ELECTRONIC SUPPLEMENTARY INFORMATION

Integrated diesel production from lignocellulosic sugars via oleaginous yeast

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Experimental

Pretreatment of corn stover biomass and hydrolysate preparation

Corn stover biomass was provided by Idaho National Laboratory in October 2010 and was processed as described by Shekiro *et al.*[1] Briefly, the biomass was hammer-milled and filtered through a rejection screen before de-acetylation with 0.4% (w/w) sodium hydroxide solution to achieve a 8% (w/w) total solids (TS) loading. After de-acetylation, pretreatment was carried out with dilute 0.8% H_2SO_4 (w/w) with 8% (w/w) TS. The resulting pretreated de-acetylated biomass solids were pressed, and the slurry was obtained for enzymatic hydrolysis. Novozymes Cellic CTec2 was added after adjusting the slurry to 20% (w/w) TS with water and to a pH of 5.2 with 5% sodium hydroxide. Enzymatic hydrolysis was carried out with slight agitation at 48°C for 168 h while maintaining a pH of 5.2. The de-acetylated, dilute-acid pretreated, enzymatically hydrolyzed (DDAP-EH) hydrolysate was filter-sterilized and stored at 4°C prior to use. The monomeric sugar composition of the resulting hydrolysate was: 97.47 g L^{-1} glucose, 58.02 g L^{-1} xylose, 3.51 g L^{-1} galactose, 8.56 g L^{-1} arabinose, and 0.24 g L^{-1} fructose.

Biological cultivations

Cultivation media and strains. YPD medium containing 10 g L^{-1} yeast extract (Becton, Dickinson and Company, Franklin Lakes, NJ, US), and 20 g L^{-1} peptone (Becton, Dickinson and Company) was supplemented with 20 g L^{-1} glucose (Sigma-Aldrich, St. Louis, MO, USA) as carbon source (YPD20). YNB medium (Sunrise Science Products, San Diego, CA, US) containing the following ingredients, 1 g L^{-1} KH_2PO_4, 500 mg L^{-1} $MgSO_4$, 100 mg L^{-1} NaCl, 100 mg L^{-1} $CaCl_2$, 0.5 mg L^{-1} H_3BO_3, 0.04 mg L^{-1} $CuSO_4$, 0.1 mg L^{-1} KI, 0.2 mg L^{-1} $FeCl_3$, 0.4 mg L^{-1} $MnSO_4$, 0.2 mg L^{-1} Na_2MoO_4, 0.4 mg L^{-1} $ZnSO_4$, was supplemented as required in each of the performed experiments. Yeast strains used in the study are summarized in **Table S1**. As previously mentioned, some yeast species names have been recently updated based on ribosomal sequencing[2] and revisions on taxonomy (Dr. Kyria Boundy-Mills, personal communication). For clarity, old species names have been kept throughout the text, and the updated name is reported in Table S1. All strains were maintained in YPD agar containing YPD20 medium supplemented with 20 g L^{-1} agar (Sigma-Aldrich), and were stored at -80°C in YPD20 medium supplemented with 30% (v/v) glycerol.

Table S1. Strains used in this study

Strain	# in Fig.1	Source	Origin	New species name / Synonym
Cryptococcus curvatus ATCC 10567	1	American Type Culture Collection	Sputum, The Netherlands	
Cryptococcus curvatus ATCC 20509	2	American Type Culture Collection	Dairy plant	*Trichosporon oleaginosus*
Cryptococcus curvatus CBS 5324	3	Centraalbureau voor Schimmelcultures	Sea of the Bahamas	*Cutaneotrichosporon curvatus*
Lipomyces starkeyi Y-11557	4	Agriculture Research Service Culture Collection (NRRL)	Soil, USA	
Lipomyces starkeyi DSM-70295	5	Liebniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures	Soil, New Jersey, USA	
Lipomyces starkeyi DSM-70296	6	Liebniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures	Unknown	
Lipomyces starkeyi UCDFST 78-23	7	Food Science and Technology Department, UC Davis – Phaff Yeast Culture Collection	Yarrawonga, N.S.W., Australia	

Rhodosporidium babjevae UCDFST 04-877	8	Food Science and Technology Department, UC Davis – Phaff Yeast Culture Collection	Olive fly trapped in olive tree, Davis, CA, USA	*Rhodotorula babjevae*
Rhodosporidium babjevae UCDFST 05-736	9	Food Science and Technology Department, UC Davis – Phaff Yeast Culture Collection	Olive fly walk plate, Davis, CA, USA	*Rhodotorula babjevae*
Rhodosporidium babjevae USDFST 05-775	10	Food Science and Technology Department, UC Davis – Phaff Yeast Culture Collection	Olive tree, Davis, CA, USA	*Rhodotorula babjevae*
Rhodosporidium babjevae USDFST 68-916.1	11	Food Science and Technology Department, UC Davis – Phaff Yeast Culture Collection	Insect frass of Alnus sp., Emory Creek, British Columbia, Canada	*Rhodotorula babjevae*
Rhodosporidium diobovatum USDFST 08-225	12	Food Science and Technology Department, UC Davis – Phaff Yeast Culture Collection	Sea water, South eastern barrier reef, Florida, USA	*Rhodotorula diobata*
Rhodosporidium glutinis UCDFST 05-613	13	Food Science and Technology Department, UC Davis – Phaff Yeast Culture Collection	Olive fly (Batrocera oleae), Davis, California, USA	*Rhodotorula babjevae*
Rhodosporidium glutinis UCDFST 06-542	14	Food Science and Technology Department, UC Davis – Phaff Yeast Culture Collection	Olive fly Batrocera oleae-infested olive, Winters, California, USA	*Rhodotorula babjevae*
Rhodosporidium glutinis USDFST 62-106	15	Food Science and Technology Department, UC Davis – Phaff Yeast Culture Collection	Wasp nest	*Rhodotorula babjevae*
Rhodosporidium paludigenum USDFST 09-163	16	Food Science and Technology Department, UC Davis – Phaff Yeast Culture Collection	Leaf of Desmodium repens, The Netherlands	*Rhodotorula paludigena*
Rhodosporidium sphaerocarpum USDFST 68-43	17	Food Science and Technology Department, UC Davis – Phaff Yeast Culture Collection	Auxotrophic mutant of strain UCDFST 48-23	*Rhodotorula sphaerocarpa*
Rhodosporidium toruloides DSM-10134	18	Liebniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures	China	
Rhodosporidium toruloides ATCC 10788	19	American Type Culture Collection	Wood pulp from conifer, Sweden	
Rhodosporidium toruloides Y-17902	20	Agriculture Research Service Culture Collection (NRRL)	The Netherlands	
Rhodosporidium toruloides Y-27012	21	Agriculture Research Service Culture Collection (NRRL)	Geothermal solids, Yellowstone National Park, USA	
Rhodosporidium toruloides Y-27013	22	Agriculture Research Service Culture Collection (NRRL)	Geothermal solids, Yellowstone National Park, USA	
Rhodosporidium toruloides DSM-4444	23	Liebniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures	Japan	
Rhodosporidium toruloides USDFST 68-264	24	Food Science and Technology Department, UC Davis – Phaff Yeast Culture Collection	Air in Tokyo, Japan	*Rhodotorula toruloides*
Rhodosporidium toruloides DSM-70398	25	Liebniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures	Phenol-containing water	
Saccharomyces cerevisiae D5A	26	American Type Culture Collection	Cheese whey, Colorado, USA	
Sporopachydermia opuntiana USDFST 78-190	27	Food Science and Technology Department, UC Davis – Phaff Yeast Culture Collection	Yarrawonga, N.S.W., Australia	
Trichosporon guehoae USDFST 60-59	28	Food Science and Technology Department, UC Davis – Phaff Yeast Culture Collection	Slime flux of a chestnut tree, Winschoten, The Netherlands	*Cutaneotrichosporon guehoae*
Yarrowia lipolytica YB-387	29	Agriculture Research Service Culture Collection (NRRL)	Steep water, Corn Products, Pekin, IL, USA	
Yarrowia lipolytica YB-421	30	Agriculture Research Service Culture Collection (NRRL)	Milled corn fiber tailings, Corn Processing plant, Pekin, IL, USA	
Yarrowia lipolytica YB-423	31	Agriculture Research Service Culture Collection (NRRL)	Milled corn fiber tailings, Corn Processing plant, Pekin, IL, USA	

Yeast screening. Seed cultures were generated by inoculating cells from YPD agar into 250 mL baffled shake flasks containing 50 mL YPD20 medium. After 24 hours of cultivation, seed cultures were used to inoculate, at an initial Optical Density (OD_{600}) of 1.0, a 500 mL baffled shake flask containing 100 mL

DDAP-EH corn stover hydrolysate diluted with sterile water to obtain an initial sugar composition of 100 g L^{-1} of monomeric sugars (glucose, galactose, xylose, and arabinose). The hydrolysate was supplemented with 2 g L^{-1} yeast extract, 4 g L^{-1} peptone, and 1.6 g L^{-1} YNB (YNB-vitamins, Sunrise Science Products, San Diego, CA, USA), and buffered with 50 mM potassium hydrogen phthalate.[3] Seed cultures were centrifuged and re-suspended in DDAP-EH. Cultivations were performed in a rotary shaker incubator (Excella E24, New Brunswick Scientific, Eppendorf, Hauppauge, NY, USA) where the temperature was kept at 30°C and shaking was set at 225 rpm. Samples for OD_{600} measurement and sugars analysis were taken daily. When glucose and xylose were depleted, cells were harvested for cell dry weight determination and baseline lipid profiling.

DDAP-EH corn stover hydrolysate cultivations. Cells of each yeast strain, propagated for 24 hours in YPD20 medium, were used to inoculate a seed culture at an initial OD_{600} of 0.3. Seed cultures were incubated in 500 mL baffled shake flasks containing 100 mL YNB medium supplemented with 50 g L^{-1} glucose, 1 g L^{-1} yeast extract, and 2 g L^{-1} peptone. Incubation of pre-cultures and seed cultures were performed in a rotary shaker incubator where the temperature was kept at 30°C and shaking was set at 225 rpm (Excella E24). After 24 hours of cultivation, the seed culture was harvested by centrifugation. Cultivations were carried out in 500 mL bioreactors (BioStat-Q Plus, Sartorius, Goettingen, Germany), at an initial OD_{600} of 1.0, containing 300 mL DDAP-EH corn stover hydrolysate, diluted with sterile water to obtain an initial sugar composition of 100 g L^{-1} of monomeric sugars (glucose, galactose, xylose, and arabinose). The hydrolysate was supplemented with 2 g L^{-1} yeast extract, 4 g L^{-1} peptone, and 1.6 g L^{-1} YNB. Temperature was maintained at 30°C, and pH was kept constant at 5.2 by addition of 1M NaOH. Aerobic conditions were obtained by continuously sparging air at 1vvm, and the dissolved oxygen (DO) level was set at 25% by adjusting the agitation speed. Samples for OD_{600} measurements, metabolite analysis, cell dry weight determination, and baseline lipid profiling were taken regularly until sugar depletion. Cells were quickly separated by centrifugation; the supernatant was filtered through a 0.2 nylon μm membrane filter and stored at -20°C until analysis. In case of incomplete sugar depletion, cultivations were considered ended after 216 hours.

Mock hydrolysate cultivations. Seed cultures were generated by inoculating cells from YPD agar into 250 mL baffled shake flasks containing 50 mL YPD20 medium. After 24 hours of cultivation, seed cultures were used to inoculate, at an initial OD_{600} of 1.0, a 1 L baffled shake flask containing 200 mL of mock hydrolysate buffered at pH 5.5 with 50 mM potassium hydrogen phthalate,[3] and supplemented with 2 g L^{-1} yeast extract, and 4 g L^{-1} peptone. Mock hydrolysate had the following composition: 58.2 g L^{-1} glucose, 34.5 g L^{-1} xylose, 5.3 g L^{-1} arabinose, 2.0 g L^{-1} galactose, 66 mg L^{-1} 5-(hydroxymethyl)furfural (5-HMF), 147.8 mg L^{-1} 4-hydroxybenzaldehyde, 12.7 mg L^{-1} vanillic acid, 1.0 mg L^{-1} caffeic acid, 6.7 mg L^{-1} syringic acid, 8 mg L^{-1} vanillin, 33.5 mg L^{-1} *p*-coumaric acid, and 98.5 mg L^{-1} ferulic acid. Seed cultures were centrifuged and re-suspended in mock hydrolysate. Cultivations were performed in a rotary shaker incubator (Excella E24), temperature was kept at 30°C and shaking was set at 225 rpm. Samples for OD_{600} measurement, sugar analysis, and inhibitors conversion determination were taken over the course of the cultivation.

Aromatics cultivations. Seed cultures were generated by inoculating cells from YPD agar into 250 mL baffled shake flasks containing 50 mL YPD20 medium. After 24 hours of cultivation, seed cultures were used to inoculate, at an initial OD_{600} of 1.0, a 500 mL baffled shake flask containing 100 mL of YNB medium buffered at pH 5.5 with 50 mM potassium hydrogen phthalate,[3] and supplemented with either 20 mM *p*-coumaric acid, ferulic acid, vanillic acid or 4-hydroxybenzoic acid as carbon source. Seed cultures

were centrifuged and washed with sterile water prior inoculation. Cultivations were performed in a rotary shaker incubator (Excella E24), temperature was kept at 30°C and shaking was set at 225 rpm. Samples for OD_{600} measurement and aromatic compound determination were taken over the course of the cultivation.

***R. toruloides* (DSM 4444) cultivation for lipid conversion.** Cultivations were carried out in a 10 L bioreactor (New Brunswick Bioflo & Celligen, Eppendorf), at an initial OD_{600} of 1.0, containing 10L YNB medium supplemented with an initial sugar composition of 100 g L^{-1}, maintaining the same sugar ratio found in DDAP-EH corn stover hydrolysate (58.2 g L^{-1} glucose, 34.5 g L^{-1} xylose, 5.3 g L^{-1} arabinose, 2.0 g L^{-1} galactose). Temperature was maintained at 30°C, and pH was kept constant at 5.2 by addition of 1N NaOH. Aerobic conditions were obtained by continuously sparging air at 1.5 vvm, and the DO level was set at 25% by adjusting the agitation speed. Upon sugar depletion, cultivation broth was centrifuged (Sorval LYNX 6000, Thermo Fisher Scientific), the yeast pellet was washed with deionized water, and stored at -80°C until pretreatment and extraction.

Cultivation monitoring and sample collection. Cell concentrations were determined from absorbance measurements at 600 nm on samples diluted with water to give an OD_{600} on the linear range (Genesys, Thermo Scientific, Waltham, MA, USA).

Over the course of cultivations, aliquots were taken at regular time points to monitor sugar utilization, inhibitor compound conversion, and aromatic compound assimilation. Immediately following collection, cells were separated from the fermentation broth by centrifugation (accuSpin Micro 17R, Thermo Fisher Scientific, Waltham, MA, US), the supernatant was filtered through a 0.2 μm nylon membrane filter (SY25GN, mdi Membrane Technologies INC, Harrisburg, PA, USA), and stored at -20°C until analysis.

Cell pellet for dry weight determination and baseline lipid profiling was obtained by centrifugation of fermentation broth (Sorval ST 16R, Thermo Fisher Scientific). Cells were washed twice with deionized water, the supernatant discarded, and the pellet kept at -80°C until further analysis. Cell lyophilization process was achieved using a Freeze Dry System (FreeZone 2.5, Labconco, Kansas City, MO, USA).

Lipid conversion into diesel blendstock

Yeast pretreatment. Pretreatment was carried out in a Zipperclave reactor in batch mode. To a predetermined amount of yeast slurry, enough DI water and sulfuric acid were added to establish an initial concentration of ~8 wt % yeast, 1 wt % H_2SO_4, and 300 or 400 mL total working volume. The mixture was reacted at 170°C for 60 min, after which the reactor vessel was depressurized and quenched in ice. The slurry was then transferred to round bottom flasks and an equal volume of hexane to the initial yeast slurry (at 8 wt%) was added. This mixture was stirred overnight to facilitate extraction of the lipids into the hexane phase, and then allowed to settle for several hours before pipetting out the hexane phase into a second round bottom flask and evaporating the hexane solvent under vacuum to yield a viscous, orange, crude lipid. Extracts from five separate Zipperclave runs were combined to produce enough lipid feedstock for hydrotreating.

Hydrotreating. The two-step process comprised of hydrodeoxygenation (HDO) and hydroisomerization (HI) was performed by the method of Kruger et al.[4] Experiments were conducted in a fixed bed tubular flow reactor. For each stage, the catalyst material was sieved to 60-120 mesh (125-250 μm) and diluted 1:5 with 60-80 mesh SiC. For the HDO step, the reactor bed was ~6 mL, and about 5.2 mL for the HI step. In both cases, the remainder of the reactor tube was filled with 40-50 mesh quartz particles to facilitate mixing

and heat transfer to the reactants. A thermocouple was positioned in the center of the catalyst bed and interfaced with the furnace control software. Heated zones before and after the reactor maintained the reactant and product temperatures at 200°C, after which products approximately C6 and higher were condensed using a tube-in-shell heat exchanger. Uncondensed products were sampled by online GC; liquid products were collected periodically and analyzed by offline GC.

For the HDO step, the catalyst was Pd/C, obtained from Johnson Matthey and used as-received except for crushing and sieving to 60-120 mesh. The oil was diluted to 25% in hexane to facilitate pumping. Reaction conditions were 450°C and 1300 psig, using a mixture of 95% H_2/5% Ar. The Ar served as an internal standard for quantification of gas-phase products. The H_2/feed ratio was 1000 Nm^3/m^3 and the LHSV was 1 h^{-1}. The catalyst was reduced in-situ during the process of bringing the reactor up to operating conditions. Liquid phase products from the HDO step were combined and fractionated into an organic phase, an aqueous phase, and a small amount of waxy solid by centrifugation. The organic fraction contained most of the residual hexane and most of the deoxygenated lipid product. The hexane was evaporated from this phase and the concentrated, deoxygenated lipid product was used as the feedstock in the HI step.

For the HI step, the catalyst was 1 wt% Pt/SAPO-11. The SAPO-11 support was synthesized as part of a previous collaboration between NREL and Utah State University,[5] and contained 10% boehmite as a binder. The crude extrudates were crushed and sieved to 60-120 mesh before loading Pt as an aqueous solution of H_2PtCl_6 by incipient wetness impregnation. After loading the precursor, the catalyst was dried overnight under vacuum at 40°C. Before feeding HDO product, the catalyst was reduced at operating conditions (350°C, 500 psig, using the same H_2/Ar mixture as for the HDO step) before liquid feed commenced. In the HI step, the H_2/feed ratio was 2325 Nm^3/m^3 and the LHSV was 0.5 h^{-1}.

Analytical procedures

Sugar analysis. Analysis of glucose, galactose, xylose, arabinose, and sucrose was performed using an ICS-5000+ system consisting of an AS-AP autosampler, and a pulsed electrochemical detector with a gold electrode and an Ag/AgCl reference electrode (Dionex Corp., Sunnyvale, CA, USA). Samples were diluted to a quantifiable range and 10 µL was injected on to a CarboPac SA-10 Dionex carbohydrates column (4 x 250 mm) paired with a CarboPac SA-10 guard column (4 x 50mm). Sugars were separated with an isocratic flow of 1 mM potassium hydroxide at 1.5 mL min^{-1} for 15 min at 45°C. Sugar standards of glucose, galactose, xylose, arabinose, and sucrose (99% purity) used to construct calibration curves between the range of 1 – 60 mg L^{-1} were purchased from Absolute Standards (Hamden, CT, USA).

Inhibitors conversion and aromatic compounds assimilation analysis. Inhibitor conversion tracking and characterization analysis of cell-free filtered mock hydrolysate samples was performed on an Agilent 1100 LC system equipped with a G1315B diode array detector (DAD) and an Ion Trap SL (Agilent Technologies, Palo Alto, CA, USA) mass spectrometer (MS) with in-line electrospray ionization (ESI). Each sample was injected undiluted at a volume of 25 µL into the LC/MS system. Aromatic compounds and conversion products were separated using reverse-phase chromatography on a Develosil C30 RPaqueous, 5µm, 4.6 x 250 mm column (Phenomenex, Torrance, CA, USA) at an oven temperature of 30°C. The HPLC solvent gradient was performed using eluents of A) water modified with 0.03% formic acid, and B) 9:1 acetonitrile and water also modified with 0.03% formic acid. At a flow rate of 0.7 mL min^{-1}, the gradient chromatography was as follows: starting at 0% B to 7% B in 13 min; 18 min, 7% B; 21 min, 8.5% B; 31 min, 10% B; 55 min, 15% B; and held at 30% B between 64-67 min, for a total run time of 80 min including

equilibrium. Flow from the HPLC-DAD was directly routed to the ESI-MS ion trap for characterizing conversion compounds. The DAD was used to monitor chromatography at 210, 265, and 315 nm for a direct comparison to MS data. MS and MS/MS tuned parameters are as follows: smart parameter setting with target mass set to 165 Da, compound stability 10%, trap drive 50%, capillary at 3500 V, fragmentation amplitude of 0.75 V with a 30 to 200 % ramped voltage implemented for 50 msec, 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^{-1} held at 350°C. MS scans and precursor isolation-fragmentation scans were performed across the range of 40-350 Da in positive- and negative-ion alternating mode.

Vanillin (≥ 99%, purity), vanillic acid (≥ 97%), caffeic acid (≥ 98%), syringic acid (≥ 97%), *p*-coumaric acid (≥ 98%), ferulic acid (≥ 99%), 4-hydroxybenzaldehyde (≥ 98%), furfural (99%), furfuryl alcohol (98%), 5-(hydroxymethyl)furfural (≥ 99%), and 2-furoic acid (≥ 99%) were purchased from Sigma-Aldrich, while 5-(hydroxymethyl)furfuryl alcohol (≥ 98%) was obtained from Santa Cruz Biotechnology (Dallas, TX, USA) and 5-hydroxymethyl-2-furancarboxylic acid (≥ 98%) was purchased from Cayman Chemicals (Ann Arbor, MI, USA). All authentic standards were used to produce external calibration curves in the range of 1 – 100 mg L^{-1}. An internal standard of α-oxo-2-furanacetic acid (≥ 97%, Sigma-Aldrich) was included in all samples and standards at a concentration of 50 mg L^{-1} for normalization purposes.

Similarly, for the evaluation of aromatic compounds assimilation, quantitation of *p*-coumaric acid, vanillin, ferulic acid, and 4-hydroxybenzoic acid (≥ 98%; Sigma-Aldrich) was performed using an HPLC-DAD system. Separations were conducted on a Rezex RFQ Fast Acid H+ (8%), 7.8 × 100 mm column (Phenomenex, Torrance, CA, USA) using an isocratic flow of 5 mM H_2SO_4 at 1 mL min^{-1} for a total run time of 35 mins. Samples were injected at 10 µL onto the column, which was held at a temperature of 85°C. Aromatic compounds were quantitated at an absorbance of 325 nm (*p*-coumaric and ferulic acids) and 225 nm (vanillin and 4-hydroxybenzoic acid).

Fatty acid methyl ester analysis. Baseline lipid profiling of yeast biomass over fermentation time was measured as fatty acid methyl esters (FAME) using an *in-situ*, acid catalyzed, transesterification procedure.[6] Briefly, 7 – 10 mg of lyophilized yeast biomass was transesterified using 0.6 M hydrochloric acid in methanol. FAME were analyzed by GC-FID on an Agilent 7890B system (Agilent Technologies Inc., Santa Clara, CA, USA) and a DB-Wax column, 30 m x 0.25 mm i.d. and 0.25 µm film thickness (J & W Scientific Inc., Folsom, CA, USA). The oven temperature program was as follows: 100°C held for 1 min, then 25°C min^{-1} up to 200°C, and held for 1 min, 5°C min^{-1} up to 250°C, and held for 7 min for a total run time of 23 min with a constant flow of helium as a carrier gas at 1 mL min^{-1}. Quantitation of FAME was based on integration of individual fatty acid peaks using representative external standard calibration curves; additionally, concentrations were normalized against a tridecanoic acid methyl ester internal standard.

Lipid extraction. Lyophilized yeast samples were extracted by accelerated solvent extraction (ASE). Extraction was performed using a Dionex ASE 200 system. Yeast samples (approximately 0.1 g) were loaded into 11 mL stainless steel vessels and extracted with a mixture of chloroform/methanol (2:1, v/v) at a pressure of 10.3 MPa and a temperature of 50°C. Within three static cycles, extractions were held at temperature and pressure for 5 min after equilibrium. Vessels were purged for 60 s with gaseous nitrogen to expel solvents and lipid extractives. Crude lipid extracts were dried under nitrogen gas and stored at -20°C until analysis.

Neutral lipid and free fatty acid analysis. The saponifiable lipid profile of yeast and the quantitation of neutral and free fatty acid lipid classes in the crude lipid extracts were investigated by a separation on a

Waters Acquity UPLC system (Waters Inc., Milford, MA, USA) with MassLynx 4.1 software. An injection volume of 5 µL was loaded onto a Luna Silica(2), 150 mm × 2 mm i.d., 3 µm column (Phenomenex, Torrance, CA, USA). Crude lipid extracts and standards were dissolved in chloroform/methanol (2:1, v/v). Separations were performed at 30°C at a flow of 0.3 mL min^{-1} until 10 min into the chromatography, at which time, the flow was adjusted to 0.2 mL min^{-1} to keep pressure consistent while using a gradient. The separation gradient was as follows: (A) hexane/isopropanol/acetic acid (99.5:0.4:0.1, v/v/v) and (B) ethanol/acetic acid (99.9:0.1, v/v) 10 min, 100% A; 20-21 min, 91% A; 25 min, 0% A, which was held for 5 min before re-equilibrating. Separations were monitored by an evaporative light scattering detector (ELSD) at 35°C with a gain of 16 and 2.5 standard L min^{-1} nitrogen gas. Each lipid class was quantified using external standards purchased from Nu-Chek Prep, Inc. (Elysian, MN, USA) representative of each free fatty acid and neutral lipid class with a purity of ≤ 99%: cholesteryl stearate, cholesterol, palmitic acid, stearic acid, oleic acid, monopalmitin, monostearin, monoolein, dipalmitin, distearin, diolein, tripalmitin, tristearin, and triolein.

Species identification of free fatty acids and neutral lipids from crude lipid extracts was performed using LC/ESI-tandem mass spectrometry (MS/MS). Species separation was achieved via a Waters Acquity UPLC system on an Acquity BEH C8 column, 100 mm × 2.1 mm i.d., 1.7 µm (Waters Corp., Milford, MA) at 60°C. Lipid samples were injected at 1 µL volumes onto the column and eluted with a gradient of (A) acetonitrile/methanol/isopropyl alcohol/acetic acid (500:300:100:4, v/v/v/v) and (B) 4 mM ammonium formate in methanol/water/acetic acid (750:250:4, v/v/v) at a flow rate of 0.2 mL min^{-1}. The gradient was as follows: 0-25.2 min, 55% A; 25.2-33.0 min, 60% A; 33.0-51.5 min, 70% A; and lastly, 51.5-54.8 min, 100% A for a total run time of 60 min including equilibration.

Positive- and negative-ion ESI-MS/MS in centroid data collection mode was performed using a Micromass Q-Tof micro with MassLynx V4.1 software (Waters Corp., Milford, MA). The nebulization gas was set to 600 L h^{-1} at a temperature of 250°C, the cone gas was set to 10 L h^{-1} and the source temperature was set to 100°C. The capillary and cone voltages were set to 3000 V and 35 V, respectively. For MS experiments, data was collected between *m/z* 100-1500 with collision energy of 8 eV and an acquisition rate of 0.4 sec $spectrum^{-1}$. The MS/MS experiments were performed by increasing the collision energy to 30 eV. All analyses were acquired using an independent reference standard of triheptadecanoin (*m/z* 848.7833) via the LockSpra interface. A concentration of 2 pmol μL^{-1} triheptadecanoin in methanol/chloroform (2:1) with 4 mM ammonium formate was infused at a flow rate of 0.2 µL min^{-1} to ensure mass accuracy of the mass spectrometer. The LockSpray frequency was set at 10 sec and averaged over 10 spectra to provide a correction factor. Additionally, each sample was normalized to an internal standard of trinonadecanoin (*m/z* 932.8772), added at a concentration of 50 µg mL^{-1}.

Polar lipid analysis. Flow injection of crude lipid extracts for the characterization of polar lipids was performed using an Agilent system including a 1290 Infinity II high speed pump and a 6470 triple quadrupole mass spectrometer coupled with dual Agilent jet stream electrospray ionization (AJS ESI) (Agilent Technologies, Palo Alto, CA, USA). Each polar lipid class was quantified using available external standards representative of each polar lipid. Standards of 1,2-distearoyl-*sn*-glycero-3-phosphate (PA), 1,2-distearoyl-*sn*-glycerol-3-phosphoethanolamine (PE), L-*α*-phosphatidylinositol from soy (PI), 1,2-distearoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol) (PG), 1,2-distearoyl-*sn*-glycero-3-phospho-L-serine (PS), and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-(2,4-dinitrophenyl) (DNP-PE) were purchased from Avanti Polar Lipids (Alabaster, AL, USA), with the exception of 1,2-distearoyl-*sn*-glycerol-3-phosphcholine (PC) which was purchased from Sigma Aldrich (St. Louis, MO, USA). Crude

lipid extracts and polar lipid standards were dissolved in methanol and spiked with 10 μg mL^{-1} of DNP-PE as an internal standard. Each sample (10 mg ml^{-1}) or standard solution (10 μg mL^{-1} to 200 μg mL^{-1}) was injected at 10 μL into a constant flow of 0.5 mL min^{-1} methanol/acetonitrile (3:1, v/v) with 4 mM ammonium formate from the UPLC pump for a total run time of 2 min. The following source parameters were used for ionization and analysis: capillary voltage 4 kV, nozzle voltage 500 V, drying gas temperature 250°C, sheath gas temperature 300°C, drying gas flow 8 L min^{-1}, sheath gas flow 12 L min^{-1} and the ESI nebulizer gas 25 psi. The fragmentor voltage varied from 124-198 V corresponding with polar lipid class, while collision energy remained constant at 24 eV, with the exception of PA and PC at 20 and 36 eV, respectively. All data were collected within a mass range of 100-1100 Da. **Table S2** depicts the polar lipid class scan types used for identification and quantitation.

Table S2. Summary of molecular ion adduct and fragmentation scan of polar lipid classes used for identification and quantitation

Compound	Adduct	Scan type	Mass (Da)
DPN-PE	$[M+NH4]^+$	Precursor Ion	551
DNP-PE	$[M+NH4]^+$	Neutral Loss	324
PG	$[M+NH4]^+$	Neutral Loss	189
PI	$[M+NH4]^+$	Neutral Loss	277
PA	$[M+NH4]^+$	Neutral Loss	115
PE	$[M+NH4]^+$	Neutral Loss	141
PS	$[M+H]^+$	Neutral Loss	185
PC	$[M+H]^+$	Precursor Ion	184

Lipid hydrotreating product analysis. HDO and HI liquid-phase products were characterized by gas chromatography with mass spectrometry (GC/MS) to identify components and qualitatively assess contents of component classes (**Figure S1**). An Agilent 7890A GC coupled with an Agilent 5975C mass selective detector (MSD) equipped with a DB-5MS column (dimensions: 30 m x 0.25 mm, 0.25μm df) was used for GC/MS analyses. The injection port temperature was set at 275°C with a column flow rate of 1 mL min^{-1} and an injection split ratio of 200:1. Injection volume was 1 μL. Oven temperature was held at 50°C for 2 minutes followed by a ramp of 10°C min^{-1} to a final temperature of 325°C held for 10 min. The MSD was operated in continuous scan mode from m/z 35 to 500 and the transfer line temperature was held at 325°C. Peaks detected were tentatively identified by comparison to the NIST 2011 library of mass spectra with NIST MS Search 2.0 software. A standard mixture of n-alkanes ranging from C5 to C20 was analyzed with the samples to confirm proper operation of the GC/MS system and assignments of compounds identified. Samples were diluted 1:10 volumetrically with dichloromethane for GC/MS analysis.

Cloud points of HI products were determined by differential scanning calorimetry (DSC). Distillation temperatures and n-alkane compositions were measured by GC-FID simulated distillation following ASTM method D2887.

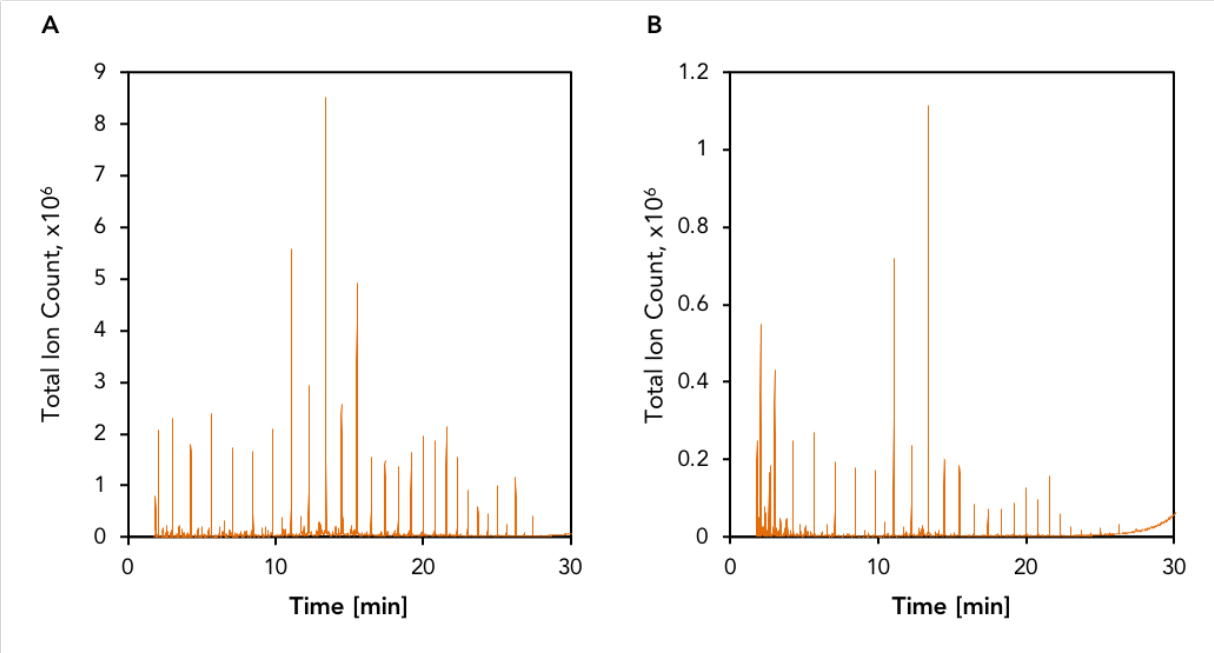


Figure S1. GC chromatogram for HDO (A) and HI (B) products.

TAG and polar lipid speciation

Of the neutral lipid classing, TAG was the most prevalent lipid and the main form of lipid storage, therefore TAG was further speciated by liquid chromatography – mass spectrometry (LC/MS), despite the fact that yeast oils are typically characterized by breaking down TAG and other intact lipids into their fatty acid counterpart, followed by derivatization into FAME. However, knowledge of the intact lipid profile of oleaginous yeast may offer insights for early stage biofuel research, such as strain selection or optimal growth conditions. Additionally, FAME profiling by gas chromatography-flame ionization detection (GC-FID) generally does not account for characterization of odd numbered acyl chains, since authentic standards are not readily available. Although important to understanding TAG synthesis, intact lipid speciation is not comprehensively performed or rarely reported[7, 8] and none to our knowledge using process relevant lignocellulosic feedstocks for cultivations. In this work, a dynamic profiling of TAG over the harvest time was performed using bioreactor cultivations of *C. curvatus*, *R. toruloides*, and *T. guehoae* on DDAP-EH (**Figure S2**). Each TAG species was identified by tandem mass spectra produced from the LC/MS system, where each fragment corresponding to a DAG was back-calculated to elucidate the fatty acyl chain released for identification. Of important note, the order of acyl chains on the glycerol backbone (ie. *sn*-1, *sn*-2, or *sn*-3) was not known since chiral chromatography was not performed; therefore, species were reported from lowest to highest carbon number and highest to lowest unsaturation number. TAG species from 45:0 to 63:3 (carbon:unsaturation, including the carbon from the glycerol backbone) were found in at least one strain at one of the harvest points. Generally, unsaturation levels greater than six and TAG species that contained odd acyl chains were low in relative ion abundance (data not shown), but were nevertheless present in all three strains. As a prevailing trend, each yeast strain began early exponential growth phase with a specific set TAG species, and the species appeared to diversify over time. Exceptions to this were *C. curvatus*, which, at the beginning of the harvest, notably contained a set of 45-49 total carbon TAG species and also *T. guehoae*, which exhibited a set of 61 and 63 total carbon TAG species that were redistributed over harvest time. A total of 314 unique TAG species were identified between the three strains over three harvest time points, with the most abundant ions pertaining to TAG species with the fatty acyl combination of POO, PPO, PLS, POS, PLO, PoOO, OOO, LOO, LLS, LnOS, LLO, LnOO, and LnLS, with P=palmitic (16:0), Po=palmitoleic (16:1), S=stearic (18:0), O=oleic (18:1), L=linoleic (18:2), and Ln=linolenic (18:3). This main acyl chain composition of the TAG species corresponds with the main acyl chains of the FAME profile; however, the FAME profile is limited in comparison. From the FAME profile and ultimately the TAG composition, all three yeast strains paralleled an oil composition similar to pili nut oil.[9] Although, not appropriate for a biofuel stock due to its low lipid content and food application, pili nut oil utilization as a biodiesel has been evaluated to comply with ASTM standards.[10] Additionally, select TAG species and the intact polar lipid species profile (**Figure S3**) consist of odd acyl chain moieties. Odd number fatty acids have been reported in yeasts, where in the biosynthetic pathway these can be synthesized from odd numbered chain precursors, such as propionyl-CoA, or by chain shortening of even chain fatty acids by α-oxidation.[11] Since odd carbon number substrates were not available in the DDAPH-EH corn stover hydrolysate media, the latter is a more probable explanation. Ultimately, intact lipid classing and TAG speciation offers complimentary data to traditional fatty acid profiling of FAME by GC and can be additionally used to design culturing conditions, optimized specifically to promote TAG production as opposed to overall fatty acid content.

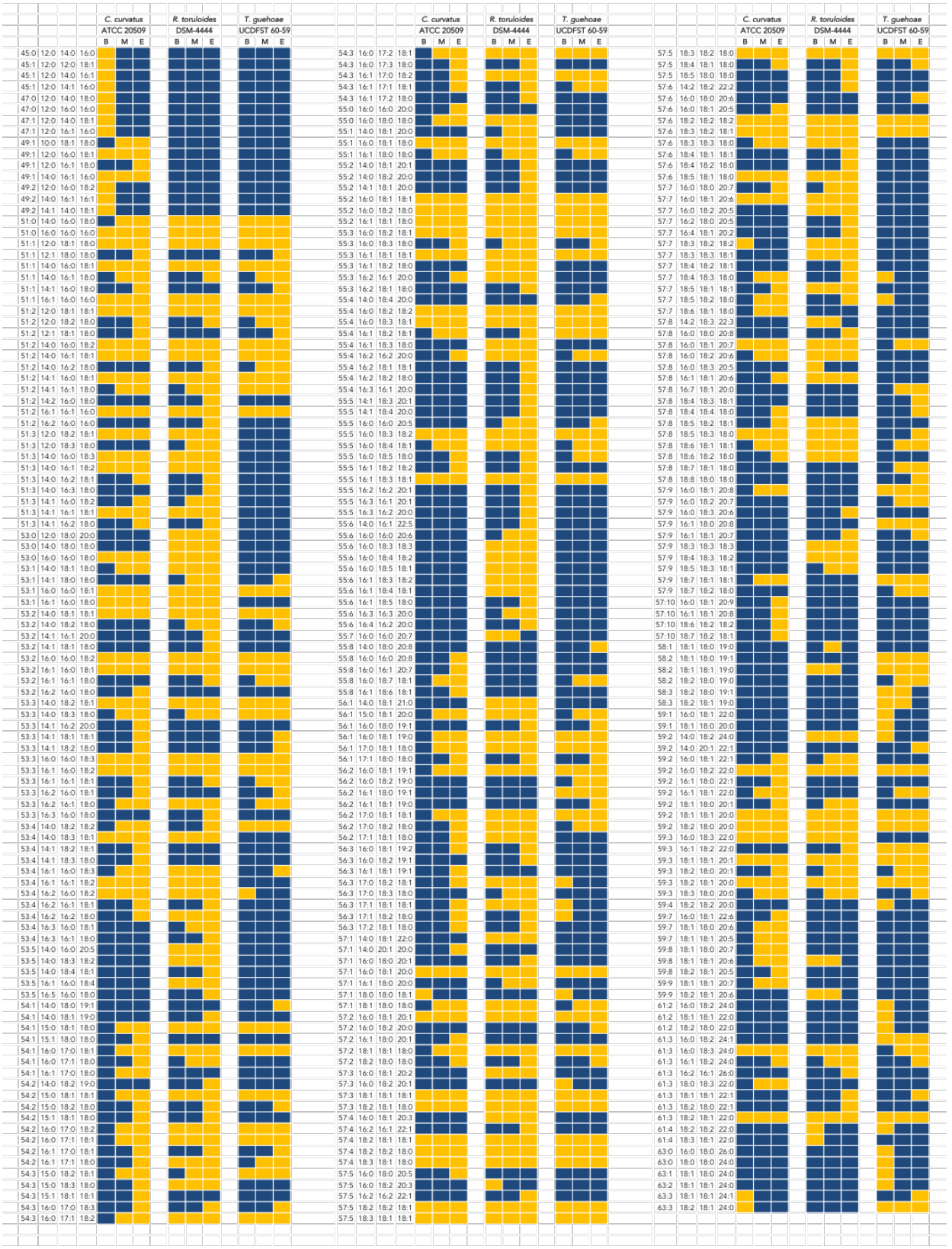
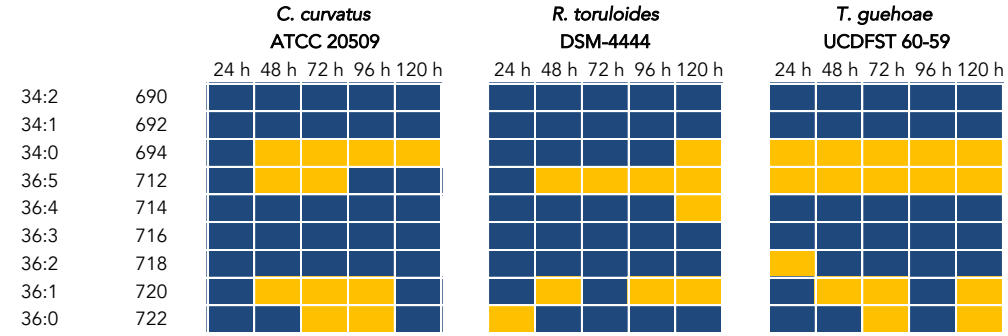
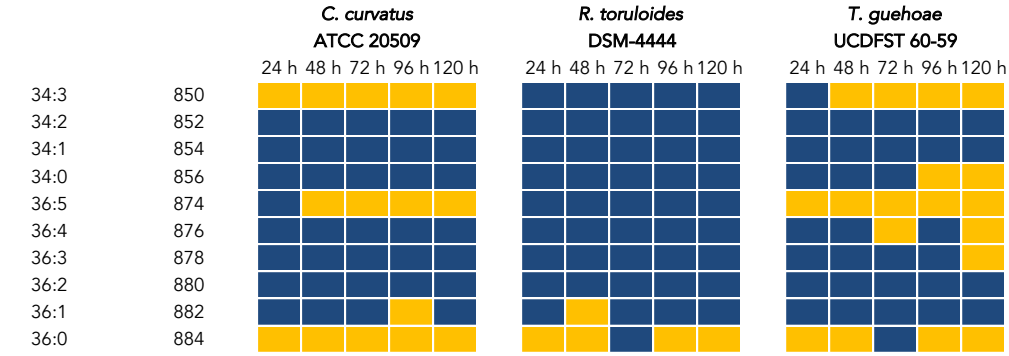


Figure S2. TAG speciation in bioreactor cultivations on DDAP-EH corn stover hydrolysate for *C. curvatus* ATCC 20509, *R. toruloides* DSM-4444, and *T. guehoae* UCDFST 60-59 strains. Presence (blue) or absence (yellow) of each TAG specie at the beginin (B), middle (M), and end (E) of the cultivation.

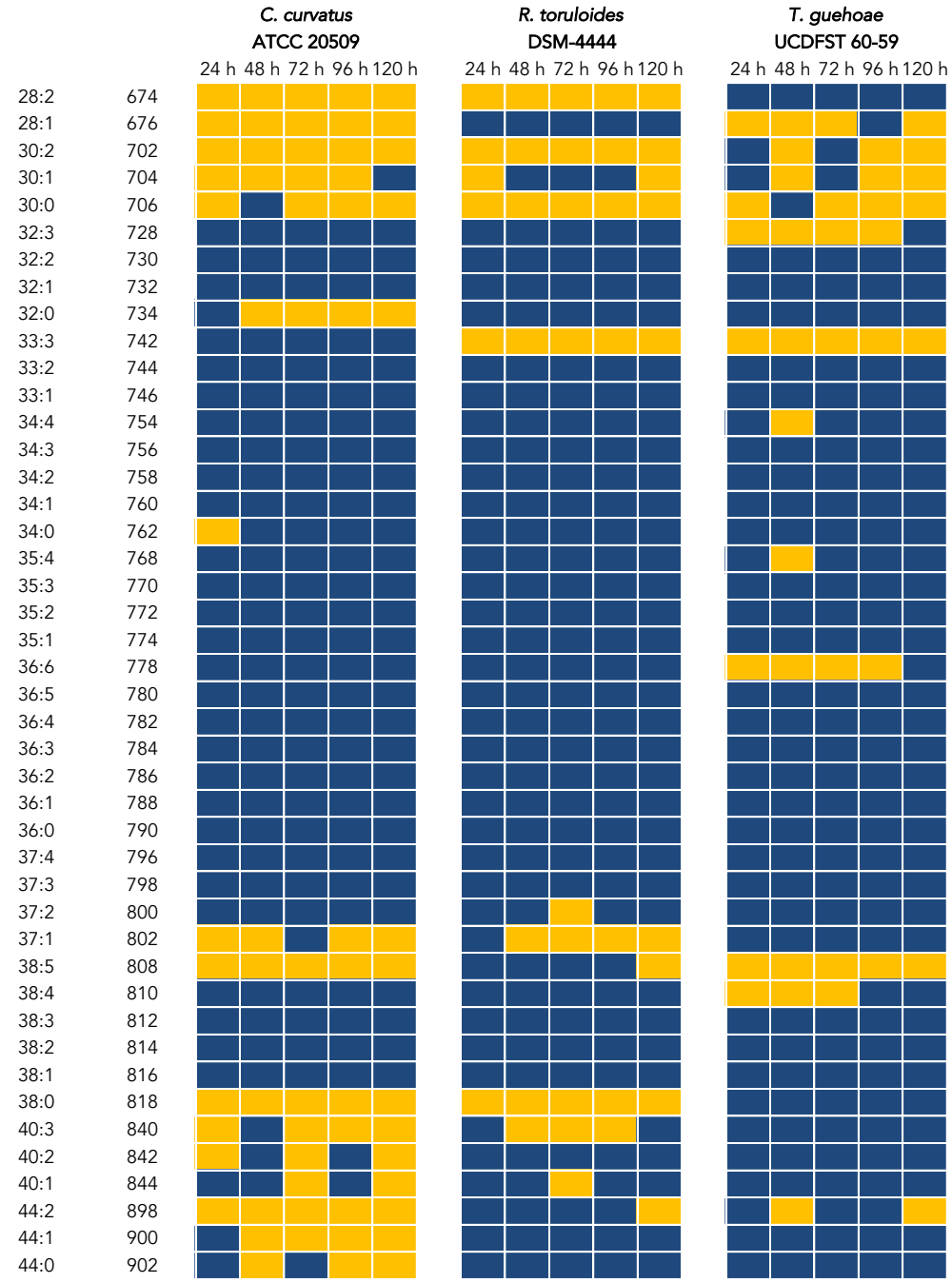
A) Phosphatidic acid (PA)



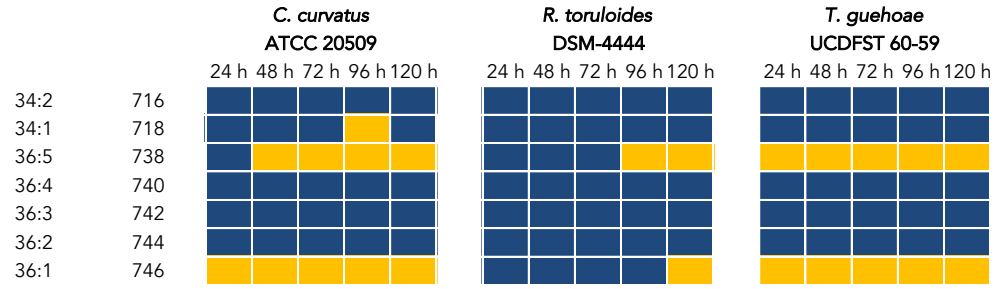
B) Phosphatidylinositol (PI)



C) Phosphatidylcholine (PC)



D) Phosphatidylethanolamine (PE)



E) Phosphatidylserine (PS)

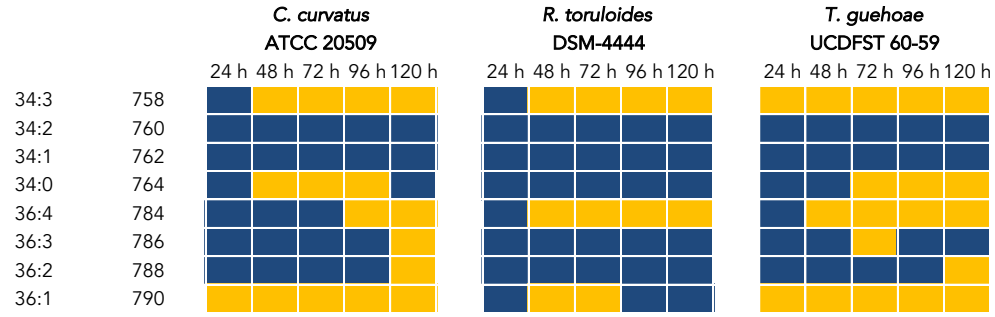


Figure S3. Polar lipid classes speciation in bioreactor cultivations on DDAP-EH corn stover hydrolysate for *C. curvatus* ATCC 20509, *R. toruloides* DSM-4444, and *T. guehoae* UCDFST 60-59 strains. Presence (blue) or absence (yellow) of each PL specie.

Cultivations on mock hydrolysate

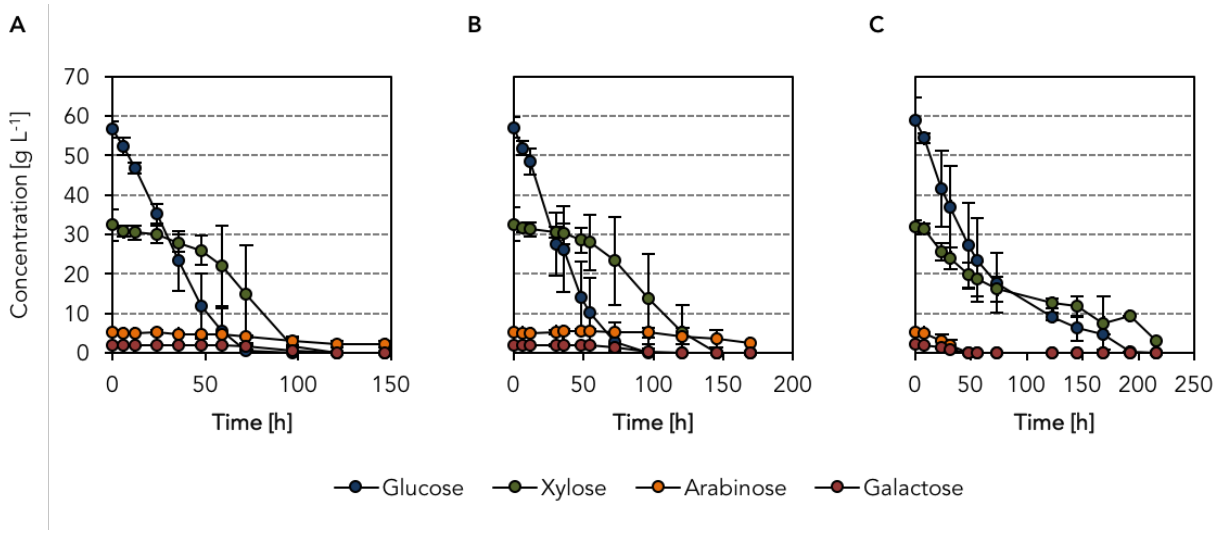


Figure S4. Sugar consumption profiles for shake flask cultivations on mock hydrolysate for *C. curvatus* ATCC 20509 (A), *R. toruloides* DSM-4444 (B), and *T. guehoae* UCDFST 60-59 (C) strains.

Cultivations on mineral medium with aromatic compounds as carbon source

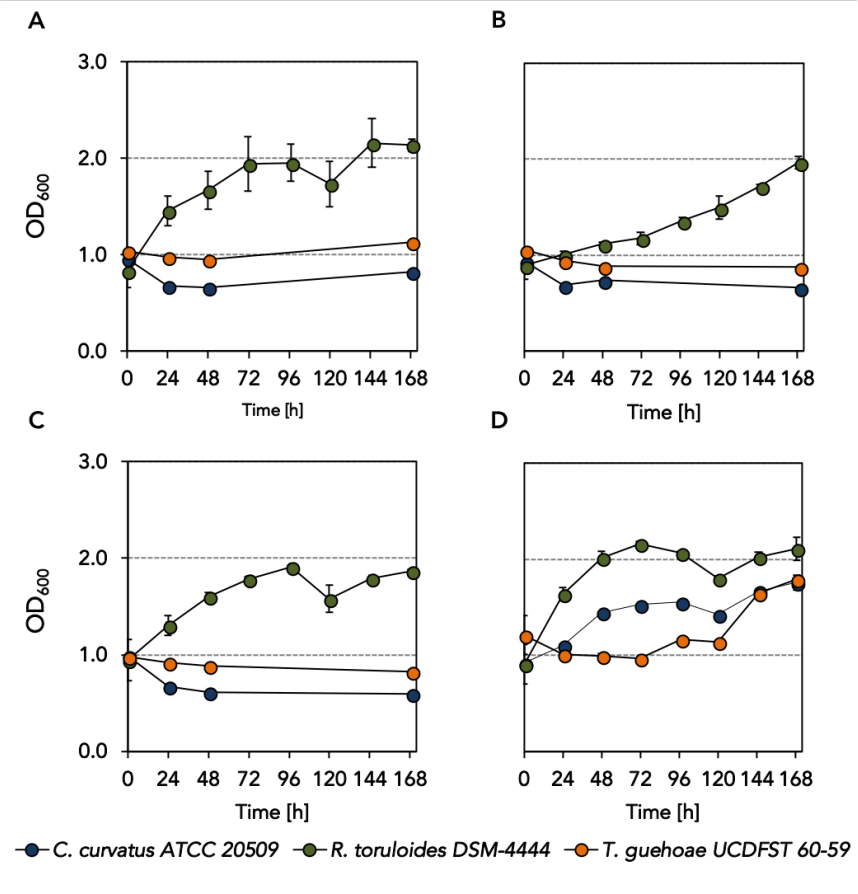


Figure S5. OD_{600} for shake flask cultivations on YNB media supplemented with *p*-coumaric acid (A), ferulic acid (B), vanillic acid (C), or 4-hydroxybenzoic acid (D) as sole carbon source for *C. curvatus* ATCC 20509, *R. toruloides* DSM-4444, and *T. guehoae* UCDFST 60-59 strains.

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