

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

Corresponding Author: Professor Yongjin Zhou

**This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.**

**Attachments originally included by the reviewers as part of their assessment can be found at the end of this file.**

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The manuscript "Methanol biotransformation for the production of the biodegradable plastic monomer L-lactate in yeast" by Yu et al. is well-written and easy to follow. This study explores L-lactic acid production in the methylotrophic yeast *Ogataea polymorpha* using methanol as a substrate. The authors demonstrate that balancing the NADH/NAD<sup>+</sup> and NADPH/NADP<sup>+</sup> cofactor ratio significantly enhances L-lactate synthesis. Additional metabolic engineering strategies included mitochondrial compartmentalization of LDH and improving cell viability by deleting *IZH3*. Adaptive laboratory evolution (ALE) experiments improved strain robustness toward methanol but did not enhance lactic acid production. In a fed-batch fermentation setup using CO<sub>2</sub>-derived methanol, the engineered strain produced 25 g/L L-lactate. A key strength of this study is the combination of genetic engineering approaches—such as optimizing reductive power and compartmentalization—to improve lactic acid production from methanol. Furthermore, the authors integrate their experimental data into techno-economic analysis (TEA) and life cycle assessment (LCA), providing valuable industrial context for future applications.

I suggest the following points for improvement:

- Lines 101-102: The authors tested five Ldh enzymes in *O. polymorpha*, with two (*Lactobacillus delbrueckii* and *Lactobacillus helveticus*) being non-functional. Could the authors clarify why these enzymes did not work in *O. polymorpha*? Gene expression analysis of all five Ldh enzymes would help confirm whether they were actively expressed.
- Line 31: "Highest titer" is more appropriate than "yield" in this context.
- Line 120: The manuscript states that HPLA06's growth decreased by 32.6% compared to the wild-type strain and refers to two separate figures (Fig. 2b and 2d). Presenting this phenotype in a single diagram—rather than requiring readers to compare two figures—would improve clarity. Consider adding a consolidated figure to the Supplementary Information section.
- Lines 154-155: It would be helpful to specify that pGAP-driven LDH constructs were used for this analysis. Otherwise, readers might assume that the previously mentioned pAOX1 strains were cultivated under glucose conditions.
- Discussion: One of the manuscript's key strengths is incorporating TEA and LCA to contextualize the experimental findings. However, this aspect is not sufficiently discussed. Adding a paragraph on TEA/LCA in the discussion section would strengthen the manuscript.

Supplementary Information (SI) Suggestions:

- SI Appendix Figure S1 (a) and (b) should be explicitly mentioned in the figure description.
- SI Appendix Figure S11: Ensure a consistent font size in the figure description.
- SI Appendix Figures S4 and S9: The phrase *in situ* does not need to be italicized, as it is commonly used in this research field. Please check for consistency throughout the manuscript.

Reviewer #4

(Remarks to the Author)

This manuscript describes the engineering of the methylotrophic yeast *O. polymorpha* for efficient production of L-lactate from CO<sub>2</sub>-derived methanol, integrating systematic metabolic engineering with comprehensive techno-economic and life cycle assessments. The study is notable not only for successfully developing a methanol-utilizing yeast strain with reasonably high lactate production, but also for providing a pioneering, systematic evaluation of the economic and

environmental impacts associated with this CO<sub>2</sub>-to-chemical scenario. However, several issues need to be addressed before the manuscript can be recommended for publication:

1. Although yeasts generally tolerate acidic conditions, the lactate tolerance limit of *O. polymorpha* should be studied and discussed.
2. Lines 127-128: The authors observed improved cell viability upon disruption of *IZH3* but not *LPL1*. Please discuss the potential mechanisms underlying the differential effects of these disruptions.
3. Lines 142-143: The authors suggest that NADPH is more abundant in methanol metabolism compared to glucose metabolism, which is very interesting. However, the stated "methanol assimilation via the pentose phosphate pathway (PPP) and the Xu5P cycle" seems to imply a metabolic cycle predominantly generating NADPH while releasing CO<sub>2</sub>, which suggests limited or no net C1 assimilation. Please provide a detailed explanation to clarify this aspect.
4. Lines 152-153: The NAD(P)H/NAD(P)<sup>+</sup> ratio can significantly fluctuate under different cultivation conditions. Given that this ratio was measured during the log growth phase, please clarify whether the high NADPH/NADP<sup>+</sup> ratio observed also applies during later stages of production.
5. The term "cofactor balance" should be used cautiously. It may be valid to claim improved cofactor balance in strain HPLA13 relative to HPLA06; however, it seems questionable to state that HPLA13 outperforms the WT strain in terms of cofactor balance, since the WT strain inherently maintains cofactor balance through evolution.
6. Lines 182-183: How was the "CO<sub>2</sub>-derived methanol solution" produced to compose methanol and water at a precise 3:1 ratio? Could the authors clarify how this exact ratio was achieved and confirm whether the phrase "without byproduct" specifically means "without impurities"?
7. Lines 201-202: When discussing the economic contributions of individual modules, please include specific percentage values.
8. For TEA and LCA, can the reuse of residual biomass (e.g., for single-cell protein, SCP) be considered to further valorize the CO<sub>2</sub>-to-lactate manufacturing process?
9. Lines 286-289: The authors should be cautious in drawing conclusions about the engineered strain's ALE performance without explicitly examining genetic stability.

Reviewer #5

(Remarks to the Author)

Biodegradable plastics such as polylactic acid (PLA) are gaining attention as sustainable alternatives to fossil-derived polymers with emerging C1-fuelled bioprocesses offering a promising route for efficient and eco-friendly lactate production. In this study, *O. polymorpha* was metabolically engineered to significantly increase the L-lactate production yield using methanol alone. The authors propose a promising strategy to combine liquid sunshine technology with biotransformation of methanol using metabolically engineered *O. polymorpha* yeast to increase L-lactate yield and evaluate its economic feasibility and GHG mitigation performance.

However, it is unable to decide to be published in Nature Communications for some major and minor points while It has no doubt that the author's research will be of interest to fellow specialists working in the field of metabolic engineering. There are serious concerns about assessment methods and results that have a significant impact on the clarity of the paper.

Major points

- 1) In Table S12, the reference paper presenting the cost of solar power provides a cost of \$0.05/kWh for solar power in 2017. However, the source of the cost remains unclear as it is uncertain whether it originates from photovoltaics or not. A range of statistics indicates that the price of electricity from the grid varies between \$0.08 and \$0.25 per kWh. As stated in the NREL report (Kazi et al., National Renewable Energy Lab (NREL), 2010), the electricity price was assumed to be \$0.054/kWh in 2007. In consideration of these reports, the cost of solar power in this study cannot be considered a viable cost. Since the cost of solar power shows a high sensitivity in the techno-economic analysis, further explanation for the decrease of the cost of solar power from 2017 to 2024 and a reference to the cost of solar derived power in 2024 are needed.
- 2) The mass and energy balance and specific estimation of capital expenditures (CapEx) of each equipment from A100 to A500 should be presented in the supporting information file, respectively. Table S6 mentions the amounts of the primary materials but the mass and energy balance for each subsystem is not available. Table S4 mentions capital expenditures for each area, but more detailed information for each equipment is not available too.
- 3) The biofertilizer produced by anaerobic digestion of the biomass is considered economic credit in Table S12, but not included in the environmental impact assessment in Table S6-S7. It is crucial to address the different methodology between the economic assessment, which incorporated biofertilizer credits, and the environmental assessment, which made no consideration.
- 4) It is necessary to specify the impact assessment methodology such as ReCiPe 2016 Midpoint (H), Environmental Footprint 3.1 and TRACI 2.1 on which the life cycle assessment is based. In Table S6, the specified life cycle inventory data for the source of the material and energy are also required. It should include the information where the data origins from (GLO, RER, RoW, ...) and whether the data is cut-off (APOS, Cut-off, ...)
- 5) A detailed description of the thermodynamic model selected to simulate the process is required.

Minor points

- 1) The manuscript should provide a reason to support the assumption of an L-lactate production capacity of 18,500 tons/year.
- 2) It is recommended to improve the visibility of the text and numbers in Figure 6 to enhance overall clarity.
- 3) There is an inconsistency in the use of the terms 'lactate' and 'lactic acid' between the main text and the supporting information file.
- 4) In table S4, there is a typo. 'Total operating cost' should be corrected to 'Total capital cost'

5) In table S5, which presents the operating expenditures, the use of the term 'investment' should be appropriately revised.

#### Reviewer #6

(Remarks to the Author)

The present manuscript combined three diverse areas in a single paper, lacking thorough justice to individual topics, especially TEA. The TEA used a completely wrong approach for process design, which makes the results unreliable and unacceptable, I recommend removing TEA work from the manuscript to avoid passing the wrong information to the readers. Below are my few comments.

- Supporting Information: The reaction stoichiometry for methanol production ( $2 \text{ CO}_2 + 4 \text{ H}_2 \rightarrow 2 \text{ CH}_3\text{OH} + \text{O}_2$ ) seems to be vague. Also, there are no references to support the reaction conditions for methanol production. Also, I could not find the oxygen as a byproduct.
- What is the basis of plant capacity?
- TEA: There is no mention of low pH or neutral fermentation.
- L-Lactate is mentioned in the manuscript. However, the reaction stoichiometry shows L-lactic acid.
- L-lactate purification (A300): It looks to be completely vague. It may be noted that LA separation is the major cost contributor to the overall process in terms of capital investment and energy consumption. If this kind of downstream separation is considered, the TEA results become completely unreliable. Please refer to recent papers on LA production. Otherwise, I recommend removing TEA completely from the manuscript as it will pass wrong information to the readers.
- AD: Reaction stoichiometry and conditions are not mentioned.
- The complete Aspen Plus flowsheet is missing. The details of the equipment and their design parameters are also missing. As a result, it is difficult to judge the reliability of TEA results.
- The energy cost is completely missing from TEA, which is one of the main cost contributors in this process.
- "The estimation of CAPEX, OPEX, and MSP was conducted via an in-house model created by the National Renewable Energy Laboratory (NREL)." Is it an open-source software? Where is the link?

#### Reviewer #7

(Remarks to the Author)

Please see the attached file

Version 1:

Reviewer comments:

#### Reviewer #1

(Remarks to the Author)

The authors have addressed all questions and comments satisfactorily. I am fine with the current version and support its publication. There are just a few minor writing issues that should be corrected.

Minor corrections

Line 111: in situ should not be italicized.

Line 160: Please be mindful of the tense in this sentence ("It is worth mentioning...").

#### Reviewer #4

(Remarks to the Author)

After careful review, the authors have satisfactorily addressed all questions raised by the reviewers. The manuscript is now suitable for publication in Nature Communications.

#### Reviewer #5

(Remarks to the Author)

#### Reviewer #6

(Remarks to the Author)

Accept

Version 2:

Reviewer comments:

#### Reviewer #5

(Remarks to the Author)

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The article entitled “Methanol biotransformation for the production of the biodegradable plastic monomer L-lactate in yeast” discusses a promising approach to producing L-lactate, a biodegradable plastic monomer via methanol biotransformation in yeast *Ogataea polymorpha*. The topic is highly relevant and of significant interest, particularly in the context of use of C1 feedstock for sustainable biomanufacturing of plastics. However, the Manuscript needs lots of clarity as there are several loopholes. Below are my comments and suggestions to enhance the quality of the Manuscript.

1. Throughout the Manuscript, the argument of author is that methanol is an ideal feedstock for biomanufacturing lactate (LA) as it can be produced from CO<sub>2</sub> in massive quantities and this approach will help in mitigating the carbon emissions. In that case why not to use CO<sub>2</sub> directly via gas fermentation instead of methanol. I would suggest adding some text for comparing LA production using CO<sub>2</sub> and methanol with pros and cons of each and demonstrate which one could be more suitable to take forward.
2. Usually most of the microbial cultures have lactate dehydrogenase enzymes. Does genome of *Ogataea polymorpha* has no copy of this enzyme? Add few lines on Biochemistry and Microbiology of yeast.
3. The role of *LPL1* (encoding a putative lipase) and *IZH3* (encoding a membrane protein) in context of LA production is not clear?
4. Why and how ALE was done, what was the selection pressure. It was not clear how ALE cause reduction in LA production despite substantial improvement in biomass.
5. If LA production is to be taken place in mitochondria where plenty of NADH is available from TCA cycle, then why do we need NADPH as cofactor?
6. First of methanol is a reduced carbon source and should generate more reducing equivalents than glucose. Even if it is NADPH, transhydrogenase can easily convert them into NADH.
7. It is difficult to buy this argument that due to methanol metabolism, NADH is not available for LA production.
8. Usually LA production takes place in cytoplasm as pyruvate comes from Glycolysis which happens in cytoplasm. In current study, it is not clear why LA production was shifted to mitochondria.
9. The Manuscript does not about oxygen levels during cultivation. For example, I could not find any information about aeration conditions during fed-batch culture.
10. The batch and fed-batch fermentation were performed only on methanol or some glucose was added during the cultivation?
11. What is the theoretical yield of LA on methanol. Were there any byproducts formed? What was the pH profile in flask and was pH controlled in bioreactor?
12. The details of software used for LCA and TEA is not provided. Despite a LA titer of 25 g/L, MSP is coming out to be \$2.25/kg which is better than many sugar based bioprocesses. I am not sure how they are ending up with such a low number.

## Responses to Reviewer 5

**General comment:** Biodegradable polymers like polylactic acid (PLA) are increasingly recognized as eco-friendly substitutes for petroleum-based plastics, and novel single-carbon (C<sub>1</sub>) bioprocesses present an efficient pathway for lactate synthesis. The yeast *Ogataea polymorpha* was genetically modified to achieve high yields of L-lactate solely from methanol. The authors introduce an integrated approach that combines “liquid sunshine” photochemical conversion with engineered *O. polymorpha* fermentation to enhance L-lactate titers, and assess the system’s techno-economic viability alongside its greenhouse-gas reduction potential. I think this paper can be published in Nature Communications after the second revision for one major point.

### Decision: Major revision

#### Major points:

**Comment 1:** In Table S12, the reference paper presenting the cost of solar power provides a cost of \$0.05/kWh for solar power in 2017. However, the source of the cost remains unclear as it is uncertain whether it originates from photovoltaics or not. A range of statistics indicates that the price of electricity from the grid varies between \$0.08 and \$0.25 per kWh. As stated in the NREL report (Kazi et al., National Renewable Energy Lab (NREL), 2010), the electricity price was assumed to be \$0.054/kWh in 2007. In consideration of these reports, the cost of solar power in this study cannot be considered a viable cost. Since the cost of solar power shows a high sensitivity in the techno-economic analysis, further explanation for the decrease of the cost of solar power from 2017 to 2024 and a reference to the cost of solar derived power in 2024 are needed.

**Reply 1:** Thank you for your detailed feedback. We have updated the cost of solar power to reflect the most recent data available for the high-efficiency regions (e.g., United States, Europe, and China) in 2024. According to the latest reports, the levelized cost of electricity for utility-scale photovoltaic solar power in 2024 is approximately \$0.031-0.075 /kWh<sup>3,4</sup>. This updated value has been incorporated into our analysis and

is now cited with an appropriate reference in Supplementary Table S21. The results of operating expenditures, minimum selling price, and sensitivity analysis have been revised in the Supplementary Tables S5-S6, Fig. 6c, and Fig. 6d. The updates are detailed in Line 503-504:

“The levelized cost of electricity for utility-scale photovoltaic solar power in the base scenario is \$0.031 KWh<sup>-1</sup>, based on the latest data for high-efficiency regions in 2024<sup>70</sup>.”

#### References:

3. Chong, S., Wu, J. & Chang, I. S. Cost accounting and economic competitiveness evaluation of photovoltaic power generation in China-based on the system levelized cost of electricity. *Renewable Energy* 222, 119940, 119940 (2024).
4. SolarPACES. Qinghai sets CSP tariffs at 0.55 yuan/kWh (7.5 cents/kWh), (2025).

#### Reviewer's response:

Thank you for the revision and efforts to address the comment. The authors explained that the levelized cost of electricity for utility-scale photovoltaic solar power in 2024 is approximately \$0.031-0.075/kWh with references. However, some references, such as the Fraunhofer report (2024), present the levelized cost of electricity for photovoltaic solar power as a range of \$0.04-0.24/kWh. It is recommended to consider the various ranges of the levelized cost of photovoltaic solar power, rather than focusing solely on the minimum cost. Considering that photovoltaic solar power is a form of renewable energy, its price varies greatly depending on the region, making it infeasible to reflect the minimum price in a specific region. The authors should consider the price of photovoltaic solar power in various regions and perform the techno-economic analysis for two cases: when the solar power cost is minimum and when it is maximum.

The Fraunhofer report (2024): Christoph Kost et al., Levelized Cost of Electricity Renewable Energy Technologies, Fraunhofer, 2024

**Comment 2:** The mass and energy balance and specific estimation of capital expenditures (CapEx) of each equipment from A100 to A500 should be presented in the supporting information file, respectively. Table S6 mentions the amounts of the primary materials but the mass and energy balance for each subsystem is not available.

Table S4 mentions capital expenditures for each area, but more detailed information for each equipment is not available too.

**Reply 2:** Thank you for your constructive comments. We have incorporated the mass and energy balance from A100 to A500, obtained from Aspen Plus, offering an additional and transparent overview on the material and energy flows inside our system (Supplementary Tables S14-S20). Additionally, we have provided a comprehensive breakdown of the equipment and installation costs for each individual item, as depicted in Supplementary Fig. S13, to complement the area-level data previously shown in Supplementary Tables S4, which aligns with existing literature<sup>1</sup>. The detailed revisions can be found in Line 212-213 and 491-493:

“Comprehensive investment details for equipment and installation corresponding to each individual component are available in Supplementary Fig. S14. Notably, the bioreactors accounted for the predominant share of expenditures associated with equipment and installation in CAPEX.”

“Fig. 6a shows the mass balance of the L-lactate production process simulated in Aspen Plus, with comprehensive mass and energy balance data available in Supplementary Table S14-S20.”

#### References:

1. Zhang, C. et al. An upcycling bioprocess for sustainable aviation fuel production from food waste-derived greenhouse gases: Life cycle assessment and techno-economic analysis. *Chemical Engineering Journal* 486, 150242, 150242 (2024).

#### Reviewer's response:

This comment has been appropriately addressed in the revised supplementary file with a clear explanation.

**Comment 3:** The biofertilizer produced by anaerobic digestion of the biomass is considered economic credit in Table S12, but not included in the environmental impact assessment in Table S6-S7. It is crucial to address the different methodology between the economic assessment, which incorporated biofertilizer credits, and the environmental assessment, which made no consideration.

**Reply 3:** Thank you for your valuable comment. To ensure consistency with the system boundaries used in the techno-economic analysis (TEA), we have now included the environmental credits from biofertilizer in the life cycle assessment (LCA). These credits were calculated using the economic allocation method<sup>5</sup>, aligning the environmental assessment with the approach taken in the economic analysis. Based on the economic allocation method, the biofertilizer permits a deduction of 0.277 kg CO<sub>2</sub>-eq from the overall GWP of the process. The revised LCA results are displayed in Fig. 6e-6f and Supplementary Table S8. Comprehensive updates of results and methodology are included in Line 263-265 and 530-533:

“It is worth mentioning that from the standpoint of economic value allocation, the by-product of fertilizer could result in a GWP decrease of 0.277 kg CO<sub>2</sub>-eq, so further diminishing the carbon footprint.”

“The economic allocation method is chosen to assess the GWP of the by-product (biofertilizer)<sup>73</sup>. The cost of L-lactate is believed to correspond with the MSP of the basic scenario from this study, while Supplementary Table S21 encompasses the price of biofertilizer.”

#### References:

5. Ijassi, W., Ben Rejeb, H. & Zwolinski, P. Environmental impact evaluation of co-products: decision-aid tool for allocation in LCA. *The International Journal of Life Cycle Assessment* 26, 2199-2214 (2021).

#### Reviewer's response:

This comment has been appropriately addressed in the revised supplementary file and manuscript with a clear explanation of the LCA allocation method and results.

**Comment 4:** It is necessary to specify the impact assessment methodology such as ReCiPe 2016 Midpoint (H), Environmental Footprint 3.1 and TRACI 2.1 on which the life cycle assessment is based. In Table S6, the specified life cycle inventory data for the source of the material and energy are also required. It should include the information where the data origins from (GLO, RER, RoW, ...) and whether the data is cut-off (APOS, Cut-off, ...)

**Reply 4:** Thank you for your valuable suggestions. We have specified the impact assessment methodology used in the LCA as the TRACI version 2.1. Besides, the life cycle inventory data are derived from our Aspen Plus models, with comprehensive mass and energy data available in Supplementary Tables S14-S20. We also added the information where the data origins from and whether the data is cut-off into Supplementary Table S22, which details categories and providers from the Ecoinvent v3.6 database and corresponding references. Specific information regarding this methodology has been incorporated into the LCA methodologies section of the manuscript in Line 519-527.

“Supplementary Table S7 provides a comprehensive inventory of the life cycle associated with L-lactate production, derived from Aspen Plus. Moreover, we assessed the GWP (kg CO<sub>2</sub>-eq) of the L-lactate production process. The life cycle evaluation included background data from Ecoinvent v3.6 and references<sup>72</sup>, encompassing detailed categories and providers (Supplementary Table S22). The tool for reduction and assessment of chemicals and other environmental impacts (TRACI) version 2.1, created by the environmental protection agency (EPA), was utilized to provide characterization elements for evaluating the possible consequences of inputs and emissions from production processes.”

**Reviewer’s response:**

This comment has been appropriately addressed in the revised supplementary file with a clear explanation.

**Comment 5:** A detailed description of the thermodynamic model selected to simulate the process is required.

**Reply 5:** Thank you for your suggestion. The NRTL (non-random two-liquid) model was chosen to characterize the thermodynamic behavior of the system in our research. This model is widely recognized for its accuracy in predicting liquid-phase activity coefficients and phase equilibria in non-ideal mixtures, particularly those involving polar compounds and hydrogen bonding—key characteristics of our process (e.g., L-lactate fermentation broths, methanol-water systems)<sup>6</sup>. This model was implemented in

Aspen Plus, and its suitability for our process is further supported by prior studies on biomanufacturing<sup>7-9</sup>. A detailed description of the thermodynamic model selection can be found in Line 478-482.

“This study entailed the design of an L-lactate production process using Aspen Plus v14 software, which was utilized to conduct calculations for mass and energy balance. The phase equilibrium and thermodynamic model has been constructed using the NRTL (non-random two-liquid), in accordance with a previous study<sup>69</sup>.”

#### References:

6. Haydary, J. Chemical process design and simulation: Aspen Plus and Aspen Hysys applications. (John Wiley & Sons, 2019).
7. Méndez-Alva, J. A. et al. in Computer Aided Chemical Engineering Vol. 44 463-468 (Elsevier, 2018).
8. Gasca-González, R. et al. in Computer Aided Chemical Engineering Vol. 46 (eds Anton A. Kiss, Edwin Zondervan, Richard Lakerveld, & Leyla Özkan) 457-462 (Elsevier, 2019).
9. Kruschitz, A. & Nidetzky, B. Biocatalytic production of 2- $\alpha$ -d-glucosyl-glycerol for functional ingredient use: integrated process design and techno-economic assessment. ACS Sustainable Chemistry & Engineering 10, 1246-1255 (2022).

#### Reviewer's response:

This comment has been appropriately addressed in the revised manuscript with a clear explanation.

#### Minor points:

**Comment 1:** The manuscript should provide a reason to support the assumption of an L-lactate production capacity of 18,500 tons/year.

**Reply 1:** Thank you for your valuable comment. The projected production capacity of 18,500 tons annually is derived from typical scales used in the commercial lactic acid industry. Most commercial lactic acid production facilities run at capacities ranging from 15,000 to 30,000 tones every year, a range that represents prevailing industry practices, with the exception of a few manufacturers like NatureWorks LLC and

Corbion Purac<sup>10</sup>. Besides, this capacity is frequently employed in techno-economic evaluations of third-generation lactic acid biomanufacturing processes, such as those that utilize carbon dioxide or methane as feedstocks<sup>11,12</sup>. Overall, the chosen production capacity conforms to industry standards and guarantees comparability and relevance to existing TEA research. Detailed revision can be found in Line 202-205:

“It is worth noting that projected production capacity corresponds with standard industrial lactic acid plant scales (15,000-30,000 tons per year)<sup>36</sup> and is in accordance with capacities frequently utilized in published TEA studies on third-generation biomanufacturing<sup>37,38</sup>.”

#### References:

10. Adom, F. K. & Dunn, J. B. Life cycle analysis of corn-stover-derived polymer-grade l-lactic acid and ethyl lactate: Greenhouse gas emissions and fossil energy consumption. *Biofuels, Bioproducts and Biorefining* 11, 258-268 (2017).
11. Fei, Q. et al. Biological valorization of natural gas for the production of lactic acid: techno-economic analysis and life cycle assessment. *Biochemical Engineering Journal* 158, 107500 (2020).
12. Park, J. et al. Merging biomass and CO<sub>2</sub> utilization; process design and assessment on simultaneous production of lactic acid and formic acid from glycerol and CO<sub>2</sub>. *Chemical Engineering Journal* 463, 142410 (2023).

#### Reviewer's response:

This comment has been appropriately addressed in the revised manuscript with a clear explanation and statistical data.

**Comment 2:** It is recommended to improve the visibility of the text and numbers in Figure 6 to enhance overall clarity.

**Reply 2:** Thank you for your valuable suggestion. In response to your feedback, we have adjusted the font size and formatting of the text and numbers in Fig. 6. The revised version ensures that all elements are easily readable, reducing the potential for misinterpretation.

#### Reviewer's response:

This comment has been appropriately addressed in the revised manuscript with a clear visualization.

**Comment 3:** There is an inconsistency in the use of the terms ‘lactate’ and ‘lactic acid’ between the main text and the supporting information file.

**Reply 3:** Thank you for pointing out this detail. We have checked the main text and the supporting information file, and changed “lactic acid” to “lactate” in microbial fermentation for consistency. Some specific terms such as poly-lactic acid (PLA) and lactic acid bacteria (LAB) are not changed.

**Reviewer’s response:**

This comment has been appropriately addressed in the revised manuscript with a clear explanation.

**Comment 4:** In table S4, there is a typo. ‘Total operating cost’ should be corrected to ‘Total capital cost’

**Reply 4:** Thank you so much for your advice. As suggested, we have promptly corrected the typo in Supplementary Table S4, changing "Total operating cost" to "Total capital cost". The revised table has been thoroughly double-checked to ensure its accuracy.

**Reviewer’s response:**

This comment has been appropriately addressed in the revised supplementary file with a clear explanation.

**Comment 5:** In table S5, which presents the operating expenditures, the use of the term ‘investment’ should be appropriately revised.

**Reply 5:** Thank you for your comment. Considering that the values in the Supplementary Table S5 represent annual operational expenses, we have carefully amended the term to "annual costs" to accurately reflect the nature of these expenditures.

**Reviewer’s response:**

This comment has been appropriately addressed in the revised supplementary file with

a clear explanation.

## Responses to the reviewers' comments (NCOMMS-25-14369)

### Reviewer #5:

#### General comment

Biodegradable polymers like polylactic acid (PLA) are increasingly recognized as eco-friendly substitutes for petroleum-based plastics, and novel single-carbon (C1) bioprocesses present an efficient pathway for lactate synthesis. The authors introduce an integrated approach that combines “liquid sunshine” photochemical conversion with engineered *O. polymorpha* fermentation to enhance L-lactate titers, and assess the system's techno-economic viability alongside its greenhouse-gas reduction potential. The assessment methodologies have been appropriately addressed in the revised manuscript and supplementary file with a clear explanation, and the results are clearly presented and discussed in the context of relevant literature. The authors have addressed all the major and minor comments appropriately, and I think this paper can be published in *Nature Communications* now.

#### Decision: Accept

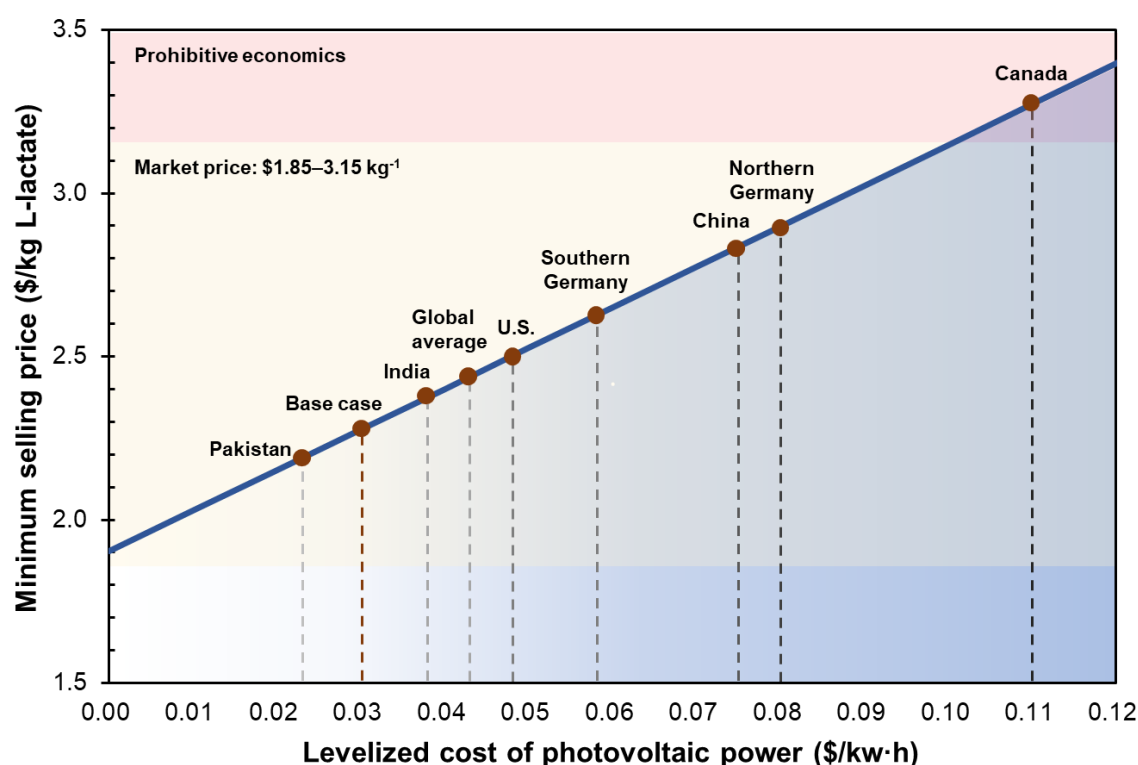
#### Major points

**Comment 1.** Thank you for the revision and efforts to address the comment. The authors explained that the levelized cost of electricity for utility-scale photovoltaic solar power in 2024 is approximately \$0.031-0.075/kWh with references. However, some references, such as the Fraunhofer report (2024), present the levelized cost of electricity for photovoltaic solar power as a range of \$0.04-0.24/kWh. It is recommended to consider the various ranges of the levelized cost of photovoltaic solar power, rather than focusing solely on the minimum cost. Considering that photovoltaic solar power is a form of renewable energy, its price varies greatly depending on the region, making it infeasible to reflect the minimum price in a specific region. The authors should consider the price of photovoltaic solar power in various regions and perform the techno-economic analysis for two cases: when the solar power cost is minimum and when it is maximum. The Fraunhofer report (2024): Christoph Kost et al., Levelized Cost of Electricity Renewable Energy Technologies, Fraunhofer, 2024.

**Reply 1:** Thank you for your detailed comment. In the revised manuscript, we incorporated sensitivity analysis scenarios by using utility-scale photovoltaic power

prices from different regions, drawing on multiple references including Fraunhofer (2025)<sup>1-6</sup>. A corresponding sensitivity curve is provided in Supplementary Fig. S15. We also explicitly discussed the regional variations in photovoltaic electricity prices in the manuscript (page 10, lines 248-255):

“Considering the regional variation in solar power costs, the MSP fluctuates significantly with the geographical location of the plant<sup>43</sup> (Supplementary Fig. S15). For example, countries with abundant solar resources such as Pakistan show solar power prices close to the global minimum (<0.025 \$/kWh), resulting in comparatively lower MSP, whereas regions with higher solar power prices such as Canada (>0.10 \$/kWh) may increase the MSP by over 13%. These results emphasize the critical role of site selection in improving the economic competitiveness of CO<sub>2</sub>-to-L-lactate biomanufacturing.”



**Supplementary Fig. S15** 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<sup>1</sup>, India<sup>2</sup>, global average<sup>2</sup>, United States<sup>3</sup>, Southern Germany<sup>4</sup>, China<sup>5</sup>, Northern Germany<sup>4</sup>, Canada<sup>6</sup>.

1 Tahir, M. H., Saleem, M. W., Bicer, Y., Ikram, M. & Iqbal, S. Optimizing Electrical Efficiency and Levelized Cost of Energy in Photovoltaic Systems Through Thermal Management Using Microchannel Heat Sinks. *International Journal of Energy Research* 2025, 2433429, doi: <https://doi.org/10.1155/er/2433429> (2025).

2 PV Tech. IRENA: Global solar PV LCOE increases by 0.6 % in 2024 to US

\$0.043/kWh. PV Tech News. 25 Jul 2025. Available from: <https://www.pv-tech.org/irena-global-solar-pv-lcoe-increases-by-0-6-in-2024-to-us0-043-kwh/>

3 Ramasamy, V. et al. Documenting 15 Years of Reductions in US Solar Photovoltaic System Costs. (National Renewable Energy Laboratory (NREL), Golden, CO (United States), 2025). Available from: <https://research-hub.nrel.gov/en/publications/documenting-15-years-of-reductions-in-us-solar-photovoltaic-syste>

4 Fraunhofer ISE. Levelized Cost of Electricity: Renewable Energy Technologies. (Fraunhofer Institute for Solar Energy Systems, 2025). Available from: <https://www.ise.fraunhofer.de/en/publications/studies/cost-of-electricity.html>

5 Chong, S., Wu, J. & Chang, I. S. Cost accounting and economic competitiveness evaluation of photovoltaic power generation in China — based on the system levelized cost of electricity. *Renewable Energy* 222, 119940, doi: <https://doi.org/10.1016/j.renene.2024.119940> (2024).

6 Sadat, S. A. & Pearce, J. M. Techno-economic evaluation of electricity pricing structures on photovoltaic and photovoltaic-battery hybrid systems in Canada. *Renewable Energy* 242, 122456, doi: <https://doi.org/10.1016/j.renene.2025.122456> (2025).

### **Reviewer's response:**

This comment has been appropriately addressed in the revised manuscript with a clear explanation.

## Responses to the reviewers' comments (NCOMMS-25-14369)

Dear reviewers,

We would like to express our thanks to the constructive suggestions regarding to our manuscript “Methanol biotransformation for the production of the biodegradable plastic monomer L-lactate in yeast”. These comments are helpful for us to revise and further improve our paper. The main corrections and responses to the reviewers' comments are as follows:

### Responses to Reviewer 1

**General comment:** The manuscript “Methanol biotransformation for the production of the biodegradable plastic monomer L-lactate in yeast” by Yu et al. is well-written and easy to follow. This study explores L-lactic acid production in the methylotrophic yeast *Ogataea polymorpha* using methanol as a substrate. The authors demonstrate that balancing the NADH/NAD<sup>+</sup> and NADPH/NADP<sup>+</sup> cofactor ratio significantly enhances L-lactate synthesis. Additional metabolic engineering strategies included mitochondrial compartmentalization of *LDH* and improving cell viability by deleting *IZH3*. Adaptive laboratory evolution (ALE) experiments improved strain robustness toward methanol but did not enhance lactic acid production. In a fed-batch fermentation setup using CO<sub>2</sub>-derived methanol, the engineered strain produced 25 g/L L-lactate. A key strength of this study is the combination of genetic engineering approaches—such as optimizing reductive power and compartmentalization—to improve lactic acid production from methanol. Furthermore, the authors integrate their experimental data into techno-economic analysis (TEA) and life cycle assessment (LCA), providing valuable industrial context for future applications.

**Reply:** We appreciate your positive comments on our work. Here, we provide a point-by-point reply on all your comments, which we hope to address your concerns.

**Comment 1:** Lines 101-102: Could the authors clarify why these enzymes did not work in *O. polymorpha*? Gene expression analysis of all five Ldh enzymes would help confirm whether they were actively expressed.

**Reply 1:** Thanks for your constructive comments. As suggested, qPCR was carried out in engineered strains with these five Ldh enzymes. As shown in Fig. R1-1B, all five *LDH* genes were functionally expressed at the transcriptional level. Particularly, strains failing to produce LA (HpLd and HpLh) showed higher *LDH* transcriptional levels, probably owing to the better growth behavior at the sampling points. Therefore, no LA detected in strain HpLd and HpLh could be attributed to that these two Ldh enzymes did not work in *O. polymorpha*, which is commonly observed in heterologous expression (Bioresour. Technol., 2025, 415, 131730). Related information was also involved in the revised main text (Line 101-102) and Fig S3a.

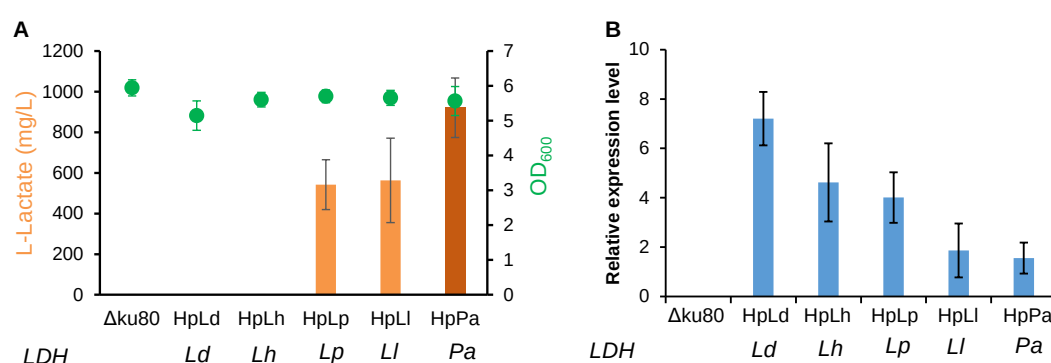


**Figure R1-1 Screening functional Ldh enzymes in *O. polymorpha*.**

**Comment 2:** Line 31: "Highest titer" is more appropriate than "yield" in this context.

**Reply 2:** Thank you for pointing out this detail. We have replaced “yield” with “titer” in Line 30.

**Comment 3:** Line 120: The manuscript states that HPLA06’s growth decreased by 32.6% compared to the wild-type strain and refers to two separate figures (Fig. 2b and 2d). Presenting this phenotype in a single diagram—rather than requiring readers to compare two figures—would improve clarity. Consider adding a consolidated figure to the Supplementary Information section.

**Reply 3:** Thank you for your valuable suggestion. We compared wild-type strain with HPLA05 and HPLA06 in a single experiment. Cell growth and LA were detected at different time points. Similar to the previous results, HPLA05 and HPLA06 with efficient LA production showed obviously decreased growth (Figure R1-2). We have added a consolidated figure in Supplementary Fig. S5, and replaced the figure annotation in the manuscript with Supplementary Fig. S5.

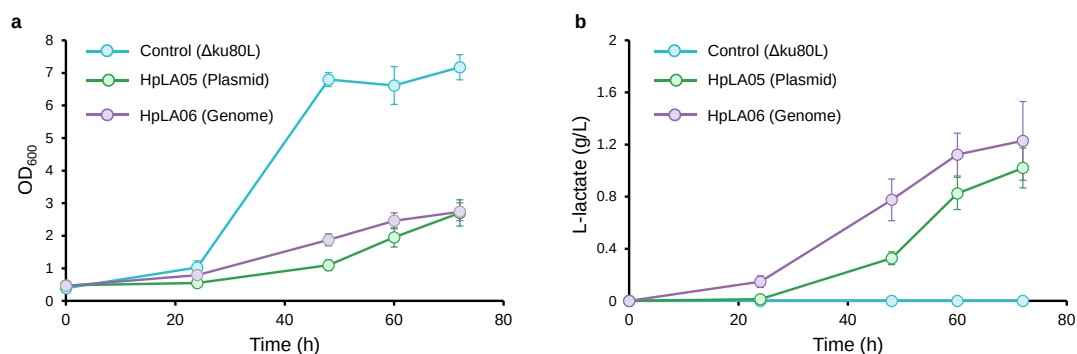


**Figure R1-2 Engineered strain HPLA05 and HpLA06 for L-lactate production, significantly decreased biomass relative to that of the wild-type strain.**

**Comment 4:** Lines 154-155: It would be helpful to specify that pGAP-driven LDH constructs were used for this analysis. Otherwise, readers might assume that the previously mentioned pAOX1 strains were cultivated under glucose conditions.

**Reply 4:** Thank you for your valuable suggestion. To avoid the misunderstanding for readers, we have specified the use of pGAP-driven *LDH* constructs in glucose cultivation in Line 160-161.

**Comment 5:** Discussion: One of the manuscript's key strengths is incorporating TEA and LCA to contextualize the experimental findings. However, this aspect is not sufficiently discussed. Adding a paragraph on TEA/LCA in the discussion section would strengthen the manuscript.

**Reply 5:** Thank you for your insightful suggestion regarding the insufficient discussion. We have added a dedicated paragraph in the discussion section to explore the implications and prospects of L-lactate production via the CO<sub>2</sub>-derived methanol pathway, in relation to our TEA and LCA findings. The key points are summarized below. A comprehensive revision can be found on Line 355-371:

“The integration of TEA and LCA facilitated a comprehensive assessment of the CO<sub>2</sub>-to-L-lactate biomanufacturing process, revealing essential insights beyond basic experimental performance. Although the competitive potential is inadequate to that of established sugar-based and lignocellulosic processes (Supplementary Table S9), this study emphasize the importance of third-generation biomanufacturing — particularly regarding reduced carbon source costs, optimized process utility, and carbon credit considerations — which collectively improve economic viability,

resulting in an MSP lower than the market price of L-lactate. Furthermore, it is imperative to develop the TEA to identify key factors affecting return on investment, thereby guiding experiments, and mitigating investment risks<sup>67</sup>. This research demonstrated that production characteristics, including yield and productivity, are key cost determinants, providing explicit targets for metabolic engineering and process intensification to reduce MSP and enhance scalability. The LCA demonstrated that utilizing CO<sub>2</sub>-derived methanol as a carbon source facilitated a net GHG capture of 7.29 tones CO<sub>2</sub> per ton of product, establishing this pathway as both carbon-negative and economically feasible. The TEA and LCA confirm the viability and sustainability of L-lactate production through CO<sub>2</sub>-derived methanol and provide strategic direction for the future advancement of carbon-based biomanufacturing.”

**Comment 6:** SI Appendix Figure S1 (a) and (b) should be explicitly mentioned in the figure description.

**Reply 6:** Thank you for your valuable suggestion. We have revised the figure description of Figure S1 and added explicit explanation of Fig. S1 (a) and (b).

**Comment 7:** SI Appendix Figure S11: Ensure a consistent font size in the figure description.

**Reply 7:** Thank you for your valuable feedback. We have carefully reviewed the figure description and ensured that all textual elements conform to a consistent font size standard. The amended version has been included in the Supplementary Information (now Supplementary Fig. S12).

**Comment 8:** SI Appendix Figures S4 and S9: The phrase *in situ* does not need to be italicized, as it is commonly used in this research field. Please check for consistency throughout the manuscript.

**Reply 8:** Thank you for pointing out this detail. We have checked and revised all the “*in situ*” throughout the manuscript and Supplemental Information.

#### **Responses to Reviewer 4**

**General comment:** This manuscript describes the engineering of the methylotrophic yeast *O. polymorpha* for efficient production of L-lactate from CO<sub>2</sub>-derived methanol, integrating systematic metabolic engineering with comprehensive techno-economic

and life cycle assessments. The study is notable not only for successfully developing a methanol-utilizing yeast strain with reasonably high lactate production, but also for providing a pioneering, systematic evaluation of the economic and environmental impacts associated with this CO<sub>2</sub>-to-chemical scenario. However, several issues need to be addressed before the manuscript can be recommended for publication:

**Reply:** We appreciate your encouraged comments on our manuscript, and we provide a point-by-point reply to address your concerns.

**Comment 1:** Although yeasts generally tolerate acidic conditions, the lactate tolerance limit of *O. polymorpha* should be studied and discussed.

**Reply 1:** Thank you for your valuable suggestion. We have conducted a tolerance experiment, where *O. polymorpha* wild type strain k-L was cultivated in various concentrations of L-lactate with an initial OD<sub>600</sub>=0.2. The results showed that 5 g/L L-lactate exhibited no obvious growth inhibition to *O. polymorpha* (Supplemental Fig. S6). In practical fermentation where L-lactate is produced and secreted to the medium gradually, especially in stationary phase, the tolerance may reach 10-fold (50 g/L). Just as James C. Liao et al. (Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels, Nature, 2008, 451, 3) reported that the *E. coli* strain with tolerance of 15 g/L isobutanol can produce about 20 g/L isobutanol in shake flask, which may reach 10-fold in bioreactor. We have added this description in Discussion in Line 349-354.

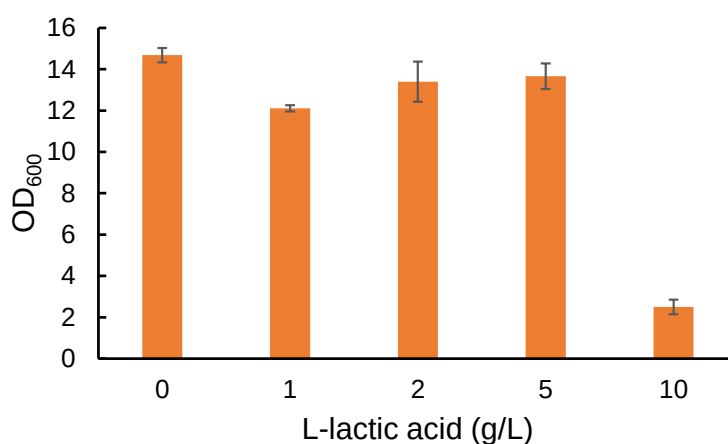


**Figure R4-1** L-lactate tolerance of wild-type *O. polymorpha*.

**Comment 2:** Lines 127-128: The authors observed improved cell viability upon disruption of *IZH3* but not *LPLI*. Please discuss the potential mechanisms underlying the differential effects of these disruptions.

**Reply 2:** Thank you for your valuable suggestion. In our previous study, we found that mutation of *IZH3* and *LPLI* was responsible for growth recovery of fatty acid producing strain which cannot grow in methanol. *LPLI* possesses the activity of phospholipase/lysophospholipase for degrading various phospholipid substrates and *IZH3* is a membrane protein related to zinc metabolism. *izh3Δ* mutation was found to alleviate the DNA damage caused by antimicrobial peptide (Molecular Microbiology, 2007, 65: 494-507), and it is speculated that the *izh3Δ* mutation may also attenuate the damaging effects of formaldehyde during L-LA production. In contrast, the disruption of *LPLI* blocked phospholipid degradation, which may direct more carbon flux from pyruvate to acetyl-CoA to synthesize lipid, therefore the flux from pyruvate to L-lactate was reduced. We have added this description to the Discussion in Line 320-329.

**Comment 3:** Lines 142-143: The authors suggest that NADPH is more abundant in methanol metabolism compared to glucose metabolism, which is very interesting. However, the stated “methanol assimilation via the pentose phosphate pathway (PPP) and the Xu5P cycle” seems to imply a metabolic cycle predominantly generating NADPH while releasing CO<sub>2</sub>, which suggests limited or no net C1 assimilation. Please provide a detailed explanation to clarify this aspect.

**Reply 3:** Thank you for your advice. NADPH generated from the pentose phosphate pathway that release CO<sub>2</sub>, which is a fact in both methanol and glucose metabolism. And Xu5P is necessary for formaldehyde assimilation.

**Comment 4:** Lines 152-153: The NAD(P)H/NAD(P)<sup>+</sup> ratio can significantly fluctuate under different cultivation conditions. Given that this ratio was measured during the log growth phase, please clarify whether the high NADPH/NADP<sup>+</sup> ratio observed also applies during later stages of production.

**Reply 4:** Thanks for your reasonable suggestion. As detailed in the manuscript, the cofactor ratios were measured during the log growth phase (~36 h), when lactate dehydrogenase (Ldh) activity peaked. Specifically, time-course analysis of growth and

lactate (LA) production (Figure R4-2) revealed that LA biosynthesis was primarily confined to the log phase. This temporal correlation directly determined our focus on cofactor ratios during this critical period. It is true that cofactor may exhibit variability under different cultivation parameters (e.g., stationary phase), such fluctuations do not appear to impact the LA production under our experimental conditions.

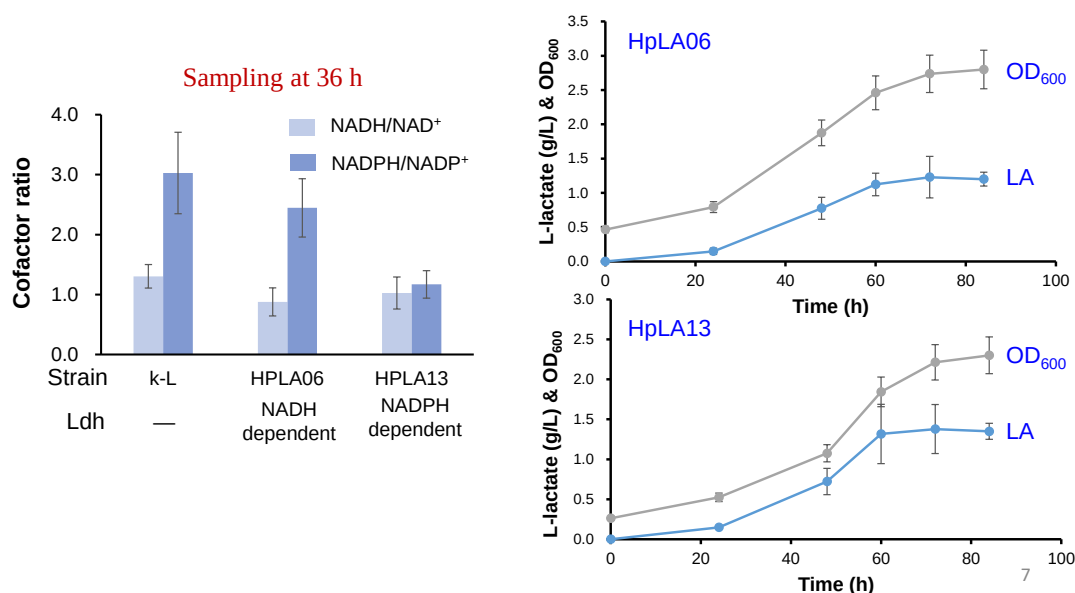


**Figure R4-2** L-lactate was primarily produced during the log growth phase, when the cofactor ratios were analyzed.

**Comment 5:** The term "cofactor balance" should be used cautiously. It may be valid to claim improved cofactor balance in strain HPLA13 relative to HPLA06; however, it seems questionable to state that HPLA13 outperforms the WT strain in terms of cofactor balance, since the WT strain inherently maintains cofactor balance through evolution.

**Reply 5:** Thank you for your valuable suggestion. Cofactor balance is indeed not applicable to the WT strain, therefore we deleted the comparison of HPLA13 with WT strain.

**Comment 6:** Lines 182-183: How was the "CO<sub>2</sub>-derived methanol solution" produced to compose methanol and water at a precise 3:1 ratio? Could the authors clarify how this exact ratio was achieved and confirm whether the phrase "without byproduct" specifically means "without impurities"?

**Reply 6:** Thank you for pointing out this detail. The "CO<sub>2</sub>-derived methanol solution"

was produced via CO<sub>2</sub> hydrogenation by High-Performance MaZrOx (Ma = Cd, Ga) Solid-Solution Catalysts, under the reaction conditions of H<sub>2</sub>/CO<sub>2</sub> = 3/1 (Wang et al. ACS Catal, 2019, 9, 10253-10259). The reaction is 3H<sub>2</sub>+CO<sub>2</sub>=CH<sub>3</sub>OH+H<sub>2</sub>O, therefore the molecular ratio of methanol and water is 1:1, and the mass ratio is 32:18. Therefore the v/v of methanol and water is 32/0.791:18/1=2.25:1. We are so sorry for making this mistake, and we have revised the ratio from 3:1 to 2.25:1 (v/v) in the manuscript (Line 188).

And the phrase "without byproduct" specifically means "without impurities". That is to say, the solution contains nothing but methanol and water. We have revised this description in the manuscript (Line 189).

**Comment 7:** Lines 201-202: When discussing the economic contributions of individual modules, please include specific percentage values.

**Reply 7:** Thank you for your constructive feedback. We have added specific percentage values to clearly present the economic contributions of each module, covering both capital expenditures (Supplementary Table S4) and minimum selling price (Supplementary Table S6). These revisions are detailed in Line 209-213, and 226-227 of the revised manuscript:

“Among the five modules of the process (methanol production, L-lactate production, L-lactate purification, wastewater treatment, and utilities), the L-lactate production subsystem was determined to necessitate the greatest installation cost for equipment. The CAPEX for equipment and installation of five modules were 2.3%, 59.5%, 12.3%, 7.3%, and 18.6%, respectively (Supplementary Table S4).”

“When we assumed operation of the L-lactate production process for 30 years with an internal rate of return (IRR) of 10%, the net present value (NPV) became zero at an L-lactate minimum selling price (MSP) of \$2.29 kg<sup>-1</sup>, which was comparable to the prevailing market price of \$1.85-3.15 kg<sup>-1</sup> 35, 42. The cost distribution for the MSP of five modules was 31.2%, 43.9%, 9.5%, 3.0%, and 12.4%, respectively (Supplementary Table S6).”

**Comment 8:** For TEA and LCA, can the reuse of residual biomass (e.g., for single-cell protein, SCP) be considered to further valorize the CO<sub>2</sub>-to-lactate manufacturing process?

**Reply 8:** Thank you for your insightful inquiry. We have evaluated the valorization of residual biomass in our TEA and LCA. In our research, residual biomass is employed through anaerobic digestion, and the solid digestate is sold as biofertilizer<sup>1, 2</sup>. The approach yields economic and environmental advantages, producing an anticipated annual profit of around \$2.14 million (Supplementary Table S5) and allowing for a reduction of 0.277 kg CO<sub>2</sub>-eq from the total GWP of the process (Supplementary Table S8). Detailed revision can be found in Line 222-223 and 263-265:

“Furthermore, the residual biomass can be marketed as nitrogen fertilizer through anaerobic digestion.”

“It is worth mentioning that from the standpoint of economic value allocation, the by-product of fertilizer could result in a GWP decrease of 0.277 kg CO<sub>2</sub>-eq, so further diminishing the carbon footprint.”

#### References:

1. Zhang, C. et al. An upcycling bioprocess for sustainable aviation fuel production from food waste-derived greenhouse gases: Life cycle assessment and techno-economic analysis. *Chemical Engineering Journal* 486, 150242, 150242 (2024).
2. Yan, X. et al. Paradigm of engineering recalcitrant non-model microorganism with dominant metabolic pathway as a biorefinery chassis. *Nature Communications* 15, 10441 (2024).

**Comment 9:** Lines 286-289: The authors should be cautious in drawing conclusions about the engineered strain's ALE performance without explicitly examining genetic stability.

**Reply 9:** Thank you for the valuable suggestion. Actually, ALE decreased L-lactate production, but the mechanism was still unknown, especially the genome stability was not explored. Therefore, to be cautious, we deleted the conclusion about trade-off between lactate production and biomass accumulation. We just stated that fact that ALE decreased L-lactate production from methanol, and the mechanism is worth being explored in the future (Line 138-139).

## Responses to Reviewer 5

**General comment:** Biodegradable plastics such as polylactic acid (PLA) are gaining attention as sustainable alternatives to fossil-derived polymers with emerging C1-fuelled bioprocesses offering a promising route for efficient and eco-friendly lactate production. In this study, *O. polymorpha* was metabolically engineered to significantly increase the L-lactate production yield using methanol alone. The authors propose a promising strategy to combine liquid sunshine technology with biotransformation of methanol using metabolically engineered *O. polymorpha* yeast to increase L-lactate yield and evaluate its economic feasibility and GHG mitigation performance.

However, it is unable to decide to be published in Nature Communications for some major and minor points while it has no doubt that the author's research will be of interest to fellow specialists working in the field of metabolic engineering. There are serious concerns about assessment methods and results that have a significant impact on the clarity of the paper.

**Reply:** We appreciate your positive evaluation on our work. Here, we provide a point-by-point reply on all your comments, especially to clarify assessment methods and results of TEA and LCA.

### Major points:

**Comment 1:** In Table S12, the reference paper presenting the cost of solar power provides a cost of \$0.05/kWh for solar power in 2017. However, the source of the cost remains unclear as it is uncertain whether it originates from photovoltaics or not. A range of statistics indicates that the price of electricity from the grid varies between \$0.08 and \$0.25 per kWh. As stated in the NREL report (Kazi et al., National Renewable Energy Lab (NREL), 2010), the electricity price was assumed to be \$0.054/kWh in 2007. In consideration of these reports, the cost of solar power in this study cannot be considered a viable cost. Since the cost of solar power shows a high sensitivity in the techno-economic analysis, further explanation for the decrease of the cost of solar power from 2017 to 2024 and a reference to the cost of solar derived power in 2024 are needed.

**Reply 1:** Thank you for your detailed feedback. We have updated the cost of solar

power to reflect the most recent data available for the high-efficiency regions (e.g., United States, Europe, and China) in 2024. According to the latest reports, the levelized cost of electricity for utility-scale photovoltaic solar power in 2024 is approximately \$0.031-0.075 /kWh<sup>3,4</sup>. This updated value has been incorporated into our analysis and is now cited with an appropriate reference in Supplementary Table S21. The results of operating expenditures, minimum selling price, and sensitivity analysis have been revised in the Supplementary Tables S5-S6, Fig. 6c, and Fig. 6d. The updates are detailed in Line 503-504:

“The levelized cost of electricity for utility-scale photovoltaic solar power in the base scenario is \$0.031 KWh<sup>-1</sup>, based on the latest data for high-efficiency regions in 2024<sup>70</sup>.”

#### References:

3. Chong, S., Wu, J. & Chang, I. S. Cost accounting and economic competitiveness evaluation of photovoltaic power generation in China-based on the system levelized cost of electricity. *Renewable Energy* 222, 119940, 119940 (2024).
4. SolarPACES. Qinghai sets CSP tariffs at 0.55 yuan/kWh (7.5 cents/kWh), (2025).

**Comment 2:** The mass and energy balance and specific estimation of capital expenditures (CapEx) of each equipment from A100 to A500 should be presented in the supporting information file, respectively. Table S6 mentions the amounts of the primary materials but the mass and energy balance for each subsystem is not available. Table S4 mentions capital expenditures for each area, but more detailed information for each equipment is not available too.

**Reply 2:** Thank you for your constructive comments. We have incorporated the mass and energy balance from A100 to A500, obtained from Aspen Plus, offering an additional and transparent overview on the material and energy flows inside our system (Supplementary Tables S14-S20). Additionally, we have provided a comprehensive breakdown of the equipment and installation costs for each individual item, as depicted in Supplementary Fig. S13, to complement the area-level data previously shown in Supplementary Tables S4, which aligns with existing literature<sup>1</sup>. The detailed revisions can be found in Line 212-213 and 491-493:

“Comprehensive investment details for equipment and installation corresponding to each individual

component are available in Supplementary Fig. S14. Notably, the bioreactors accounted for the predominant share of expenditures associated with equipment and installation in CAPEX.”

“Fig. 6a shows the mass balance of the L-lactate production process simulated in Aspen Plus, with comprehensive mass and energy balance data available in Supplementary Table S14-S20.”

#### References:

1. Zhang, C. et al. An upcycling bioprocess for sustainable aviation fuel production from food waste-derived greenhouse gases: Life cycle assessment and techno-economic analysis. *Chemical Engineering Journal* 486, 150242, 150242 (2024).

**Comment 3:** The biofertilizer produced by anaerobic digestion of the biomass is considered economic credit in Table S12, but not included in the environmental impact assessment in Table S6-S7. It is crucial to address the different methodology between the economic assessment, which incorporated biofertilizer credits, and the environmental assessment, which made no consideration.

**Reply 3:** Thank you for your valuable comment. To ensure consistency with the system boundaries used in the techno-economic analysis (TEA), we have now included the environmental credits from biofertilizer in the life cycle assessment (LCA). These credits were calculated using the economic allocation method<sup>5</sup>, aligning the environmental assessment with the approach taken in the economic analysis. Based on the economic allocation method, the biofertilizer permits a deduction of 0.277 kg CO<sub>2</sub>-eq from the overall GWP of the process. The revised LCA results are displayed in Fig. 6e-6f and Supplementary Table S8. Comprehensive updates of results and methodology are included in Line 263-265 and 530-533:

“It is worth mentioning that from the standpoint of economic value allocation, the by-product of fertilizer could result in a GWP decrease of 0.277 kg CO<sub>2</sub>-eq, so further diminishing the carbon footprint.”

“The economic allocation method is chosen to assess the GWP of the by-product (biofertilizer)<sup>73</sup>. The cost of L-lactate is believed to correspond with the MSP of the basic scenario from this study, while Supplementary Table S21 encompasses the price of biofertilizer.”

#### References:

5. Ijassi, W., Ben Rejeb, H. & Zwolinski, P. Environmental impact evaluation of co-

products: decision-aid tool for allocation in LCA. *The International Journal of Life Cycle Assessment* 26, 2199-2214 (2021).

**Comment 5:** It is necessary to specify the impact assessment methodology such as ReCiPe 2016 Midpoint (H), Environmental Footprint 3.1 and TRACI 2.1 on which the life cycle assessment is based. In Table S6, the specified life cycle inventory data for the source of the material and energy are also required. It should include the information where the data origins from (GLO, RER, RoW, ...) and whether the data is cut-off (APOS, Cut-off, ...)

**Reply 5:** Thank you for your valuable suggestions. We have specified the impact assessment methodology used in the LCA as the TRACI version 2.1. Besides, the life cycle inventory data are derived from our Aspen Plus models, with comprehensive mass and energy data available in Supplementary Tables S14-S20. We also added the information where the data origins from and whether the data is cut-off into Supplementary Table S22, which details categories and providers from the Ecoinvent v3.6 database and corresponding references. Specific information regarding this methodology has been incorporated into the LCA methodologies section of the manuscript in Line 519-527.

“Supplementary Table S7 provides a comprehensive inventory of the life cycle associated with L-lactate production, derived from Aspen Plus. Moreover, we assessed the GWP (kg CO<sub>2</sub>-eq) of the L-lactate production process. The life cycle evaluation included background data from Ecoinvent v3.6 and references<sup>72</sup>, encompassing detailed categories and providers (Supplementary Table S22). The tool for reduction and assessment of chemicals and other environmental impacts (TRACI) version 2.1, created by the environmental protection agency (EPA), was utilized to provide characterization elements for evaluating the possible consequences of inputs and emissions from production processes.”

**Comment 5:** A detailed description of the thermodynamic model selected to simulate the process is required.

**Reply 5:** Thank you for your suggestion. The NRTL (non-random two-liquid) model was chosen to characterize the thermodynamic behavior of the system in our research. This model is widely recognized for its accuracy in predicting liquid-phase activity

coefficients and phase equilibria in non-ideal mixtures, particularly those involving polar compounds and hydrogen bonding—key characteristics of our process (e.g., L-lactate fermentation broths, methanol-water systems)<sup>6</sup>. This model was implemented in Aspen Plus, and its suitability for our process is further supported by prior studies on biomanufacturing<sup>7-9</sup>. A detailed description of the thermodynamic model selection can be found in Line 478-482.

“This study entailed the design of an L-lactate production process using Aspen Plus v14 software, which was utilized to conduct calculations for mass and energy balance. The phase equilibrium and thermodynamic model has been constructed using the NRTL (non-random two-liquid), in accordance with a previous study<sup>69</sup>.”

#### References:

6. Haydary, J. Chemical process design and simulation: Aspen Plus and Aspen Hysys applications. (John Wiley & Sons, 2019).
7. Méndez-Alva, J. A. et al. in Computer Aided Chemical Engineering Vol. 44 463-468 (Elsevier, 2018).
8. Gasca-González, R. et al. in Computer Aided Chemical Engineering Vol. 46 (eds Anton A. Kiss, Edwin Zondervan, Richard Lakerveld, & Leyla Özkan) 457-462 (Elsevier, 2019).
9. Kruschitz, A. & Nidetzky, B. Biocatalytic production of 2- $\alpha$ -d-glucosyl-glycerol for functional ingredient use: integrated process design and techno-economic assessment. ACS Sustainable Chemistry & Engineering 10, 1246-1255 (2022).

#### Minor points:

**Comment 1:** The manuscript should provide a reason to support the assumption of an L-lactate production capacity of 18,500 tons/year.

**Reply 1:** Thank you for your valuable comment. The projected production capacity of 18,500 tons annually is derived from typical scales used in the commercial lactic acid industry. Most commercial lactic acid production facilities run at capacities ranging from 15,000 to 30,000 tones every year, a range that represents prevailing industry practices, with the exception of a few manufacturers like NatureWorks LLC and Corbion Purac<sup>10</sup>. Besides, this capacity is frequently employed in techno-economic

evaluations of third-generation lactic acid biomanufacturing processes, such as those that utilize carbon dioxide or methane as feedstocks<sup>11,12</sup>. Overall, the chosen production capacity conforms to industry standards and guarantees comparability and relevance to existing TEA research. Detailed revision can be found in Line 202-205:

“It is worth noting that projected production capacity corresponds with standard industrial lactic acid plant scales (15,000-30,000 tons per year)<sup>36</sup> and is in accordance with capacities frequently utilized in published TEA studies on third-generation biomanufacturing<sup>37,38</sup>.”

#### References:

10. Adom, F. K. & Dunn, J. B. Life cycle analysis of corn-stover-derived polymer-grade l-lactic acid and ethyl lactate: Greenhouse gas emissions and fossil energy consumption. *Biofuels, Bioproducts and Biorefining* 11, 258-268 (2017).
11. Fei, Q. et al. Biological valorization of natural gas for the production of lactic acid: techno-economic analysis and life cycle assessment. *Biochemical Engineering Journal* 158, 107500 (2020).
12. Park, J. et al. Merging biomass and CO<sub>2</sub> utilization; process design and assessment on simultaneous production of lactic acid and formic acid from glycerol and CO<sub>2</sub>. *Chemical Engineering Journal* 463, 142410 (2023).

**Comment 2:** It is recommended to improve the visibility of the text and numbers in Figure 6 to enhance overall clarity.

**Reply 2:** Thank you for your valuable suggestion. In response to your feedback, we have adjusted the font size and formatting of the text and numbers in Fig. 6. The revised version ensures that all elements are easily readable, reducing the potential for misinterpretation.

**Comment 3:** There is an inconsistency in the use of the terms ‘lactate’ and ‘lactic acid’ between the main text and the supporting information file.

**Reply 3:** Thank you for pointing out this detail. We have checked the main text and the supporting information file, and changed “lactic acid” to “lactate” in microbial fermentation for consistency. Some specific terms such as poly-lactic acid (PLA) and lactic acid bacteria (LAB) are not changed.

**Comment 4:** In table S4, there is a typo. ‘Total operating cost’ should be corrected to

‘Total capital cost’

**Reply 4:** Thank you so much for your advice. As suggested, we have promptly corrected the typo in Supplementary Table S4, changing "Total operating cost" to "Total capital cost". The revised table has been thoroughly double-checked to ensure its accuracy.

**Comment 5:** In table S5, which presents the operating expenditures, the use of the term ‘investment’ should be appropriately revised.

**Reply 5:** Thank you for your comment. Considering that the values in the Supplementary Table S5 represent annual operational expenses, we have carefully amended the term to "annual costs" to accurately reflect the nature of these expenditures.

## **Responses to Reviewer 6**

**General comment:** The present manuscript combined three diverse areas in a single paper, lacking thorough justice to individual topics, especially TEA. The TEA used a completely wrong approach for process design, which makes the results unreliable and unacceptable, I recommend removing TEA work from the manuscript to avoid passing the wrong information to the readers. Below are my few comments.

**Reply:** Thank you for your critical feedback. The TEA and LCA evaluate the entire "cradle-to-grave" process of a product or an organization of a certain scale from the perspectives of environmental impact and economic viability. They observe issues from a broad scope, including regional, national, and global levels, and from the perspective of sustainable development, which provide detailed process analysis, argumentation, and assessment for optimization. As a matter of fact, our TEA framework follows to the approach established by the US National Renewable Energy Laboratory (NREL), utilizing publicly accessible models, which are widely used in many TEA-related research<sup>1, 2, 13, 14</sup>. We sincerely appreciate your comments and hope to address them properly to ensure reliable results for readers.

References:

1. Zhang, C. et al. An upcycling bioprocess for sustainable aviation fuel production from food waste-derived greenhouse gases: Life cycle assessment and techno-

economic analysis. Chemical Engineering Journal 486, 150242, 150242 (2024).

2. Yan, X. et al. Paradigm of engineering recalcitrant non-model microorganism with dominant metabolic pathway as a biorefinery chassis. Nature Communications 15, 10441, doi:10.1038/s41467-024-54897-5 (2024).

13. Carpenter, D. et al. Design of an Integrated Solids Handling System to Maximize Syngas Process Reliability (Cooperative Research and Development Final Report). (National Renewable Energy Laboratory (NREL), Golden, CO (United States, 2023).

14. Kobos, P. H. et al. Techno-economic analysis: Best practices and assessment tools. (Sandia National Lab. (SNL-CA), Livermore, CA (United States); Sandia National, 2020).

**Comment 1:** Supporting Information: The reaction stoichiometry for methanol production ( $2\text{ CO}_2 + 4\text{ H}_2 \rightarrow 2\text{ CH}_3\text{OH} + \text{O}_2$ ) seems to be vague. Also, there are no references to support the reaction conditions for methanol production. Also, I could not find the oxygen as a byproduct.

**Reply 1:** We are very sorry for making this serious mistake. The correct stoichiometry for methanol production<sup>15,16</sup>:  $\text{CO}_2 + 3\text{ H}_2 \rightarrow \text{CH}_3\text{OH} + \text{H}_2\text{O}$ .

References:

15. Wang J. et al. A highly selective and stable ZnO-ZrO<sub>2</sub> solid solution catalyst for CO<sub>2</sub> hydrogenation to methanol, Science Advance, 2017, 3, e1701290.

16. Wang J. et al. High-Performance MaZrO<sub>x</sub> (Ma = Cd, Ga) Solid-solution catalysts for CO<sub>2</sub> hydrogenation to methanol, ACS Catalysis, 2019, 9, 10253-10259.

**Comment 2:** What is the basis of plant capacity?

**Reply 2:** The projected production capacity of 18,500 tons annually is derived from typical scales used in the commercial lactic acid industry. Most commercial lactic acid production facilities run at capacities ranging from 15,000 to 30,000 tones every year, a range that represents prevailing industry practices, with the exception of a few manufacturers like NatureWorks LLC and Corbion Purac<sup>10</sup>. Besides, this capacity is frequently employed in techno-economic evaluations of third-generation lactic acid biomanufacturing processes, such as those that utilize carbon dioxide or methane as feedstocks<sup>11, 12</sup>. Overall, the chosen production capacity conforms to industry standards

and guarantees comparability and relevance to existing TEA research. Detailed revision can be found in Line 202-205:

“It is worth noting that projected production capacity corresponds with standard industrial lactic acid plant scales (15,000-30,000 tons per year)<sup>36</sup> and is in accordance with capacities frequently utilized in published TEA studies on third-generation biomanufacturing<sup>37, 38</sup>.”

#### References:

10. Adom, F. K. & Dunn, J. B. Life cycle analysis of corn-stover-derived polymer-grade l-lactic acid and ethyl lactate: Greenhouse gas emissions and fossil energy consumption. *Biofuels, Bioproducts and Biorefining* 11, 258-268 (2017).
11. Fei, Q. et al. Biological valorization of natural gas for the production of lactic acid: techno-economic analysis and life cycle assessment. *Biochemical Engineering Journal* 158, 107500 (2020).
12. Park, J. et al. Merging biomass and CO<sub>2</sub> utilization; process design and assessment on simultaneous production of lactic acid and formic acid from glycerol and CO<sub>2</sub>. *Chemical Engineering Journal* 463, 142410 (2023).

**Comment 3:** TEA: There is no mention of low pH or neutral fermentation.

**Reply 3:** Thank you for pointing out this detail. The initial pH was controlled at 5.6 and not further adjusted during cultivation in shake flask. In bioreactor, however, the pH was controlled as constant 5.6 with feeding 4M KOH to neutralize the produced L-lactate. This information was added to Methods section in Line 402-403 and 410-414.

**Comment 4:** L-Lactate is mentioned in the manuscript. However, the reaction stoichiometry shows L-lactic acid.

**Reply 4:** Thank you for your comment. L-lactate denotes the conjugate base of L-lactic acid. These terms are frequently used interchangeably in biological contexts depending on pH<sup>17</sup>. In this study, the fermentation pH was maintained at 5.6. Given that the pKa of lactic acid is 3.86, the produced L-lactic acid was predominantly in its dissociated form, L-lactate. Similar usage occurs in many related studies<sup>2,18</sup>. Therefore, we used "L-lactic acid" in the reaction stoichiometric equations. This distinction has been clarified in the amended "Supplementary Method" section.

#### References:

2. Yan, X. et al. Paradigm of engineering recalcitrant non-model microorganism with dominant metabolic pathway as a biorefinery chassis. *Nature Communications* 15, 10441 (2024).

17. Pohanka, M. D - lactic acid as a metabolite: toxicology, diagnosis, and detection. *BioMed Research International* 2020, 3419034 (2020).

18. Parra-Ramírez, D., Martínez, A. & Cardona, C. A. Lactic acid production from glucose and xylose using the lactogenic *Escherichia coli* strain JU15: Experiments and techno-economic results. *Bioresource Technology* 273, 86-92 (2019).

**Comment 5:** L-lactate purification (A300): It looks to be completely vague. It may be noted that LA separation is the major cost contributor to the overall process in terms of capital investment and energy consumption. If this kind of downstream separation is considered, the TEA results become completely unreliable. Please refer to recent papers on LA production. Otherwise, I recommend removing TEA completely from the manuscript as it will pass wrong information to the readers.

**Reply 5:** Thank you for your comments. Our study's design for the L-lactate separation and purification process corresponds to the recent literature — including a dewatering system and a membrane-based separation system (Supplementary Fig. S12) — mirrors approach used in sugar-based and methane-based lactic acid bioprocesses<sup>2, 11</sup>. Notably, the capital expenditure contribution from the L-lactate separation unit accounts for 12.30% of the total, which falls within the 10 – 30% range commonly reported in the literature<sup>11, 19, 20</sup>. Compared to conventional, energy-intensive purification processes (e.g., involving acidification, neutralization, crystallization, evaporation, ion exchange, etc.), membrane separation approach operates under milder conditions with fewer steps, which reduces both energy demand and complexity<sup>21, 22</sup>. To further clarify this point, we have updated the Supplementary Method and Supplementary Table S2 to provide additional technical details and operational parameters on the L-lactate separation process<sup>23-29</sup>. Detailed revisions and explanation can be found in Line 202-210:

Moreover, in numerous high-impact and authoritative international publications<sup>30-33</sup>, TEA has been extensively utilized to support experimental research by assessing economic viability, hence corroborating findings from an industrial and practical

standpoint. Furthermore, it is essential to build the TEA to identify key factors influencing return on investment, thereby directing experiments, and decreasing investment risks<sup>34</sup>. Consequently, we have preserved the TEA section, including additional clarification and methodological transparency in the updated Supplementary Information to guarantee its rigor and alignment with contemporary research. To further on the significance of TEA, we incorporated the necessary details to the Discussion section on in Line 209-218, 355-357, and 362-363:

“The CAPEX for equipment and installation of five modules were 2.3%, 59.5%, 12.3%, 7.3%, and 18.6%, respectively (Supplementary Table S4). Comprehensive investment details for equipment and installation corresponding to each individual component are available in Supplementary Fig. S14. Notably, the bioreactors accounted for the predominant share of expenditures associated with equipment and installation in CAPEX. The investment in the purification unit is less extensive due to compared to the traditional separation process, the membrane separation approach operates under milder conditions and involves fewer steps, hence decreasing both energy requirements and complexity<sup>39, 40</sup>.”

“The integration of TEA and LCA facilitated a comprehensive assessment of the CO<sub>2</sub>-to-L-lactate biomanufacturing process, revealing essential insights beyond basic experimental performance.”

“Furthermore, it is imperative to develop the TEA to identify key factors affecting return on investment, thereby guiding experiments, and mitigating investment risks<sup>67</sup>.”

## References:

2. Yan, X. et al. Paradigm of engineering recalcitrant non-model microorganism with dominant metabolic pathway as a biorefinery chassis. *Nature Communications* 15, 10441 (2024).
11. Fei, Q. et al. Biological valorization of natural gas for the production of lactic acid: techno-economic analysis and life cycle assessment. *Biochemical Engineering Journal* 158, 107500 (2020).
19. Pal, P., Sikder, J., Roy, S. & Giorno, L. Process intensification in lactic acid production: A review of membrane based processes. *Chemical Engineering and Processing: Process Intensification* 48, 1549-1559 (2009).
20. Tejayadi, S. & Cheryan, M. Lactic acid from cheese whey permeate. *Productivity*

and economics of a continuous membrane bioreactor. *Applied microbiology and biotechnology* 43, 242-248 (1995).

21. Kumar, R., Basak, B. & Jeon, B.-H. Sustainable production and purification of succinic acid: A review of membrane-integrated green approach. *Journal of Cleaner Production* 277, 123954 (2020).

22. Taleghani, H. G., Ghoreyshi, A. A. & Najafpour, G. D. Thin film composite nanofiltration membrane for lactic acid production in membrane bioreactor. *Biochemical Engineering Journal* 132, 152-160 (2018).

23. Henderson, R., Parsons, S. A. & Jefferson, B. The impact of algal properties and pre-oxidation on solid–liquid separation of algae. *Water research* 42, 1827-1845 (2008).

24. Grima, E. M., Belarbi, E.-H., Fernández, F. A., Medina, A. R. & Chisti, Y. Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnology advances* 20, 491-515 (2003).

25. Chen, G., Zhao, L., Qi, Y. & Cui, Y.-L. Chitosan and its derivatives applied in harvesting microalgae for biodiesel production: An outlook. *Journal of Nanomaterials* 2014, 217537 (2014).

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27. Kumar, A., Thakur, A. & Panesar, P. S. Lactic acid and its separation and purification techniques: A review. *Reviews in Environmental Science and Bio/Technology* 18, 823-853 (2019).

28. Davis, R. E. et al. Process Design and economics for the conversion of lignocellulosic biomass to hydrocarbon fuels and coproducts: 2018 biochemical design case update; biochemical deconstruction and conversion of biomass to fuels and products via integrated biorefinery pathways. (National Renewable Energy Lab. (NREL), Golden, CO (United States), 2018).

28. Humbird, D., Davis, R. & McMillan, J. Aeration costs in stirred-tank and bubble column bioreactors. *Biochemical engineering journal* 127, 161-166 (2017).

30. Luo, Z. et al. Carbon–carbon bond cleavage for a lignin refinery. *Nature Chemical*

Engineering 1, 61-72 (2024).

31. Choi, K. R., Luo, Z. W., Kim, G. B., Xu, H. & Lee, S. Y. A microbial process for the production of benzyl acetate. *Nature Chemical Engineering* 1, 216-228 (2024).

32. Crandall, B. S. et al. Transforming CO<sub>2</sub> into advanced 3D printed carbon nanocomposites. *Nature Communications* 15, 10568 (2024).

33. Chi, H. et al. Electrosynthesis of ethylene glycol from biomass glycerol. *Nature Communications* 16, 979 (2025).

34. Zhang, C. et al. Economic and sustainable revolution to facilitate one-carbon biomanufacturing. *Nature Communications* 16, 1-10 (2025).

**Comment 6:** AD: Reaction stoichiometry and conditions are not mentioned.

**Reply 6:** Thank you for your comment. This study modelled the anaerobic digestion (AD) unit according to established methodologies reported in the literature, utilizing the RYield block in Aspen Plus, a prevalent technique employed in various process modelling investigations of AD systems<sup>35-37</sup>. The yield distributions of the AD products were calculated using Fortran code performed by calculator blocks, with residual biomass and waste converted into biogas (CH<sub>4</sub> and CO<sub>2</sub>), NH<sub>3</sub>, H<sub>2</sub>O, and digestate, in accordance with existing AD process design studies (Supplementary Table S2)<sup>35-37</sup>. In addition, the AD process operates under mesophilic conditions at 37°C and atmospheric pressure, which are standard conditions for medium-temperature AD<sup>37,38</sup>. Detailed AD unit design and parameters are provided in the revised Supplementary Method.

References:

37. Leal Moraes Luna, N. A., Lopes da Silva, M. G., Cezar Menezes, R. S., Dutra, E. D. & Albuquerque, A. A. Simulation refinement of the biogas production process via anaerobic digestion: Validation of Properties, PHASE equilibrium and database construction. *Chemical Engineering Science*, 121908 (2025).

38. Wang, T. et al. Biogas-to-methanol: A new green methanol production process based on anaerobic digestion of biomass. *Energy Conversion and Management* 321, 119065 (2024).

39. Kelif Ibro, M., Ramayya Ancha, V. & Beyene Lemma, D. Biogas Production Optimization in the Anaerobic Codigestion Process: A Critical Review on Process

Parameters Modeling and Simulation Tools. *Journal of Chemistry* 2024, 4599371 (2024).

40. Zhang, Z., Zhang, R., Ma, Y. & Sun, Y. Improved volatile fatty acid production in anaerobic digestion via simultaneous temperature regulation and persulfate activation by biochar: Chemical and biological response mechanisms. *Environmental Research* 264, 120271, doi:<https://doi.org/10.1016/j.envres.2024.120271> (2025).

**Comment 7:** The complete Aspen Plus flowsheet is missing. The details of the equipment and their design parameters are also missing. As a result, it is difficult to judge the reliability of TEA results.

**Reply 7:** Thank you very much for your constructive comments. We have added the complete Aspen Plus process flowsheet in the Supplementary Fig. S12 to enhance transparency, including essential units and material streams. Additionally, all equipment sizes are directly derived from mass and energy balance data generated by our Aspen Plus model (Supplementary Tables S14-S20). Costs scale with implementation size using a power law model relative to original reference prices, ensuring consistency with industry-standard scaling practices<sup>1</sup>. Original equipment prices are sourced from the National Renewable Energy Laboratory (NREL) reports and published research<sup>30, 31, 41, 42</sup>. A detailed breakdown of costs for each unit is provided in Supplementary Fig. S13. The investment ratio in equipment aligns with that of existing biomanufacturing studies and reports<sup>1, 43</sup>. The detailed revisions can be found in Line 198-202 and 210-215:

“the proposed production scenario was indicative of the capacity of a plant to produce 18,500 tons of L-lactate annually through three major conversion steps, as illustrated in Supplementary Fig. S12-S13, which depicts the primary carbon flow within the various subsystems and the detailed process design in Aspen plus, respectively.”

“The CAPEX for equipment and installation of five modules were 2.3%, 59.5%, 12.3%, 7.3%, and 18.6%, respectively (Supplementary Table S4). Comprehensive investment details for equipment and installation corresponding to each individual component are available in Supplementary Fig. S14. Notably, the bioreactors accounted for the predominant share of expenditures associated with equipment and installation in CAPEX.”

**References:**

1. Zhang, C. et al. An upcycling bioprocess for sustainable aviation fuel production from food waste-derived greenhouse gases: Life cycle assessment and techno-economic analysis. *Chemical Engineering Journal* 486, 150242 (2024).
30. Davis, R. E. et al. Process Design and economics for the conversion of lignocellulosic biomass to hydrocarbon fuels and coproducts: 2018 biochemical design case update; biochemical deconstruction and conversion of biomass to fuels and products via integrated biorefinery pathways. (National Renewable Energy Lab. (NREL), Golden, CO (United States), 2018).
31. Humbird, D., Davis, R. & McMillan, J. Aeration costs in stirred-tank and bubble column bioreactors. *Biochemical engineering journal* 127, 161-166 (2017).
41. Davis, R., Wiatrowski, M., Kinchin, C. & Humbird, D. Conceptual Basis and Techno-Economic Modeling for Integrated Algal Biorefinery Conversion of Microalgae to Fuels and Products (2019 NREL TEA Update: Highlighting Paths to Future Cost Goals via a New Pathway for Combined Algal Processing). (National Renewable Energy Lab. (NREL), Golden, CO (United States), 2020).
42. Davis, R., McNamara, I. & Bartling, A. Techno-Economic Case Study: Low-Temperature Conversion Performance Based on Isolated Anatomical Fractions of Corn Stover. (National Renewable Energy Laboratory (NREL), Golden, CO (United States), 2023).
43. Shaji, A., Shastri, Y., Kumar, V., Ranade, V. V. & Hindle, N. Economic and environmental assessment of succinic acid production from sugarcane bagasse. *ACS Sustainable Chemistry & Engineering* 9, 12738-12746 (2021).

**Comment 8:** The energy cost is completely missing from TEA, which is one of the main cost contributors in this process.

**Reply 8:** Thank you for your comment. The energy costs are incorporated into the operating expenditures (OPEX). We calculated power and energy requirements based on the Aspen Plus simulation, and the associated data have been included in Supplementary Tables S19-S20. Following the implementation of the heat integration method, energy consumption is diminished by more than 95%, consistent with prior studies<sup>42, 43</sup>. The combined heat and power (CHP) unit generates 24,502 kW of

electricity, satisfying 46.15% of the plant's operational energy needs. Hence, we considered the cost of power consumption, and the facility's overall annually power expenditures reach 7.02 million dollars (Supplementary Tables S5).

#### References:

42. Kim, H., Baek, S. & Won, W. Integrative technical, economic, and environmental sustainability analysis for the development process of biomass-derived 2,5-furandicarboxylic acid. *Renewable and Sustainable Energy Reviews* 157, 112059 (2022).
43. Jeong, S., Kim, J., Liu, J. J. & Won, W. A lignocellulosic 2,3-butanediol strategy: Process development and integrative analyses. *Journal of Environmental Chemical Engineering* 11, 110085 (2023).

**Comment 9:** “The estimation of CAPEX, OPEX, and MSP was conducted via an in-house model created by the National Renewable Energy Laboratory (NREL).” Is it an open-source software? Where is the link?

**Reply 9:** Thanks for your comments. The estimation of CAPEX, OPEX, and MSP in our study is based on the original calculation spreadsheets and models developed by the NREL, which are widely used in TEA-related research<sup>1, 2, 11, 13, 14</sup>. These tools are open-source and publicly accessible and the resources<sup>14</sup> can be downloaded from the NREL website at: <https://www.nrel.gov/analysis/techno-economic.html>. The relevant description is detailed in the Methods section of the manuscript in Line 490-491 as follows:

“The estimation of CAPEX, OPEX, and MSP was conducted via an in-house model created by the National Renewable Energy Laboratory (NREL).”

#### References:

1. Zhang, C. et al. An upcycling bioprocess for sustainable aviation fuel production from food waste-derived greenhouse gases: Life cycle assessment and techno-economic analysis. *Chemical Engineering Journal* 486, 150242 (2024).
2. Yan, X. et al. Paradigm of engineering recalcitrant non-model microorganism with dominant metabolic pathway as a biorefinery chassis. *Nature Communications* 15, 10441 (2024).

11. Fei, Q. et al. Biological valorization of natural gas for the production of lactic acid: techno-economic analysis and life cycle assessment. *Biochemical Engineering Journal* 158, 107500 (2020).
13. Carpenter, D. et al. Design of an Integrated Solids Handling System to Maximize Syngas Process Reliability (Cooperative Research and Development Final Report). (National Renewable Energy Laboratory (NREL), Golden, CO (United States), 2023).
14. Kobos, P. H. et al. Techno-economic analysis: Best practices and assessment tools. (Sandia National Lab. (SNL-CA), Livermore, CA (United States), 2020).

## Responses to Reviewer 7

**General comment:** The article entitled “Methanol biotransformation for the production of the biodegradable plastic monomer L-lactate in yeast” discusses a promising approach to producing L-lactate, a biodegradable plastic monomer via methanol biotransformation in yeast *Ogataea polymorpha*. The topic is highly relevant and of significant interest, particularly in the context of use of C1 feedstock for sustainable biomanufacturing of plastics. However, the Manuscript needs lots of clarity as there are several loopholes. Below are my comments and suggestions to enhance the quality of the Manuscript.

**Reply:** We appreciate your positive comments on our work. Here, we provide a point-by-point reply to address your concerns.

**Comment 1:** Throughout the Manuscript, the argument of author is that methanol is an ideal feedstock for biomanufacturing lactate (LA) as it can be produced from CO<sub>2</sub> in massive quantities and this approach will help in mitigating the carbon emissions. In that case why not to use CO<sub>2</sub> directly via gas fermentation instead of methanol. I would suggest adding some text for comparing LA production using CO<sub>2</sub> and methanol with pros and cons of each and demonstrate which one could be more suitable to take forward.

**Reply 1:** Thank you for your valuable suggestion. Although CO<sub>2</sub> can be used directly to produce lactate, the titer and productivity are much lower than that from methanol (Table 1). The main reason is the low gas-to-liquid transfer rate in gas fermentation. In

comparison, the high liquid-to-liquid transfer rate in methanol fermentation greatly facilitates microbial cell growth and chemicals production. We have added this description in the Discussion (Line 290-293).

**Comment 2:** Usually most of the microbial cultures have lactate dehydrogenase enzymes. Does genome of *Ogataea polymorpha* has no copy of this enzyme? Add few lines on Biochemistry and Microbiology of yeast.

**Reply 2:** Thank you for your valuable suggestion. While *O. polymorpha*, like other yeasts such as *Saccharomyces cerevisiae* and *Pichia pastoris*, possesses a *LDH* gene in its genome, these yeasts naturally produce no lactate. Lactate production only occurs when a bacterial *LDH* gene is introduced and expressed. We speculate that the native yeast *LDH* may encode an enzyme catalyzing the reverse reaction (lactate to pyruvate), consistent with their known ability to degrade lactate. To enable lactate production in *O. polymorpha* from methanol, we screened five *LDH* genes from different LAB. Among these, the *Ldh* from *P. acidilactici* demonstrated the highest performance for L-lactate production. We have added this description to the Discussion in Line 309-315.

**Comment 3:** The role of *LPL1* (encoding a putative lipase) and *IZH3* (encoding a membrane protein) in context of LA production is not clear?

**Reply 3:** Thank you for pointing out this detail. In our previous study, we found that mutation of *IZH3* and *LPL1* was responsible for growth recovery of fatty acid producing strain which cannot grow in methanol. *LPL1* possesses the activity of phospholipase/lysophospholipase for degrading various phospholipid substrates and *IZH3* is a membrane protein related to zinc metabolism. *izh3Δ* mutation was found to alleviate the DNA damage caused by antimicrobial peptide (Molecular Microbiology, 2007, 65: 494-507), and it is speculated that the *izh3Δ* mutation may also attenuate the damaging effects of formaldehyde during L-LA production. In contrast, the disruption of *LPL1* blocked phospholipid degradation, which may direct more carbon flux from pyruvate to acetyl-CoA to synthesize lipid, therefore the flux from pyruvate to L-lactate was reduced. We have added this description to the Discussion in Line 320-329.

**Comment 4:** Why and how ALE was done, what was the selection pressure. It was not clear how ALE cause reduction in LA production despite substantial improvement in

biomass.

**Reply 4:** The detailed process of ALE was described in Methods in Line 386-392. To be specific, the selection pressure is cell growth under methanol stress (10 g/L methanol). We intended to increase methanol tolerance of the L-lactate producing strain, so as to increase cell growth, as well as L-lactate titer. While the methanol tolerance of *O. polymorpha* was easy to increase, which was inflected by the obviously improved cell growth, the L-lactate titer dramatically decreased. We supposed that the better cell growth redirected more carbon flux from pyruvate to TCA cycle, therefore the flux to L-lactate was severely reduced. In other words, the cell growth competes pyruvate with L-lactate synthesis in methanol fermentation.

**Comment 5:** If LA production is be taken place in mitochondria where plenty of NADH is available from TCA cycle, then why do we need NADPH as cofactor?

**Reply 5:** The engineered strain HPLA22 has dual L-lactate producing pathways in cytoplasm (using NADPH as cofactor) and mitochondria (using NADH as cofactor). Therefore, both NADPH in cytoplasm and NADH in mitochondria were needed to produce L-lactate production from methanol.

**Comment 6:** First of methanol is a reduced carbon source and should generate more reducing equivalents than glucose. Even if it is NADPH, transhydrogenase can easily convert them into NADH.

**Reply 6:** Indeed, methanol has higher reduction degree than glucose. However, in methylotrophic yeast, alcohol oxidase (Aox) oxidizes methanol to formaldehyde, and the H reacts with O<sub>2</sub> to form H<sub>2</sub>O<sub>2</sub>. Then H<sub>2</sub>O<sub>2</sub> is catalyzed by catalase (Cat) to H<sub>2</sub>O and O<sub>2</sub>. Therefore, the reduction equivalent (H) in methanol metabolism is wasted. Since the cofactors NADH and NADPH drive numerous cellular reactions, introducing transhydrogenase to convert NADPH to NADH could disrupt native cofactor distribution and trigger destructive effects on overall metabolism. To isolate the impact of Ldh and avoid unknown interactions from altered redox balances, we focused exclusively on expressing Ldh without co-expressing transhydrogenase.

**Comment 7:** It is difficult to buy this argument that due to methanol metabolism, NADH is not available for LA production.

**Reply 7:** Sorry for the confusing description. We wish to clarify the cofactor distribution in our system. Under methanol metabolism, the NADPH/NADP<sup>+</sup> pool is substantially more abundant than the NADH/NAD<sup>+</sup> pool. This NADPH abundance makes it a more suitable cofactor for L-lactate production via Ldh. Indeed, while strain HPLA06 (utilizing NADH) produced 1.2 g/L L-lactate, strain HPLA13 (engineered to utilize NADPH) achieved a significantly higher titer of ~1.6 g/L L-lactate. This confirms NADPH's superior driving force for lactate synthesis in this study.

**Comment 8:** Usually LA production takes place in cytoplasm as pyruvate comes from Glycolysis which happen in cytoplasm. In current study, it is not clear why LA production was shifted to mitochondria.

**Reply 8:** In this study, we introduced Ldh enzyme which is fused with mitochondrial targeting signal (MTS) to *O. polymorpha*. The MTS peptide can target Ldh from cytoplasm to mitochondria, thus enabling L-lactate production in mitochondria. As the final engineered strain HPLA22 has both Ldh and MTS-Ldh, the L-lactate production from methanol take place in both cytoplasm and mitochondria.

**Comment 9:** The Manuscript does not about oxygen levels during cultivation. For example, I could not find any information about aeration conditions during fed-batch culture.

**Reply 9:** Thank you for pointing out this detail. In the fed-batch fermentation in bioreactor, the pH, temperature, and DO were set as 5.6, 37°C, and 30%, respectively. Initial agitation was set to 400 rpm, and increased to maximal 800 rpm, depending on the DO level. Aeration was initially provided at 18 sL/h and increased to maximally 48 sL/h depending on DO level. We have added these conditions to Methods in Line 410-414.

**Comment 10:** The batch and fed-batch fermentation were performed only on methanol or some glucose was added during the cultivation?

**Reply 10:** All fermentations for L-lactate production in this study used methanol as the sole carbon source without any auxiliary carbon sources.

**Comment 11:** What is the theoretical yield of LA on methanol. Were there any byproducts formed? What was the pH profile in flask and was pH controlled in

bioreactor?

**Reply 11:** We calculated the theoretical yield of L-lactate on methanol is 0.804 (g/g). A key advantage of *O. polymorpha* is its clean metabolic profile: it produces minimal byproducts, with only trace amounts of intermediates (e.g., TCA cycle organic acids or formate) detectable. The pH was controlled initially at 5.6 and not further adjusted during cultivation in shake flask. In bioreactor, the pH was controlled at constant 5.6 with feeding 4M KOH to neutralize the produced L-lactate.

**Comment 12:** The details of software used for LCA and TEA is not provided. Despite a LA titer of 25 g/L, MSP is coming out to be \$2.25/kg which is better than many sugar based bioprocesses. I am not sure how they are ending up with such a low number.

**Reply 12:** The software used for LCA and TEA was described in the Methods section of the original manuscript (“TEA and LCA analysis”) in Line 478-480, 490-491 and 530-531. All data used in both analyses were derived from a rigorously developed process simulation in Aspen Plus v14. LCA was performed using openLCA v2.2 ([www.openlca.org](http://www.openlca.org)), while TEA was conducted using an in-house model based on the open-source National Renewable Energy Laboratory TEA framework (<https://www.nrel.gov/analysis/techno-economic.html>)<sup>14</sup>.

Regarding the minimum selling price (MSP) of L-lactate, we have included a comparison with sugar-based lactic acid production in the Supplementary Table S9. The MSP for sugar-based lactic acid ranges from \$0.35-1.57 /kg<sup>2, 44-46</sup>, which is generally lower than the value reported in our study. A detailed analysis of the comparison between MSP and sugar-based lactic acid can be found in Line 357-361.

References:

2. Yan, X. et al. Paradigm of engineering recalcitrant non-model microorganism with dominant metabolic pathway as a biorefinery chassis. *Nature Communications* 15, 10441, doi:10.1038/s41467-024-54897-5 (2024).
14. Kobos, P. H. et al. Techno-economic analysis: Best practices and assessment tools. (Sandia National Lab. (SNL-CA), Livermore, CA (United States), 2020).
44. Liu, G., Sun, J., Zhang, J., Tu, Y. & Bao, J. High titer L-lactic acid production from corn stover with minimum wastewater generation and techno-economic evaluation

based on Aspen plus modeling. *Bioresource Technology* 198, 803-810 (2015).

45. Kwan, T. H., Hu, Y. & Lin, C. S. K. Techno-economic analysis of a food waste valorisation process for lactic acid, lactide and poly (lactic acid) production. *Journal of cleaner production* 181, 72-87 (2018).

46. Li, Y. et al. Sustainable lactic acid production from lignocellulosic biomass. *ACS Sustainable Chemistry & Engineering* 9, 1341-1351 (2021).

“This study entailed the design of an L-lactate production process using Aspen Plus v14 software, which was utilized to conduct calculations for mass and energy balance.”

“The estimation of CAPEX, OPEX, and MSP was conducted via an in-house model created by the National Renewable Energy Laboratory (NREL).”

“All calculations and estimations are predicated on openLCA software version 2.2.”

“Although the competitive potential is inadequate to that of established sugar-based and lignocellulosic processes (Supplementary Table S9), this study emphasize the importance of third-generation biomanufacturing — particularly regarding reduced carbon source costs, optimized process utility, and carbon credit considerations — which collectively improve economic viability, resulting in an MSP lower than the market price of L-lactate.”

## Responses to the reviewers' comments (NCOMMS-25-14369)

Dear reviewers,

We would like to express our thanks to the constructive suggestions regarding to our manuscript “Methanol biotransformation for the production of the biodegradable plastic monomer L-lactate in yeast”. These comments are helpful for us to revise and further improve our paper. The main corrections and responses to the reviewers' comments are as follows:

### Reviewer #5:

#### General comment

Biodegradable polymers like polylactic acid (PLA) are increasingly recognized as eco-friendly substitutes for petroleum-based plastics, and novel single-carbon (C1) bioprocesses present an efficient pathway for lactate synthesis. The yeast *Ogataea polymorpha* was genetically modified to achieve high yields of L-lactate solely from methanol. The authors introduce an integrated approach that combines “liquid sunshine” photochemical conversion with engineered *O. polymorpha* fermentation to enhance L-lactate titers, and assess the system's techno-economic viability alongside its greenhouse-gas reduction potential. I think this paper can be published in *Nature Communications* after the second revision for one major point.

**Response:** We sincerely appreciate the reviewer for the constructive and encouraging comments on our manuscript. We have carefully addressed the major concern raised and revised the manuscript accordingly. We hope that these improvements will address your concerns.

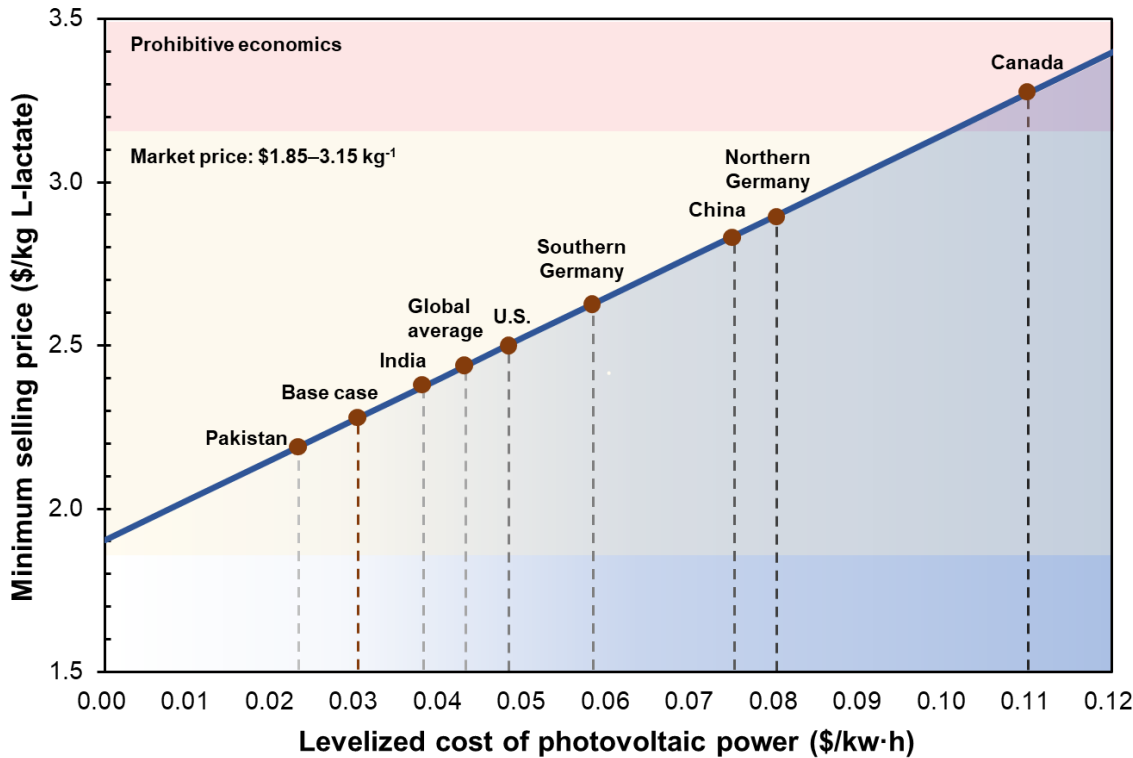
#### Major points

**Comment 1.** Thank you for the revision and efforts to address the comment. The authors explained that the levelized cost of electricity for utility-scale photovoltaic solar power in 2024 is approximately \$0.031-0.075/kWh with references. However, some references, such as the Fraunhofer report (2024), present the levelized cost of electricity for photovoltaic solar power as a range of \$0.04-0.24/kWh. It is recommended to consider the various ranges of the levelized cost of photovoltaic solar power, rather than focusing solely on the minimum cost. Considering that photovoltaic solar power is a form of renewable energy, its price varies greatly depending on the region, making it infeasible to reflect the minimum price in a specific region. The authors should consider the price of photovoltaic solar power in various regions and perform the techno-

economic analysis for two cases: when the solar power cost is minimum and when it is maximum. The Fraunhofer report (2024): Christoph Kost et al., Levelized Cost of Electricity Renewable Energy Technologies, Fraunhofer, 2024.

**Reply 1:** Thank you for your detailed comment. In the revised manuscript, we incorporated sensitivity analysis scenarios by using utility-scale photovoltaic power prices from different regions, drawing on multiple references including Fraunhofer (2025)<sup>1-6</sup>. A corresponding sensitivity curve is provided in Supplementary Fig. S15. We also explicitly discussed the regional variations in photovoltaic electricity prices in the manuscript (page 10, lines 248-255):

“Considering the regional variation in solar power costs, the MSP fluctuates significantly with the geographical location of the plant<sup>43</sup> (Supplementary Fig. S15). For example, countries with abundant solar resources such as Pakistan show solar power prices close to the global minimum (<0.025 \$/kWh), resulting in comparatively lower MSP, whereas regions with higher solar power prices such as Canada (>0.10 \$/kWh) may increase the MSP by over 13%. These results emphasize the critical role of site selection in improving the economic competitiveness of CO<sub>2</sub>-to-L-lactate biomanufacturing.”



**Supplementary Fig. S15** 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<sup>1</sup>, India<sup>2</sup>, global average<sup>2</sup>, United States<sup>3</sup>, Southern Germany<sup>4</sup>, China<sup>5</sup>, Northern Germany<sup>4</sup>, Canada<sup>6</sup>.

- 1 Tahir, M. H., Saleem, M. W., Bicer, Y., Ikram, M. & Iqbal, S. Optimizing Electrical Efficiency and Levelized Cost of Energy in Photovoltaic Systems Through Thermal Management Using Microchannel Heat Sinks. *International Journal of Energy Research* 2025, 2433429, doi: <https://doi.org/10.1155/er/2433429> (2025).
- 2 PV Tech. IRENA: Global solar PV LCOE increases by 0.6 % in 2024 to US \$0.043/kWh. PV Tech News. 25 Jul 2025. Available from: <https://www.pv-tech.org/irena-global-solar-pv-lcoe-increases-by-0-6-in-2024-to-us0-043-kwh/>
- 3 Ramasamy, V. et al. Documenting 15 Years of Reductions in US Solar Photovoltaic System Costs. (National Renewable Energy Laboratory (NREL), Golden, CO (United States), 2025). Available from: <https://research-hub.nrel.gov/en/publications/documenting-15-years-of-reductions-in-us-solar-photovoltaic-syste>
- 4 Fraunhofer ISE. Levelized Cost of Electricity: Renewable Energy Technologies. (Fraunhofer Institute for Solar Energy Systems, 2025). Available from: <https://www.ise.fraunhofer.de/en/publications/studies/cost-of-electricity.html>
- 5 Chong, S., Wu, J. & Chang, I. S. Cost accounting and economic competitiveness evaluation of photovoltaic power generation in China —— based on the system levelized cost of electricity. *Renewable Energy* 222, 119940, doi: <https://doi.org/10.1016/j.renene.2024.119940> (2024).
- 6 Sadat, S. A. & Pearce, J. M. Techno-economic evaluation of electricity pricing structures on photovoltaic and photovoltaic-battery hybrid systems in Canada. *Renewable Energy* 242, 122456, doi: <https://doi.org/10.1016/j.renene.2025.122456> (2025).

**Reviewer #1 (Remarks to the Author):****General comment**

The authors have addressed all questions and comments satisfactorily. I am fine with the current version and support its publication. There are just a few minor writing issues that should be corrected.

**Minor corrections**

Line 111: *in situ* should not be italicized.

Line 160: Please be mindful of the tense in this sentence ("It is worth mentioning...").

**Reply:** Thank you for your valuable suggestions. We have corrected the mistakes in Line 111 and 160.