dryer at 135°F for 8 hours, before being ground to pass a 2-inch sieve using a Vermeer BG480 grinder. They were further refined in a bliss hammermill to pass through a ¼" sieve.[1] The final milled particles were sieved through a 2 mm screen.

Catalyst preparation

A 15 wt% Ni/C catalyst was prepared as described by Anderson *et al*.[2] One modification was made to this procedure to ensure the catalyst was fully reduced: namely, the catalyst was reduced under 4% H_2 and 96% N_2, relative to the pure N_2 as described in the original procedure. Briefly summarizing this procedure, nickel nitrate on carbon was heated to 450°C at a rate of 7°C min^{-1} under a flow of 100 mL min^{-1} N_2. Then, the catalyst was held at 450°C for 2 hours under a flow of 4 mL min^{-1} H_2 and 96 mL min^{-1} N_2. The temperature was reduced to 30°C under a flow of 100 mL min^{-1} N2. To passivate the catalyst surface, the catalyst was held under a flow of 5 mL min^{-1} zero air and 95 mL min^{-1} N_2 for 1 hour, followed by a 1-hour hold under flow of 100 mL min^{-1} N_2. This process was repeated twice. A final hold under 5 mL min^{-1} zero air and 95 mL min^{-1} N_2 was carried out until the catalyst was retrieved from the tube furnace.

In situ RCF

Flow

In situ RCF experiments (**Figure 1A** in the main text) were prepared by loading the flow reactor with two 5 g beds of hybrid poplar and one 0.9 g bed of 15 wt% Ni/C. The Ni/C was diluted in 2.1 g of fused silica (Dupre Minerals 30/50 grade) to avoid a significant pressure drop across the catalyst bed. Each bed was plugged with glass wool at both ends. The biomass and catalyst were located in the center of the respective reactor tube with residual space packed with coarse SiO_2 (Sigma-Aldrich fused 4-20 mesh). Additional glass wool plugs were used to separate the inert packing from the reactive material. Each bed was loaded vertically utilizing VCR fittings, and pressure tested to 1.2X reaction operating pressure.

Once the reactors were loaded and pressure tested, one biomass bed was filled with methanol (Sigma-Aldrich reagent ≥99.6%) to a pressure of 1,600 psi. The second biomass bed remained idle for this experiment. Simultaneously, the remainder of the reactor was filled with H_2 to a pressure of 1,600 psi, while heating to 225°C. Once pressure was equilibrated, the biomass bed was heated to 225°C while open to the system with a methanol flow rate of 2.0 mL min^{-1} and H_2 flow rate of 200 SCCM. The biomass bed reached reaction temperature typically in one hour. Initial time points (denoted as time zero in the main text figures) was noted as the time when the biomass bed reached 225°C. Samples of the effluent were then collected every 30 minutes. Upon completion, the reactor was depressurized and cooled under 200 SCCM of N_2. Replicate experiments were completed in the same fashion using the alternate biomass bed.

Batch

In situ RCF reactions were conducted by loading 75 mL Parr reactors with 0.313 g poplar, 50 mg of 15 wt% Ni/C, and 30 mL methanol. Parrs were sealed, flushed 3x with 30 bar He, and pressure tested prior to charging with 30 bar H_2. The reactors were heated to 225°C (usually over 30 mins) and held at temperature for 3 hours, all while stirring at 800 rpm. The pressure at 225°C was ~85 bar. The reactors were quenched in a cold water bath and cooled to room temperature for 30 mins. Finally, the RCF liquor was filtered through 0.2 μm filter.

***Ex situ* RCF**

Flow Solvolysis

Ex situ solvolysis (**Figure 1B** in the main text) was conducted in flow to produce solvolysis liquor by packing the biomass beds as described above. The biomass beds were subsequently filled to 1,600 psi with methanol then heated to 225°C with 2 mL min^{-1} methanol flow. The effluent was directed immediately to knockout pots for collection. A total of twelve 5 g beds of poplar were used to produce 5.5 L of solvolysis liquor.

Batch Solvolysis

Batch solvolysis was conducted in the same manner as batch RCF with two exceptions. No catalyst was loaded to the reactor and the headspace was purged with He but not charged with H_2. The resulting reaction pressure at 225°C was approximately 62.5 bar.

Flow Hydrogenolysis

Ex situ hydrogenolysis experiments were conducted by loading the catalyst bed as described for *in situ* RCF. However, instead of loading the biomass beds, the HPLC pump was used to deliver 2 mL min^{-1} of *ex situ* solvolysis liquor once the catalyst bed has reached 225°C. This feed continued for 3 hours with sampling every 30 minutes as described above. At the three hour mark, the feed was changed from *ex situ* solvolysis liquor to fresh methanol, which was fed for an additional 2 hours to ensure all residual solvolysis liquor was flushed through the reactor. Lignin content for these reactions were calculated based on the difference between the initial and final mass of the solvolysis feed times the equivalent biomass lignin content per unit volume of solvolysis liquor.

Batch Hydrogenolysis

For *ex situ* batch hydrogenolysis, 23.76 g solvolysis liquor was loaded into the Parr reactors, along with 50 mg 15% Ni/C. The reaction was then conducted as described for batch *in situ* RCF.

Solvolysis liquor aging study

As-made

The 5.5 L of solvolysis liquor produced in batch were combined in a 20 L translucent LDPE container. This container was stored, sealed, and placed in secondary containment in a walk-in hood at ambient temperature and pressure. Samples were taken from this container at subsequent aging time points to test in either batch or flow hydrogenolysis experiments.

Reconstituted

To make the reconstituted liquor, 23.76 g aliquots of solvolysis oil was dried by rotary evaporation and stored in clear glass vials at room temperature. To prepare the samples for hydrogenolysis, each was brought up in enough MeOH to have a final mass of 23.76 g. This sample was then run according to the batch hydrogenolysis procedure.

GC-FID analysis

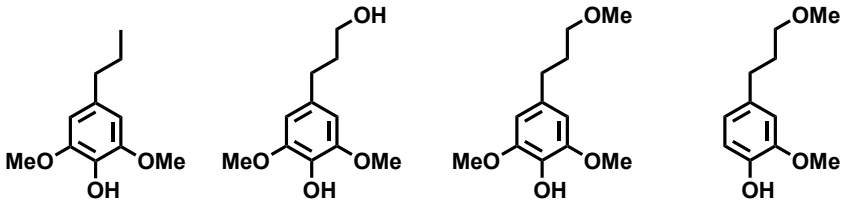
A 200 µL aliquot from each sample was diluted 1:1 with 2 g/L 1,3,5-tri-*tert*-butylbenzene (Sigma Aldrich 97%) as an internal standard. Linear calibrations were created for each hydrogenolysis monomer using authentic standards. All available standards were purchased from Sigma Aldrich. 4-propenylsyringol was purchased from AKos GmbH. Several standards, 4-(3-methoxy)propylguaiacol, 4-propylsyringol, 4-(3-methoxy)propylsyringol, and 4-propanolsyringol, were synthesized in house (*vida infra*) and purity was verified by ^{1}H NMR spectroscopy and GC-MS (data not shown).

Due to the low absolute concentration of monomers in the final hydrogenolysis liquor, the variability of quantification across multiple calibration curves was high. Accordingly, we utilized one calibration curve injected shortly after all standards were synthesized. Calibration verification standards (CVSs) were used to verify that no instrument drift occurred over the course of this study. Freshly injected CVSs proved to be stable over the time of this study, excluding 4-propenylsyringol which appears to degrade rapidly.

Compositional analysis

Compositional analysis on the solids followed the NREL Laboratory Analytical Procedure (LAP).[3,4] Due to a small quantity of sample, this procedure was scaled down to 100 mg opposed to 300 mg as stated in the LAP. Sulfuric acid and water volumes were also scaled down proportionally.

Monomer syntheses for standards



4-Propylsyringol (**1**)

10 wt% Pd/C (0.38 g) was added to a solution of 4-allylsyringol (2.62g, 13.5 mM) in methanol (6 mL). The reaction mixture was kept stirring under H_2 atmosphere. After 19 hours, the Pd/C catalyst was removed by filtration. The solvent in the filtration was then removed by a rotary evaporator. Crude product was purified by flash chromatography (Teledyne CombiFlash equipped with Teledyne 80 and 120 g prepared column) with EtOAc-hexane (1:4) as an eluent to isolate 4-propylsyringol (**1**) (2.30 g, 86.8 mol%).

4-Propanolsyringol (**2**)

BH_3 (10.6 mM) was added dropwise over 0.5 hour at 0°C to a solution of 4-allylsyringol (1.91 g, 9.83 mM) in THF (90 mL). After stirring the mixture for 2.5 hours, H_2O (3.8 mL) was added slowly to quench the reaction. NaOH aq (3 M, 4.71 mL) and H_2O_2 aq (30 wt%, 2.74 mL) were added at the same temperature. After 1.0 hour stirring, HCl (3 M) was added to acidify the reaction mixture. The product was then extracted with EtOAc (4 x 60 mL), washed with brine and dried under Na_2SO_4. Following evaporation of the solvent, the residue was purified by a flash chromatography to obtain 4-propanolsyringol (**2**) (1.14 g, 54.7 mol%).

4-(3-Methoxy)-propylsyringol (**3**)

Compound **3** was prepared from compound **(2)** in 3 steps. In the first step, BnBr (0.17 mL, 1.41 mM) and K_2CO_3 (0.52 g, 3.77 mM) were added at ambient temperature to a solution of 4-propanolsyringol (**2**) (0.2 g, 0.94 mM) in DMF (2 mL). After stirring the contents for 12 hours, the reaction solution was diluted with H_2O (20 mL) and then acidified with 3 M HCl. The reaction mixture was then extracted with EtOAc (3 x 30 mL), washed with brine and dried under Na_2SO_4. The solvent was removed by evaporation, and then the residue was purified by preparative TLC with EtOAc-hexane (1:1) to produce 3-(4-O-benzyl-3,5-dimethoxyphenyl)-1-propanol **(5)** (0.11 g, 39.0 mol%). In the second step, iodomethane (0.4 mL, 6.26 mM) and silver oxide (I) (174 mg, 0.75 mM) were added at ambient temperature to a solution of compound **(5)** (94.6 mg, 0.31 mM) in acetonitrile (10 mL). After stirring the contents at 70°C for 29 hours, silver oxide was removed by filtration. The filtrate was diluted with H_2O (30 mL) and then extracted with EtOAc. The solid residue was purified by a p-TLC to obtain 3-(4-O-benzyl-3,5-dimethoxyphenyl)-1-methoxypropanol **(6)** (76.8 mg, 77.6 mol%). In the third step, 10 wt% Pd/C (30 mg) was added and then the reaction mixture was kept stirring under H_2 atmosphere to a solution of compound **(6)** (75.0 mg, 0.24 mM). After 2.5 hours, the Pd/C catalyst was removed by filtration. The solvent in the filtration was removed under reduced pressure. The solid residue was purified by a p-TLC to isolate 4-(3-methoxy)-propylsyringol **(3)** (27.0 mg, 50.3 mol%).

4-(3-Methoxy)-propylguaiacol (**4**)

Compound **(4)** was prepared from eugenol in 4 steps. In the first steps, BH_3 (60 mM) was added dropwise over 1 hour at 0°C to a solution of eugenol (7.78 g, 46.9 mM) in THF (50 mL). After stirring the contents for 1.5 hours, H_2O (8 mL) was added slowly, and then NaOH aq (3 M, 20 mL) and H_2O_2 aq (30 wt%, 20 mL) were added at the same temperature. After 1.5 hours stirring, HCl (3 M) was added to acidify the reaction mixture which was then extracted with EtOAc (3 x 100 mL), washed with brine and dried under Na_2SO_4. After removal of the solvent by evaporation, the residue was purified by flash chromatography to obtain 4-propanolguaiacol **(7)** (2.61 g, 30.3 mol%). In the second step, BnBr (0.24 mL, 1.99 mM) and K_2CO_3 (1.14 g, 8.25 mM) were added at 0°C to a solution of compound **(7)** (0.3 g, 1.65 mM) in DMF (3 mL). After stirring the contents for 44 hours, K_2CO_3 was removed by filtration. The reaction mixture was extracted with EtOAc, washed with brine and dried under Na_2SO_4. The solvent was removed by evaporation, and then the residue was purified by a preparative TLC with EtOAc-hexane (1:1) to produce 3-(4-O-benzyl-3-methoxyphenyl)-1-propanol **(8)** (0.32 g, 71.0 mol%). In the third step, iodomethane (0.12 mL, 2.0 mM) and silver oxide (I) (54.2 mg, 0.23 mM) were added to a solution of compound **(8)** (53.0 mg, 0.20 mM) in acetonitrile (7 mL). After refluxing for 23 hours, silver oxide was removed by filtration. The filtrate was acidified with 1 N HCl and then

extracted with EtOAc. The solid residue was purified by a p-TLC to obtain 3-(4-O-benzyl-3-methoxyphenyl)-1-methoxypropanol **(9)** (36.3 mg, 65.2 mol%). In the fourth step, the product from step three was dissolved in methanol (2mL) and stirred with 10% Pd/C (30 mg) under H_2 atmosphere. After 2.5 hours, the Pd/C catalyst was removed by filtration. The solvent in the filtration was removed under reduced pressure to yield 4-(3-methoxy)-propylguaiacol (**4**) (23.1 mg, 93.6 mol%).

2D HSQC NMR spectroscopy

Heteronuclear single quantum coherence (HSQC) NMR spectra were acquired on 6 mL aliquots of solvolysis and hydrogenolysis liquors dried to oils and solubilized in 500 µL acetone-d_6 at 25°C on a Bruker Avance III 600 MHz spectrometer at 11.7 T using a room temperature broadband probe. Spectra were acquired with 1,024 points and a SW of 12 ppm in the F2 (^{1}H) dimension and 128 points and SW of 220 ppm in the F1 (^{13}C) dimension using a standard phase sensitive, gradient selected pulse sequence. Native poplar biomass was prepared as describe previously (Happs et al 2021). Briefly, spectra were acquired on 50 mgs of ball milled sample dissolved in DMSO-d6 and pyridine-d5 (4:1, 500 µL) at 25°C on a Bruker Avance Neo 300 MHz spectrometer at 7.05 T with a room-temperature broadband probe using a standard adiabatic HSQC pulse sequence.[5] The spectral processing parameters from Mansfield *et al*.[6] were used and integrations were performed using TopSpin 3.6. Peaks of interest, most notably those arising from β-O-4 bonds, were identified based on previous work and available databases.[7,8]

Gel permeation chromatography

An appropriate amount of solvolysis or hydrogenolysis liquor was dried down to produce 15-20 mg of oil. Samples are then acetylated using 0.5 mL pyridine (Sigma-Aldrich anhydrous 99.8%) and 0.5 mL of acetic anhydride (Sigma-Aldrich reagent plus ≥99%) sealed and heated to 40°C for 24 hours while stirring. Subsequently, 1 mL aliquots of methanol were then added to each sample and dried under N_2. This was repeated five times. Samples are then dried under vacuum at 40°C overnight. Samples are then diluted in THF and stirred for 30 minutes. The THF solution is filtered through a 0.2 µm syringe filter into an HPLC vial. 20 µL of sample is injected on an HPLC fitted with three PLgel 7.5 x 300 mm columns in series: 10 µm x 50 Å, 10 µm x 10^3 Å, 10 µm x 10^4 Å (Agilent Technologies, Stockport, UK) at ambient temperature with an isocratic 1 mL min^{-1} 100% tetrahydrofuran (Sigma-Aldrich inhibitor-free ≥99.9%) for 45 minutes. Analytes are monitored at 210 nm, 260 nm, and 270 nm on the DAD.

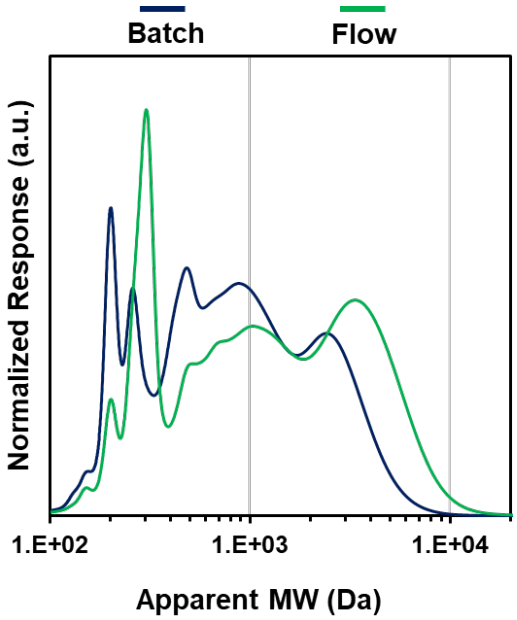


Figure S1. GPC traces for batch and flow solvolysis liquors. Conditions for batch solvolysis: 0.03 g poplar in 30 mL methanol, He purged headspace, 225°C, 3 h (not including 35 min temperature ramp). Conditions for flow solvolysis: 5 g poplar, 2 mL min^{-1} methanol, 225°C, 3 h (not including 1 h heating ramp). All data are normalized by total area.

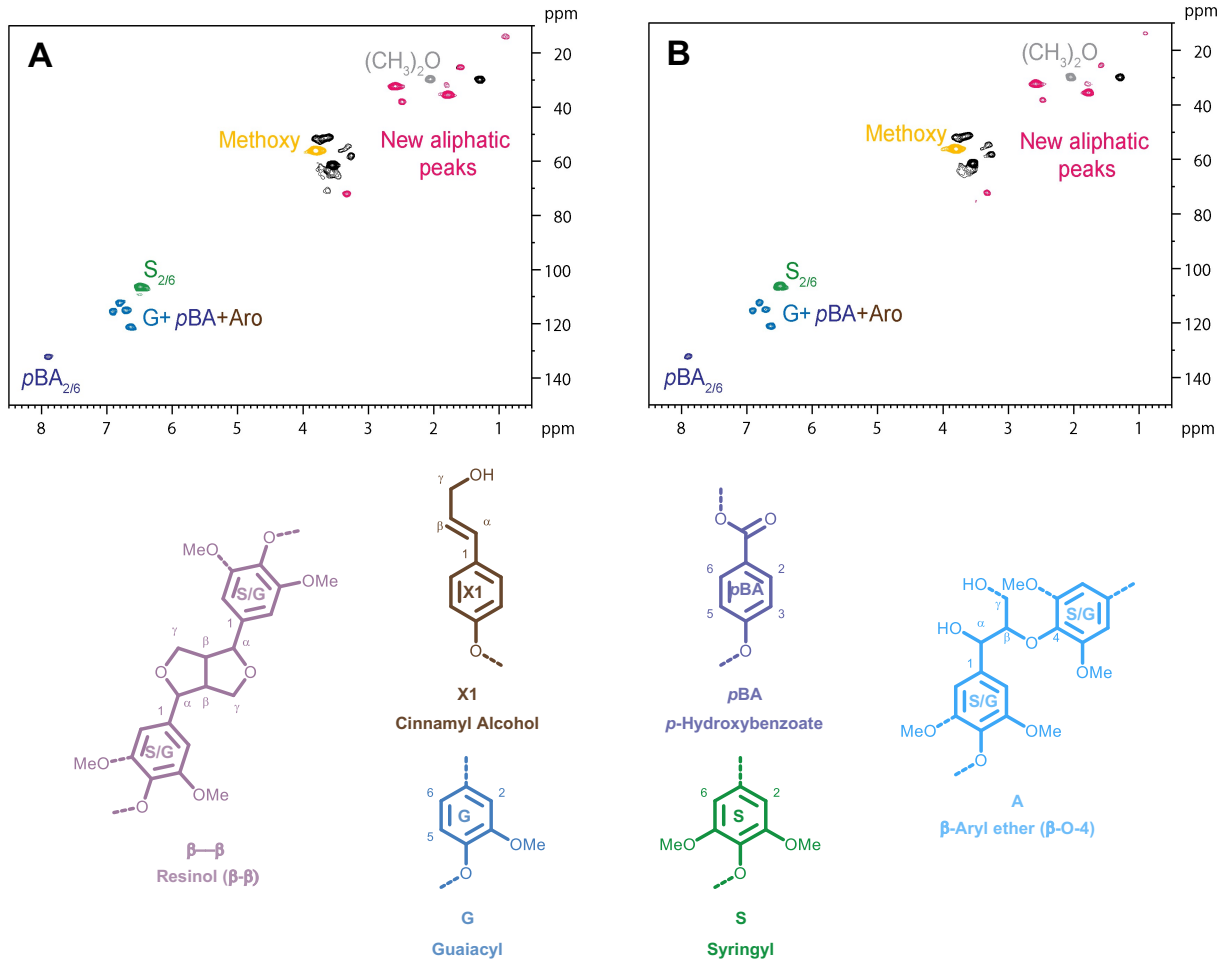


Figure S2. 2D HSQC NMR spectra of *in situ* **(A)** and *ex situ* **(B)** flow RCF. Representative samples collected from 60-90 minutes illustrating the disappearance of β-O-4 linkage. Conditions for flow RCF: 5 g poplar (*in situ*), 2 mL min^{-1} methanol (*in situ*) or solvolysis liquor (*ex situ*), 225°C, 3 h (not including 1 h temperature ramp).

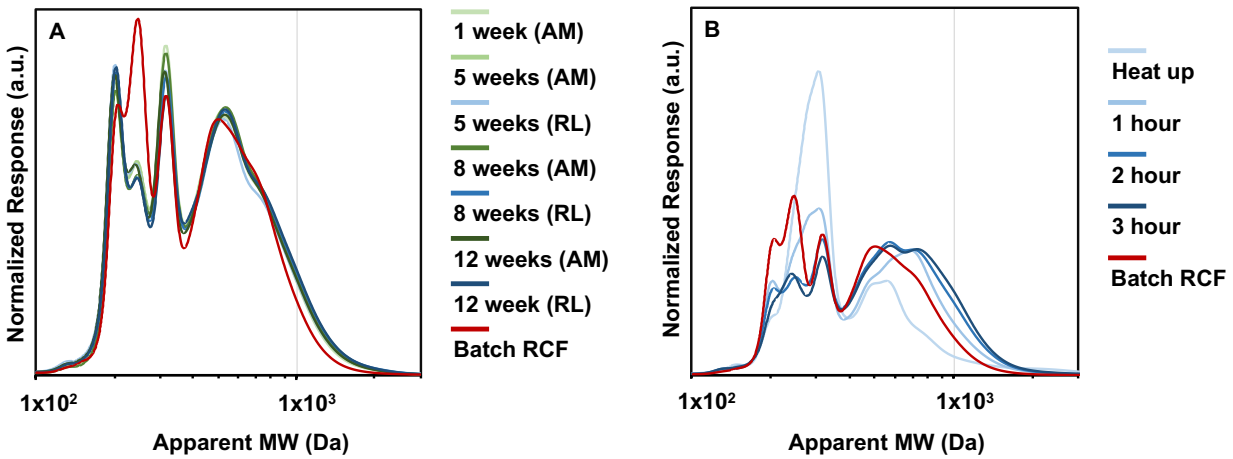


Figure S3. GPC traces of RCF product oils from **(A)** as-made (AM) and reconstituted (RC) solvolysis liquor at different room-temperature aging time points. **(B)** hourly sample time points of *in* situ flow through RCF and *in situ* batch RCF. Conditions for *ex situ* batch RCF: 30 mL *ex situ* solvolysis liquor, 0.1 g 15 wt% Ni/C catalyst, 30 bar H_2 at room temperature, 225°C, 3 h (not including 35 min temperature ramp). Conditions for *in situ* batch RCF, 0.03 g poplar in 30 mL methanol, 30 bar H_2 at room temperature, 225°C (not including 35 min heating ramp), 3 h. Conditions for *in situ* flow RCF: 5 g poplar, 2 mL min^{-1} methanol, 0.9 g 15 wt% Ni/C catalyst, 225°C, 3 h (not including 1 h heating ramp). All data are normalized by total area.

Table S1. Compositional analysis of parent and post-solvolysis poplar.

Run	Substrate	Ash	Extractives	Lignin	Glucan	Xylan	Galactan	Arabinan	Mannan	Acetyl	Total
1	Poplar	0.69	3.54	25.95	45.31	13.24	1.34	0.14	2.79	3.84	97.43
2	Post-Solvolysis Poplar	0.77	0	13.72	65.42	19.69	1.43	0	3.41	0.07	104.51

Table S2. Monomer yield and selectivity data from *in situ* and *ex situ* RCF experiments in batch and flow-through modes. All experiments were conducted in duplicate, and the error bars are the average and range of the data.

Run	Substrate	PG	IEG	P(OMe)G	PS	P(OH)G	P(ene)S	P(OMe)S	P(OH)S	Total	Error +/-
1	*In situ* Batch RCF	6.5%	3.3%	0.0%	11.4%	6.6%	0.0%	0.0%	9.1%	36.8%	0.2%
2	*In situ* Flow RCF	2.8%	1.9%	0.4%	4.8%	6.9%	0.2%	1.4%	13.2%	31.6%	1.3%
3	*In situ* Batch Control	0.0%	4.6%	0.0%	0.0%	0.0%	6.7%	0.0%	0.0%	11.3%	0.3%
4	Batch *ex situ* (Aged 1 week)	2.9%	4.3%	0.0%	5.4%	8.0%	0.0%	0.0%	14.5%	35.2%	0.0%
5	Flow *ex situ* (Aged 4 weeks)	2.1%	2.2%	0.1%	3.3%	8.3%	0.0%	0.0%	16.2%	32.3%	0.0%
6	Flow *ex situ* Control (Aged 6 weeks)	0.0%	3.7%	0.0%	0.0%	0.0%	2.9%	0.0%	0.0%	6.6%	0.4%
7	*Ex situ* Batch RCF	0.0%	3.6%	0.0%	1.7%	3.7%	0.0%	0.0%	9.6%	18.6%	0.2%

Table S3. Time-resolved study of ex situ batch RCF reactions on aged solvolysis liquor produced in flow that is stored in the methanol solvent as well as reconstituted after solvent evaporation. All experiments were conducted in duplicate, and the error bars are the average and range of the data.

Run	Substrate	PG	IEG	P(OMe)G	PS	P(OH)G	P(ene)S	P(OMe)S	P(OH)S	Total	Error +/-
1	1 week	2.9%	4.3%	0.0%	5.4%	8.0%	0.0%	0.0%	14.5%	35.2%	0.0%
2	2 weeks	2.9%	4.2%	0.0%	5.3%	7.8%	0.0%	0.0%	14.1%	34.4%	0.3%
3	3 weeks	3.2%	4.1%	0.0%	5.5%	7.5%	0.0%	0.0%	13.6%	33.9%	0.2%
4	5 weeks	3.2%	4.2%	0.0%	5.7%	7.8%	0.0%	0.0%	13.8%	34.6%	0.3%
5	5 weeks (reconstituted)	2.8%	4.2%	0.0%	5.3%	7.9%	0.0%	0.0%	13.8%	34.0%	0.3%
6	8 weeks	3.0%	4.1%	0.0%	5.3%	8.0%	0.0%	0.0%	14.1%	34.5%	0.1%
7	8 weeks (reconstituted)	2.7%	4.2%	0.0%	5.1%	7.8%	0.0%	0.0%	13.1%	32.7%	0.1%
8	12 weeks	3.1%	4.1%	0.0%	5.5%	8.1%	0.0%	0.0%	14.1%	34.8%	0.1%
9	12 weeks (reconstituted)	2.8%	4.3%	0.0%	5.0%	7.6%	0.0%	0.0%	12.7%	32.5%	0.0%

Table S4. Transient measure of cumulative monomer yields for *in situ* and *ex situ* hydrogenolysis. All experiments were conducted in duplicate, and the error bars are the average and range of the data.

Time (hours)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0
In situ	6.7 +/- 0.4%	20 +/- 0.3%	24.9 +/- 0.5%	27.6 +/- 0.9%	29.3 +/- 1%	30.9 +/- 1.1%	32.2 +/- 1.2%	32.2 +/- 1.2%	32.2 +/- 1.2%	32.2 +/- 1.2%	32.2 +/- 1.2%
Ex situ (4 weeks)	0 +/- 0%	2.2 +/- 0.1%	7.2 +/- 0.3%	12 +/- 0.2%	16.9 +/- 0.1%	21.9 +/- 0.2%	26.6 +/- 0%	30.6 +/- 0%	32.3 +/- 0%	32.3 +/- 0%	32.3 +/- 0%
Ex situ Reconstituted	0 +/- 0%	1.7 +/- 0.2%	6.4 +/- 0.3%	11.7 +/- 0.1%	16.5 +/- 0%	21.3 +/- 0.3%	26.1 +/- 0.4%	29.8 +/- 0.7%	31.6 +/- 0.7%	31.6 +/- 0.7%	31.6 +/- 0.7%
Ex situ Aged (7 weeks)	0 +/- 0%	2.1 +/- 0%	6.8 +/- 0.1%	11.5 +/- 0.1%	16.3 +/- 0.1%	21.5 +/- 0.2%	26.1 +/- 0.4%	30.1 +/- 0.1%	32 +/- 0.2%	32 +/- 0.2%	32 +/- 0.2%

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