Supporting Information for

Methanol biotransformation for the production of the biodegradable plastic monomer L-lactate in yeast

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The detailed process of the of the techno-economic analysis of L-lactate production from CO_2-derived methanol

Methanol production (A100)

The synthesis of methanol from CO_2 and hydrogen (H_2) achieves a CO_2 single-pass conversion exceeding 95% at a pressure of 5.0 MPa, an H_2: CO_2 ratio of 3:1, and a temperature of 315°C (A100.1) (31). H_2 required for methanol manufacturing is produced via solar electrolysis of water. The maximum efficiency achieved in large-scale alkaline water electrolysis for hydrogen production is around 87%, and the energy consumption for each unit of hydrogen generated remains below 4.1 $kW \cdot (h \cdot m^3)^{-1}$ (32).

$2\ CO_2 + 4\ H_2 \rightarrow 2\ CH_3OH + O_2$ (A100.1)

L-lactate production (A200)

Methanol acts as the exclusive carbon source for the growth of recombinant *O. polymorpha* HPLA22u, with oxygen (O_2) being provided via alkaline water electrolysis and pressure swing adsorption (PSA). The L-lactate production process involves recombinant *O. polymorpha* HPLA22u being cultured in 300 m^3 fermenters, and each bioreactor has a working capacity of 240 m^3. Under the assumptions for L-lactate production in *SI Appendix* Table S2, the total fermentation volume was 2,587 m^3. The stoichiometric equations for bioreactors in the Aspen Plus simulations are derived from Equation A200.1 and A200.2 (33).

$3\ CH_3OH + 1.5\ O_2 \rightarrow$ L-lactate ($C_3H_6O_3$) + $3\ H_2O$ (A200.1)

$CH_3OH + 0.118\ NH_3 + 0.9985\ O_2 \rightarrow 0.118$ Biomass ($C_4H_8O_2N$) + $0.528\ CO_2 + 1.705\ H_2O$ (A200.2)

L-lactate purification (A300)

The integration of chitosan flocculation with dissolved air flotation (DAF) results in biomass recovery efficiencies surpassing 90%. The liquid stream is passed through an ultrafiltration membrane separator to eliminate residual solids and contaminants. The L-lactate-rich stream from the separator undergoes further concentration by an evaporation process, achieving an expected overall L-lactate recovery efficiency of 90% for the base scenario.

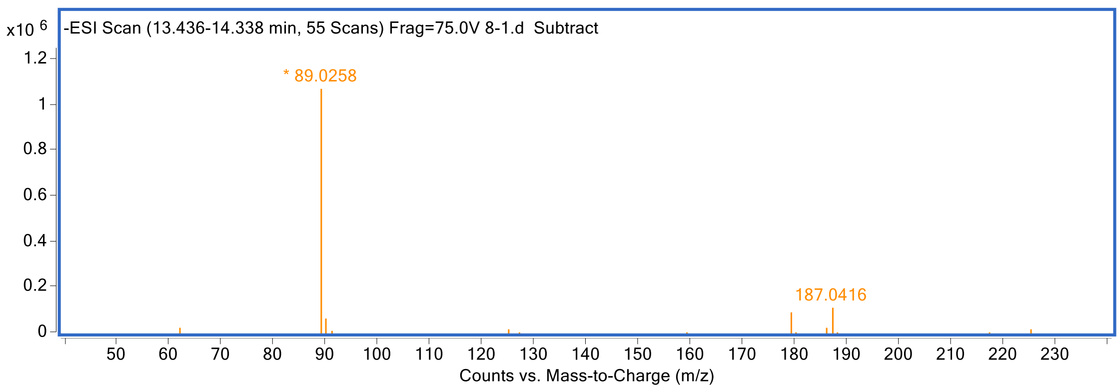
Wastewater treatment (A400)

All wastewater is directed to the anaerobic digestion units to enhance the energy efficiency of the feedstocks and to recycle the cooling water. The methane (CH_4) obtained from the anaerobic digestion process is fully combusted in an on-site combustor to generate energy on the premises. The liquid effluent resulting from anaerobic digestion comprises carbonates, solid sludge, nitrogen, and water. The material produced from the anaerobic digestion process is rich in nitrogen nutrients, making it a valuable fertiliser by-product.

Utilities (A500)

Area 500 facilitates overall energy, water, and power integration, including a cooling water system, chilled water system, process water manifold, and power systems. In this area, the total power requirements of the system are determined in this area.

a



b

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
$C_3H_6O_3$	TRUE	90.0331	90.0317	-15.5	$C_3H_5O_3$	82.74
$C_6H_{12}O_6$	TRUE	180.063	180.0634	1.89	$C_6H_{11}O_6$	99.37
$C_{11}H_8O_3$	TRUE	188.0489	188.0473	-8.36	$C_{11}H_7O_3$	79.76

Fig. S1. LC-MS determination of lactic acid synthesized from sole methanol in *O. polymorpha* HpPa.

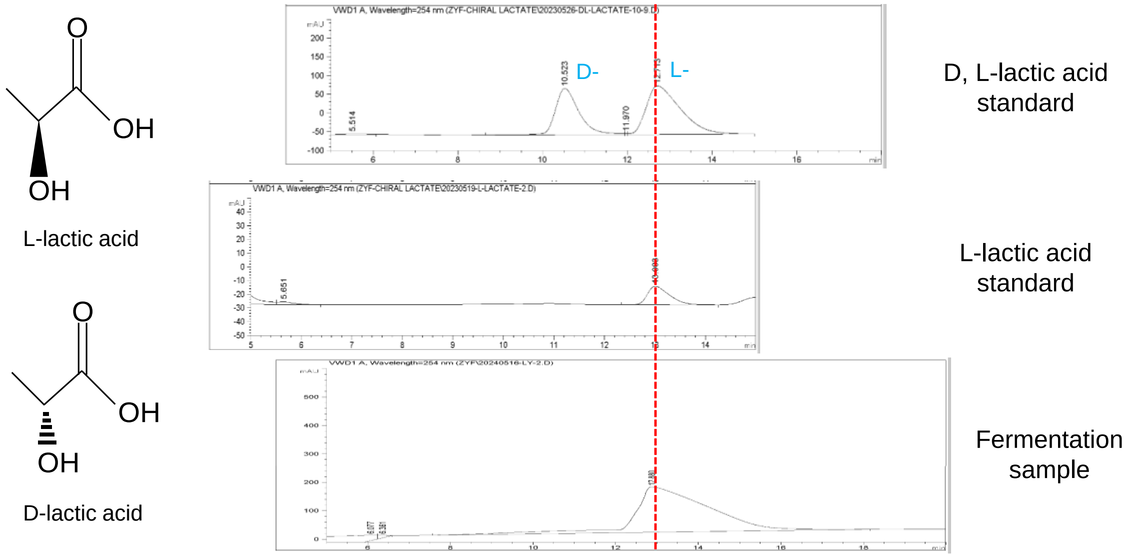


Fig. S2. Determination of the optical isomer of L-lactate produced from methanol in *O. polymorpha* by using a chiral column on HPLC equipped with a UV detector.

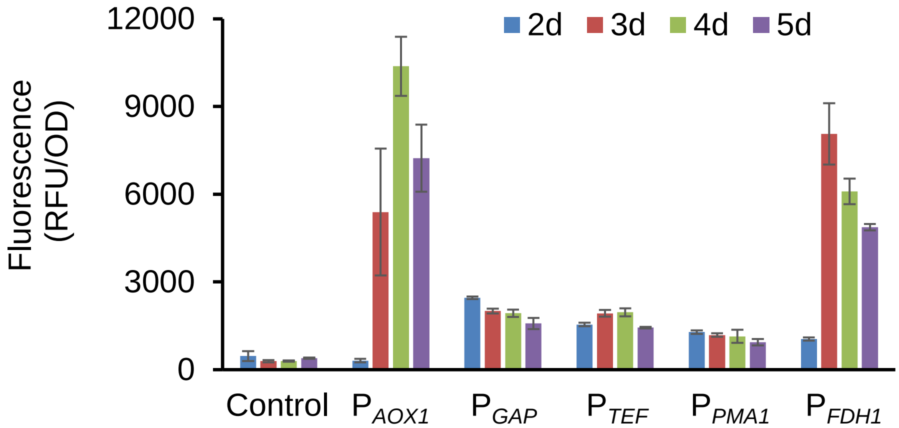


Fig. S3. Promoter characterization in methanol metabolism process of *O. polymorpha*. *AOX* and *FDH1* promoters belong to methanol inducible ones, while *GAP*, *TEF* and *PMA1* promoters belong to constitutive ones.

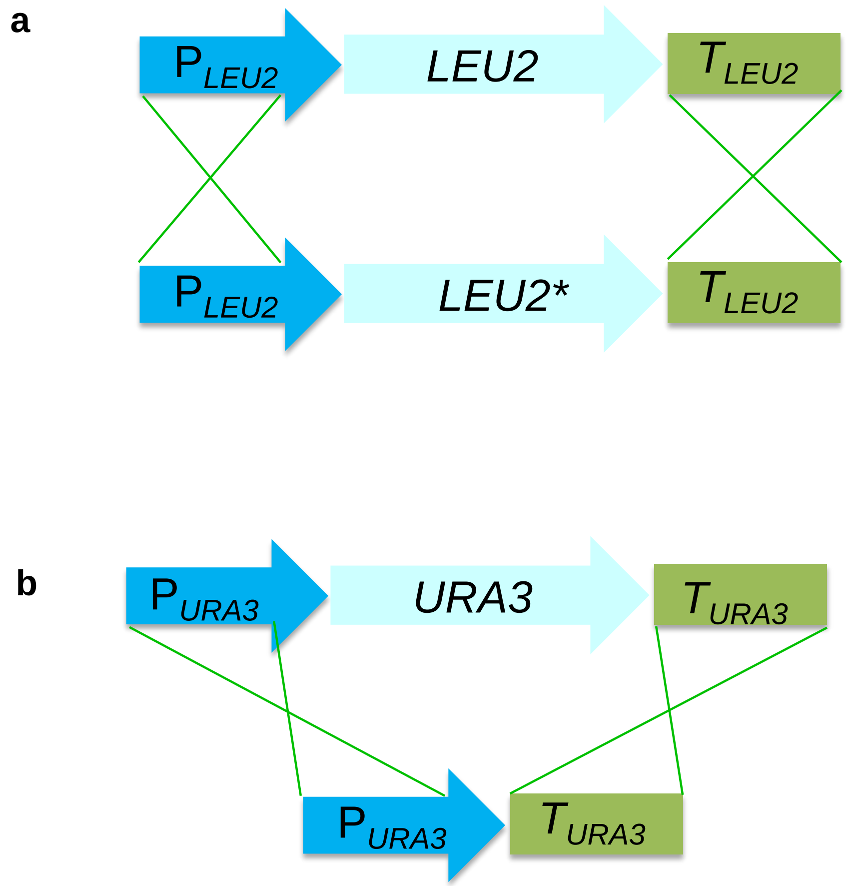


Fig. S4. *In situ* recovery of the auxotrophic gene *LEU2* (a) and *URA3* (b).

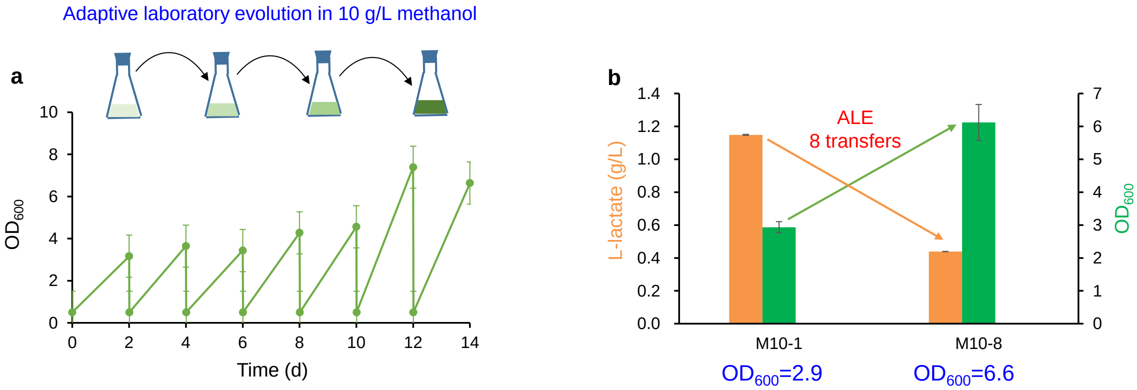


Fig. S5. Adaptive laboratory evolution (ALE) of the engineered strain HPLA06. a. The process of ALE in 10 g/L methanol. b. ALE improved cell growth but dramatically decreased L-lactate production.

GXGXXG * NAD+

PaLdh MSNIQNHQKVVLVGDGAVGSSYAFAMAQQGIAEEFVIVDV

V40R NADP+

Fig. S6. Single amino acid of V40R close to the cofactor binding site (GXGXXG) to change the cofactor preference of PaLdh from NADH to NADPH.

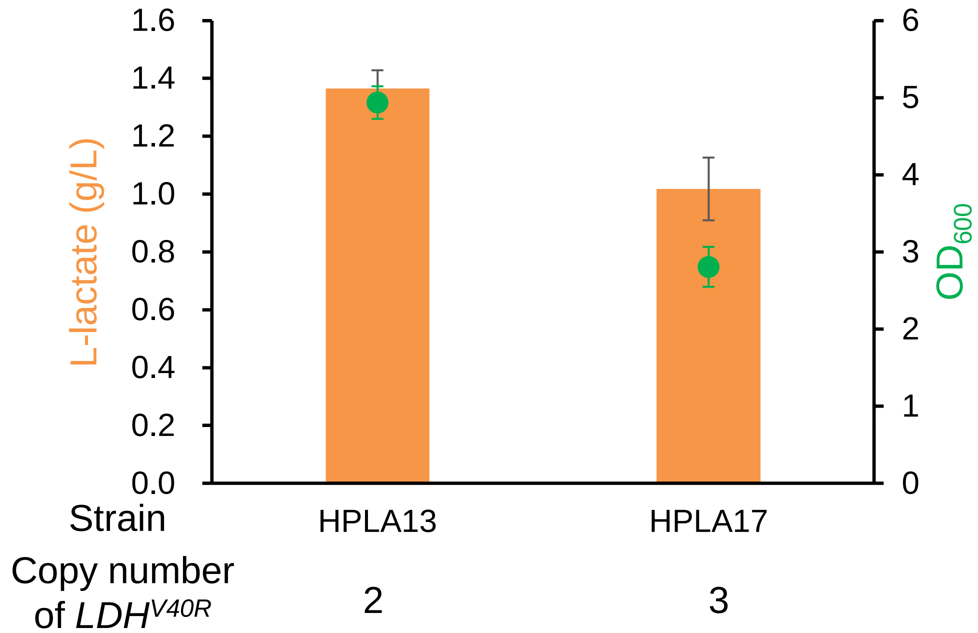


Fig. S7. Further increasing another copy of *LDH*^{V40R} in the strain HPLA13 decreased L-lactate production.

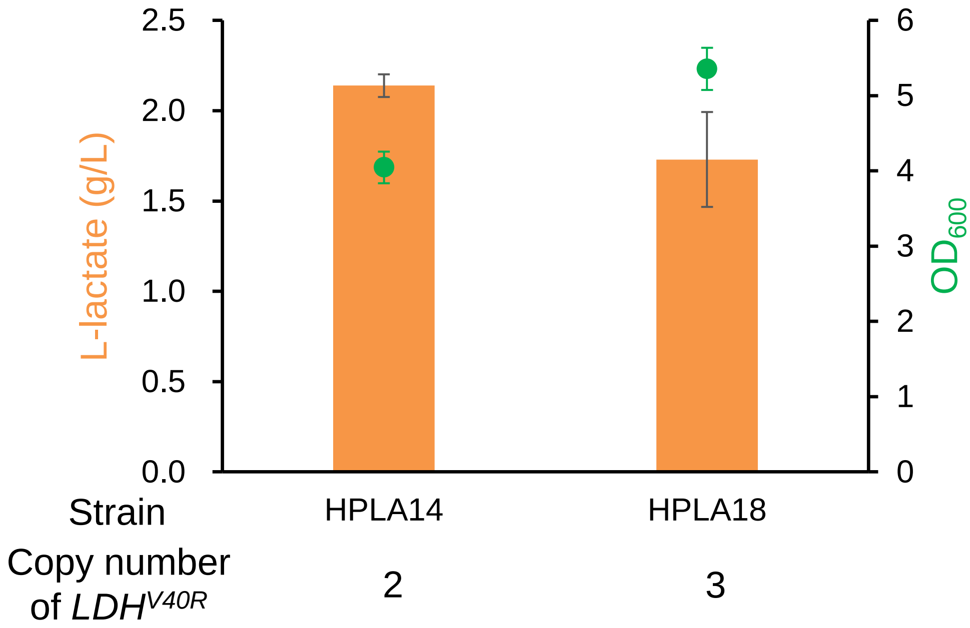


Fig. S8. Further increasing another copy of *LDH*^{V40R} in the strain HPLA14 decreased L-lactate production.

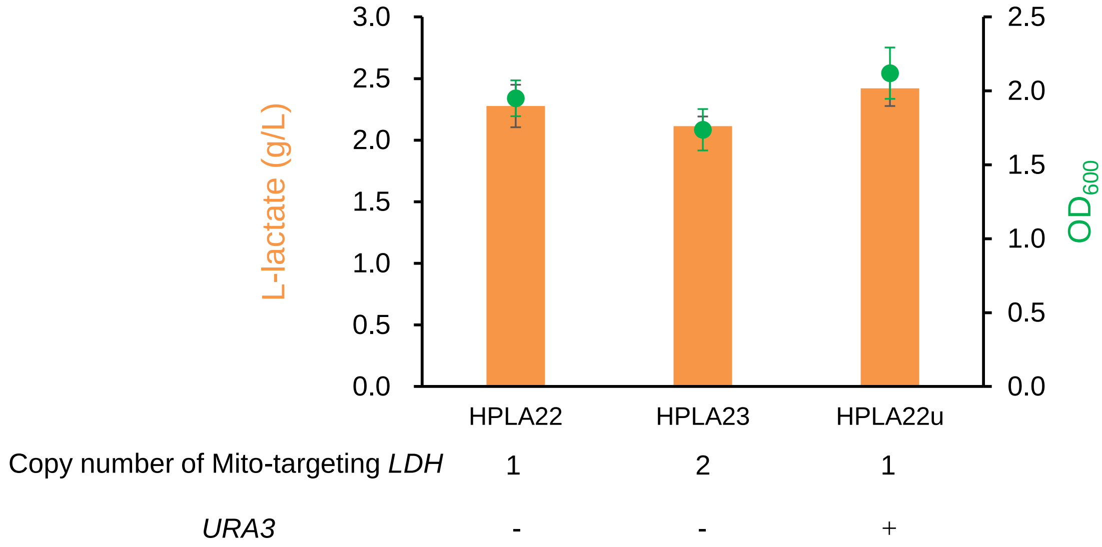


Fig. S9. Increasing copy number of Mito-targeting *LDH* and *in situ* recovery of selection marker gene *URA3* for improving L-lactate production.

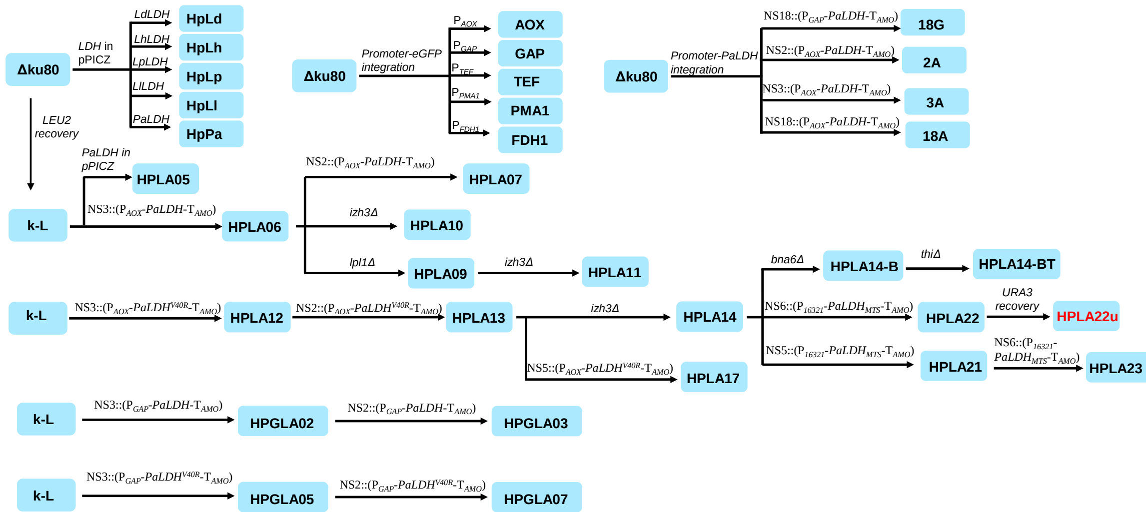


Fig. S10. Flowchart of *O. polymorpha* strain construction for L-lactate production. The strain used for fed-batch fermentation was marked with red.

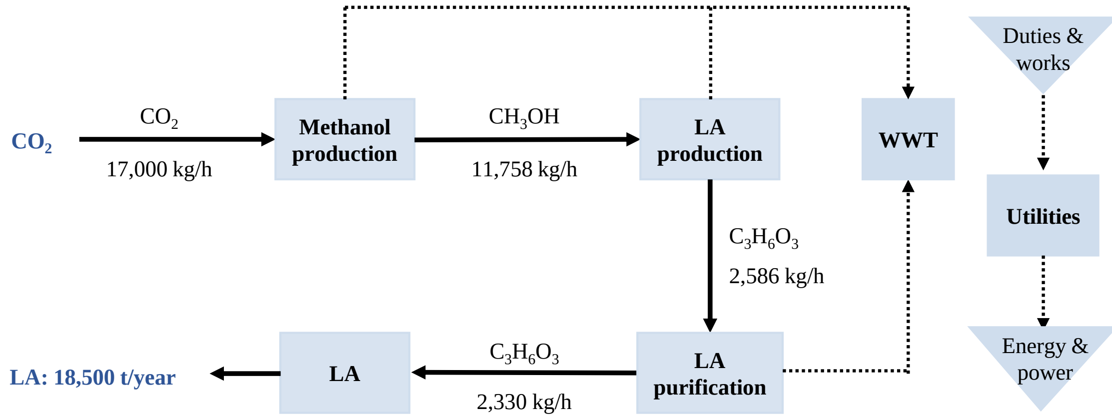


Fig. S11. Simplified carbon flow diagram for CO_2-derived methanol conversed into lactic acid. LA: lactic acid.

Table S1. Detailed information of Ldh from different sources.

Genera	Km (mM)	Kcat (s^{-1})	Titer (g/L)	Host	Reference
Lactobacillus delbrueckii	-	-	135	*Lactobacillus delbrueckii*	(1)
Lactobacillus helveticus	0.25	643	92	*Candida sonorensis*	(2)
Lactobacillus plantarum	-	-	18	*Schizosaccharomyces pombe*	(3)
Lactococcus lactis	-	-	50	*Lactococcus lactis*	(4)
Pediococcus acidilactici	-	-	97	*Escherichia coli*	(5)

Table S2. Assumptions for the lactic acid production from CO_2-derived methanol.

Parameter	Assumption	References
CO_2 inflow	17,000 kg/h	Aspen plus
CO_2 to methanol efficiency	95%	(6)
Electricity to H_2 efficiency	4.1 kW·h/m^3	(7)
Water electrolysis efficiency	87%	(7)
Lactic acid titer	25 g/L	This study
Lactic acid productivity	0.5 g/(L·h)	This study
Lactic acid yield	0.22 g/g	This study
Fermentation condition	*Ogataea polymorpha*	This study
Culture temperature	37 ℃	
Nominal volume of bioreactors	300 m^3	Aspen plus
Maximum working volume	240 m^3	Aspen plus
Total fermentation liquid volume	2,587 m^3	Aspen plus
Bioreactor required	22	Aspen plus
Lactic acid recovery rate	90%	(8)
Annual production capacity of lactic acid	18,500 t	Aspen plus

Table S3. n^{th}-plant assumptions required by techno-economic analysis (8).

Assumption	**Parameters**
Cost year for analysis	2024
Equity for plant	50%
Loan interest and terms	5% annually / 10 years
General plant depreciation period	7 years
Bioreactors depreciation period	10 years
Construction period	2.5 years
Start-up time	0.5 years
Revenues during startup	50%
Variable costs during startup	75%
Fixed costs during startup	100%
Internal rate of return	10%
Income tax rate	21%
Annual operating time	330 day/year (7920 hours/year)
Design years	30 years
Inside battery limit (ISBL)	A100, A200, A300
Warehouse	4% of ISBL
Site development	9% of ISBL
Additional piping	4.5% of ISBL
Indirect costs	**percentage of total direct costs**
Prorateable expenses	10%
Field expenses	10%
Home office and construction fee	20%
Project contingency	10%
Other costs (Start-Up, Permits, etc.)	10%
Fixed operating costs	**percentage of total direct costs**
Employees	13 positions
Labor burden	60% of total salaries
Maintenance	2% of ISBL
Insurance and taxes	0.5% of fixed capital investment

Table S4. Capital expenditures for the annual production of 18,500-ton lactic acid from CO_2.

Category		Classification	Items	Investment	Total investment
FCI	TDC	Equipment & installation	A100	1.43 MM$	71.20 MM$
			A200	37.53 MM$	
			A300	7.75 MM$	
			A400	4.59 MM$	
			A500	11.73 MM$	
		Warehouse		1.87 MM$	
		Site development		4.20 MM$	
		Additional piping		2.10 MM$	
	TIC	Prorated expenses		7.12 MM$	42.72 MM$
		Field expenses		7.12 MM$	
		Office & construction fee		14.24 MM$	
		Project contingency		7.12 MM$	
		Other costs (Start-Up, permits, etc.)		7.12 MM$	
Land				1.14 MM$	1.14 MM$
Working capital				5.70 MM$	5.70 MM$
Total operating cost			**120.76 MM$**		

Table S5. Operating expenditures for the annual production of 18,500-ton lactic acid from CO_2.

Classification	Items	Investment	Total investment
Raw material	CO_2 from capture	12.45 MM$/yr	23.65 MM$/yr
	Ammonia	1.77 MM$/yr	
	Flocculant	2.77 MM$/yr	
	Water for H_2	0.07 MM$/yr	
	Makeup water	2.21 MM$/yr	
	Ultrafilter replacement	0.03 MM$/yr	
	Electricity	4.35 MM$/yr	
Sludge disposal	Sludge disposal	1.16 MM$/yr	1.16 MM$/yr
By-product's credit	Biomass credit	-2.14 MM$/yr	-11.16 MM$/yr
	Carbon tax	-9.02 MM$/yr	
Labor	Salaries	1.24 MM$/yr	1.99 MM$/yr
	Labor burden	0.75 MM$/yr	
Others	Maintenance	0.93 MM$/yr	1.50 MM$/yr
	Property insurance	0.57 MM$/yr	
Total operating cost		**17.14 MM$/yr**	

Table S6. Inventory data used in the life cycle assessment.

Input data	**Unit**	**Aspen Plus results**
CO_2 from capture	kg/kg lactic acid	7.2948
Tap water	kg/kg lactic acid	6.8603
Nitrogen source	kg/kg lactic acid	0.1580
Flocculant	kg/kg lactic acid	0.0111
Solar power	kwh/kg lactic acid	44.1584
Output data	**Unit**	**Aspen Plus results**
Lactic acid	kg/kg lactic acid	1.0000
Sludge	kg/kg lactic acid	2.8237
O_2	kg/kg lactic acid	17.9059
CO_2	kg/kg lactic acid	3.0360
N_2	kg/kg lactic acid	53.3864
NH_3	kg/kg lactic acid	0.1558

Table S7. Global warming potential for producing 1 kg of lactic acid.

Impact category	GWP (kg CO_2-eq)
CO_2 from capture	-7.2948
Tap water	0.0020
Nitrogen source	1.3606
Flocculant	0.0510
Solar power	4.8574
Sludge	0.0300
CO_2 emission	3.0360
Total GWP (kg CO_2-eq)	**2.0421**

Table S8. List of plasmids used in this study.

Plasmid	Relevant characteristics	Resource
pLdLDH	*AmpR, URA3,* P_{AOX}*-LdLDH*	This study
pLhLDH	*AmpR, URA3,* P_{AOX}*-LHLDH*	This study
pLpLDH	*AmpR, URA3,* P_{AOX}*-LpLDH*	This study
pLlLDH	*AmpR, URA3,* P_{AOX}*-LlLDH*	This study
pPaLDH	*AmpR, URA3,* P_{AOX}*-PaLDH*	This study
pgRNA-*NS2*	*AmpR, URA3,* gRNA- *NS2*	(9)
pgRNA-*NS3*	*AmpR, URA3,* gRNA- *NS3*	(9)
pgRNA-*NS5*	*AmpR, URA3,* gRNA- *NS5*	(9)
pgRNA-*NS6*	*AmpR, URA3,* gRNA- *NS6*	(9)
pgRNA-*NS18*	*AmpR, URA3,* gRNA- *NS18*	(9)
pgRNA-*izh3*	*AmpR, URA3,* gRNA-*izh3*	(10)
pgRNA-*lpl1*	*AmpR, URA3,* gRNA-*lpl3*	(10)

Table S9. List of strains used in this study.

Strain	Genotype	Resource
Δku80	*MATa*; *leu1.1*; *ura3Δ*; *ku80Δ*; (P_{GAP}-*hCAS9*-T_{AOX})	Lab stock
k-L	*MATa*; *ura3Δ*; *ku80Δ*; (P_{GAP}-*hCAS9*-T_{AOX}); *leu2*::*LEU2*	This study
HpLd	*MATa*; *leu1.1*; *ura3Δ*; *ku80Δ*; (P_{GAP}-*hCAS9*-T_{AOX}); pLdLDH =[Δku80]+(pLdLDH)	This study
HpLh	*MATa*; *leu1.1*; *ura3Δ*; *ku80Δ*; (P_{GAP}-*hCAS9*-T_{AOX}); pLhLDH =[Δku80]+(pLhLDH)	This study
HpLp	*MATa*; *leu1.1*; *ura3Δ*; *ku80Δ*; (P_{GAP}-*hCAS9*-T_{AOX}); pLpLDH =[Δku80]+(pLpLDH)	This study
HpLl	*MATa*; *leu1.1*; *ura3Δ*; *ku80Δ*; (P_{GAP}-*hCAS9*-T_{AOX}); pLlLDH =[Δku80]+(pLlLDH)	This study
HpPa	*MATa*; *leu1.1*; *ura3Δ*; *ku80Δ*; (P_{GAP}-*hCAS9*-T_{AOX}); pPaLDH =[Δku80]+(pPaLDH)	This study
AOX	*MATa*; *leu1.1*; *ura3Δ*; *ku80Δ*; (P_{GAP}-*hCAS9*-T_{AOX}); NS3::(P_{AOX}-*eGFP*-T_{AMO}) =[Δku80]+[NS3::(P_{AOX}-*eGFP*-T_{AMO})]	This study
GAP	*MATa*; *leu1.1*; *ura3Δ*; *ku80Δ*; (P_{GAP}-*hCAS9*-T_{AOX}); NS3::(P_{GAP}-*eGFP*-T_{AMO}) =[Δku80]+[NS3::(P_{GAP}-*eGFP*-T_{AMO})]	This study
TEF	*MATa*; *leu1.1*; *ura3Δ*; *ku80Δ*; (P_{GAP}-*hCAS9*-T_{AOX}); NS3::(P_{TEF}-*eGFP*-T_{AMO}) =[Δku80]+[NS3::(P_{TEF}-*eGFP*-T_{AMO})]	This study
PMA1	*MATa*; *leu1.1*; *ura3Δ*; *ku80Δ*; (P_{GAP}-*hCAS9*-T_{AOX}); NS3::(P_{PMA1}-*eGFP*-T_{AMO}) =[Δku80]+[NS3::(P_{PMA1}-*eGFP*-T_{AMO})]	This study
FDH1	*MATa*; *leu1.1*; *ura3Δ*; *ku80Δ*; (P_{GAP}-*hCAS9*-T_{AOX}); NS3::(P_{FDH1}-*eGFP*-T_{AMO}) =[Δku80]+[NS3::(P_{FDH1}-*eGFP*-T_{AMO})]	This study
18G	*MATa*; *leu1.1*; *ura3Δ*; *ku80Δ*; (P_{GAP}-*hCAS9*-T_{AOX}); NS18::(P_{GAP}-*PaLDH*-T_{AMO}) =[Δku80]+[NS18::(P_{GAP}-*PaLDH*-T_{AMO})]	This study
2A	*MATa*; *leu1.1*; *ura3Δ*; *ku80Δ*; (P_{GAP}-*hCAS9*-T_{AOX}); NS2::(P_{AOX}-*PaLDH*-T_{AMO}) =[Δku80]+[NS2::(P_{AOX}-*PaLDH*-T_{AMO})]	This study
3A	*MATa*; *leu1.1*; *ura3Δ*; *ku80Δ*; (P_{GAP}-*hCAS9*-T_{AOX}); NS3::(P_{AOX}-*PaLDH*-T_{AMO})	This study

	=[Δku80]+[NS3::(P_{AOX}-*PaLDH*-T_{AMO})]	
18A	*MATa*; *leu1.1*; *ura3Δ*; *ku80Δ*; (P_{GAP}-*hCAS9*-T_{AOX}); NS18::(P_{AOX}-*PaLDH*-T_{AMO}) =[Δku80]+[NS18::(P_{AOX}-*PaLDH*-T_{AMO})]	This study
HPLA05	*MATa*; *ura3Δ*; *ku80Δ*; (P_{GAP}-*hCAS9*-T_{AOX}); *leu2*::*LEU2*; pPaLDH =[k-L]+(pPaLDH)	This study
HPLA06	*MATa*; *ura3Δ*; *ku80Δ*; (P_{GAP}-*hCAS9*-T_{AOX}); *leu2*::*LEU2*; NS3::(P_{AOX}-*PaLDH*-T_{AMO}) =[k-L]+[NS3::(P_{AOX}-*PaLDH*-T_{AMO})]	This study
HPLA07	*MATa*; *ura3Δ*; *ku80Δ*; (P_{GAP}-*hCAS9*-T_{AOX}); *leu2*::*LEU2*; NS3::(P_{AOX}-*PaLDH*-T_{AMO}); NS2::(P_{AOX}-*PaLDH*-T_{AMO}) =[HPLA06]+[NS2::(P_{AOX}-*PaLDH*-T_{AMO})]	This study
HPLA09	*MATa*; *ura3Δ*; *ku80Δ*; *lpl1*Δ; (P_{GAP}-*hCAS9*-T_{AOX}); *leu2*::*LEU2*; NS3::(P_{AOX}-*PaLDH*-T_{AMO}) =[HPLA06]+(*lpl1*Δ)	This study
HPLA10	*MATa*; *ura3Δ*; *ku80Δ*; *izh3*Δ; (P_{GAP}-*hCAS9*-T_{AOX}); *leu2*::*LEU2*; NS3::(P_{AOX}-*PaLDH*-T_{AMO}) = [HPLA06]+(*izh3*Δ)	This study
HPLA11	*MATa*; *ura3Δ*; *ku80Δ*; *lpl1*Δ; *izh3*Δ; (P_{GAP}-*hCAS9*-T_{AOX}); *leu2*::*LEU2*; NS3::(P_{AOX}-*PaLDH*-T_{AMO}) =[HPLA09]+(*izh3*Δ)	This study
HPLA12	*MATa*; *ura3Δ*; *ku80Δ*; (P_{GAP}-*hCAS9*-T_{AOX}); *leu2*::*LEU2*; NS3::(P_{AOX}-*PaLDH*^{V40R}-T_{AMO}) =[k-L]+(*PaLDH*^{V40R})	This study
HPGLA02	*MATa*; *ura3Δ*; *ku80Δ*; (P_{GAP}-*hCAS9*-T_{AOX}); *leu2*::*LEU2*; NS3::(P_{GAP}-*PaLDH*-T_{AMO}) =[k-L]+[NS3::(P_{GAP}-*PaLDH*-T_{AMO})]	This study
HPGLA03	*MATa*; *ura3Δ*; *ku80Δ*; (P_{GAP}-*hCAS9*-T_{AOX}); *leu2*::*LEU2*; NS3::(P_{GAP}-*PaLDH*-T_{AMO}); NS2::(P_{GAP}-*PaLDH*-T_{AMO}) =[HPGLA02]+[NS2::(P_{GAP}-*PaLDH*-T_{AMO})]	This study
HPGLA05	*MATa*; *ura3Δ*; *ku80Δ*; (P_{GAP}-*hCAS9*-T_{AOX}); *leu2*::*LEU2*; NS3::(P_{GAP}-*PaLDH*^{V40R}-T_{AMO}) =[k-L]+[NS3::(P_{GAP}-*PaLDH*^{V40R}-T_{AMO})]	This study
HPGLA07	*MATa*; *ura3Δ*; *ku80Δ*; (P_{GAP}-*hCAS9*-T_{AOX}); *leu2*::*LEU2*; NS3::(P_{GAP}-*PaLDH*^{V40R}-T_{AMO}); NS2::(P_{GAP}-*PaLDH*^{V40R}-T_{AMO}) =[HPGLA05]+[NS2::(P_{GAP}-*PaLDH*^{V40R}-T_{AMO})]	This study

HPLA13	*MATa*; *ura3Δ*; *ku80Δ*; (P$_{GAP}$-*hCAS9*-T$_{AOX}$); *leu2*::*LEU2*; NS3::(P$_{AOX}$-*PaLDH*^{V40R}-T$_{AMO}$); NS2::(P$_{AOX}$-*PaLDH*^{V40R}-T$_{AMO}$) =[HPLA12]+[NS2::(P$_{AOX}$-*PaLDH*^{V40R}-T$_{AMO}$)]	This study
HPLA14	*MATa*; *ura3Δ*; *ku80Δ*; *izh3*Δ; (P$_{GAP}$-*hCAS9*-T$_{AOX}$); *leu2*::*LEU2*; NS3::(P$_{AOX}$-*PaLDH*^{V40R}-T$_{AMO}$); NS2::(P$_{AOX}$-*PaLDH*^{V40R}-T$_{AMO}$) =[HPLA13]+(*izh3*Δ)	This study
HPLA17	*MATa*; *ura3Δ*; *ku80Δ*; (P$_{GAP}$-*hCAS9*-T$_{AOX}$); *leu2*::*LEU2*; NS3::(P$_{AOX}$-*PaLDH*^{V40R}-T$_{AMO}$); NS2::(P$_{AOX}$-*PaLDH*^{V40R}-T$_{AMO}$) ; NS5::(P$_{AOX}$-*PaLDH*^{V40R}-T$_{AMO}$) =[HPLA13]+[NS5::(P$_{AOX}$-*PaLDH*^{V40R}-T$_{AMO}$)]	This study
HPLA21	*MATa*; *ura3Δ*; *ku80Δ*; *izh3*Δ; (P$_{GAP}$-*hCAS9*-T$_{AOX}$); *leu2*::*LEU2*; NS3::(P$_{AOX}$-*PaLDH*^{V40R}-T$_{AMO}$); NS2::(P$_{AOX}$-*PaLDH*^{V40R}-T$_{AMO}$); NS5::(P$_{16321}$-*PaLDH*$_{MTS}$-T$_{AMO}$) =[HPLA14]+[NS5::(P$_{16321}$-*PaLDH*$_{MTS}$-T$_{AMO}$)]	This study
HPLA22	*MATa*; *ura3Δ*; *ku80Δ*; *izh3*Δ; (P$_{GAP}$-*hCAS9*-T$_{AOX}$); *leu2*::*LEU2*; NS3::(P$_{AOX}$-*PaLDH*^{V40R}-T$_{AMO}$); NS2::(P$_{AOX}$-*PaLDH*^{V40R}-T$_{AMO}$); NS6::(P$_{16321}$-*PaLDH*$_{MTS}$-T$_{AMO}$) =[HPLA14]+[NS6::(P$_{16321}$-*PaLDH*$_{MTS}$-T$_{AMO}$)]	This study
HPLA23	*MATa*; *ura3Δ*; *ku80Δ*; *izh3*Δ; (P$_{GAP}$-*hCAS9*-T$_{AOX}$); *leu2*::*LEU2*; NS3::(P$_{AOX}$-*PaLDH*^{V40R}-T$_{AMO}$); NS2::(P$_{AOX}$-*PaLDH*^{V40R}-T$_{AMO}$); NS5::(P$_{16321}$-*PaLDH*$_{MTS}$-T$_{AMO}$); NS6::(P$_{16321}$-*PaLDH*$_{MTS}$-T$_{AMO}$) =[HPLA21]+[NS6::(P$_{16321}$-*PaLDH*$_{MTS}$-T$_{AMO}$)]	This study
HPLA22u	*MATa*; *ura3Δ*; *ku80Δ*; *izh3*Δ; *ura3*::*URA3*; (P$_{GAP}$-*hCAS9*-T$_{AOX}$); *leu2*::*LEU2*; NS3::(P$_{AOX}$-*PaLDH*^{V40R}-T$_{AMO}$); NS2::(P$_{AOX}$-*PaLDH*^{V40R}-T$_{AMO}$); NS6::(P$_{16321}$-*PaLDH*$_{MTS}$-T$_{AMO}$) =[HPLA22]+[*ura3*::*URA3*]	This study

Table S10. List of primers used in this study.

Primer	Sequence (5'-3')
BBLA-F	GTATATAGTACACGACAATCTAGTAATCTCC
BBLA-R	TTTGTTTTTGTACTTTAGATTGATGTCACC
LdLDH-F	ATCTAAAGTACAAAAACAAAATGTCGAG
LdLDH-R	GATTGTCGTGTACTATATACTTAGCCC
LhLDH-F	ATCTAAAGTACAAAAACAAAATGGCC
LhLDH-R	GATTGTCGTGTACTATATACTTATTGACGTAC
LpLDH-F	ATCTAAAGTACAAAAACAAAATGTCGTC
LpLDH-R	GATTGTCGTGTACTATATACTTACTTGTTC
LlLDH-F	ATCTAAAGTACAAAAACAAAATGGCC
LlLDH-R	GATTGTCGTGTACTATATACTTAGTTCTTC
PaLDH-F	ATCTAAAGTACAAAAACAAAATGTCGAAC
PaLDH-R	GATTGTCGTGTACTATATACTTACTTGTC
NS3up-F	CAAATAAATACTTTTTTCGCACAGCCAG
NS3up-R-gap	GGTGCACGGATGTGGTCTAGACTCGTTCCAGTTTATACGTCA GCTG
GAPp-F	TCTAGACCACATCCGTGCACC
GAPp-R	TTTGTTTCTATATTATCTTTGTACTAAAGAGCAATTG
gap-GFP-F	CAATTGCTCTTTAGTACAAAGATAATATAGAAACAAAATGGT GAGCAAGGGCGAGGAG
GFP-R-tdh2	CTAAATCATTAAAGTAACTTAAGGAGTTAAATTTACTTGTAC AGCTCGTCCATGC
TDH2t-F	ATTTAACTCCTTAAGTTACTTTAATGATTTAG
TDH2t-R	GCGAAAAGCCAATTAGTGTGATAC
tdh2-NS3dw-F	CGTTTTCAAGCAGCTCGCTCATCGCGAAAAGCCAATTAGTGT GATAC
NS3dw-R	GAGACCTCATCAGGTCCTTTAGGTC
NS3-JDF	CGGATTACATTTTTTTTAAGGGTAAAAGTAGC
NS3-JDR	CGAGTTTTTCCTGTAAACTCACCAGC
NS3up-R-pma1	CTTTGCAACTCATCCCCAGCTTCTACTCGTTCCAGTTTATACG TCAGCTG
PMA1p-F	TAGAAGCTGGGGATGAGTTGCAAAGAG
PMA1p-R	GATGAGTCTCTATTCTAGCAATTGAACTG
pma1-GFP-F	GTTCAATTGCTAGAATAGAGACTCATCATGGTGAGCAAGGGC GAGGAGCTG

NS3up-R-tef	GGCAACGGTGTCTTTAGGGCTACTCGTTCCAGTTTATACGTCAGCTG
TEFp-F	TACGAATGTACTAATTAAAAGATCA
TEFp-R	TAGCCCTAAAGACACCGTTGCCATA
tef-GFP-F	GAGATTTTTTCTGATCTTTTAATTAGTACATTCGTAATGGTGAGCAAGGGCGAGGAG
NS3up-R-aox	CCATCCGAGATCGCAAGCTGCAGCCTCGTTCCAGTTTATACGTCAGCTG
AOXp-F	GCTGCAGCTTGCGATCTCGG
AOXp-R	TTTGTTTTTGTACTTTAGATTGATGTCACCACC
aox-GFP-F	GGTGGTGACATCAATCTAAAGTACAAAAACAAAATGGTGAGCAAGGGCGAGGAG
NS3up-R-fdh1	CGCCGGTAACATTTTTCCAGCTCTCGTTCCAGTTTATACGTCAGCTG
FDH1p-F	AGCTGGAAAAATGTTACCGGCG
FDH1p-R	GATTTGATTGATGAAGGCAGAGAGCG
fdh1-GFP-F	CGCTCTCTGCCTTCATCAATCAAATCATGGTGAGCAAGGGCGAGGAG
NS18-JDF	GTTGAGGCTTACAGTCCATTCGG
NS18-JDR	CTTCTCATATCGCTCGGCAAGTC
NS18up-F	GGTCCTGGAGCTTCCTCTGG
NS18up-R-gap	GGTGCACGGATGTGGTCTAGAGTGAAGGTGGTCATACTGGACTCAG
gap-LDH-F	CAATTGCTCTTTAGTACAAAGATAATATAGAAACAAAATGTCGAACATCCAGAACCACC
LDH-R-amo	GTGGAGATTACTAGATTGTCGTGTACTATATACTTACTTGTCCTGCTTCTCGGCTAAAG
AMOt-F	GTATATAGTACACGACAATCTAGTAATCTCCAC
AMOt-R	GTTTGGATCAACAGACGACCTGATGAAC
amo-NS18dw-F	GTTCATCAGGTCGTCTGTTGATCCAAACGTACATGGTTTGAACGGGCTTTCG
NS18dw-R	CTCACATGGATTTAGATGATTGCACG
NS18-up-R-aox	CCGAGATCGCAAGCTGCAGCGTGAAGGTGGTCATACTGGACTCAGTC
aox-LDH-F	GGTGACATCAATCTAAAGTACAAAAACAAAATGTCGAACATCCAGAACCACCAG
NS2-JDF	CGAGCAATTCATCAGTTTTTG

NS2-JDR	CCTCTTTCCTAAACCTTCGC
NS2up-F	GAGAACGACTGACAACGTGCTAC
NS2up-R-aox	CCGAGATCGCAAGCTGCAGCCTGTAGTTGTGATTTTAATGCACACTTG
amo-NS2dw-F	CATCAGGTCGTCTGTTGATCCAAACGTTGCTGGTGCCATCTTTTGGC
NS2dw-R	CACTTCAAATTTGCTACCACTGAACTGG
NS3up-R-aox	CCATCCGAGATCGCAAGCTGCAGCCTCGTTCCAGTTTATACGTCAGCTG
amo-NS3dw-F	GTTCATCAGGTCGTCTGTTGATCCAAACGATGAGCGAGCTGCTTGAAAACG
LPL1up-F	CTTTGCTGGGCTGTCAAATGAGTC
LPL1up-R	GATAGATTTACTCTCTATAAATGCTGAATGCGTGGCGGGCTAGGGAATTTACAG
LPL1dw-F	ACGCATTCAGCATTTATAGAGAGTAAATCTATCAG
LPL1dw-R	CTGCGAAACACAGCTAACTCGG
IZH3up-F	TCGGACAATCTGCTGGAACAGG
IZH3up-R	GATGAGAGTATCGCAAACAAAAATATTCACC
IZH3dw-F	GGTGAATATTTTTGTTTGCGATACTCTCATCATCAAACAATGATGCATTGTGCTGTC
IZH3dw-R	GATCTCGTGCTGGACTATCTCTACG
NS5-JDF	CATCGTTGTGACAGAAAAGATCGAAG
NS5up-F	GGCCTTAGTGTCAACTTGGGTTTAC
NS5up-R-aox	CCATCCGAGATCGCAAGCTGCAGCCTGAATAGTTTGAGCGCTATTAGGTCC
amo-NS5dw-F	GTTCATCAGGTCGTCTGTTGATCCAAACGCCATTCTCGACCGATAATTACGATAATC
NS5dw-R	CGAGACATCCATTGCCGAAAC
NS5-JDR	CGTTTTTGTGCTCTGAAGAAGC
gap-eGFP-F	CTCTTTAGTACAAAGATAATATAGAAACAAAATGGTGAGCAAGGGCGAGG
eGFP-R-amo	GGAGATTACTAGATTGTCGTGTACTATATACTTACTTGTACAGCTCGTCCATGCC
GAPp-R-mts1-egfp	cttatagattgacgtagtgaaagcatCATTTTGTTTCTATATTATCTTTGTACTAAAG
MTS1-eGFP-F	atgctttcactacgtcaatctataagatttttcaagccagccacaagaactttgtgtagctctagatatctgcttcagGTGAGCAAGGGCGAGGAG

NS5up-R-p16321	CTGCATTGTTGATACCACTGACACCCTGAATAGTTTGAGCGCTATTAGGTCC
P16321-F	GGTGTCAGTGGTATCAACAATGCAG
P16321-R-mts1	ttgaaaaatcttatagattgacgtagtgaaagcatCATCTTGTTCGCGCTTTGTGATTG
mts1-LDH-F	atctataagatttttcaagccagccacaagaactttgtgtagctctagatatctgcttcagTCGAACATCCAGAACCACCAG
LDH-R-amo	GGAGATTACTAGATTGTCGTGTACTATATACTTACTTGTCCTGCTTCTCGGC
NS6up-F	CCAGTTCCTCCAGAAACTGTAAATC
NS6up-R-p16321	CTGCATTGTTGATACCACTGACACCGTCAGGCGTTATTTCAAGACATACACC
P16321-R-mts1	ttgaaaaatcttatagattgacgtagtgaaagcatCATCTTGTTCGCGCTTTGTGATTG
amo-NS6dw-F	CATCAGGTCGTCTGTTGATCCAAACGGGTCGATGACGACACACC
NS6dw-R	CATAGCTGATGGTGCTCCG
HpURA3-F	TCAACTGATGTTCAGCAACGC
HpURA3-R	ATAAAATTCAAAAGGTCCACCTGAC

Table S11. List of codon-optimized genes used in this study.

Gene	Sequence (5’-3’)
eGFP	ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCC CATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGT TCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGC AAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCC GTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTG CAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGAC TTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGC ACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCC GAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGA GCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGG GGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTAT ATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTT CAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCG CCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCC GTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCC CTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCT GCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGA CGAGCTGTACAAGTAA
LdLDH	ATGTCGAGAAAGGTGCTGCTGGTGGGCGACGGCGCCGTGGG CTCGAACTTCGCCAACGACCTGCTGCAGACCACCAGAGTGGA CGAGCTGGTGATCTGCGACCTGAACAAGGACAGAGCCGCCG GCGACTGCCTGGACCTGGAGGACATGACCTACTTCACCGGCC AGACCAAGCTGAGAGCCGGCGACTACTCGGACGCCGCCGAC GCCGACGTGGTGGTGATCACCGCCGGCGTGCCAAGAAAGCC AGGCGAGTCGAGACTGGACCTGATCAAGAAGAACGAGGCCA TCCTGAGATCGATCGTGGACCCAGTGGTGGCCTCGGGCTTCT CGGGCATCTTCGTGGTGTCGGCCAACCCAGTGGACATCCTGA CCACCCTGACCCAGAAGCTGTCGGGCTTCCCAAAGAAGAGA GTGATCGGCACCGGCACCTCGCTGGACTCGGCCTCGCTGAGA GTGGAATTGGCTAAACGTTTACAGGTGCCAATCGAGTCGGTG AACGCCTGGGTGCTGGGCGAGCACGGCGACTCGTCGTTCGA GAACTTCTCGTCGGCCGTGGTGAACGGCAAGCCACTGCTGGA CTACCCAGGCATGACCGAGGCCGCCCTGGACGAGATCGAGG CCCACGTGAGAGAGAAGGGCTCGGAGATCATCGTGAAGAAG GGCGCCACCTACTACGGCGTGGCCATGATGTTAGCGAAGATT

	GTTACCGCCATCCTGGAGAACAACGACCTGGCCCTGCCACTG TCGGCACCGTTGCACGGCGAGTACGGCATCAAGGACGAGAT CTACCTGGGCACCCTGGCCATCATCAACGGCCAGGGCATCTC GCACGTGCTGGAGCTGCCACTGAACGACTCGGAACTGGCAA AGATGCGAGCCTCGGCCGCCACCATCAAGGCCACCCTGGACT CGCTGGGCTAA
Lh-LDH	ATGGCCAGAGAGGAGAAGCCAAGAAAGGTGATCCTGGTGGG CGACGGCGCCGTGGGCTCGACCTTCGCCTTCTCGATGGTGCA GCAGGGCATCGCCGAGGAGCTGGGCATCATCGACATCGCCA AGGAGAGAGTGGAGGGCGACGCCATCGACCTGGCCGACGCC ACTCCGTGGACCTCGCCAAAGAACATCTACGCCGCCGACTAC CCAGACTGCAAGGACGCCGACCTGGTGGTGATCACCGCCGG CGCTCCACAGAAACCAGGCGAGACCAGACTGGACCTGGTGA ACAAGAACCTGAAGATCCTGTCGTCGATCGTGGAGCCAGTG GTGGAGTCGGGCTTCGAGGGCATCTTCCTGGTGGTGGCCAAC CCAGTGGACATCCTGACCCACGCCACCTGGAGAATGTCGGGC TTCCCAAAGGACAGAGTGATCGGCTCGGGCACCTCGCTGGAC ACCGGCAGACTGCAGAAGGTGATCGGCAAGATGGAGAACGT GGACCCATCGTCGGTGAACGCCTACATGCTGGGCGAGCACG GCGACACCGAGTTCCCAGCCTGGTCGTACAACAACGTGGGC GGAGTAAAGGTGGCCGATTGGGTGAAGGCCCACAACATGCC AGAGTCGAAGCTGGAGGACATCCACCAGGAGGTGAAGGACA TGGCCTACGACATCATCAACAAGAAGGGCGCCACCTTCTACG GCATCGGCACTGCATCAGCAATGATAGCCAAGGCCATCCTGA ACGACGAGCACAGAGTGCTACCGTTGAGTGTTCCGATGGAC GGCGAGTACGGCCTGCACGACCTGCACATCGGCACTCCGGC GGTGGTGGGCAGAAAGGGCCTGGAGCAGGTTATTGAAATGC CGCTTTCAGACAAGGAACAAGAATTGATGACGGCGTCCGCT GACCAGCTTAAGAAGGTGATGGACAAGGCCTTCAAGGAGAC CGGTGTGAAGGTACGTCAATAA
LpLDH	ATGTCGTCGATGCCAAACCACCAGAAGGTGGTGCTGGTGGG CGACGGCGCCGTGGGCTCGTCGTACGCCTTCGCCATGGCCCA GCAGGGCATCGCCGAGGAGTTCGTGATCGTGGACGTGGTGA AGGACAGAACCAAGGGCGACGCCCTGGACCTGGAGGACGCC CAGGCCTTCACCGCGCCGAAGAAGATCTATTCGGGCGAGTAC TCGGACTGCAAGGACGCCGACCTGGTGGTGATCACCGCCGG CGCACCTCAGAAACCAGGCGAGTCGAGACTGGACCTGGTGA

	ACAAGAACCTGAACATCCTGTCGTCGATCGTGAAGCCAGTGG TGGACTCGGGCTTCGATGGGATATTCCTAGTCGCCGCCAACC CAGTGGACATCCTGACCTACGCCACCTGGAAGTTCTCGGGCT TCCCAAAGGACAGAGTGATCGGCTCGGGCACCTCGCTGGACT CGTCGAGACTGAGAGTGGCCCTGGGCAAGCAGTTCAACGTG GACCCAAGATCGGTGGACGCCTACATCATGGGCGAGCACGG CGACTCGGAGTTCGCCGCCTACTCGACCGCCACCATCGGCAC CAGACCAGTGAGAGACGTGGCCAAGGAGCAGGGCGTGTCGG ACGAGGACCTGGCCAAGCTGGAGGACGGCGTGAGAAACAAG GCCTACGACATCATCAACCTGAAGGGCGCCACTTTCTACGGC ATAGGAACCGCCCTGATGAGAATCTCGAAGGCCATCCTGAG AGACGAGAACGCCGTGCTGCCAGTGGGCGCCTACATGGACG GCCAGTACGGCCTGAACGACATCTACATCGGCACACCTGCAG TGATCGGCGGCACCGGCCTGAAGCAGATCATCGAGTCGCCA CTGTCGGCCGACGAGCTGAAGAAGATGCAGGACTCGGCCGC CACCCTGAAGAAGGTGCTGAACGACGGCCTGGCCGAGCTGG AGAACAAGTAA
LlLDH	ATGGCCGACAAGCAGAGAAAGAAGGTGATCCTGGTGGGCGA CGGCGCCGTGGGCTCGTCGTACGCCTTCGCCCTGGTGAACCA GGGCATCGCCCAGGAGCTGGGCATCGTGGACCTGTTCAAGG AGAAGACCCAGGGCGACGCCGAGGACCTGTCGCACGCCCTG GCCTTCACCTCGCCAAAGAAGATCTACTCGGCCGACTACTCG GACGCCTCGGACGCCGACCTGGTGGTGCTGACTAGTGGCGCA CCACAGAAGCCAGGCGAGACCAGACTGGACCTGGTGGAGAA GAACCTGAGGATTACGAAAGATGTTGTGACCAAGATCGTGG CCTCGGGCTTCAAGGGCATCTTCCTGGTGGCCGCCAACCCAG TGGACATCCTGACCTACGCCACCTGGAAGTTCTCGGGCTTCC CAAAGAACAGAGTGGTGGGCTCGGGCACCTCGCTGGACACC GCCAGATTCAGACAGGCCCTGGCCGAGAAGGTGGACGTGGA CGCCAGATCGATCCACGCCTACATCATGGGCGAGCACGGCG ACTCGGAGTTCGCCGTGTGGTCGCACGCCAACGTGGCCGGCG TGAAGCTGGAGCAGTGGTTCCAGGAGAACGACTACCTGAAC GAGGCCGAGATCGTGGAGCTGTTCGAGTCGGTGAGAGACGC CGCCTACTCGATCATCGCCAAGAAGGGCGCCACCTTCTACGG CGTGGCCGTGGCCCTGGCCCGTATAACGAAAGCTATTCTGGA CGACGAGCACGCCGTGCTGCCAGTGTCGGTGTTCCAGGACGG CCAGTACGGCGTGTCGGACTGCTACCTGGGCCAGCCAGCCGT

	GGTGGGCGCCGAGGGCGTGGTGAACCCAATCCACATCCCAC TGAACGACGCCGAGATGCAGAAGATGGAGGCAAGTGGTGCA CAACTAAAGGCCATCATCGACGAGGCCTTCGCCAAGGAGGA GTTCGCCTCGGCCGTGAAGAACTAA
PaLDH	ATGTCGAACATCCAGAACCACCAGAAGGTGGTGCTGGTGGG CGACGGCGCCGTGGGCTCGTCGTACGCCTTCGCCATGGCCCA GCAGGGCATCGCCGAGGAGTTCGTGATCGTGGACgtgGTGAA GGACAGAACCGTGGGCGACGCCCTGGACCTGGAGGACGCCA CTCCGTTTACCGCACCCAAGAATATTTACTCGGGCGAGTACT CGGACTGCAAGGACGCCGACCTGGTGGTGATCACCGCCGGC GCACCTCAGAAACCCGGCGAGACCAGACTGGACCTGGTGAA CAAGAACCTGAACATCCTGTCGACCATCGTGAAGCCAGTGGT GGACTCGGGCTTCGACGGCATCTTCCTGGTGGCCGCCAACCC AGTGGACATCCTGACCTACGCCACCTGGAAGTTCTCGGGCTT CCCAAAGGAGAAGGTGATCGGCTCGGGCATCTCGCTGGACA CCGCCAGACTGAGAGTGGCCCTGGGCAAGAAGTTCAACGTG TCGCCAGAGTCGGTGGACGCCTACATCCTGGGCGAGCACGG CGACTCGGAGTTCGCCGCCTACTCGTCGGCCACCATCGGCAC CAAGCCACTGCTGGAGATCGCCAAGGAGGAGGGCGTGTCGA CCGACGAGCTGGCCGAGATCGAGGACTCGGTGAGAAACAAG GCCTACGAGATCATCAACAAGAAGGGCGCCACCTTCTACGG CGTGGGCACCGCCCTGATGAGAATCTCGAAGGCCATCCTGAG AGACGAGAACGCCGTGCTGCCAGTGGGCGCCTACATGGACG GCGAGTATGGGCTAAATGATATATACATCGGCACACCTGCGG TGATCAACGGCCAGGGCCTGAACAGAGTGATCGAGGCACCG TTATCGGACGACGAGAAGAAGAAGATGACCGACTCGGCCAC CACCCTGAAGAAGGTGCTGACCGATGGTCTAAATGCTTTAGC CGAGAAGCAGGACAAGTAA

Table S12. Raw material and utility costs and credit used in the base case.

Items	Price (2024$)	Reference
Raw material and utility		
CO_2	$92.5/ton	(11)
Ammonia	$550.5/ton	(12)
Flocculant	$12258.7/ton	(12)
Makeup Water	$0.54/ton	(12)
Power	$0.02/kWh	(12)
Sludge disposal	$20.2/ton	(12)
Credit		
Biofertilizer credit	$362.3/ton	(13)
Carbon credit	$104.1/ton	(14)

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