

Supporting Information for

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

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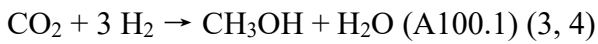
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Supplementary references

Supplementary Method 1: The detailed process of the techno-economic analysis of L-lactate production from CO₂-derived methanol.

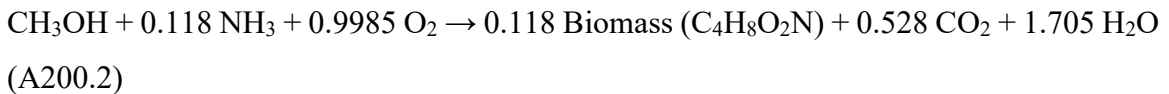
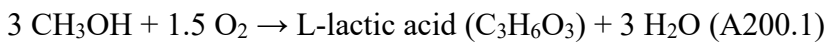
Methanol production (A100)

The synthesis of methanol from CO₂ and hydrogen (H₂) achieves a CO₂ single-pass conversion exceeding 95% at a pressure of 5.0 MPa, an H₂: CO₂ ratio of 3:1, and a temperature of 315°C (A100.1) (1). H₂ 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·(h·m³)⁻¹ (2).



L-lactate production (A200)

Methanol acts as the exclusive carbon source for the growth of recombinant *O. polymorpha* HPLA22u, with oxygen (O₂) being provided via alkaline water electrolysis. The L-lactate production process involves recombinant *O. polymorpha* HPLA22u being cultured in 300 m³ fermenters, and each bioreactor has a working capacity of 240 m³. Under the assumptions for L-lactate production in [SI Appendix Table S2](#), the total fermentation volume was 2,587 m³. The stoichiometric equations for bioreactors in the Aspen Plus simulations are derived from Equation A200.1 and A200.2 (5). To maintain adherence to established chemical formulae, we utilized L-lactic acid to represent L-lactate in the A200.1.



L-lactate purification (A300)

The basic unit of A300 comprises a dewatering system and a membrane-based separation system. The dewatering system utilizes a dissolved air flocculation (DAF) unit followed by centrifugation for solid-liquid separation. The flocculant dosage (1–50 mg/L) is chosen to enhance biomass aggregation, facilitating the separation of flocculated solids from the liquor(6-8). The solids are directed to wastewater treatment for anaerobic digestion, while the liquor proceeds to L-lactate separation. While various technologies have been developed and demonstrated on a small scale for the purification of L-lactate from

fermentation broth, membrane techniques are more practical and cost-effective for large-scale applications(9, 10). Consequently, the liquid stream is subsequently sent to an ultrafiltration membrane (UM) separator to eliminate remaining particles and macromolecular contaminants(11). The clarified stream is concentrated through evaporation, assuming an overall recovery efficiency of 90% in the baseline scenario(9, 12).

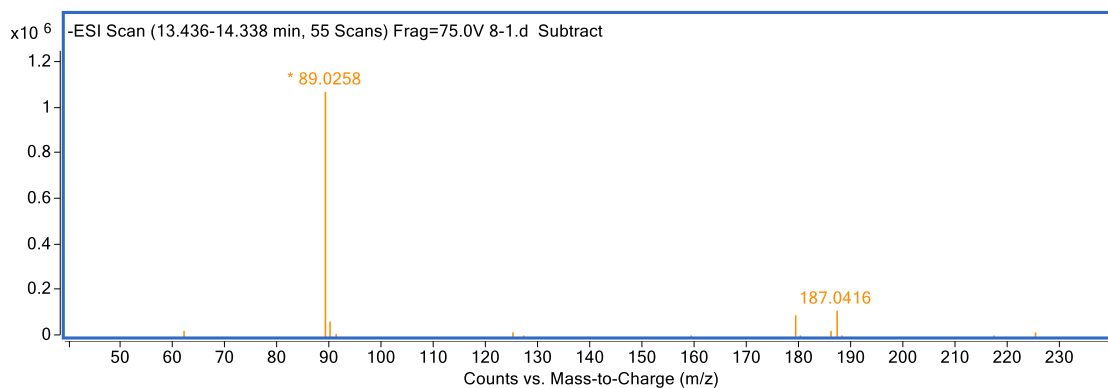
Wastewater treatment (A400)

All wastewater is directed to the anaerobic digestion units to enhance the energy efficiency of the feedstocks and to recycle the cooling water. Anaerobic digestion is modelled using the RYield block with Fortran code for predicting the decomposition products. Residual biomass can be transformed into biogas (CH_4 and CO_2), NH_3 , H_2O , and digestate by defining the yield distribution (*SI Appendix* Table S2). The digestate generated from the anaerobic digestion process is abundant in nitrogen nutrients, rendering it a valuable fertilizer 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. The CH_4 obtained from the anaerobic digestion process is fully combusted in an on-site combustor of combined heat and power system (CHP) to generate energy and heat on the premises.

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. 1. LC-MS determination of lactate synthesized from sole methanol in *O. polymorpha* HpPa. Search in the library revealed that the mass-to-charge (m/z) of 89.0258 (a) in LC-MS was the molecule of $C_3H_6O_3$ without H (b), just corresponding to lactate. In addition, the peak time of this product in HPLC coincided with the standard of lactate, rather than other isomer such as 3-hydroxypropionate, which confirmed that this fermentation product was lactate.

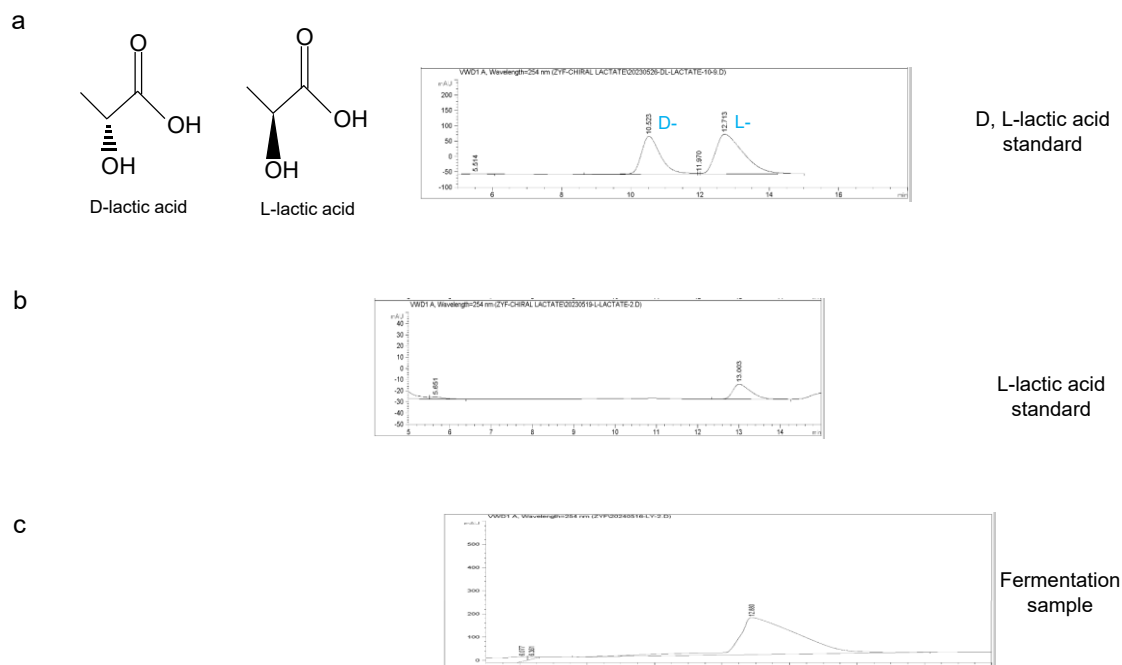


Fig. 2. Determination of the optical isomer of L-lactate produced from methanol in *O. polymorpha* by using a chiral column on HPLC equipped with a UV detector. a. The configuration of D-lactic acid and L-lactic acid, and the HPLC peak of their standard; b. The HPLC peak of L-lactic acid standard; c. The HPLC peak of the fermentation sample. It can be concluded that the optical purity of L-lactic acid in the fermentation sample was >99%.

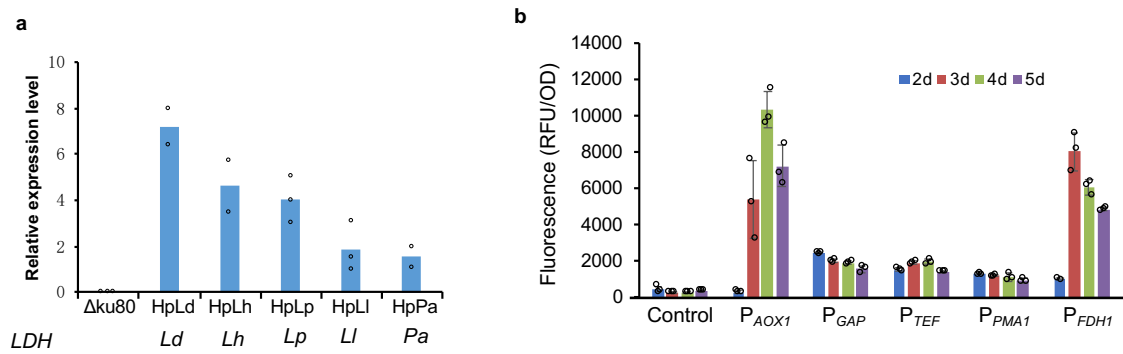


Fig. S3. Optimization of L-LA production in *O. polymorpha*. (a) Relative expression levels of five LDH genes by qPCR experiments. Data are presented as mean values $\pm$ SEM of two (for *LdLDH*, *LhLDH*, and *PaLDH*), or three (for *LpLDH* and *LILDH*) biological replicates. (b) Promoter characterization 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. Data are presented as mean values $\pm$ SEM of three biological replicates.

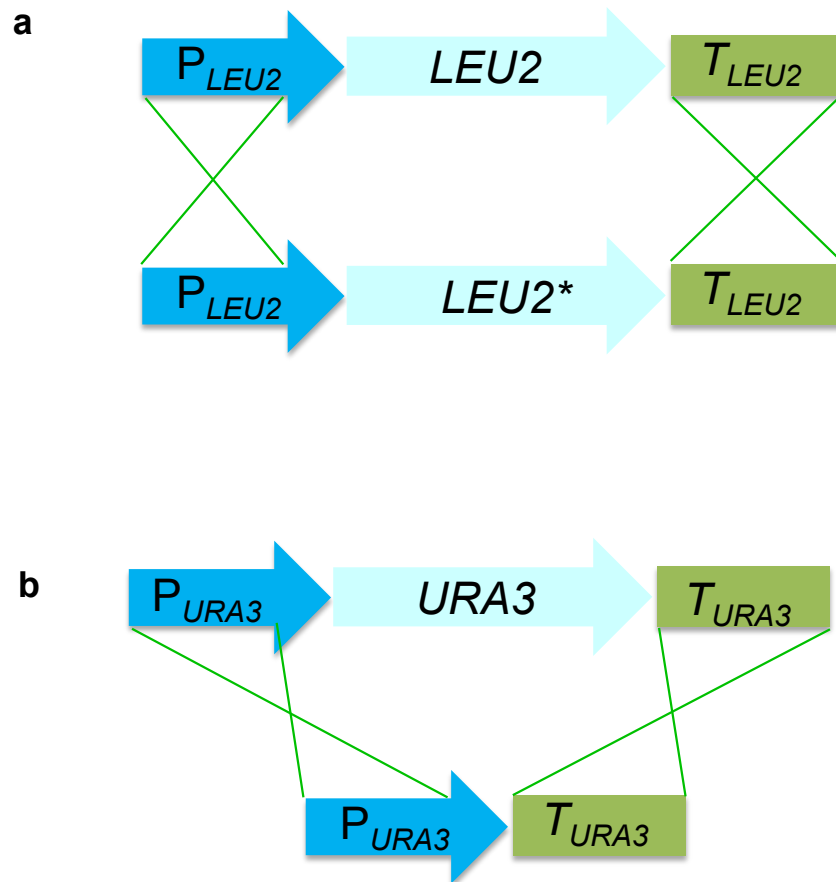


Fig. 4. In situ recovery of the auxotrophic gene *LEU2* (a) and *URA3* (b).

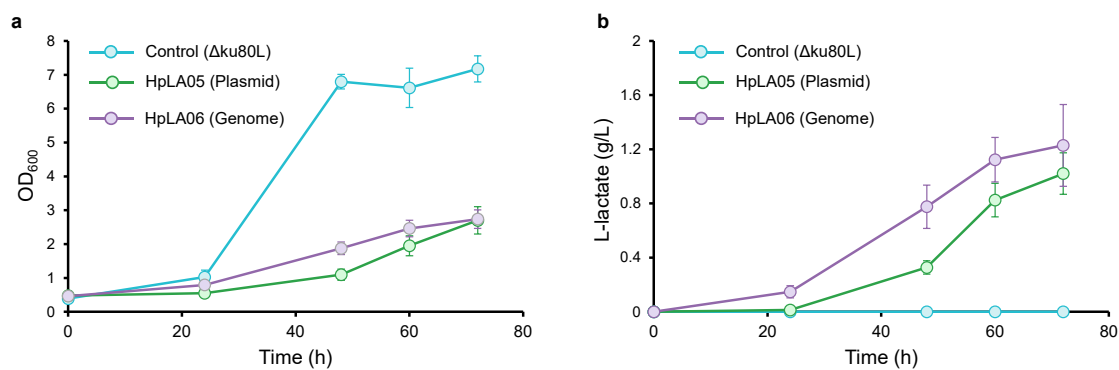


Fig. 5 Engineered strain HPLA05 and HpLA06 for L-lactate production significantly decreased biomass relative to that of the wild-type strain. (a) Growth curve of wild-type strain and engineered strain HPLA05 and HpLA06. (b) L-lactate production of wild-type strain and engineered strain HPLA05 and HpLA06. Data are presented as mean values $\pm$ SEM of three biological replicates. Source data available in the Source Data file.

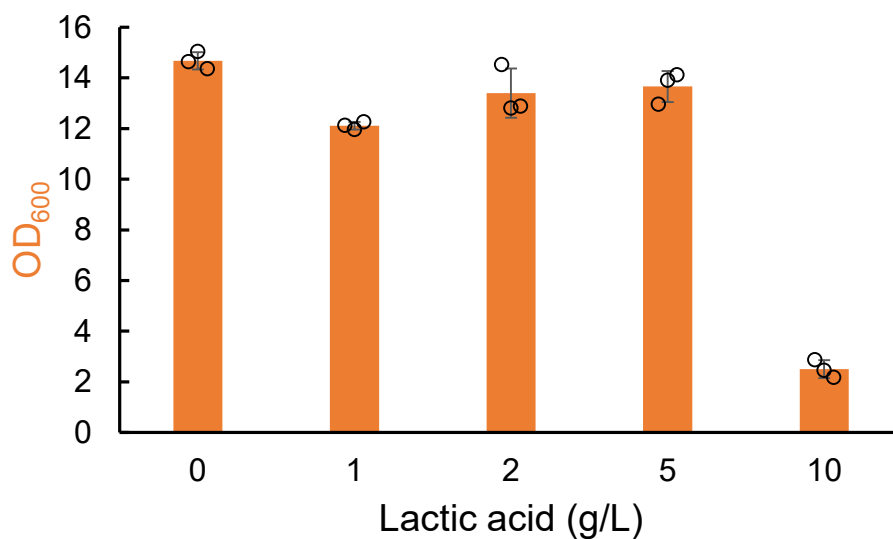


Fig. 6. L-lactate tolerance of wild-type *O. polymorpha*. 0, 1, 2, 5, and 10 g/L L-lactate was added in the culture medium. An initial OD₆₀₀=0.2 was adopted to test cell growth of *O. polymorpha* wild type strain k-L. Data are presented as mean values $\pm$ SEM of three biological replicates. Source data available in the Source Data file.

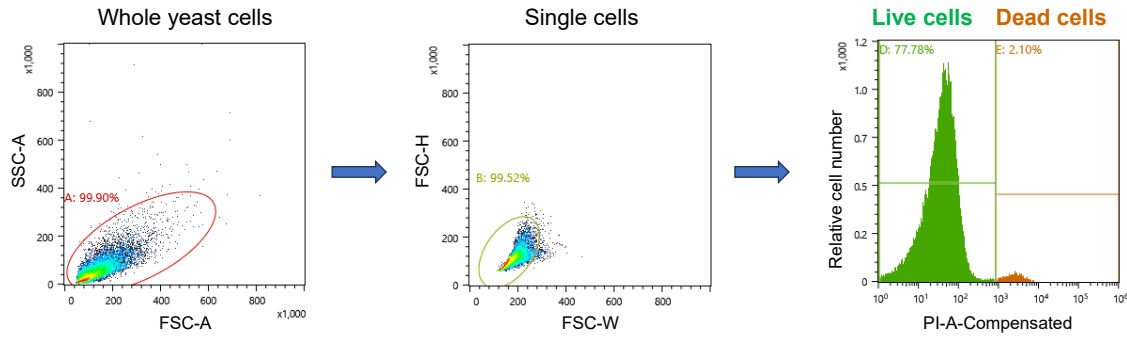


Fig. 7. Representative gating strategy for flow cytometry of Fig. 3b. In this sample gating, whole yeast cells were identified by gating on FSC-A and SSC-A to exclude debris and noise. Subsequent doublet exclusion was performed using FSC-W versus FSC-H plotting. Viability within the singlet yeast population was then determined by PI staining ($>10^3$). FSC-A, forward scatter area; SSC-A, side scatter area; FSC-W, forward scatter weight; FSC-H, forward scatter height.

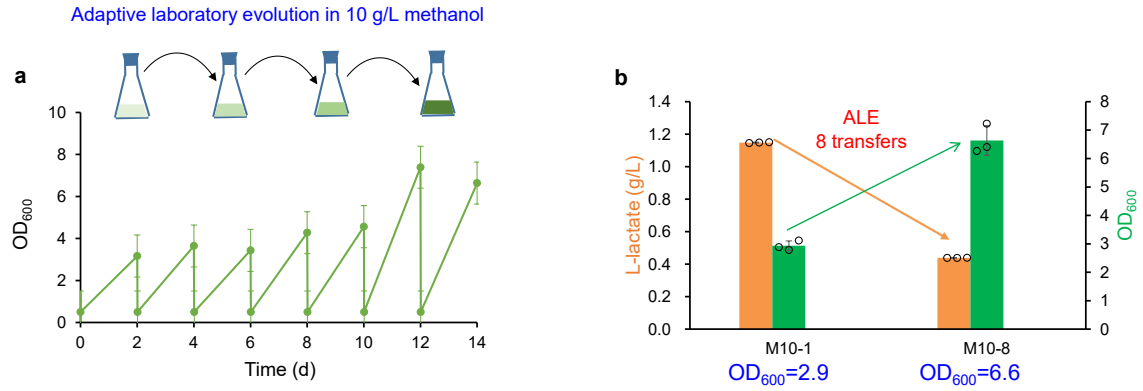


Fig. 8. Adaptive laboratory evolution (ALE) of the engineered strain HPLA06. a. The process of ALE in 10 g/L methanol. b. ALE improved cell growth but dramatically decreased L-lactate production. Data are presented as mean values $\pm$ SEM of three biological replicates. Source data available in the Source Data file.

PaLdh MSNIQNHQKVVLV **GXGXXG** **GDG**AV**G**SSYAFAMAQQGIAEEFVIV**DV** ^{*} **NAD⁺**
V40R **NADP⁺**

Fig. 9. Single amino acid of V40R close to the cofactor binding site (GXGXXG) to change the cofactor preference of PaLdh from NADH to NADPH.

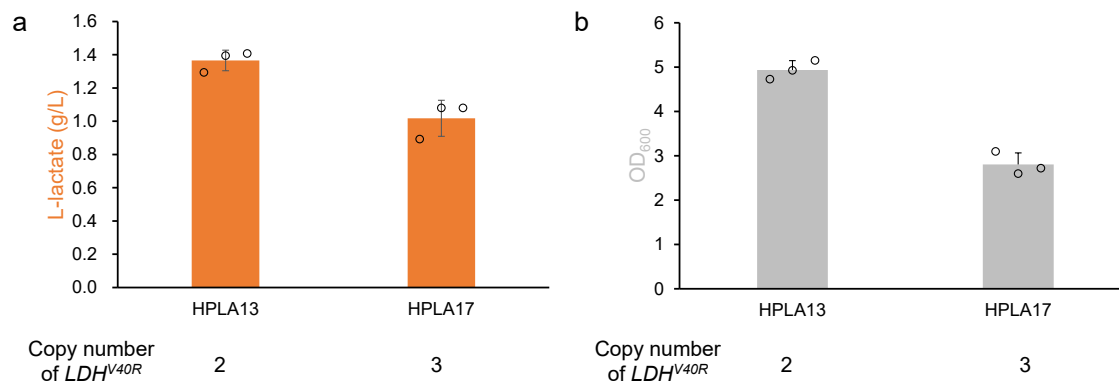


Fig. 10. Further increasing another copy of LDH^{V40R} in the strain HPLA13 decreased L-lactate production. a. The L-lactate production of the two strains. b. OD₆₀₀ of the two strains. Data are presented as mean values $\pm$ SEM of three biological replicates. Source data available in the Source Data file.

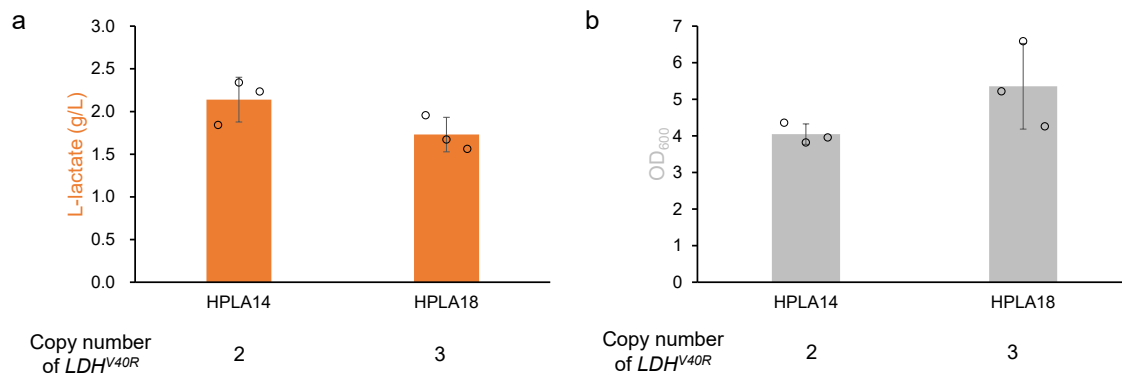


Fig. 11. Further increasing another copy of LDH^{V40R} in the strain HPLA14 decreased L-lactate production. a. The L-lactate production of the two strains. b. OD₆₀₀ of the two strains. Data are presented as mean values $\pm$ SEM of three biological replicates. Source data available in the Source Data file.

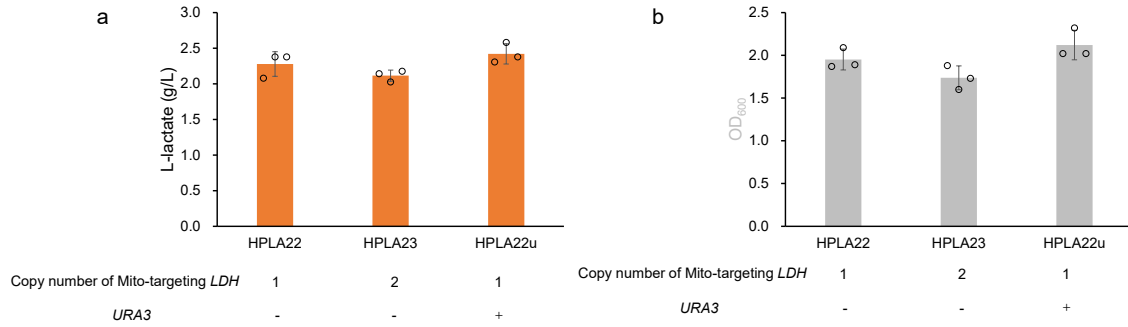


Fig. 12. Increasing copy number of Mito-targeting *LDH* and in situ recovery of selection marker gene *URA3* for improving L-lactate production. a. The L-lactate production of the three strains. b. OD₆₀₀ of the three strains. Data are presented as mean values +/- SEM of three biological replicates. Source data available in the Source Data file.

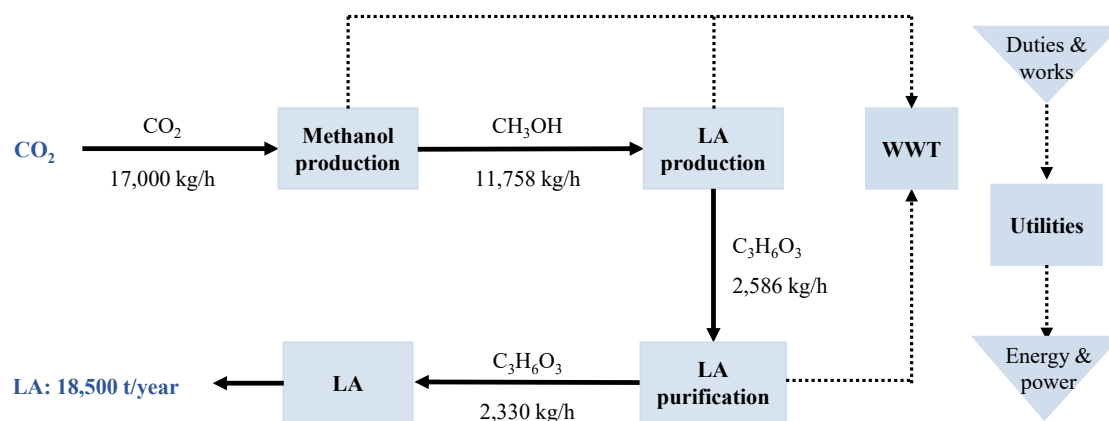


Fig. 13. Simplified carbon flow diagram for CO₂-derived methanol converted into L-lactate. LA: L-lactate.

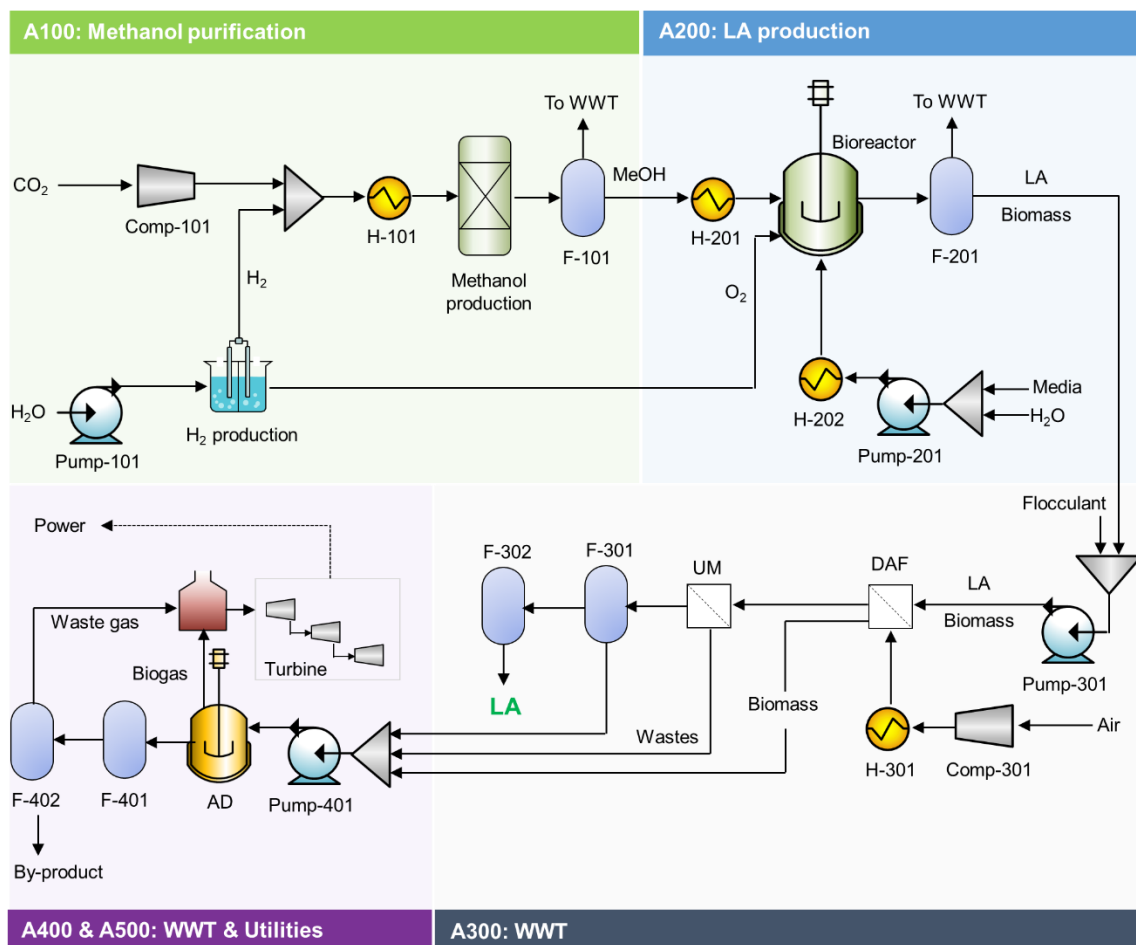


Fig. 14. Detailed process design for CO₂-derived methanol converted into L-lactate. AD: anaerobic digestion, DAF: dissolved air flotation, LA: L-lactate, UM: ultrafiltration membrane, WWT: wastewater treatment.

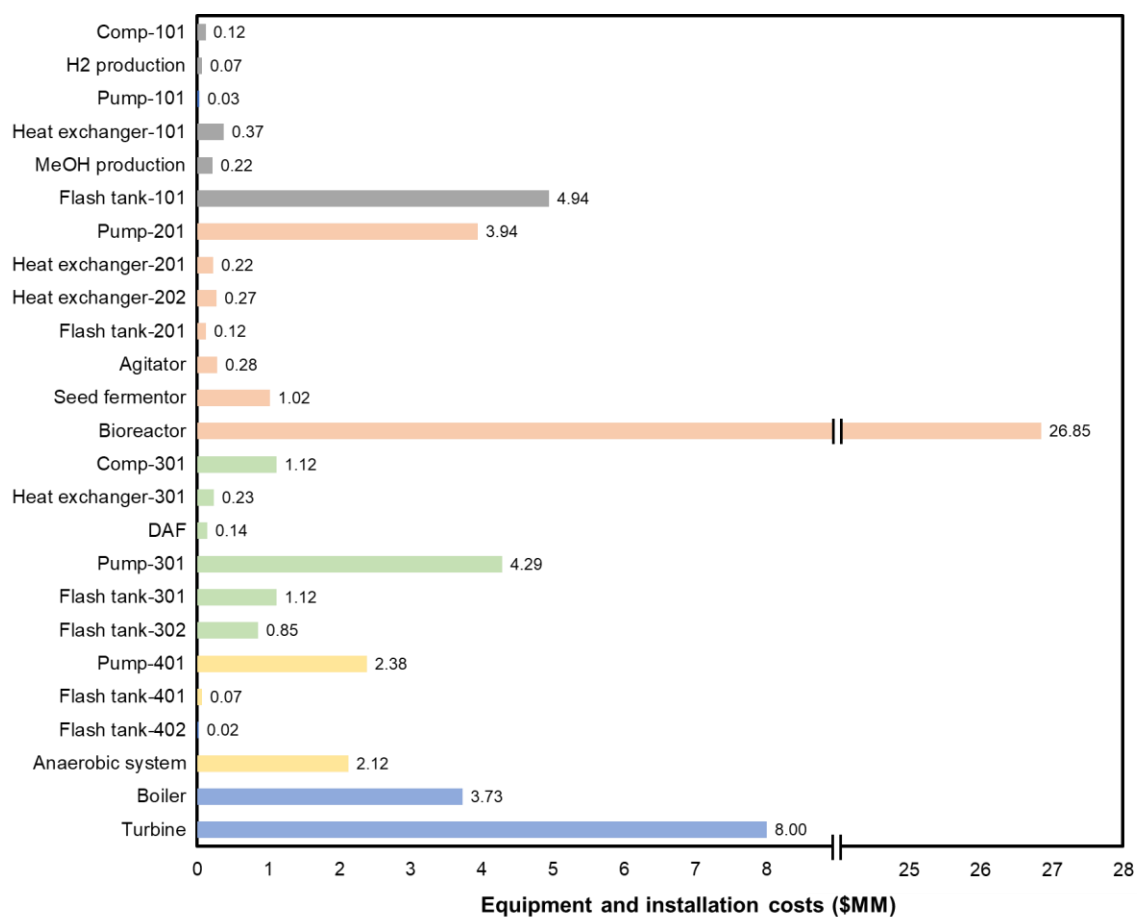


Fig. 15. Detailed cost analysis of the equipment and installation expenses for CO₂-derived methanol converted into L-lactate.

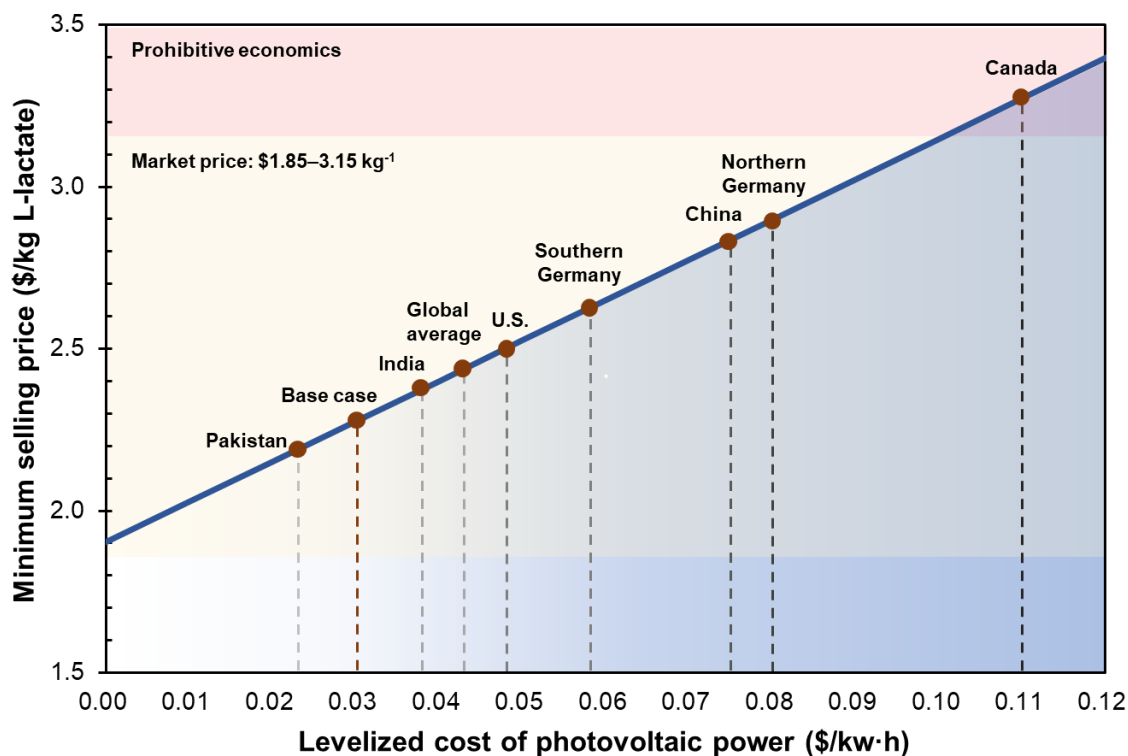


Fig. 16. Regional impact of utility-scale photovoltaic solar levelized cost of electricity (LCOE) on the minimum selling price of L-lactate in 2024. Data sources: Pakistan(13), India(14), global average(14), United States(15), Southern Germany(16), China(17), Northern Germany(16), Canada(18).

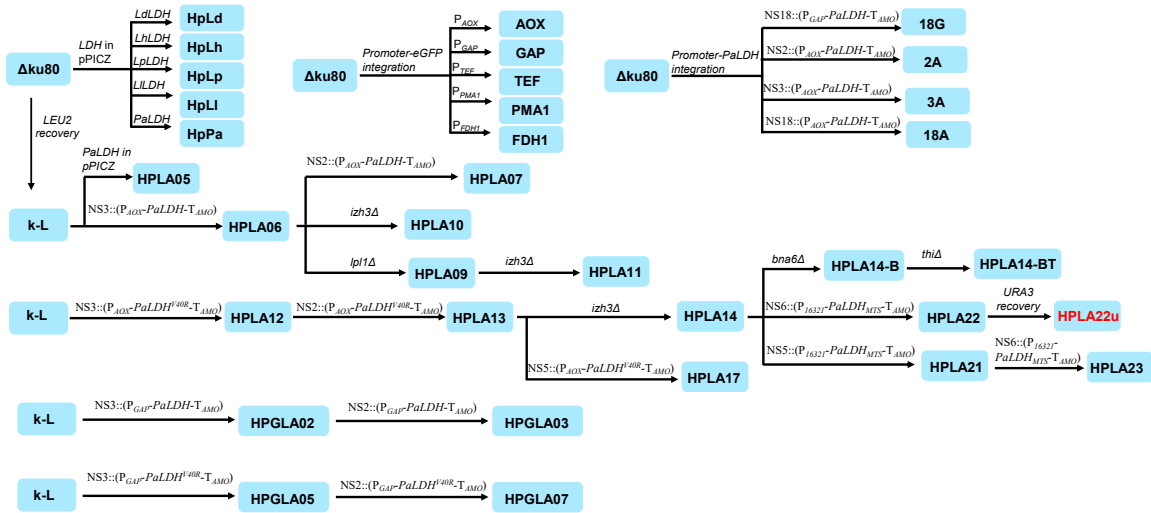


Fig. 17. Flowchart of *O. polymorpha* strain construction for L-lactate production. The strain used for fed-batch fermentation was marked with red.

Table 1. Detailed information of Ldh from different sources.

Genera	Km (mM)	Kcat (s ⁻¹)	Titer (g/L)	Host	Reference
<i>Lactobacillus delbrueckii</i>	-	-	135	<i>Lactobacillus delbrueckii</i>	(19)
<i>Lactobacillus helveticus</i>	0.25	643	92	<i>Candida sonorensis</i>	(20)
<i>Lactobacillus plantarum</i>	-	-	18	<i>Schizosaccharomyces pombe</i>	(21)
<i>Lactococcus lactis</i>	-	-	50	<i>Lactococcus lactis</i>	(22)
<i>Pediococcus acidilactici</i>	-	-	97	<i>Escherichia coli</i>	(23)

Table 2. Assumptions for the L-lactate production from CO₂-derived methanol.

Parameter	Assumption	References
CO ₂ inflow	17,000 kg/h	Aspen plus
CO ₂ to methanol efficiency	95%	(1)
Methanol production reaction condition	5.0 MPa, 315 °C	(1)
Electricity to H ₂ efficiency	4.1 kW·h/m ³	(2)
Water electrolysis efficiency	87%	(2)
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	<i>Ogataea polymorpha</i>	This study
Culture temperature	37 °C	This study
Culture pH	5.6	This study
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
L-lactate recovery rate	90%	(5)
Biogas productivity of anaerobic digestion	60%	(24)
Anaerobic digestion condition	35 °C and 1 bar	(24)
Annual production capacity of L-lactate	18,500 t	Aspen plus

Table 3. nth-plant assumptions required by techno-economic analysis (5).

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 4. Capital expenditures for the annual production of 18,500-ton L-lactate from CO₂.

Category	Classification	Items	Investment	Total investment
FCI	TDC	A100	1.43 MM\$	71.20 MM\$
		A200	37.53 MM\$	
		Equipment & installation 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\$	
	Prorated expenses		7.12 MM\$	
	Field expenses		7.12 MM\$	
	TIC	Office & construction fee	14.24 MM\$	42.72 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 capital cost		120.76 MMS		

Table 5. Operating expenditures for the annual production of 18,500-ton L-lactate from CO₂.

Classification	Items	Investment	Annually costs
Raw material	CO ₂ from capture	12.45 MM\$/yr	24.10 MM\$/yr
	Ammonia	1.77 MM\$/yr	
	Flocculant	0.55 MM\$/yr	
	Water for H ₂	0.07 MM\$/yr	
	Makeup water	2.21 MM\$/yr	
	Ultrafilter replacement	0.03 MM\$/yr	
	Solar power	7.02 MM\$/yr	
Sludge disposal	Sludge disposal	1.16 MM\$/yr	1.16 MM\$/yr
By-product's credit	Residue biomass	-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		17.59 MM\$/yr	

Table 6. Cost breakdown for the minimum selling price of 18,500-ton L-lactate from CO₂.

Area	Capital recovery	Raw materials	Byproduct credit	Fixed costs	Area totals
A100	\$0.029	\$0.679	\$0.000	\$0.006	\$0.713
A200	\$0.753	\$0.096	\$0.000	\$0.153	\$1.002
A300	\$0.156	\$0.030	\$0.000	\$0.032	\$0.217
A400	\$0.092	\$0.563	-\$0.605	\$0.019	\$0.068
A500	\$0.236	\$0.000	\$0.000	\$0.048	\$0.283
Sum	\$1.265	\$1.367	-\$0.605	\$0.257	\$2.284

Table 7. Inventory data used in the life cycle assessment.

Input data	Unit	Aspen Plus results
CO ₂ from capture	kg/kg L-lactate	7.2948
Tap water	kg/kg L-lactate	6.8603
Nitrogen source	kg/kg L-lactate	0.1580
Flocculant	kg/kg L-lactate	0.0111
Solar power	kwh/kg L-lactate	44.1584
Output data	Unit	Aspen Plus results
L-lactate	kg/kg L-lactate	1.0000
Fertilizer	kg/kg L-lactate	0.2905
O ₂	kg/kg L-lactate	17.9059
CO ₂	kg/kg L-lactate	3.0360
N ₂	kg/kg L-lactate	53.3864
NH ₃	kg/kg L-lactate	0.1558

Table 8. Global warming potential for producing 1 kg of L-lactate.

Impact category	GWP (kg CO₂-eq)
CO ₂ from capture	-7.2948
Tap water	0.0020
Nitrogen source	1.3606
Flocculant	0.0510
Solar power	4.8574
CO ₂ emission	3.0360
Total GWP (kg CO₂-eq)	2.0122
By-product (kg CO₂-eq)	0.2770
L-lactate (kg CO₂-eq)	1.7352

Table 9. Comparisons of the minimum selling price of lactic acid from different research.

Feedstock	Feedstock price	Titer	Productivity	Yield	MSP	Ref
	\$/ton	g/L	g/L/h	g/g	\$/kg	
This study	92.5	25	0.5	24%	2.29	-
Corn cob residues	38.7	104.6	2.32	97%	0.35	(5)
Corn stover	61.72	104.5	NA	72%	0.52	(25)
Food waste	77	NA	NA	92%	0.94	(26)
Lignocellulosic	71.3	97.5	0.89	76%	1.57	(27)

Table 10. List of plasmids used in this study.

Plasmid	Relevant characteristics	Resource
pLdLDH	<i>AmpR, URA3, P_{AOX}-LdLDH</i>	This study
pLhLDH	<i>AmpR, URA3, P_{AOX}-LhLDH</i>	This study
pLpLDH	<i>AmpR, URA3, P_{AOX}-LpLDH</i>	This study
pLiLDH	<i>AmpR, URA3, P_{AOX}-LiLDH</i>	This study
pPaLDH	<i>AmpR, URA3, P_{AOX}-PaLDH</i>	This study
pgRNA-NS2	<i>AmpR, URA3, gRNA- NS2</i>	(28)
pgRNA-NS3	<i>AmpR, URA3, gRNA- NS3</i>	(28)
pgRNA-NS5	<i>AmpR, URA3, gRNA- NS5</i>	(28)
pgRNA-NS6	<i>AmpR, URA3, gRNA- NS6</i>	(28)
pgRNA-NS18	<i>AmpR, URA3, gRNA- NS18</i>	(28)
pgRNA- <i>izh3</i>	<i>AmpR, URA3, gRNA-izh3</i>	(29)
pgRNA- <i>lpl1</i>	<i>AmpR, URA3, gRNA-lpl3</i>	(29)

Table 11. Power production and consumption for the L-lactate production from CO₂.

POWER	Turbine	W100	W200	W300	W500	Total work
kW	-24502	9893.64	1883.35	8658.36	32652.6	28585.49

Table 12. Energy consumption for the L-lactate production from CO₂.

Q	Q101	Q102	Q200	Q201	Q202	Q301	Q302	Q303	Q501
MMkcal/hr	4.99	0.0017	-300.3	-303.8	3.4	322.5	5.8	316.7	-132.3

Table 13. 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	(30)
Ammonia	\$550.5/ton	(31)
Flocculant	\$12258.7/ton	(31)
Makeup Water	\$0.54/ton	(31)
Solar power	\$0.031/kWh	(17)
Sludge disposal	\$20.2/ton	(31)
Credit		
Biofertilizer credit	\$362.3/ton	(32)
Carbon credit	\$104.1/ton	(33)

Supplementary references

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