Supplemental Information

Method for isopentenol fermentation and analysis

Growth and 3-methyl-3-buten-1-ol (isopentenol) production was performed in *E. coli* BW25113 harboring two plasmids (pBbA5c-MevTsa-MK-PMK (JBEI-6829); and pTrc99a-rNudB-PMD (JBEI parts ID JBx_046690)). Seed cultures were prepared by growing a single colony overnight in LB media containing 100 µg/ml ampicillin and 30 µg/ml chloramphenicol at 37 ºC. The overnight seed cultures were diluted to an OD_{600nm} of 0.07 in 4 ml fermentation media containing EZ-Rich media (10× MOPS Mixture, 0.132 M K2HPO4, 10× ACGU solution and 5× Supplement EZ solution; Teknova, USA), 10.8 g/L glucose, 6.4 g/L xylose, 100 µg/ml ampicillin and 30 µg/ml chloramphenicol with different treatments. Four treatments were 10% [Ch][Glu] + sugars, 10% [Ch][Glu] hydrolysate, 25% [Ch][Glu] + sugars, and 25% [Ch][Glu] hydrolysate. Each of these four treatments was diluted to the fermentation media 0, 2, 4, or 10-fold. All cultures were grown at 37ºC until the OD_{600nm} reached to 0.6-0.8, at which isopentenol fermentation was initiated by addition of 0.5 mM IPTG, and the fermentation was continued at 30ºC for 90 hours. Aliquots of 150-300 µL were collected for OD_{600nm}, sugars, and isopentenol quantification.

For isopentenol analysis, equal parts cell culture and ethyl acetate (containing 30 mg/L 1-butanol as an internal standard) were mixed for 15 minutes then centrifuged to separate the ethyl acetate phase from aqueous phase. The ethyl acetate phase was diluted 5-fold and 1 µL was analyzed with an Agilent GCMS equipped with Cyclosil-B column. For isopentenol production from 25% [Ch][Glu] hydrolysate, the *E. coli* host was serially adapted to the fermentation media containing 4-fold diluted 25% [Ch][Glu] hydrolysate and 2-fold diluted 25% [Ch][Glu] hydrolysate. First, overnight culture of the host strain was diluted to the fermentation media containing 4-fold diluted 25% [Ch][Glu] hydrolysate and grown at 37°C. When the cell cultures reached at OD_{600nm} of 3.6-4 they were diluted 10 times in a fresh fermentation media containing 2-fold diluted 25% [Ch][Glu] hydrolysate and grown overnight (14 hours). On the following day, the overnight cultures (OD_{600nm} of 3.7-4.4) were diluted 20 times in another fresh fermentation media containing 2-fold diluted 25% [Ch][Glu] hydrolysate, and isopentenol production was tested as described for all other fermentation conditions. As positive controls, isopentenol

fermentation of the adapted *E. coli* strains was also examined in the same fermentation media without 25% [Ch][Glu] Hydrolysate.

Major compounds in the hydrolysate

Since there was very limited inhibition of microbial growth in the hydrolysates, we test them for common inhibitors produced during pretreatment. As the ionic liquid concentration increased, the amount of dissolved lignin fragments increased. Table S1 shows some of the common inhibitors identified in the hydrolysate. Furfural and HMF were not identified in the hydrolysates and this likely contributes to the limited inhibition during fermentation. The limited production of inhibitors may be due to the narrower pH range of the choline glutamate process (pH 5 – 12), versus more extreme alkaline or acidic conditions or temperatures employed in other processes such as AFEX or dilute acid (need 2 refs).

Table S1. List of Major Compounds identified by LC/MS

Compound	Formula	RT	m/z	Peak Area	
				10 % IL	25 % IL
Furfural	$C_5H_4O_2$	-	95.01	-	-
5-Hydroxymethylfurfural	$C_6H_6O_3$	-	125.02	-	-
4-Hydroxybenzaldehyde	$C_7H_6O_2$	5.16	121.03	942,826	766,190
4-Hydroxybenzaldehyde	$C_7H_6O_2$	5.95	121.03	122,123	157,616
Syringaldehyde	$C_9H_{10}O_4$	-	181.05	-	-
Vanillin	$C_8H_8O_3$	5.60	151.04	18,617	13,920
Vanillic Acid	$C_8H_8O_4$	4.15	167.03	27,752	36,779
Levulinic Acid	$C_5H_8O_3$	3.26	115.04	28,560	30,293
4-Hydroxybenzoic Acid	$C_7H_6O_3$	4.00	137.02	177,116	126,293
p-Coumaric Acid	$C_9H_8O_3$	4.84	163.04	1,091,498	2,805,354
Syringic Acid	$C_9H_{10}O_5$	4.10	197.05	14,159	16,406
Coumaryl alcohol	$C_9H_{10}O_2$	5.52	151.08	117,574	179,352
Coumaryl alcohol	$C_9H_{10}O_2$	5.76	151.08	82,926	131,938
Coumaryl alcohol	$C_9H_{10}O_2$	7.36	151.08	164,145	92,767
Hydroquinone	$C_6H_6O_2$	6.39	111.04	23,600	44,377

Missing entries in the table were not identified by LC/MS

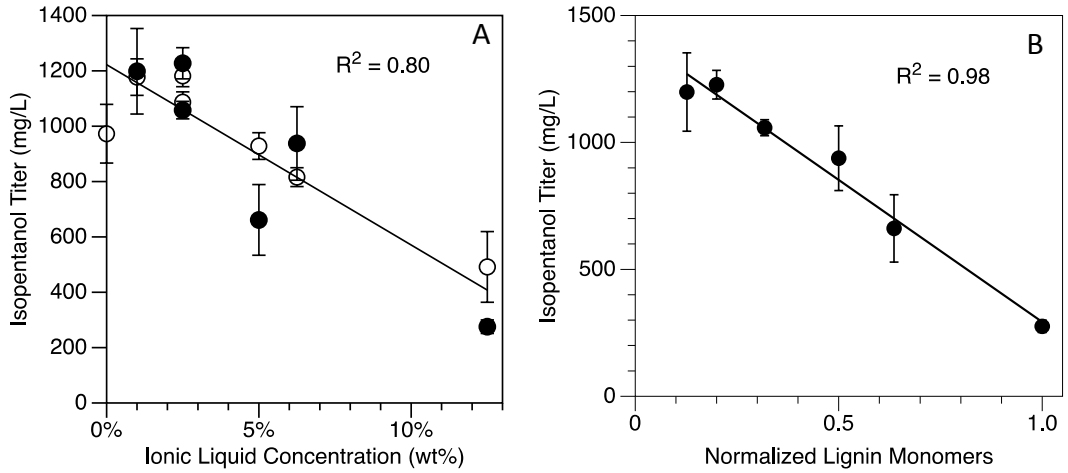
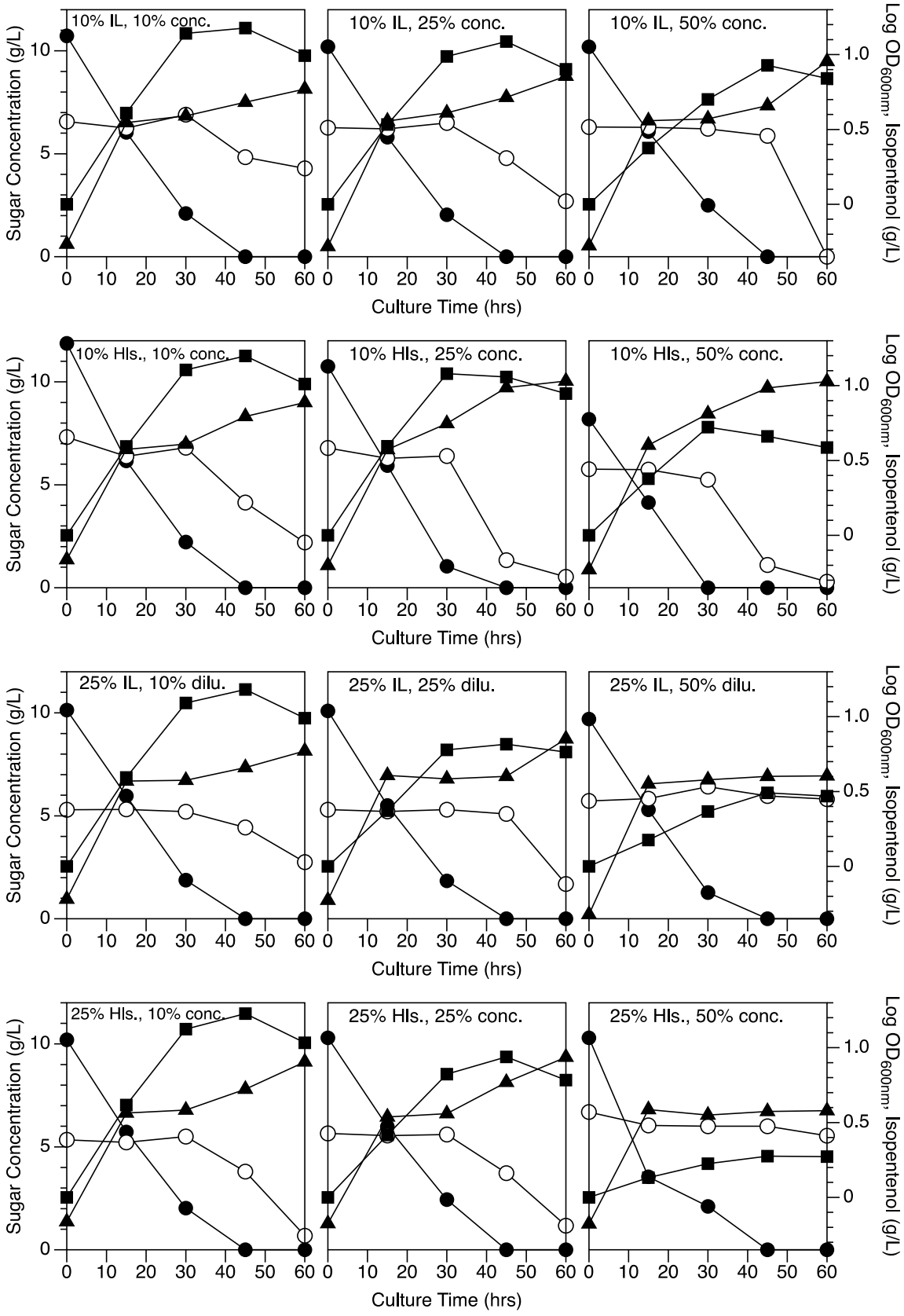


Figure S1: Panel A, Correlation of Isopentenol Production with Ionic Liquid Concentration and Lignin. Panel B, Correlation of isopentenol production with estimated lignin monomer concentration.


10% IL, 10% conc.
10% IL, 25% conc.
10% IL, 50% conc.
10% Hls., 10% conc.
10% Hls., 25% conc.
10% Hls., 50% conc.
25% IL, 10% dilu.
25% IL, 25% dilu.
25% IL, 50% dilu.
25% Hls., 10% conc.
25% Hls., 25% conc.
25% Hls., 50% conc.
Sugar Concentration (g/L)
Log OD600nm, Isopentenol (g/L)
Culture Time (hrs)
0
5
10
0.5
1.0
0 10 20 30 40 50 60

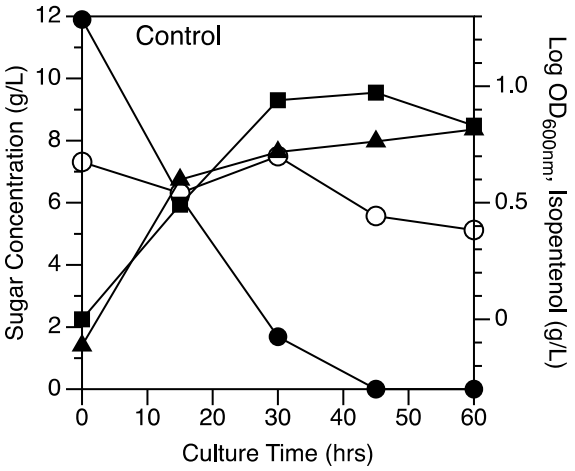


Figure S2: Growth, Sugar Consumption and Isopentenol Production Time-Courses. Glucose concentration (g/L), closed circles; xylose concentration (g/L), open circle; $\log_{10}$ culture OD, triangles; isopentenol concentration (g/L), squares.

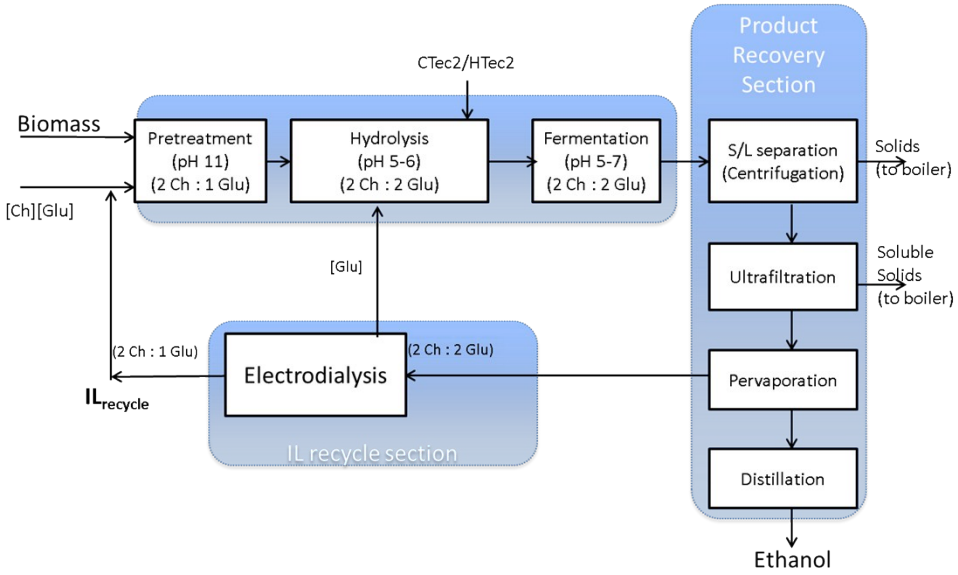


Figure S3: Simplified block flow diagram representation of the integrated biorefinery modeled

Table S2: Key process and cost data used in the techno-economic analysis (TEA)

Biomass processed (dry)	2000 dry MT/day
Biomass price (delivered at plant-gate)	$80/dry ton
Pretreatment	
IL used	[Ch][Glu]
IL purity (wt% of IL in aqueous IL solution [IL:H_2O])	25%
Biomass loading (wt% dry biomass during pretreatment)	20%
Operating temperature	120 C
Operating time	16 hr
Hydrolysis	
Enzyme loading (mg/g cellulose)	20 mg/g
IL concentration in hydrolysis reactor (wt%)	25%
Operating temperature	50 C
Operating time	72 hr
Enzyme price ($/kg protein)	$4.29/kg
Fermentation	
Co-fermentation of glucose and xylose	YES
Overall ethanol yield (gal/ton dry biomass)	76 gal/dry ton
Economic analysis data	
Plant life time	30 yr
Internal Rate of Return (IRR)	10%