Table S1. Composition of the non-treated lignocellulosic hydrolysate, a waste stream (spent liquor) from cellulose fiber production with acidic hydrolysis, used in this study for pretreatment and fermentation.

Category	Compound	Concentration (g/L)	Standard deviation (g/L)
Monomeric sugar	**total sugar**	**115.60**	**4.99**
	Xylose	77.05	2.46
	Glucose	11.51	0.39
	Mannose	8.21	0.84
	Galactose	6.47	0.21
	Rhamnose	5.87	0.24
	Arabinose	4.93	0.42
	Fucose	1.57	0.44
Organic acids	**total organic acids**	**16.84**	**1.43**
	Acetic acid	12.34	1.20
	Galactoronic acid	4.51	0.23
Sulfates	**H_2SO_4 and X_zSO_4**	**19.40**	**2.00**
Furans	**total furans**	**5.21**	**0.64**
	HMF	4.53	0.59
	Furfural	0.68	0.04
Lignols, lignans and metabolites	**total lignols, lignans and metabolites**	**89.95**	**22.97**
Ash	**total Ash**	**0.700**	**0.009**
	O	0.316	
	Ca	0.142	
	Fe	0.044	
	C	0.044	
	S	0.041	
	P	0.035	
	Mg	0.025	
	K	0.017	
	Si	0.013	
	Mn	0.010	
	Al	0.009	
	Cr	0.003	
	Na	0.002	
	N	NA	
dry mass	**total dry mass**	**247.70**	**13.90**

Table S2. Pretreatment strategies with respective growth behavior and microscope examination.

Starting material	Sugar conc. *i*n %	Neutralization	additional Treatment	Sterilization	Av. max. OD_{600}	Microscope examination	Batch
LCH	3	NaOH	non	filtered	0.93	insoluble particles	1
LCH	3	NaOH	non	70°C, 1h	0.92	insoluble particles	1
LCH	3	NaOH	non	95°C, 30 min	0.89	insoluble particles	1
LCH	3	NaOH	non	121°C, 15 min	0.86	insoluble particles	1
LCH	3	$CaCO_3$, NaOH	non	filtered	1.74	insoluble particles	1
LCH	5	$CaCO_3$, NaOH	non	filtered	1.81	insoluble particles	1
LCH	7	$CaCO_3$, NaOH	non	filtered	1.71	insoluble particles	1
LCH	3	$CaCO_3$, NaOH	overliming to pH 10 with $Ca(OH)_2$, neutral. with HCl		1.85	insoluble particles	1
XGA	3	-	non	121°C, 15 min	0.8	fine	1
XGA	5	-	non	121°C, 15 min	0.59	fine	1
XGA	7	-	non	121°C, 15 min	0.48	fine	1
LCH	3	$CaCO_3$ 1g, NaOH	non	filtered	0.84	insoluble particles	2
LCH	3	$CaCO_3$ 500mg, NaOH	non	filtered	1.10	insoluble particles	2
LCH	3	$CaCO_3$ 300mg, NaOH	non	filtered	0.94	insoluble particles	2
LCH	3	$CaCO_3$ 200mg, NaOH	non	filtered	0.94	insoluble particles	2
LCH	3	$CaCO_3$ 200mg, NaOH, EDTA	non	filtered	0.15	insoluble particles	2
LCH	3	$CaCO_3$ 1g, NaOH	no addition of medium salts	filtered	0.72	fine	3
LCH	3	$CaCO_3$ 1g, NaOH	no additions of phosphates	filtered	0.80	fine	3
LCH	3	$CaCO_3$ 1g, NaOH	only additions of KH_2PO_4, $MgSO_4$, $CaCl_2$	filtered	0.78	insoluble particles	3
LCH	3	$CaCO_3$, NaOH	KH_2PO_4, salts addition before autocl.	filtered, 121°C, 15min	0.25	insoluble particles	3
LCH	3	$CaCO_3$, NaOH	KH_2PO_4, salts addition after autocl.	filtered, 121°C, 15min	1.02	insoluble particles	3
LCH	3	$CaCO_3$, NaOH	salts addition before autocl.	filtered, 121°C, 15min	0.03	insoluble particles	3
LCH	3	$CaCO_3$, NaOH	salts addition after autocl.	filtered, 121°C, 15min	1.30	insoluble particles	3
XGA	3	-	non	filtered	1.16	insoluble particles	4
LCH	3	NaOH	non	filtered	1.07	insoluble particles	4
LCH	3	NaOH	Active charcole	filtered	0.59	insoluble particles	4
LCH	**3**	**$CaCO_3$, NaOH**	**KH_2PO_4**	**filtered**	**1.14**	**fine**	4
LCH	**3**	**$CaCO_3$, NaOH**	**H_3PO_4**	**filtered**	**0.98**	**fine**	4

Table S3. Sugar and organic acid composition of the untreated LCH and pretreated LCH.

Compound	Untreated LCH (g/L)	Pretreated LCH (g/L)
Xylose	96.56 ± 0.78	91.66 ± 1.48
Glucose	14.42 ± 0.15	13.89 ± 0.22
Mannose	10.25 ± 0.20	9.38 ± 0.34
Galactose	8.11 ± 0.07	7.70 ± 0.12
Rhamnose	7.35 ± 0.07	6.96 ± 0.12
Arabinose	6.205 ± 0.07	6.24 ± 0.09
Acetic acid	13.79 ± 0.06	18.55 ± 0.32
Galacturonic acid	5.37 ± 0.05	5.47 ± 0.32
Total sugar and organic acid	**162.05 ± 1.44**	**159.85 ± 2.80**

Table S4. Duncan groups of the statistical analysis of the fatty acid profile, ordered by fermentation condition and fatty acid; p-vale < 0.01; groups starting with the highest share in group a.

Fermentation condition	Palmitic acid C16:0 in %	Duncan groups Palmitic acid C16:0	Stearic acid C18:0 in %	Duncan groups Stearic acid C18:0	Oleic acid C18:1 in %	Duncan groups Oleic acid C18:1	Linoleic acid C18:2 in %	Duncan groups Linoleic acid C18:2
XGA, cb-feed	24.8	ab	14.8	c	54.0	a	6.2	ab
Glucose, cb-feed	22.5	b	19.4	a	51.5	b	6.4	a
Xylose, cb-feed	22.9	ab	17.0	b	54.1	a	5.7	ab
LCH, cb-feed	24.5	ab	16.0	bc	54.5	a	4.9	b
LCH, co-feed	25.1	a	14.6	c	54.6	a	5.4	b

Table S5. GC-FID relative quantification of LCH derived lignin breakdown products before and after the fermentation with *C. oleaginosus*.

Extracted LCH containing media before/after fermentation	GC-MS identified compound	Retention time GC-FID (min)	Mean of area	Standard deviation of area	Relative area to ISTD	Standard deviation relative to ISTD	n
before	Vanilin	15.52	1886754	111589	0.10666	0.00169	3
before	Vanilic acid hydrazide	17.78	139919	21747	0.00791	0.00108	3
before	Syringaldehyde	18.62	3135042	136088	0.17731	0.00134	3
before	Acetosyringone	19.40	30804	1824	0.00174	0.00010	3
before	Eicosane	21.57	17683655	836307	1.00000	0	3
after	Vanilin	15.52	NA	NA	0	0	3
after	Vanilic acid hydrazide	17.78	NA	NA	0	0	3
after	Syringaldehyde	18.62	392635	150845	0.02456	0.01110	3
after	Acetosyringone	19.40	NA	NA	0	0	3
after	Eicosane	21.57	16623897	2630691	1.00000	0	3

Table S6. Share of LCH from the overall consumed carbon for the different feeding strategies.

Feeding strategy	Total consumed carbon (g/L)	Consumed LCH carbon (g/L)	Share of LCH on total carbon (%)
Con*sumption-based feed with 50% acetic acid*	153.37 ± 20.94	4.26	2.8
Acetic acid:LCH 50:10	169.78 ± 0.3	11.32	6.7
Acetic acid:LCH 50:50	182.07 ± 9.82	22.76	12.5
LCH continuous feed 0.5 mL/h	154.0 ± 0.6	15.98	10.4
LCH continuous feed 1 mL/h	139.76 ± 16.15	29.74	21.3

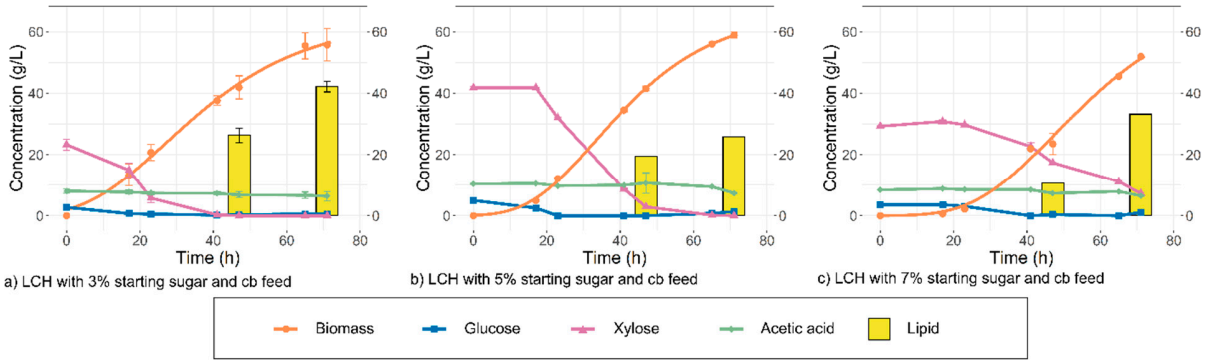

Figure S1. Comparison between three different starting concentration of LCH in the fermentation media. Regarding to the carbon source content 3% (a), 5% (b) and 7% (c) starting carbon were applied. Error bars display two-times standard deviation. LCH - Lignocellulosic hydrolysate, cb-feed – consumption-based acetic acid feed.

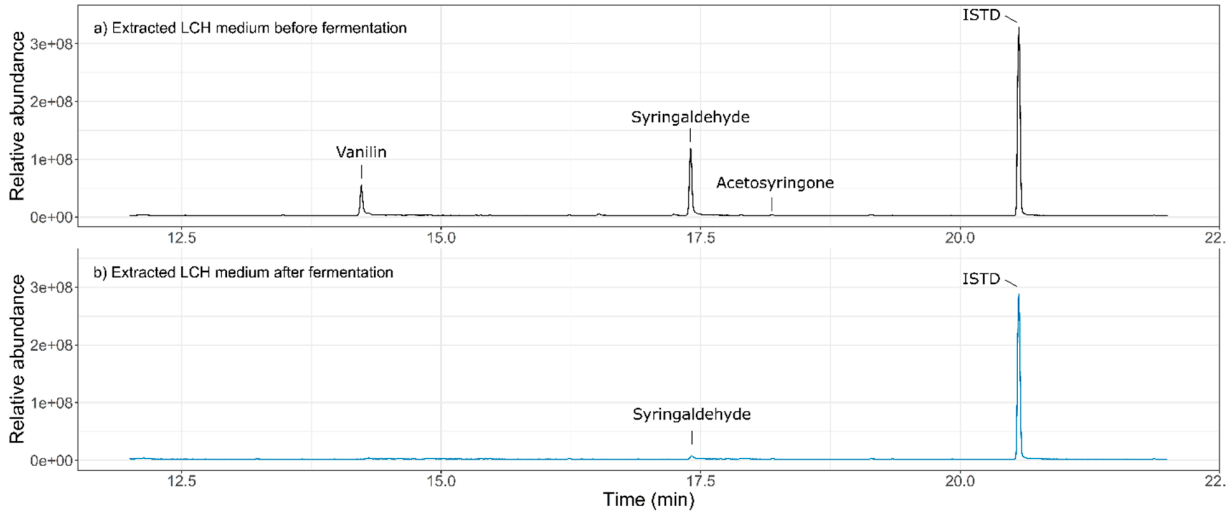

Figure S2. GC-MS chromatogrammes of fermentation medium of LCH (22.3% LCH) extracted with methyl *tert*-butyl ether, before (a) and after (b) consumption-based acetic acid fed fermentation with *C. oleaginosus*.

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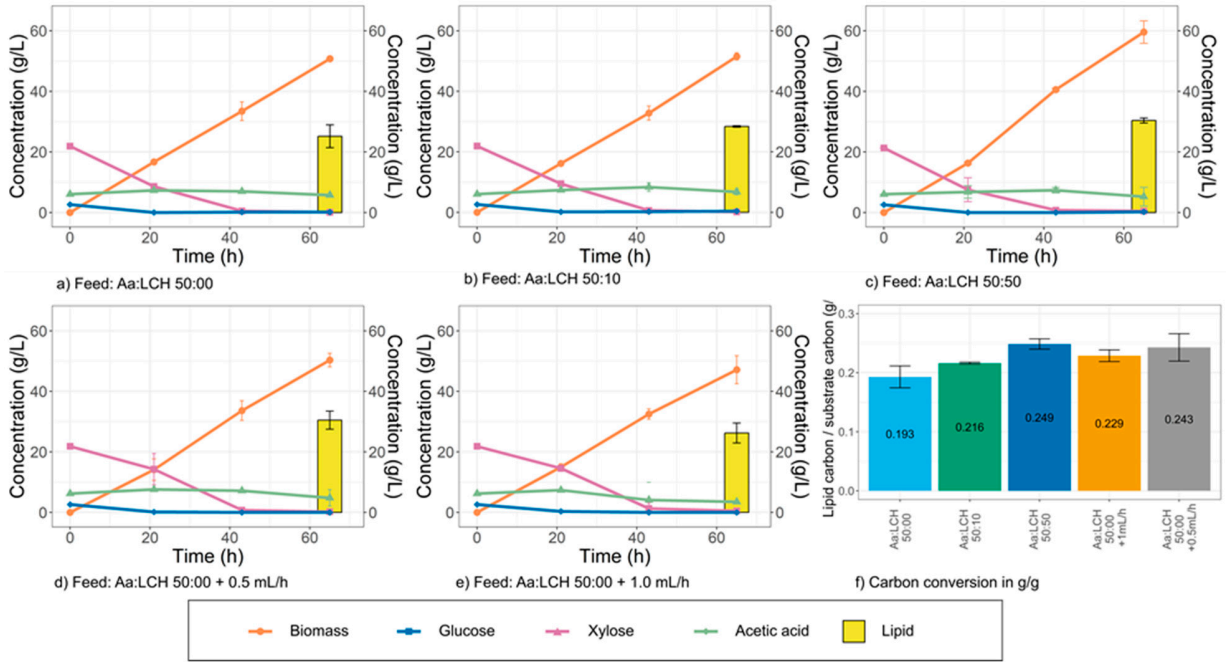

Figure S3. (a – e) Comparison between five different feeding strategies of LCH. (f) Carbon conversion rate in lipid carbon/substrate carbon (g/g), without significant differences detected, as confirmed by ANOVA. Error bars display two-times standard deviation. LCH - Lignocellulosic hydrolysate, cb-feed – consumption-based acetic acid feed.

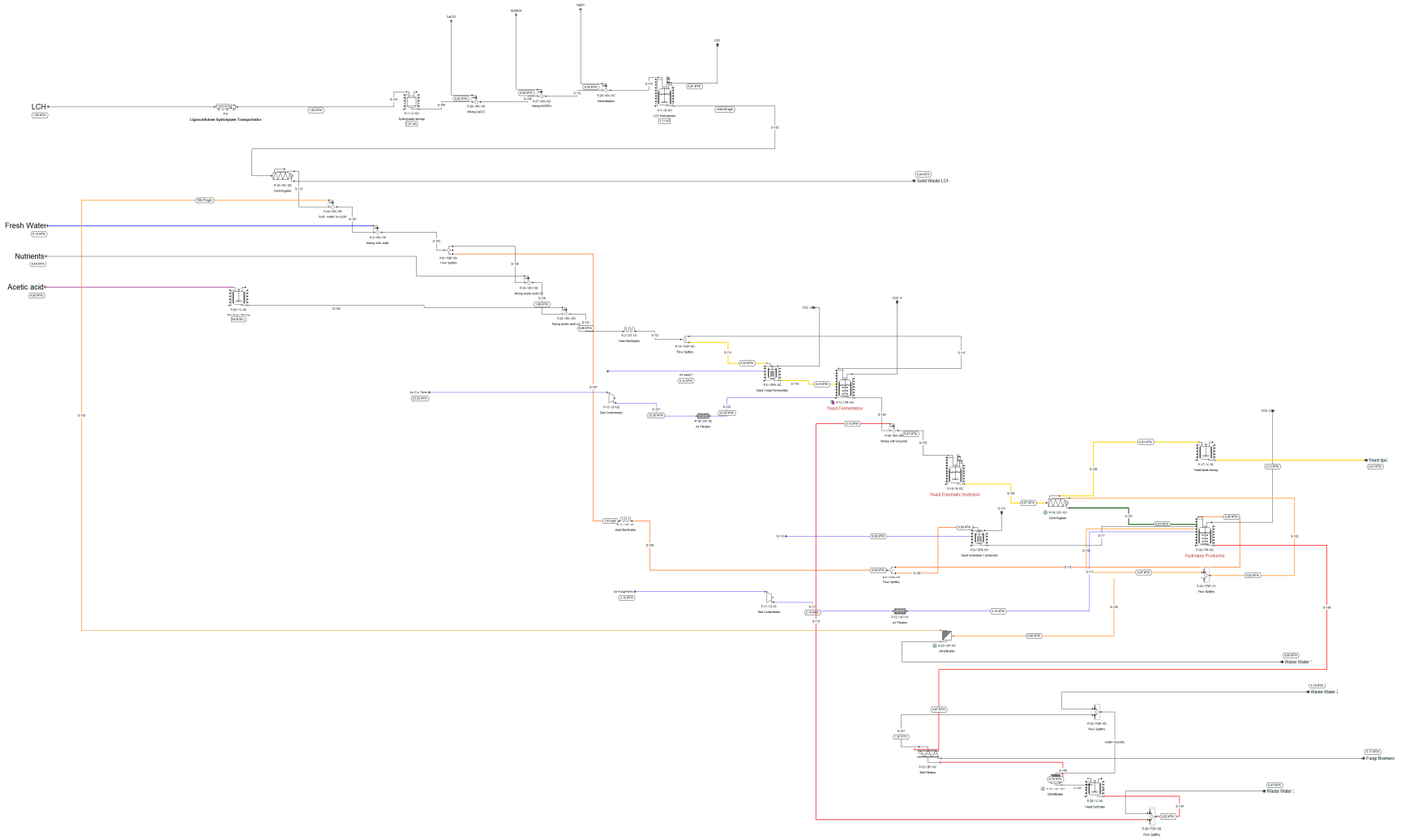

Figure S4. Super Pro Designer model for the TEA of single cell oil production on the basis of LCH and acetic acid.