A sustainable, high-performance process for the economic production of waste-free microbial oils that can replace plant-based equivalents

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Supplementary Data

1.1. Maximizing lipid productivity

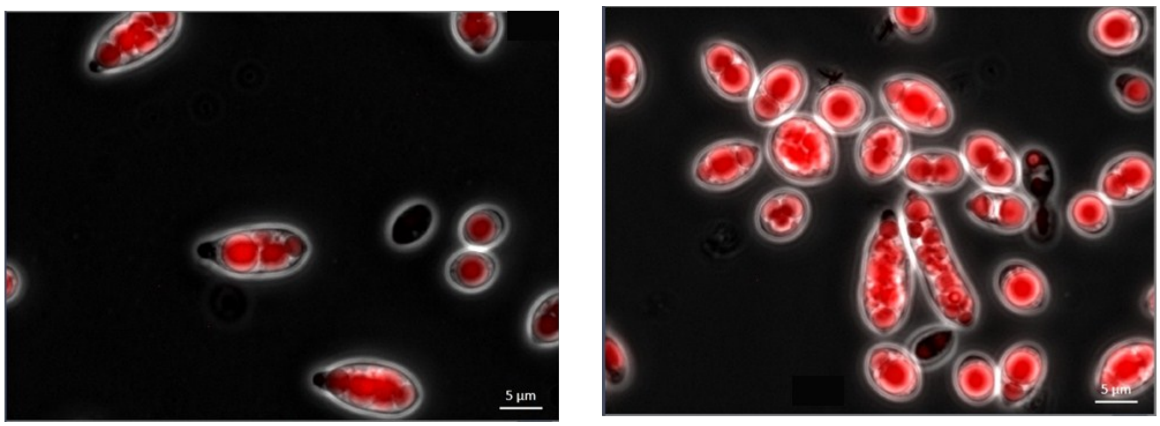


Figure S1: fluorescence microscope imaging shows a remarkable increase in the cell volume and lipid content for: Glucose-based fermentation in minimal nitrogen media (on the lift) and co-fermentation in rich nitrogen media (on the right).

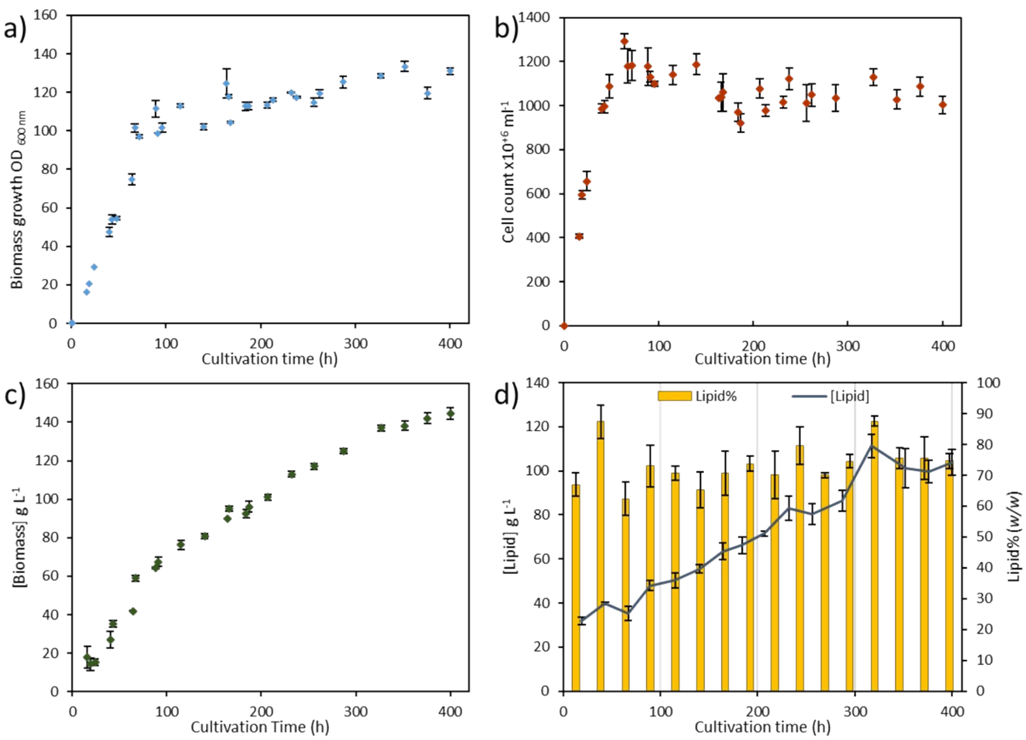


Figure S2: Growth rates and lipid accumulation during acetic acid and glucose co-fermentation in nitrogen-rich medium applying continuous fermentation mode. (a) Growth rates determined via the optical density. (b) Growth rates determined via the cell count. (c) Growth rates determined via the gravimetric method. (d) Lipid accumulation and productivity determined via the gravimetric method.

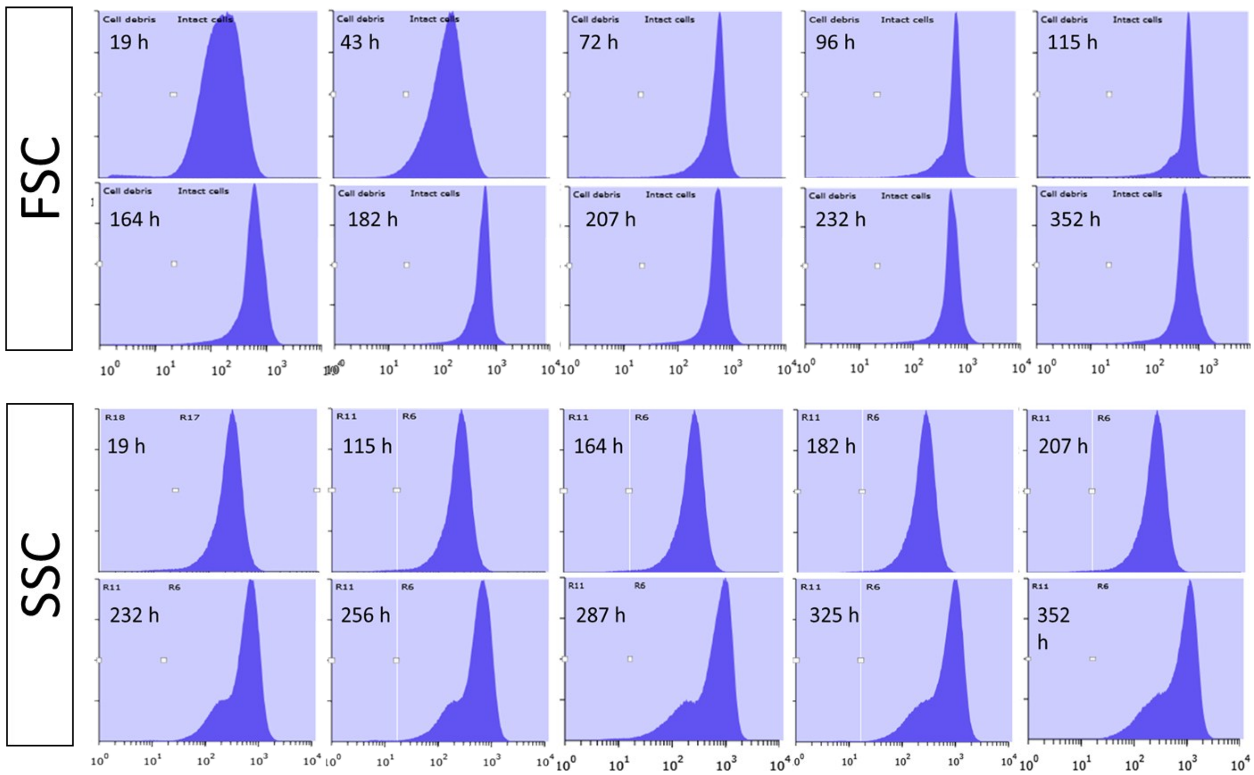


Figure S3: The increase in intensity of the forward scatter [FSC] (on x axis) and side scatter [SSC] (on y axis) over the acetic acid and glucose co-fermentation time in rich nitrogen media. The fermentation was carried out at: 25-liters, temp.: 28°C, pH 6.5 and $pO_2 \geq 50\%$.

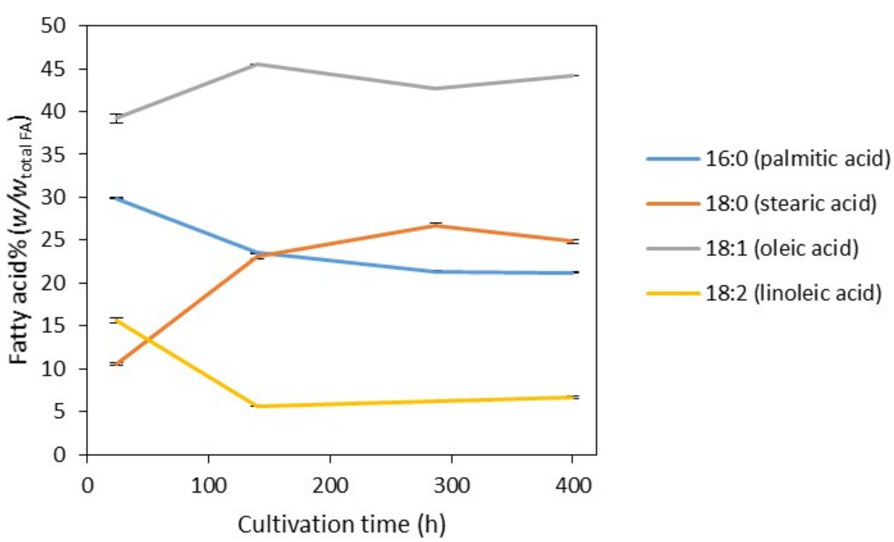


Figure S4: The changes in fatty acid profile over the acetic acid and glucose co-fermentation time in rich nitrogen media. The fermentation was carried out at: 251-liters, temp.: 28°C, pH 6.5 and $pO_2 \geq 50\%$.

Table S1: Yeast biomass analysis at the end of the semi-continuous run

	%(w/w)	Method
Sugar	8.3	Chemical hydrolysis with H_2SO_4 at 1% and 3%
Lipid	87.6	3 times extraction with Folch solution (chloroform : methanol, in a 2:1 (vol/vol) ratio
protein	3.5	Kjeldahl method, Kjeldahl factor 6.25
Ash	0.6	Incineration at 1200°C, 3h

1.2. Downstream processing and lipid recovery

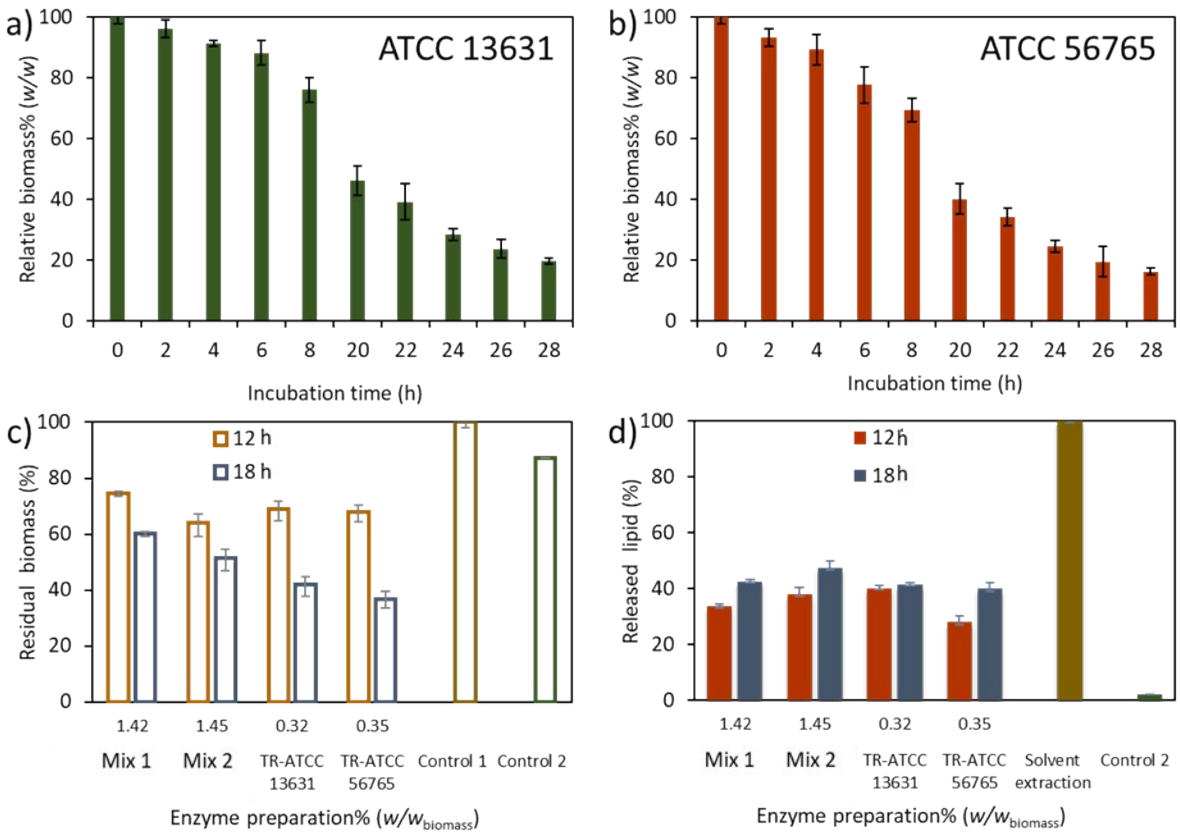


Figure S5: Evaluation of produced enzymes from T. reesei ATCC 13631 and RUT C-30 (ATCC 56765) on purified C. oleaginosus-processed biomass (a b) and fresh culture (c d). (a) Relative decrease in the C. oleaginosus biomass weight over the enzymatic hydrolysis time using T. reesei (ATCC 13631). (b) Relative decrease in the biomass weight over the enzymatic hydrolysis time using T. reesei RUT C-30 (ATCC 56765). (c) Relative residual biomass weight after 12 and 18 h of incubation with the following enzyme systems: mixes 1 and 2 (commercial mixtures), T. reesei ATCC 13631, and T. reesei RUT C-30 (ATCC 56765). (d) Relative released lipid weight after 12 and 18 h of incubation with the following enzyme systems: mixes 1 and 2 (commercial mixtures), T. reesei ATCC 13631, and T. reesei RUT C-30 (ATCC 56765).Controls: Control1(negative control): the biomass amount before treatment. Control2(positive control): Samples are incubated at same pH, tempreture and time but without addting enzyme. Solvent extreacton: amout of lipid which abtained after 3 times extraction with Folch solution (chloroform : methanol, in a 2:1 (vol/vol) ratio.

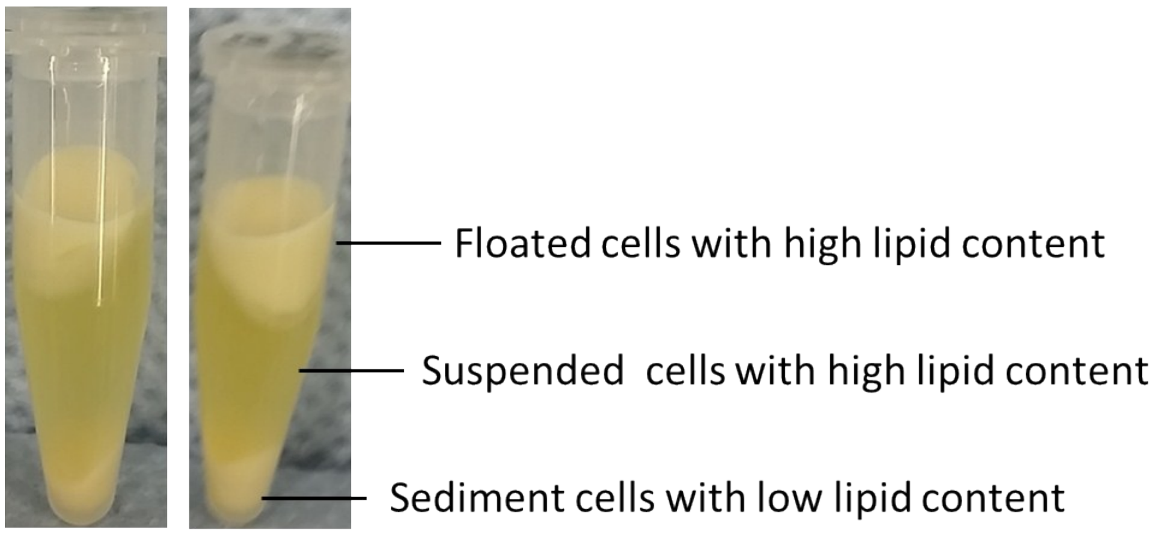


Figure S6: Yeast culture with high lipid content (75% w/w) after centrifugation at 15,000g for 30 min.

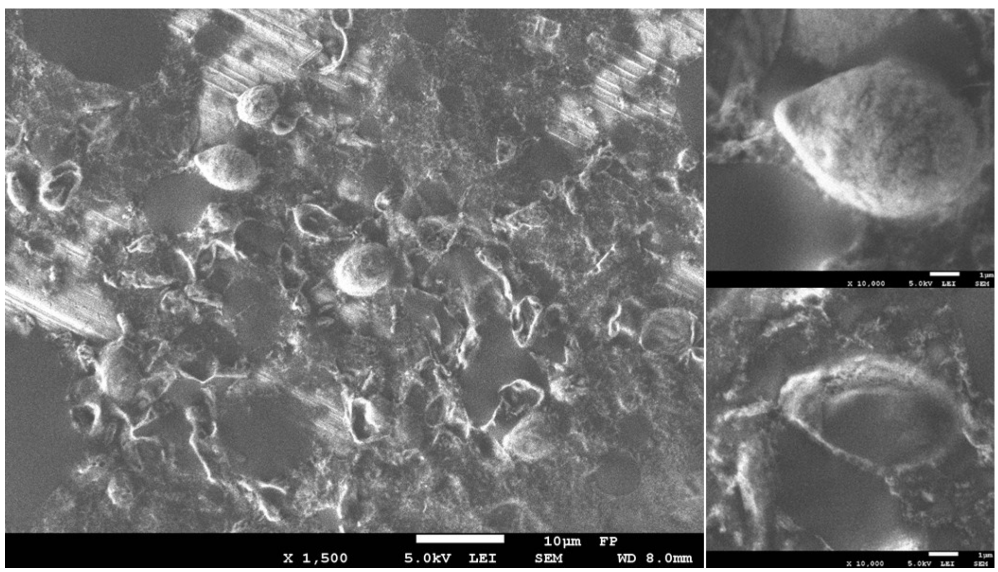


Figure S7: Electron microscope image for C. oleaginosus cells after treatment with high pressure homogenizer for 3 times at 2400 bar.

1.4. TEA

The TEA was carried out to estimate the total capital investment and operating cost for process flowsheets that could be used for the production of lipids from oleaginous yeast.

Due to the lack of major databases (such as NREL) in process design related to the oil production from oleaginous yeasts, process and economic data were collected from data generated in this study. Additionally, parameters extracted from available literature sources[1-3, 4] and intgrated mathematical function in SuperPro Designer version 10 - Intelligen, Inc. (SPD) were applied.

The Techno-Economic Analysis (TEA) was carried out for the estimation of the total capital investment and operating cost for process flowsheets that can be used for the production of yeast lipids. The industrial plant is required to operate 24 h daily, 7 days per week, which amounts to 8300 h y^{-1}. A standby fermenter and centrifuge are used to avoid maintenance-based operation downtime. With these prerequisites, the production capacity is of 23,000 t y^{-1}. The mass and energy balances as well as equipment sizing were determined using Excel spreadsheets and validated by SuperPro Designer V10 (Intelligen, Inc.).Instilation factors, additional direct and indirect capital costs were estimated as presentage based on a Provoius NREL publication about bioethanol production.[5]

Raw materials amounts were estimated based on the final lipid yield. A built-in mathematical function in SPD adjusts the equipment purchase price based on the required size of process equipment and the analysis year (2018). All the capital and operating data extracted from SPD are used to determine the minimum selling price.

A simplified process flow diagram (fig. S8) was generated based on the data presented in the paragraphs (2.1, 2.2 and 2.3). Thus, the co-fermentation in rich nitrogen media was carried out in consuming base feeding mode. The applied lipid productivity was of 1.4 g L^{-1} h^{-1} [biomass: 200 g L^{-1} with lipid content of 83% (*w*/*w*), after 120 h]. The applied lipid productivity considers a basline productivity value that was determined in the current study current for generation of a conservative TEA scenario.

As co-fermentation enables concurrent biomass growth and the lipid accumulation the mass balances were calculated by a single stoichiometric equation during the fermentation. The stoichiometric coefficients were appropriately calculated based on applied media and feed composition as well as the recoded biomass and lipid yields respectively. Lipid-biomass (biomass including the lipid, $C_{61}H_{110.5}O_{9.8}N_{1.55}$) was formulated based on the element analysis of C, H, N, and O. Amino acids formula $C_{5.35}H_{9.8}O_{2.45}N_{1.5}$ is the average molecular of amino acid and it taken form Koutinas *et al*[1]:

(1) $0.1875\ C_{5.35}H_{9.8}O_{2.45}N_{1.5} + 7.496\ CH_3COOH + 0.27778\ C_6H_{12}O_6 + 2.2536\ O_2 \rightarrow$

$\rightarrow 0.18633\ C_{61}H_{110.5}O_{9.8}N_{1.55} + 6.25\ CO_2 + 7.33\ H_2O$

By enzymatic hydrolysis, lipid-biomass is converted to glucose, amino acid, released lipid ($C_{57}H_{104}O_6$), and non-lipid biomass ($C_4H_{6.5}O_{1.9}N_{0.7}$). Glyceryl trioleate ($C_{57}H_{104}O_6$) has been used representatively for the lipid formula since the $C_{18:1}$ fatty acid comprises 53% (*w/w*) of total fatty acid profile (Based on FAMEs analysis). The non lipid-biomass formula ($C_4H_{6.5}O_{1.9}N_{0.7}$) was extracted from Babel and Muller.[6]

(2) $0.18633\ C_{61}H_{110.5}O_{9.8}N_{1.55} + 6.25\ CO_2 + 0.23340\ H_2O \rightarrow$

$\rightarrow 0.08066\ C_6H_{12}O_6 + 0.14998\ C_{5.35}H_{9.8}O_{2.45}N_{1.5} + 0.17200\ C_{57}H_{104}O_6 + 0.0804\ C_4H_{6.5}O_{1.9}N_{0.7}$

Figure S8 shows the process flow diagram for the yeast lipid production. Water, glucose and nitrogen sources (such as peptone and yeast extracts) are fed to the bioreactor (FR-101) (estimated process duration:6 h). Acetic acid is used as a feed during the fermentation time. *In-situ* sterilization is performed for 20 min at 121°C. After cooling, the fermentation starts by adding the inoculum at OD:0.1. The fermentation is controlled at 28°C, pH 6.5 (process duration:120h). The whole culture is transferred out to the enzymatic hydrolysis reactor (R-101). Transferring out, and transferring in are connected processes, running in parallel (estimated process duration:8 h). The enzymatic hydrolysis starts by adding the enzyme (process duration:20h). The final centrifugation (CD-101) will start in parallel to transferring the culture out from R-101 (estimated process duration:8 h). After centrifugation, 3 phases are generated. The upper phase contains pure lipid. The middle phase contains residual biomass. The lower phase contains the hydrolysate which is transferred (in parallel to centrifugation) into a cross-flow filtration unit (UF-101). The filtration results in a retentate fraction estimated to be 10% (*v/v*), the remaining filtrate will be about 90% (*v/v*). The filtrate will be mixed with additional amount of sugar and nutrition (in MX-103) and used for subsequent fermentation processes. Major operating and process parameters used to develop the process model and determine the required material are listed in the **Table2**.

Table S2: Major operating and process parameters used to develop the process model and determine the required material

Process parameters	Unite	Baseline	Optimal	Process parameters	Unite	Baseline	Optimal
Feed stock				**Enzymatic hydrolysis**			
Acetic acid	$ per kg	0.2	0.1	hydrolysis time	h	24	20
Sugar	$ per kg	0.3	0.2	Lipid release	% w/w	86	86
Peptone/ yeast extract	$ per kg	0.3	0.2	non-lipid biomass conversion to nutrition*	% w/w	80	95
				Enzyme load*	% ($w/w_{biomass}$)	1.4	-
Centrifugal gas compressor				Enzyme price[5]	$ per kg	4	2
volumetric throughput[7, 8]	$m^3\ min^{-1}$	380	-	Power consumption	$kw\ m^{-3}$	0.05	-
Pressure change[4]	bar	5	-	hydrolysis temperature*	°C	45/37	50/37
				Steam flow rate	$kg\ h^{-1}$	1300	-
Power consumption[9, 10]	kw	6348	-	Heat transfer efficiency	%	98	100
Efficiency	%	70	90	Power dissipation to heat	%	100	-
Fermentation				**Centrifugation**			
RM loading time[1]	h	8	6	volumetric throughput	$m^3\ h^{-1}$	160	-
Sterilization[1]	h	1.5	1.5	Lipid separation efficiency[7, 8]	% w/w	90	95
Lipid Productivity*	$g\ L^{-1}\ h^{-1}$	1.4	2.4	water contain in the non-lipid biomass [7]	% w/w	50	-
Fermentation time*	h	120	72	Centrifugation time	h	contentious	
Biomass content*	$g\ L^{-1}$	200	190	Power consumption[9]	$kw\ m^{-2}$	0.2	-
Lipid content*	% (w/w)	83	85	Sedimentation efficiency[7, 8]	%	30	50
transfer out to reactor[1]	h	8	6				
Power consumption[9, 10]	$kw\ m^{-3}$	3	-	**Filtration**			
Aeration rate[1, 11]	vvm	0.8	0.5	Rejection co-efficient for protein[7, 8]	% w/w	95	-
Power dissipation to heat[12]	%	50	-	Filtration time	h	10	8
Pressure inside fermenter**	bar	1.25 - 1.5		Recovery (filtrate/feed)***	% w/w	90	95
Yeast Hydrolysate				Power consumption	kw	156	-
Sugar content*	$g\ L^{-1}$	18	24	Power dissipation to heat[12]	%	10	-
Nitrogen content*	$g\ L^{-1}$	8	15	Filtrate flux	$L\ m^{-2}\ h$	32	

*Based on the current process paramneters and current results. ** Based on the fermentation paramenters at 25-L to red. ***Based on the recycling experiments.

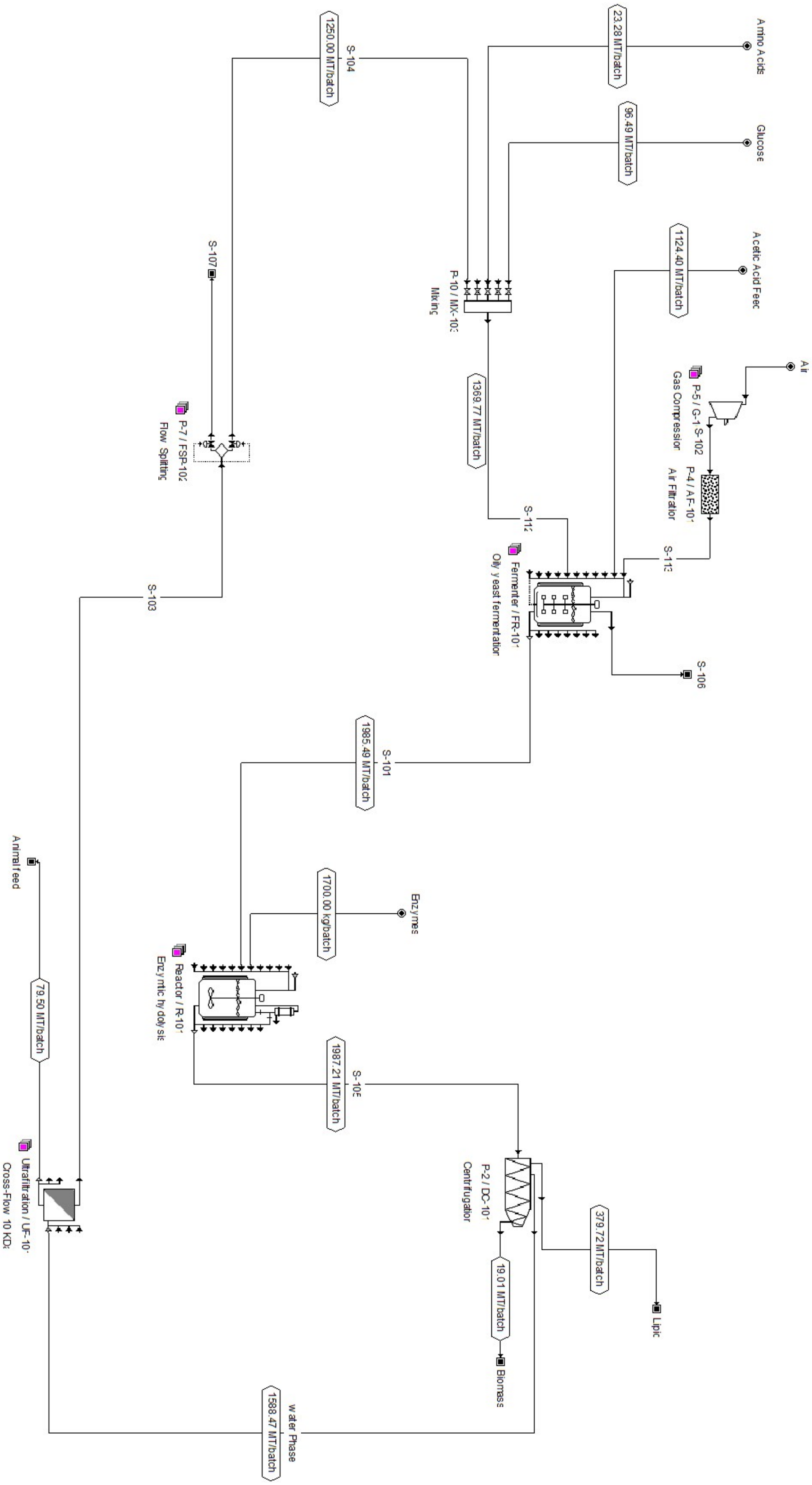


Figure S8: The simplified process flow diagram for the lipid production from yeast.

Table S3: Capital cost-list of equipment specification and PC cost according to market analysis in 2018-SuperPro designer V10.

EQUIPMENT SPECIFICATION AND FOB COST (2018 prices)	#	Item	Unite Purchased Cost(PC)	Quantity			Total Purchased Cost (PC)	Installation factor*	Installed cost	Ref.
				In process	Standby	Total				
	1	Fermenter, Vessel Volume = 250.00 m3	2,333,000	10	1	11	25,663,000	1.60	41,060,800	SuperPro Designer (V10) Built-in cost model (Year analysis 2018)
	2	Stirred Reactor Vessel Volume = 248.76 m3	1,773,000	3	1	4	7,092,000	1.60	11,347,200	SuperPro Designer (V10) Built-in cost model (Year analysis 2018)
	3	Bending Tank, Vessel Volume = 150.00 m3	178,000	2	-	2	356,000	1.60	569,600	SuperPro Designer (V10) Built-in cost model (Year analysis 2018)
	4	Storage/ Receiving Tank, Vessel Volume = 250.00 m3	78,000	3	-	3	234,000	1.70	397,800	SuperPro Designer (V10) Built-in cost model (Year analysis 2018)
	5	Decanter Centrifuge, Throughput = 159919.85 L/h	243,000	1	1	2	486,000	1.60	777,600	SuperPro Designer (V10) Built-in cost model (Year analysis 2018)
	7	Centrifugal Compressor Compressor Power = 2115.79 kW	2,212,000	3	-	3	6,636,000	1.60	10,617,600	SuperPro Designer (V10) Built-in cost model (Year analysis 2018)
	8	Ultrafilter Membrane Area = 77.69 m2, shell & tube, stainless steel	124,000	27	-	27	3,348,000	1.8	6,026,400	SuperPro Designer (V10) Built-in cost model (Year analysis 2018)
	9	Air Filter Rated Throughput = 12112043.16 L/h, shell & tube, stainless steel	43,000	1	1	2	86,000	1.80	154,800	SuperPro Designer (V10) Built-in cost model (Year analysis 2018)
		Total cost					**43,901,000**		**70,951,800**	
		Interest							4,257,108	Estimated as: 6% of the PC
		Total Installed Costs							**75,208,908**	

*The installation Factor is obtained from Provoius NREL publication about bioethanol production.[5]

Table S4: Capital cost-list of additional direct capital cost.

	#	Item	Description	Installed Cost	Factor	Cost	Ref.
Additionall direct capital cost	1	Warehouse ,	On-site storage of equipment and supplies.	70,951,800	0.04	2,838,072	Estimated as: 4.0%% of the ISBL
	2	Site development	Includes fencing, curbing, parking lot, roads, well drainage, rail system, soil borings, and general paving. This factor allows for minimum site development assuming a clear site with no unusual problems such as right-of-way, difficult land clearing, or unusual environmental problems.	70,951,800	0.090	6,385,662	Estimated as: 9%% of the ISBL
	3	Additional piping	To connect ISBL equipment to storage and utilities outside the battery limits	70,951,800	0.045	3,192,831	Estimated as: 4.5%% of the ISBL
		Total				12,416,565	
		Total Direct Costs (TDC)				83,368,365	

Table 5: Capital cost-list of additional indirect capital cost.

	#	Item	Description	Total Direct Costs	Factor	Cost	Ref.
Indirect capital cost	1	Prorateable costs	This includes fringe benefits, burdens, and insurance of the construction contractor.	83,368,365	0.10	8,336,837	Estimated as: 10% of total direct cost (TDC)
	2	Field expenses	Consumables, small tool and equipment rental, field services, temporary construction facilities, and field construction supervision.	83,368,365	0.10	8,336,837	Estimated as: 10% of total direct cost (TDC)
	3	Home office and construction	Engineering plus incidentals, purchasing, and construction	83,368,365	0.20	16,673,673	Estimated as: 20% of total direct cost (TDC)
	4	Project contingency	Extra cash on hand for unforeseen issues during construction.	83,368,365	0.10	8,336,837	Estimated as: 10% of total direct cost (TDC)
	5	Other costs	Start-up and commissioning costs. Land, rights-of-way, permits, surveys, and fees. Piling, soil compaction/dewatering, unusual foundations. Sales, use, and other taxes. Freight, insurance in transit, and import duties on equipment, piping, steel, instrumentation, etc. Overtime pay during construction. Field insurance. Project team. Transportation equipment, bulk shipping containers, plant vehicles, etc.	8,336,837	0.10	8,336,837	Estimated as: 10% of total direct cost (TDC)
		Total Indirect Costs				**50,021,019**	
	Total Capital Investment (TCI)		Total Direct Costs (TDC) + Indirect costs				**133,389,384**

Table S6: Operation cost-list of labor cost according to German TV-L tariff.

	#	Item	Annual cost per person	Amount	Cost	Ref.
LABOR COST	1	Operator (Full Cost) E10	83,305	30	2,499,150	Official service, full-cost list based on TV-L E10
	2	QC Analyst (Full Cost) E13	114,599	9	1,031,391	Official service, full-cost list based on TV-L E13
	3	Management/ Administration (Full Cost) E14	119,415	3	358,245	Official service, full-cost list based on TV-L E14
		Total			**3,888,786**	

Table S7: Operation cost-list of Facility-dependent cost.

	#	Item	Annual cost	Factor	Cost	Ref.
FACILITY-DEPENDENT COST	1	Maintenance	83,368,365	0.03	2,128,554	Estimated as 3% of total direct fixed capital.
	2	Property insurance	133,389,384	0.007	496,663	Estimated as 0.7% of total capital investment (TCI).
		Facility-dependent Total			**2,625,217**	
		Total fixed operating costs	Labor cost + facility-dependent		**6,514,003**	

Table S8: Operation cost-list of Utility cost, Ref.: SuperPro designer V10

	#	Item	Unite cost	Quantity	Ref. Units	Total	Cost	Ref.
UTILITIES COST (Germany 2018 prices in $)	1	Std Power	0.1	81,588,099	kW-h/year	81,588,099	8,158,809.90	The consumption amount based on SuperPro Designer (V10). The cost of kW-h is based on German price of Std power
	2	Steam	5.0	101,320	MT/year	101,320	506,600	
	3	Cooling water	0.05	11,356,146	MT/year	11,356,146	567,807	SuperPro Designer (V10)
	4	Chilled water	0.40	5,693,853	MT/year	5,693,853	2,277,541	
	5	**Saving (Exist in the process due to heat recovery)**	**1.00**	**1,573,600**	-	**1,573,600**	**1,573,600**	uperPro Designer (V10)
	6	**Saving (Steam Recycling)**	**5.00**	**99,588**	MT/year	**99,588**	**497,940**	uperPro Designer (V10)
	7	**Saving (Cooling water Recycling)**	**0.05**	**8,327,840**	MT/year	**8,327,840**	**416,392**	SuperPro Designer (V10)
		Total					**9,022,826**	

Table S9: RM cost according to market analysis in 2018.

RAW MATRIALS	#	Item	Unite cost	Quantity	Cost	Ref.
	1	Acetic acid	0.2	68,588,400	13,031,796	Whole sell market "Alibaba website"
	2	Glucose	0.3	5,517,770	1,655,331	Whole sell market "Alibaba website"
	3	Enzyme	4	103,700	414,800	Novozymes company.
	4	Amino acid	0.2	915,398	183,080	Source like yeast extract or peptone. Whole sell market "Alibaba website"
		Total			**15,285,007**	

Table S10: Revenues- the annual productivity of lipid, biomass and animal feed based on designee process.

REVENUES	#	Item	Unit cost	Quantity	Total Cost	
	1	Lipid	1.60	23,163,170	37,061,072	Estimated
	2	Biomass	0.35	1,159,905	405,967	Market cost
	3	Animal Feed (concentrated protein after cross-flow)	0.35	439,787	153,925	Market cost
		Total			**37,620,964**	

Table S11: The cost summary of Capital cost (CAPX), Operation cost (OPEX) and Raw Martials cost (RM) against the revenues.

SUMMARY	#	Items	Description	Cost
	1	CAPEX	Total / 20 years depreciation	6,669,469.20
	2	OPEX	Including: Lab, maintenance and utility	15,536,828.78
	3	RM	All chemicals are included	15,285,006.60
		Total cost		**37,491,305**
		Revenues		**37,620,964**

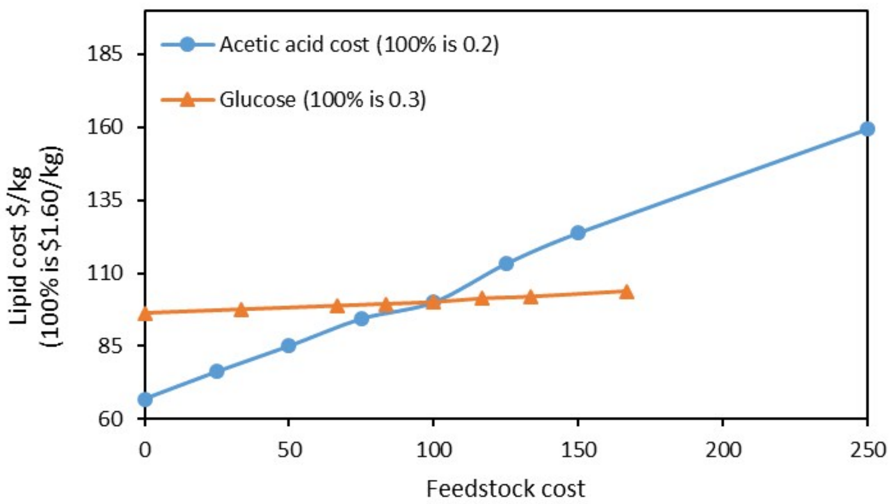


Figure S9: Lipid cost sensitivity to acetic acid and glucose cost.

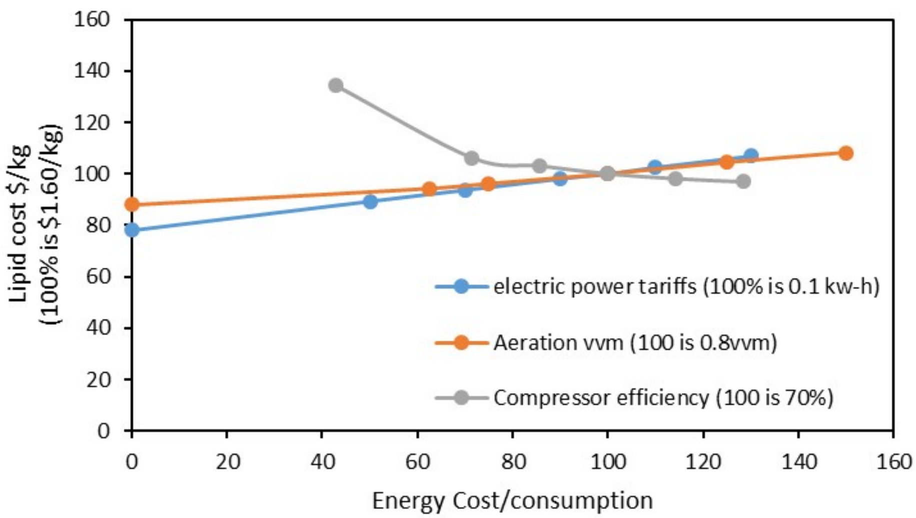


Figure 10:Lipid cost sensitivity to aerations need, electric power tariffs and compressor efficiency.

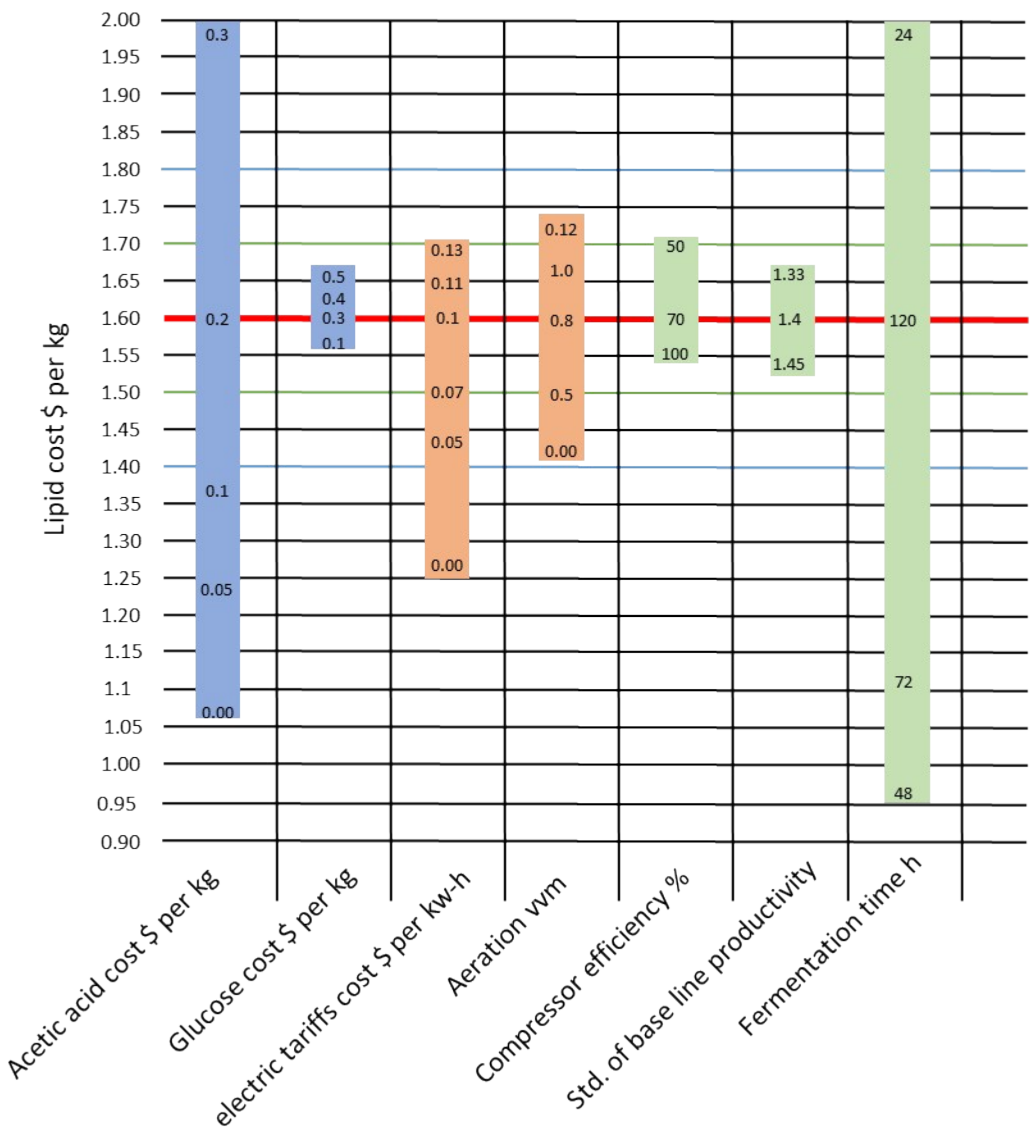
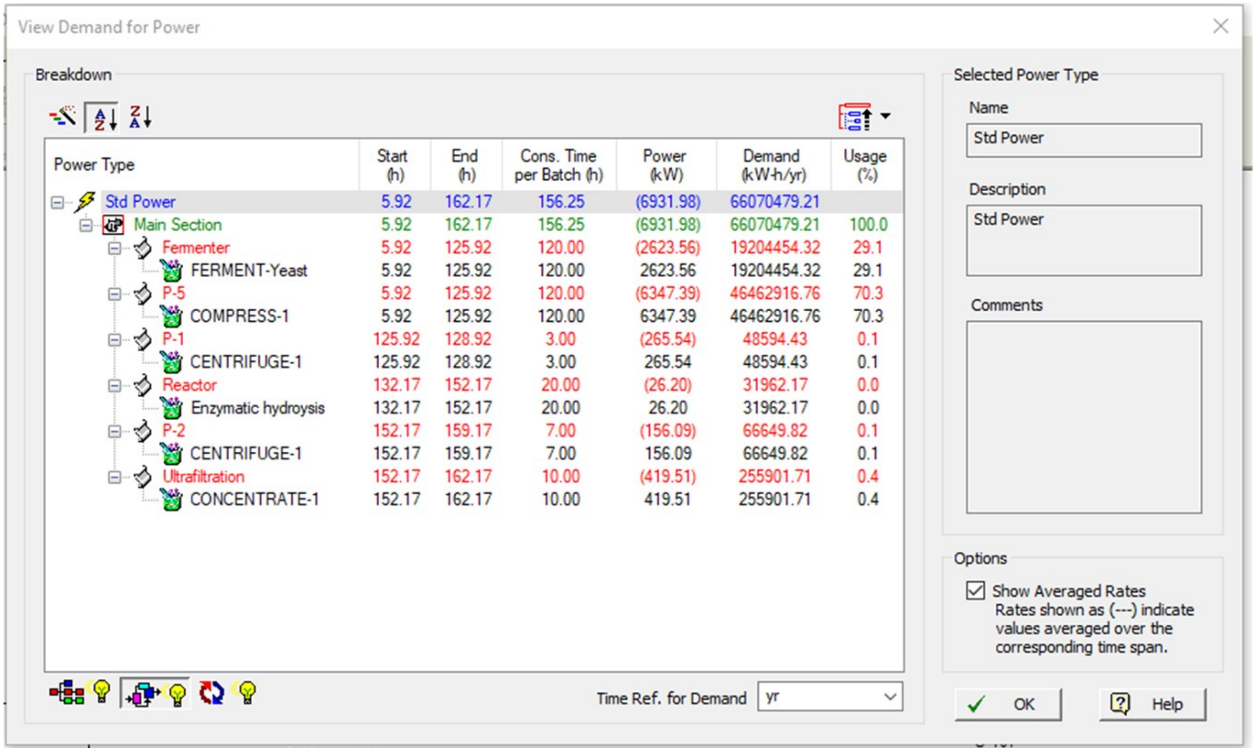


Figure S11: Lipid cost sensitivity to the main process input parameters.



View Demand for Power

Breakdown

Power Type	Start (h)	End (h)	Cons. Time per Batch (h)	Power (kW)	Demand (kW-h/yr)	Usage (%)
Std Power	5.92	162.17	156.25	(6931.98)	66070479.21	
Main Section	5.92	162.17	156.25	(6931.98)	66070479.21	100.0
Fermenter	5.92	125.92	120.00	(2623.56)	19204454.32	29.1
FERMENT-Yeast	5.92	125.92	120.00	2623.56	19204454.32	29.1
P-5	5.92	125.92	120.00	(6347.39)	46462916.76	70.3
COMPRESS-1	5.92	125.92	120.00	6347.39	46462916.76	70.3
P-1	125.92	128.92	3.00	(265.54)	48594.43	0.1
CENTRIFUGE-1	125.92	128.92	3.00	265.54	48594.43	0.1
Reactor	132.17	152.17	20.00	(26.20)	31962.17	0.0
Enzymatic hydroysis	132.17	152.17	20.00	26.20	31962.17	0.0
P-2	152.17	159.17	7.00	(156.09)	66649.82	0.1
CENTRIFUGE-1	152.17	159.17	7.00	156.09	66649.82	0.1
Ultrafiltration	152.17	162.17	10.00	(419.51)	255901.71	0.4
CONCENTRATE-1	152.17	162.17	10.00	419.51	255901.71	0.4

Selected Power Type

Name: Std Power

Description: Std Power

Comments

Options

Show Averaged Rates. Rates shown as (---) indicate values averaged over the corresponding time span.

Time Ref. for Demand: yr

OK Help

Figure S12: Eclectic power usage in the plant

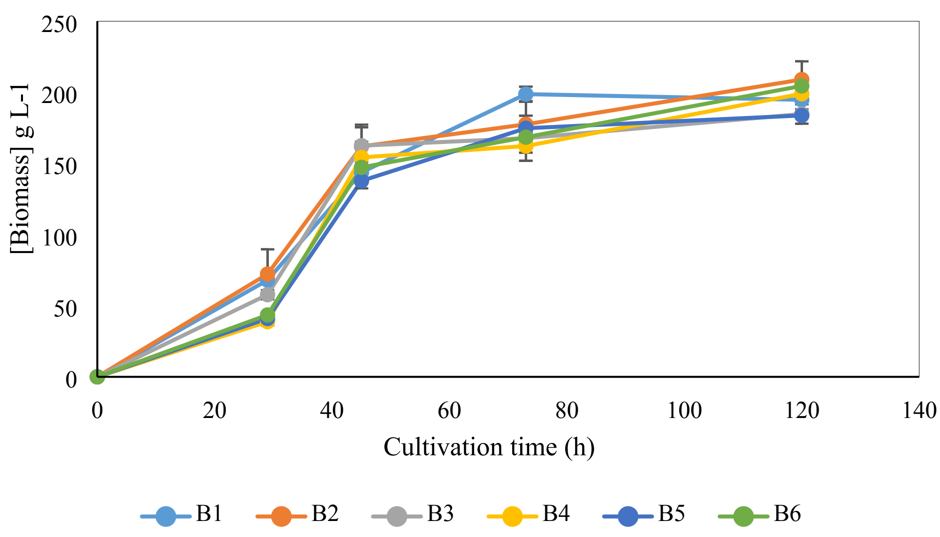


Figure S13: Six biological replication to validate the reproducibility of achieved lipid productivity.

1.5 LCA

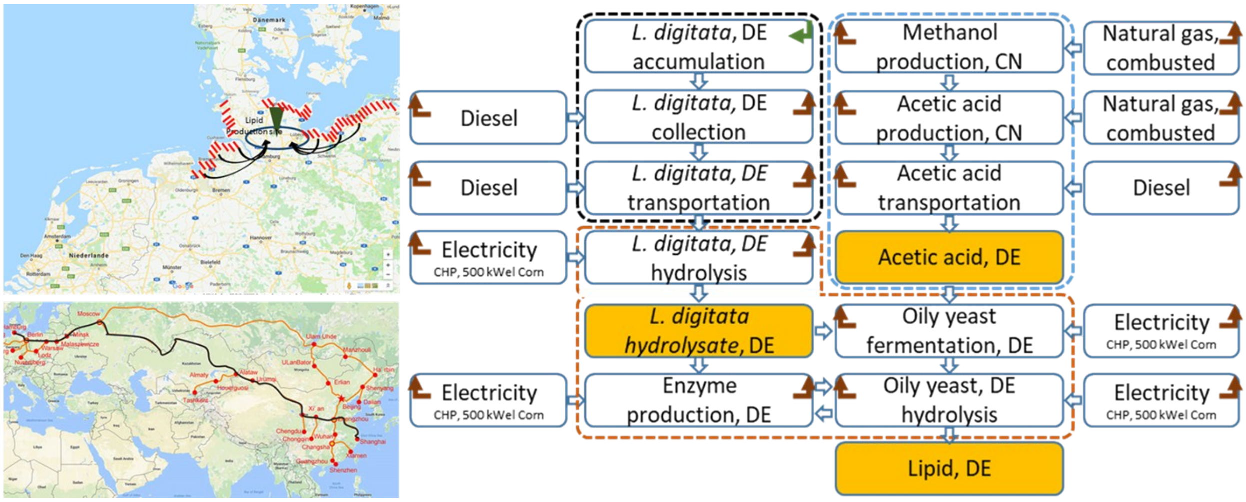


Figure S14: (a) Map showing the proposed location for the production plant. The brown algae collection area is shadowed in red. (b) Map showing the train route for acetic acid transportation from China. (c) The production system used in this study.

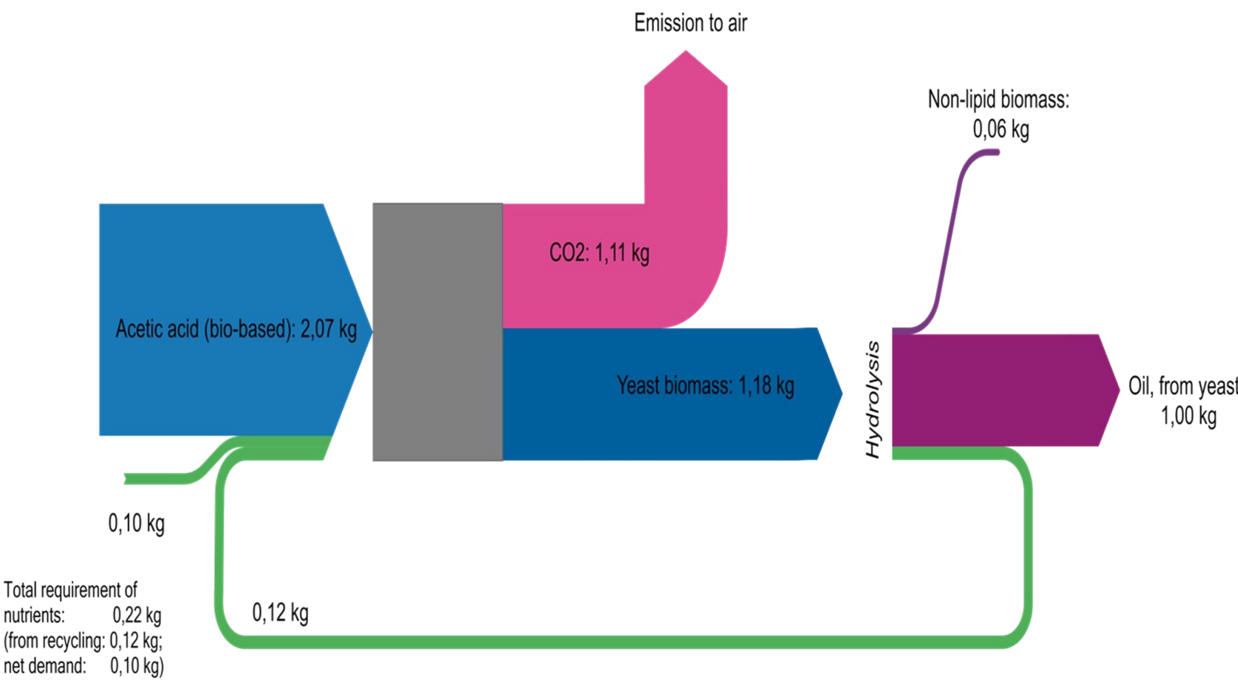


Figure S15: The Sankey diagram of the mass balance for the functional unit 1 kg Oil.

Table S12: Inventory of yeast fermentation according to the chosen productivity and current process design (This work).

Input

Flow	**Amount**	**Unit**	**Description**
Acetic acid, at fermentation plant,	2.96	kg	According to the SuperPro Designer, based on amount of acetic acid consumed during the fermentation
Calcium chloride, CaCl2, at regional storage	5.48E-06	kg	Medium formula
Yeast hydrolysate	4.16	kg	According the current process design
Digitata hydrolysate	8.2E-05	m3	According to the SuperPro Designer, based on amount of sugar consumed during the fermentation
Magnesium sulphate, at plant	1.64E-05	kg	Medium formula
Electricity, biogas CHP, at plant	1.547	kWh	According to the SuperPro Designer, the total Electricity consuming by Air compressor, Air filtration, fermenter 250m3, and centrifuge is813496 m3 , the no. of batches per year 63, mass of biomass per batch 50000 kg
Water, process and cooling, surface	0.258	m3	According to the SuperPro Designer, the total cooling water consuming by Air compressor, Air filtration, fermenter 250m3, and centrifuge is813496 m3 , the no. of batches per year 63, mass of biomass per batch 50000 kg

Output

Flow	**Amount**	**Unit**	**Description**
Acetic acid/ emission to air	1.14E-10	kg	According to the SuperPro Designer,
Carbon dioxide/emission to air	1.11	kg	According to the SuperPro Designer,
Oily-yeast biomass (200 g/L) culture	5	kg	Based on the biomass productivity 1.7 g L-1 h-1 [biomass: 200 g L-1 with lipid content of 85% ($w_{lipid}/{}^{d}w_{biomass}$), after 120 h]

Table S13: Inventory of the enzymatic hydrolysis of the yeast culture after the fermentation (This work).

Input

Flow	**Amount**	**Unit**	**Description**
Cell hydrolase	0.027	kg	Based on the enzyme dose hydrolase: 0.8% ($w/^{d}w_{biomass}$) & Protease: 0.5% (w/dw) Enzyme/ biomass
Oily-yeast biomass (200 g/L) culture	5	kg	Resulted from fermentation
Electricity, biogas CHP, at plant	1.13	kWh	According to the SuperPro Designer, the total Electricity consuming by 5 times reactor 250m3, and steam generator for (20 h at 50°C & (8 h at 37°C), then centrifuge is 813496 m3 , the no. of batches per year 63, mass of biomass per batch 50000 kg

Output

Flow	**Amount**	**Unit**	**Description**
C. oleaginosus hydrolysate	4.16	kg	According the current process design
Non-lipid biomass	0.04	kg	Based on the enzyme hydrolysis yield: 80% (w/w)
Oil, from oily yeast, TAG	1	kg	Based on the lipid productivity 1.4 g L^{-1} h^{-1} [biomass: 200 g L-1 with lipid content of 85% ($w_{lipid}/^{d}w_{biomass}$), after 120 h]

Table S14: Inventory of the enzymatic hydrolysis of L.Digitata (This work).

Input

Flow	Amount	Unit	Description
Acetic acid, at Fermentation plant,	4	kg	Used As buffer solution (50mM)
Cell hydrolase	1.05	kg	Based on the enzyme dose hydrolase: 1.3 % (w/dw). (Masri et al, 2018)
L.digitata, at plant, truck	70	kg	form The previous inventory (Table xx)
Sodium hydroxide	2	kg	Used As buffer solution (50mM)
Electricity, biogas CHP, at plant	1.325	kWh	According to the SuperPro Designer, the total Electricity consuming by 5 times reactor 250m3, and steam generator for 72h, 50°C.
Water, unspecified natural origin/m^3	0.9	m^3	According to the SuperPro Designer, the total water consuming steam generator for 72h, 50°C.

Output

Flow	Amount	Unit	Description
L.digitata Hydrolysate	1	m^3	Based on results presented in (*Masri et al, 2018*)
Residual L.digitata biomass	21.05	kg	Based on results presented in (*Masri et al, 2018*)

Table S15: Inventory of hydrolase production from T. reesei (This work).

Input

Flow	Amount	Unit	Description
L.digitata Hydrolysate	0.02	m3	Based on the media composition according to(Aftab et al 2008)
Non-Lipid Biomass	10	kg	Based on the media composition 10g L-1
Strom, Biogas BHKW, ab Anlage	1.12	kWh	According to the SuperPro Designer, the total Electricity consuming by fermenter 250m3, and steam generator for 120h, 28°C.

Output

Flow	Amount	Unit	
Carbon dioxide	1.40	kg	According to the SuperPro Designer, estimated CO2 released.
Enzyme, Mixed activities, T. reesei	1	kg	Based on the results presented in the work

Table S16: Inventory of L. digitata growth (This work).

Input

Flow	Amount	Unit	Description
Carbon dioxide/ absorption from air	1.47	kg	A theoretical calculation based on the C content in the produced biomass
Nitrate/ absorption from soil	0.031	kg	A theoretical calculation based on the N content in the produced biomass

Output

Flow	Amount	Unit
L.digitata	1	kg

Table S17: Inventory of truck (>32 ton) operation (BioEnergieDat Database).

Input

Flow	Amount	Unit	Description
Diesel, low-Sulphur, at regional storage	0.042	kg	

Output

Flow	Amount	Unit
Operation Truck >32t, EURO3_ei/Tremod	1	t*km
Acetaldehyde /emission to air	1.32E-05	kg
Ammonia /emission to air	0.15	kg
Benzene /emission to air	1.28E-06	kg
Cadmium /emission to air	6.05E-06	kg
Cadmium /emission to soil	3.61E-06	kg
Cadmium, ion /emission to water	3.61E-06	kg
Carbon dioxide /emission to air	0.14	kg
Carbon monoxide /emission to air	0.0003	kg
Chromium /emission to air	0.000064	kg
Chromium /emission to soil	1.71E-05	kg
Chromium VI /emission to air	5.12E-09	kg
Chromium, ion /emission to water	1.71E-05	kg
Copper /emission to soil	0.0002	kg
Copper /emission to air	0.004	kg
Copper, ion /emission to water	0.0002	kg
Dinitrogen monoxide /emission to air	0.22	kg
Ethane, 1,1,1,2-tetrafluoro-, HFC-134a /emission to air	3.92E-07	kg

Formaldehyde /emission to air	2.43E-05	kg	
Heat, waste /emission to air	2326.92	MJ	
Hydrocarbons, halogenated, unspecified /emission to air	6.75E-05	kg	
Lead /emission to soil	0.0002	kg	
Lead /emission to water	0.0002	kg	
Lead /emission to air	0.0002	kg	
Mercury /emission to air	1.03E-08	kg	
Methane, fossil /emission to air	1.62E-06	kg	
Nickel/emission to air	6.39E-05	kg	
Nickel /emission to soil	4.66E-05	kg	
Nickel, ion /emission to water	4.66E-05	kg	
Nitrogen oxides /emission to air	0.001	kg	
NMVOC, non-methane volatile organic compounds, unspecified origin /emission to air	6.59E-05	kg	
PAH, polycyclic aromatic hydrocarbons /emission to air	1.71E-10	kg	
Particulates, < 10 um /emission to air	9.45E-07	kg	
Particulates, < 2.5 um /emission to air	2.97E-05	kg	
Particulates, > 2.5 um, and < 10um /emission to air	8.58E-07	kg	
Selenium /emission to air	5.12E-07	kg	
Sulfur dioxide /emission to air	7.06E-07	kg	
Toluene /emission to air	5.40E-07	kg	
Xylene /emission to air	5.40E-07	kg	
Zinc /emission to air	0.002	kg	
Zinc /emission to soil	0.01	kg	
Zinc, ion /emission to water	0.01	kg	

Table 18: Inventory of tractor operation (BioEnergieDat Database).

Input

Flow	**Amount**	**Unit**	**Description**
Diesel, low-Sulphur, at regional storage	1	kg	

Output

Flow	Amount	Unit
Operation Tractor	1	kg
Carbon dioxide /emission to air	3.12	kg
Carbon monoxide /emission to air	0.003	kg
Dinitrogen monoxide /emission to air	0.22	kg
Methane, fossil /emission to air	0.000128	kg
Sulfur dioxide /emission to air	0.001	kg
Nitrogen dioxide	0.0001	kg
Nitrogen oxides	0.04	kg

Table S19: Inventory of acetic acid production and transportation from China (US LCI database).

Input

Flow	Amount	Unit	Description
CUTOFF Disposal, solid waste, unspecified, to sanitary landfill	0.001	kg	
Electricity, at cogent, for natural gas turbine	0.002	kWh	
Electricity, at grid, US, 2000	0.02	kWh	
Methanol, at plant	0.54	kg	
Natural gas, combusted in industrial boiler	0.22	m3	
Natural gas, processed, at plant	0.44	m3	
Transport, barge, diesel powered	0.01	t*km	
Transport, barge, residual fuel oil powered	0.03	t*km	
Transport, combination truck, diesel powered	0.005	t*km	
Transport, pipeline, natural gas	0.49246	t*km	
Transport, pipeline, unspecified petroleum products	0.000867	t*km	
Transport, train, diesel powered	0.004925	t*km	
Transport, train, diesel powered	12.5	t*km	Based on the suggested train way from China to Hamburg (about 12, 000 km)

Output

Flow	Amount	Unit
Acetic acid, at plant in Hamburg	1	kg
Acids, unspecified /emission to water	0.00096	kg

Ammonia/emission to air	0.00057	kg
Ammonia /emission to water	0.000052	kg
Carbon dioxide /emission to air	0.00176	kg
Carbon monoxide /emission to air	0.00397	kg
Methanol /emission to air	0.00004	kg
Recovered energy, at acetic acid production	0.18841	MJ
TOC, Total Organic Carbon /emission to air	0.00217	kg

Table 20: Inventory of methanol production in China (US LCI database).

Input

Flow	**Amount**	**Unit**	**Description**
CUTOFF Disposal, solid waste, unspecified, to sanitary landfill	0.0005	kg	
Electricity, at grid, US, 2008	0.0081	kWh	
Natural gas, combusted in industrial boiler	0.13	m3	
Natural gas, processed, for olefins production, at plant	0.62	kg	
Oxygen, in air	0.38	kg	
Transport, combination truck, diesel powered	0.01	t*km	
Transport, pipeline, natural gas	0.998	t*km	
Transport, train, diesel powered	0.00997	t*km	
Water, process, unspecified natural origin/m3	0.00054	m3	

Output

Flow	**Amount**	**Unit**
BOD5, Biological Oxygen Demand /emission to water	0.000058	kg
Carbon dioxide /emission to air	0.53	kg
Methanol, at plant	1	kg
NMVOC, non-methane volatile organic compounds, unspecified /emission to air	0.005	kg
Suspended solids, unspecified /emission to water	0.000088	kg

Table 21: Inventory of natural gas combusted in industrial equipment (US LCI database).

Input

Flow	**Amount**	**Unit**	**Description**
Natural gas, processed, at plant	1	m3	
Transport, combination truck, average fuel mix	0.20	t*km	
Transport, pipeline, natural gas	1.19	t*km	
Transport, train, diesel powered	0.01	t*km	

Output

Flow	**Amount**	**Unit**	**Description**
Acetaldehyde /emission to air	6.54E-07	kg	
Acrolein /emission to air	1.05E-07	kg	
Benzene /emission to air	1.96E-07	kg	
Benzene, ethyl- /emission to air	5.23E-07	kg	
Butadiene /emission to air	7.03E-09	kg	
Carbon dioxide, fossil /emission to air	1.96	kg	
Carbon monoxide, fossil /emission to air	0.00024	kg	
Formaldehyde /emission to air	0.000012	kg	
Methane, fossil /emission to air	0.00014	kg	
Naphthalene /emission to air	2.124E-08	kg	
Natural gas, combusted in industrial equipment	1	m3	
Nitrogen oxides /emission to air	0.0017	kg	
PAH, polycyclic aromatic hydrocarbons /emission to air	3.5945E-08	kg	
Particulates, > 2.5 um, and < 10um /emission to air	0.00011	kg	
Propylene oxide /emission to air	4.7383E-07	kg	
Sulfur oxides /emission to air	0.00001	kg	
Toluene /emission to air	0.000002	kg	
VOC, volatile organic compounds /emission to air	0.00003	kg	
Xylene /emission to air	1.0457E-06	kg	

Table 22: Life cycle assessment and impact analysis for 1 kg yeast lipid production.

		Name	Inventory result	Unit	Impact factor	Impact result	Impact co-factor
1		**Eutrophication - generic**				**0.00339**	**kg PO4--- eq.**
	1.1	**Transport, train, diesel powered - DE**				**0.00241**	**kg PO4--- eq.**
		Nitrogen oxides	0.018	kg	0.13	0.0024	kg PO4--- eq.
	1.2	**Acetic acid, at plant - CN**				**0.00065**	**kg PO4--- eq.**
		Ammonia	0.002	kg	0.35	0.00059	kg PO4--- eq.
		Ammonia	0.0001	kg	0.35	5.39E-05	kg PO4--- eq.
	1.3	**Natural gas, combusted in industrial boiler - RNA**				**0.00019**	**kg PO4--- eq.**
		Nitrogen oxides	0.001	kg	0.13	0.00018	kg PO4--- eq.
		Dinitrogen monoxide	3.13E-05	kg	0.27	8.46E-06	kg PO4--- eq.
	1.4	**L.digitata Growth**				**0.00013**	**kg PO4--- eq.**
		Nitrate	0.001	kg	0.1	0.00013	kg PO4--- eq.
2		**Photochemical oxidation - high Nox**				**0.00043**	**kg ethylene eq.**
	2.1	**Acetic acid, at plant - CN**				**0.00033**	**kg ethylene eq.**
		Carbon monoxide	0.01	kg	0.027	0.00032	kg ethylene eq.
		Methanol	0.00012	kg	0.14	1.66E-05	kg ethylene eq.
	2.2	**Transport, train, diesel powered - DE**				**4.93E-05**	**kg ethylene eq.**
		Carbon monoxide	0.002	kg	0.027	4.91E-05	kg ethylene eq.
	2.3	**CHP (gas engine) 500 kWel Mais (90), cattle manure (10), DE**				**4.52E-05**	**kg ethylene eq.**
		Methane, biogenic	0.007	kg	0.006	4.52E-05	kg ethylene eq.
3		**Terrestrial ecotoxicity - TETP inf**				**0.00026**	**kg 1,4-dichlorobenzene eq.**
	3.1	**Natural gas, combusted in industrial boiler - RNA**				**0.00017**	**kg 1,4-dichlorobenzene eq.**
		Mercury	3.62E-09	kg	2.83E+04	0.0001	kg 1,4-dichlorobenzene eq.
		Chromium	1.95E-08	kg	3031.11	5.91E-05	kg 1,4-dichlorobenzene eq.
		Nickel	2.93E-08	kg	116.04	3.40E-06	kg 1,4-dichlorobenzene eq.
	3.2	**Operation truck >32t, EURO3**				**9.42E-05**	**kg 1,4-dichlorobenzene eq.**
		Zinc	1.52E-06	kg	24.59	3.74E-05	kg 1,4-dichlorobenzene eq.
		Chromium	9.70E-09	kg	3031.12	2.94E-05	kg 1,4-dichlorobenzene eq.
		Chromium	2.56E-09	kg	6302.86	1.61E-05	kg 1,4-dichlorobenzene eq.
		Copper	5.50E-07	kg	6.99	3.85E-06	kg 1,4-dichlorobenzene eq.
		Zinc	2.32E-07	kg	11.96	2.78E-06	kg 1,4-dichlorobenzene eq.

	Nickel	6.96E-09	kg	238.55	1.66E-06	kg 1,4-dichlorobenzene eq.
	Nickel	9.55E-09	kg	116.04	1.11E-06	kg 1,4-dichlorobenzene eq.
4	**Climate change - GWP100**				**3.56179**	**kg CO2 eq.**
4.1	**Yeast Biomass production, Fermentation - DE**				**1.10924**	**kg CO2 eq.**
	Carbon dioxide	1.10	kg	1	1.10924	kg CO2 eq.
4.2	**CHP (gas engine) 500 kWel Mais (90), cattle manure (10), DE**				**0.87251**	**kg CO2 eq.**
	Carbon dioxide	0.68	kg	1	0.68407	kg CO2 eq.
	Methane, biogenic	0.007	kg	25	0.18843	kg CO2 eq.
4.3	**Methanol, at plant - CN**				**0.8466**	**kg CO2 eq.**
	Carbon dioxide	0.84	kg	1	0.8466	kg CO2 eq.
4.4	**Transport, train, diesel powered - DE**				**0.71149**	**kg CO2 eq.**
	Carbon dioxide	0.70	kg	1	0.70195	kg CO2 eq.
4.5	**Enzyme, Mixed activities, T. reesei**				**0.03711**	**kg CO2 eq.**
	Carbon dioxide	0.04	kg	1	0.03711	kg CO2 eq.
4.6	**L.digitata_Growth**				**-0.06262**	**kg CO2 eq.**
	Carbon dioxide	-0.06	kg	1	-0.06262	kg CO2 eq.
5	**Acidification potential - average Europe**				**0.01267**	**kg SO2 eq.**
5.1	**Transport, train, diesel powered - DE**				**0.00923**	**kg SO2 eq.**
	Nitrogen oxides	0.02	kg	0.5	0.00923	kg SO2 eq.
5.2	**Acetic acid, at plant - CN**				**0.0027**	**kg SO2 eq.**
	Ammonia	0.00169	kg	1.6	0.0027	kg SO2 eq.
5.3	**Natural gas, combusted in industrial boiler - RNA**				**0.0007**	**kg SO2 eq.**
	Nitrogen oxides	0.001	kg	0.5	0.0007	kg SO2 eq.
6	**Depletion of abiotic resources - fossil fuels**				**0**	**MJ**
7	**Depletion of abiotic resources - elements, ultimate reserves**				**0**	**kg antimony eq.**
8	**Human toxicity - HTP inf**				**0.03056**	**kg 1,4-dichlorobenzene eq.**
8.1	**Transport, train, diesel powered - DE**				**0.02254**	**kg 1,4-dichlorobenzene eq.**
	Nitrogen oxides	0.02	kg	1.2	0.02216	kg 1,4-dichlorobenzene eq.
	Particulates, < 10 um	0.0005	kg	0.82	0.00038	kg 1,4-dichlorobenzene eq.
8.2	**Natural gas, combusted in industrial boiler - RNA**				**0.00496**	**kg 1,4-dichlorobenzene eq.**
	Cadmium	1.53E-08	kg	1.45E+05	0.00222	kg 1,4-

						dichlorobenzene eq.
	Nitrogen oxides	0.00139	kg	1.2	0.00167	kg 1,4-dichlorobenzene eq.
	Nickel	2.93E-08	kg	3.50E+04	0.00103	kg 1,4-dichlorobenzene eq.
8.3	**Operation truck >32t, EURO3**				**0.00289**	**kg 1,4-dichlorobenzene eq.**
	Copper	5.50E-07	kg	4295.03	0.00236	kg 1,4-dichlorobenzene eq.
	Nickel	9.55E-09	kg	3.50E+04	0.00033	kg 1,4-dichlorobenzene eq.
	Cadmium	9.03E-10	kg	1.45E+05	0.00013	kg 1,4-dichlorobenzene eq.
9	**Ozone layer depletion - ODP steady state**				**0**	**kg CFC-11 eq.**
10	**Marine aquatic ecotoxicity - MAETP inf**				**0.74621**	**kg 1,4-dichlorobenzene eq.**
10.1	**Operation truck >32t, EURO3**				**0.61477**	**kg 1,4-dichlorobenzene eq.**
	Copper	5.50E-07	kg	8.93E+05	0.49159	kg 1,4-dichlorobenzene eq.
	Nickel	9.55E-09	kg	3.76E+06	0.03587	kg 1,4-dichlorobenzene eq.
	Zinc, ion	1.52E-06	kg	1.38E+04	0.02108	kg 1,4-dichlorobenzene eq.
	Nickel, ion	6.96E-09	kg	2.25E+06	0.01566	kg 1,4-dichlorobenzene eq.
	Zinc	2.32E-07	kg	6.73E+04	0.01561	kg 1,4-dichlorobenzene eq.
	Zinc	1.52E-06	kg	7208.56	0.01098	kg 1,4-dichlorobenzene eq.
	Copper, ion	3.61E-08	kg	2.33E+05	0.0084	kg 1,4-dichlorobenzene eq.
	Nickel	6.96E-09	kg	1.17E+06	0.00818	kg 1,4-dichlorobenzene eq.
10.2	**Natural gas, combusted in industrial boiler - RNA**				**0.13144**	**kg 1,4-dichlorobenzene eq.**
	Nickel	2.93E-08	kg	3.76E+06	0.10999	kg 1,4-dichlorobenzene eq.
	Cadmium	1.53E-08	kg	1.11E+06	0.01695	kg 1,4-dichlorobenzene eq.
	Mercury	3.62E-09	kg	1.20E+06	0.00435	kg 1,4-dichlorobenzene eq.
11	**Freshwater aquatic ecotoxicity - FAETP inf**				**0.00047**	**kg 1,4-dichlorobenzene eq.**
11.1	**Operation truck >32t, EURO3**				**0.00044**	**kg 1,4-dichlorobenzene eq.**
	Zinc, ion	1.52E-06	kg	91.71	0.00014	kg 1,4-dichlorobenzene eq.
	Copper	5.50E-07	kg	221.65	0.00012	kg 1,4-dichlorobenzene eq.
	Zinc	1.52E-06	kg	47.745	7.27E-05	kg 1,4-dichlorobenzene eq.
	Copper, ion	3.61E-08	kg	1157.30	4.18E-05	kg 1,4-dichlorobenzene eq.

	Nickel, ion	6.96E-09	kg	3237.61	2.25E-05	kg 1,4-dichlorobenzene eq.
	Copper	3.61E-08	kg	594.65	2.15E-05	kg 1,4-dichlorobenzene eq.
	Nickel	6.96E-09	kg	1690.25	1.18E-05	kg 1,4-dichlorobenzene eq.
	Nickel	9.55E-09	kg	629.47	6.01E-06	kg 1,4-dichlorobenzene eq.
11.2	**Natural gas, combusted in industrial boiler - RNA**				**2.41E-05**	**kg 1,4-dichlorobenzene eq.**
	Nickel	2.93E-08	kg	629.47	1.84E-05	kg 1,4-dichlorobenzene eq.
	Cadmium	1.53E-08	kg	289.43	4.44E-06	kg 1,4-dichlorobenzene eq.
	Mercury	3.62E-09	kg	316.78	1.15E-06	kg 1,4-dichlorobenzene eq.

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