

A new-to-nature photosynthesis system enhances utilization of one-carbon substrates in *Escherichia coli*

Corresponding Author: Professor Liming Liu

This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Nature Communications.

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

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors have made a great effort to address my comments, which have improved the manuscript significantly. The new additions clarify the context, explain better the experimental setup and have a better use of the references. I only have some minor comments.

-Line 66: It should be "holo-NPS have".

-The iPBRC strategy developed here is similar to the iPRCC in a recent article (Nat. Synth 2, 960–971 (2023)). It would be beneficial to highlight the differences and unique advantages of your approach to distinguish it from the existing work.

-The author needs to carefully examine the references; for example, reference 10 and 43 refer to the same article.

-The color scheme in Figure 7e may make it difficult to distinguish between different categories. I recommend using a color scheme with higher contrast or incorporating different pattern fillings to enhance the distinction.

Reviewer #2

(Remarks to the Author)

In this manuscript, the authors significantly revised the manuscript in previous peer-review process. I appreciate the extensive amounts of experiments conducted by the authors. There are not much technical issues against this work now. Nonetheless, the question is the big picture. In the end, this work develops a strain of *E. coli* that use methanol/formate as the C1 substrate (and electron donors), to reduce CO₂ and yield biomass along with other value-added small molecules. The role of sunlight is to provide some extra driving force in the process of proton translocation for ATP synthesis. While technically impressive, the work misleads the readers and have been significantly over-claimed, because what the authors presented is not a photosynthetic system and it is largely thermal neutral. For this reason, I disagree with the authors that this work is relevant towards the claimed "green and sustainable way to mitigate climate change in the future". This is a piece of work technically impressive but unsatisfying in the big picture. Nonetheless, I understand it is the editor who makes the final decision.

The claims, percentage of increase in ATP/NAPDH contents and the carbon footprint, are misleading and should not be made in such a manner for the healthy development of our research community. This is not a photosynthetic system, as the authors claimed, and the authors are not in a position to claim better quantum yields/titers in comparison to photosynthetic bacteria without noting the huge difference in thermodynamics. In photosynthetic bacteria, water is the proton donor and sunlight provides all the thermodynamic energy to fix CO₂ (C is at +4 formal oxidation state) to biomass (roughly CH₂O, C is at +0 formal oxidation state). In this work, methanol (CH₄O, C is at –2 formal oxidation state) is the predominant electron donor for CO₂ fixation and the formation of biomass, malate (C is at +1 formal oxidation state), pyruvate (C is at +2/3 formal oxidation state), and α-ketoglutarate (C is at +4/5 formal oxidation state). The role of sunlight, as the authors now clearly depicted, is to facilitate proton translocation for ATP synthesis. From a thermodynamic point of view, yielding mostly +0 C (biomass or other derivatives) from a mixture of –2 C (CH₄O) and +4 C (CO₂) is a thermoneutral process, in contrast to the hugely thermally uphill reaction that photosynthetic bacteria operate. Therefore, it is inappropriate to compare the flux/yield/carbon footprint to photosynthetic bacteria, without considering the large difference in thermodynamics. The dissemination of the authors' inappropriate claim may risk the research community losing the sights and de-emphasizing the

thermodynamic issue when finding "green and sustainable way to mitigate climate change in the future".

While the authors have avoided doing so far, the authors must provide the overall energy efficiency of the system, i.e. the ratio between the net free energy gain of the proposed reaction, assuming this is a uphill reaction (but I doubt so, it may be downhill or largely thermal neutral), and the overall energy input from sunlight and C1 substrate (methanol/formate). The authors also need to clearly calculate the percentage of solar energy's contribution towards such a chemical transformation, and hence calculate the energy efficiency of sunlight (a component of the overall energy efficiency). For example, I hope the authors agree that in the grand scheme of things solar energy may not matter much if the 99% of free energy is provided by methanol and only 1% from sunlight. Such values are needed if the authors want a fair comparison and evaluation with photosynthetic bacteria such as algae that possess 5% energy efficiency of photosynthesis.

Reviewer #4

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All my earlier comments were somewhat taken care of and additionally the authors improved the readability and made it easier to follow the text in some cases. Still I feel that parts of the text have protocol character and could be more specific at some points making it easier for the broad readership of Nature Communications to follow the logic of the authors.

Version 1:

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The authors have made a great effort to address all reviewers' comments, which have improved the manuscript significantly. This new-to-nature photosynthesis system notably widens the metabolic boundaries of sugar heterotrophs, which may boost low-carbon biomanufacturing for a more sustainable future. Thus, I believe that this paper should be of interest to a broad readership of Nature Communications. I would like to congratulate the authors on their achievements.

Reviewer #2

(Remarks to the Author)

While I appreciate the reviewers' responses towards my prior comments, I remain unsatisfied with the reviewer's claim that "it is difficult to obtain an accurate value of the light energy efficiency parameter in the quantitative extent". True, it is hard to be exactly accurate, same. But it remains valuable to provide an estimate so that researchers in the community won't be misled and can better appreciate the performance of the reported system. As Nature Communication is towards the general research community instead of a specific field, the authors two claims, (1) focusing on the synthetic biology innovation and (2) existing reports did not report such estimate, should not constitute a valid argument of NOT reporting an estimated energy efficiency. Ref. 14~16 are the literature that the authors purported the absence of a reported energy efficiency. This is incorrect, ref. 16 indeed reports the solar-to-chemical energy efficiency of 0.7%. I don't see a technical difficulty for the authors to provide a semi-quantitative if not quantitative value of the energy efficiency.

Reviewer #4

(Remarks to the Author)

I acknowledge the efforts of the authors to further improve the readability by adding specific details to follow the procedures applied.

Version 2:

Reviewer comments:

Reviewer #2

(Remarks to the Author)

I appreciate the authors' efforts that provide a semi-quantitative estimate of their energy efficiency. However, the way that the authors estimated is incorrect. The manuscript reports a light-driven process that converts formic acid and methanol into value-added process. Therefore, the Gibbs free energy difference that is gained with the provision of light is the difference of Gibbs formation energy between the product (acetone/malate/alpha-KG) and the substrate (methanol/formic acid). However, in the authors' calculation, the Gibbs energy change from CO₂ to the product is used. As the authors use the incident photon energy in their experiments as the denominator for energy efficiency calculation, the reported value of energy efficiency in their responses over-claimed the Gibbs free energy gain not from their light-driven process. The reported values are misleading to other researchers and set an inappropriate example. The authors need to recalculate and provide an updated estimate of their energy efficiency.

Version 3:

Reviewer comments:

Reviewer #2

(Remarks to the Author)

I appreciate the reviewer's patience and diligence to revise the manuscript. This manuscript is good to be published. Thank you for all the work.

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Reply to reviewers

Replies to Reviewer #1:	1
Replies to Reviewer #2:	3
Replies to Reviewer #4:	7

For your convenience, the itemized answers to the reviewers' comments are presented below (our responses are marked in blue).

Replies to Reviewer #1:

The authors have made a great effort to address my comments, which have improved the manuscript significantly. The new additions clarify the context, explain better the experimental setup and have a better use of the references. I only have some minor comments.

A: Thank you very much for your comments.

Q1: Line 66: It should be “holo-NPS have”.

A1: Thank you for your suggestion. We have carefully checked the grammar of the entire article and revised “holo-NPS has” to “holo-NPS have”, please see **page 4, line 66** in the revised manuscript.

Q2: The iPBRC strategy developed here is similar to the iPRCC in a recent article (Nat. Synth 2, 960-971 (2023)). It would be beneficial to highlight the differences and unique advantages of your approach to distinguish it from the existing work.

A2: This suggestion is greatly appreciated. We respond to your suggestions from the following two aspects:

- (1) In our study, the differences between the iPBRC strategy developed here and the previous iPRCC strategy is mainly in the following two points: (i) the iPBRC strategy involves the two stages (photosynthetic building stage and resting catalytic stage) of one engineered strain (*E. coli*), while the iPRCC strategy involves the modular utilization of two engineered strains (cyanobacterium *Synechococcus elongatus* and *E. coli*); (ii) the iPBRC strategy involves the collection of engineered strain cells between two stage transitions, while the iPRCC strategy involves the collection of culture supernatant (containing stable mediator compounds) between two modular utilizations.
- (2) Following your kind suggestion, we have supplemented the advantages of the iPBRC strategy

in **Discussion** section (as follows), please see **page 20, lines 413-416** in the revised manuscript.

Furthermore, compared to the previous iPRCC strategy¹, the iPBRC strategy developed in this study offers unique advantages, including the ability to achieve photosynthesis and resting catalysis in an engineered strain, along with a broader capability for one-carbon substrate utilization.

Q3: The author needs to carefully examine the references; for example, reference 10 and 43 refer to the same article.

A3: Thanks for your kind suggestion. We have corrected this error and checked all references in the revised manuscript.

Q4: The color scheme in Figure 7e may make it difficult to distinguish between different categories. I recommend using a color scheme with higher contrast or incorporating different pattern fillings to enhance the distinction.

A4: Thanks for your kind suggestions. We are very sorry for causing you this confusion. Following your suggestion, we have checked the color scheme of all figures and have revised the color scheme in Figure 7e to a higher contrast (as follows), please see **Figure 7e** in the revised manuscript.

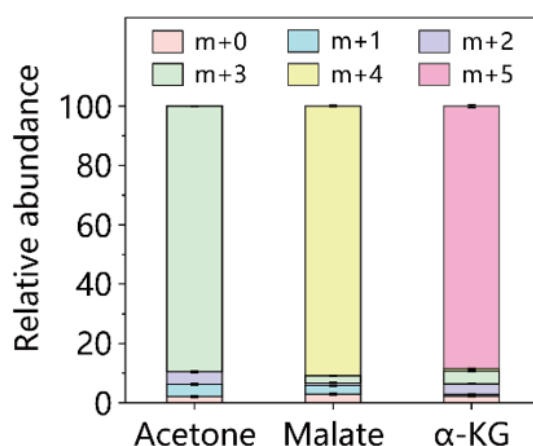


Figure 7e, Relative abundance of mass isotopomers for acetone, malate, and α -KG in the supernatant of catalytic mixture at 72 h.

Replies to Reviewer #2:

In this manuscript, the authors significantly revised the manuscript in previous peer-review process. I appreciate the extensive amounts of experiments conducted by the authors. There are not much technical issues against this work now. Nonetheless, the question is the big picture. In the end, this work develops a strain of *E. coli* that use methanol/formate as the C1 substrate (and electron donors), to reduce CO₂ and yield biomass along with other value-added small molecules. The role of sunlight is to provide some extra driving force in the process of proton translocation for ATP synthesis.

A: We greatly appreciate your recognition regarding our revision on the Manuscript.

Q1: While technically impressive, the work misleads the readers and have been significantly over-claimed, because what the authors presented is not a photosynthetic system and it is largely thermal neutral. For this reason, I disagree with the authors that this work is relevant towards the claimed “green and sustainable way to mitigate climate change in the future”. This is a piece of work technically impressive but unsatisfying in the big picture. Nonetheless, I understand it is the editor who makes the final decision.

A1: Thanks very much for your constructive comments.

Recently, artificial photosynthetic systems have been widely developed in the fields of biological science and material science, such as the material-microbe hybrid photosynthetic system with cysteine as an electron donor and CO₂/N₂ or glucose as substrates²⁻⁶. The new-to-nature photosynthesis system we developed is an artificial photosynthetic system, not a strict photosynthesis like plants and cyanobacteria. The reason for the claimed “green and sustainable way to mitigate climate change in the future” in our future outlook is that our study achieved a negative carbon footprint (-0.84~-0.23 kgCO₂e/kg product) and there might be some potential to reduce CO₂ level and thus mitigate the global greenhouse effect⁷.

Nevertheless, the statement “green and sustainable way to mitigate climate change in the future” was over-claimed. In order to express more rigorously, according to your comments, we have revised “This new-to-nature photosynthesis system provides a green and sustainable way to

mitigate climate change in the future” in the **Abstract** to “This new-to-nature photosynthesis system provides a green and sustainable approach to enhance one-carbon substrates utilization in the future” (page 2, lines 38-39 in the revised manuscript) and “thereby offering potential solutions for solving the energy crisis and mitigating climate change in the future” in **Discussion** part to “offering a green and sustainable solution for enhancing the utilization of one-carbon substrates in the future” (page 22, lines 454-455 in the revised manuscript)

Q2: (1) The claims, percentage of increase in ATP/NAPDH contents and the carbon footprint, are misleading and should not be made in such a manner for the healthy development of our research community. (2) This is not a photosynthetic system, as the authors claimed, and the authors are not in a position to claim better quantum yields/titers in comparison to photosynthetic bacteria without noting the huge difference in thermodynamics. (3) In photosynthetic bacteria, water is the proton donor and sunlight provides all the thermodynamic energy to fix CO₂ (C is at +4 formal oxidation state) to biomass (roughly CH₂O, C is at +0 formal oxidation state). In this work, methanol (CH₄O, C is at -2 formal oxidation state) is the predominant electron donor for CO₂ fixation and the formation of biomass, malate (C is at +1 formal oxidation state), pyruvate (C is at +2/3 formal oxidation state), and α-ketoglutarate (C is at +4/5 formal oxidation state). The role of sunlight, as the authors now clearly depicted, is to facilitate proton translocation for ATP synthesis. From a thermodynamic point of view, yielding mostly +0 C (biomass or other derivatives) from a mixture of -2 C (CH₄O) and +4 C (CO₂) is a thermoneutral process, in contrast to the hugely thermally uphill reaction that photosynthetic bacteria operate. Therefore, it is inappropriate to compare the flux/yield/carbon footprint to photosynthetic bacteria, without considering the large difference in thermodynamics. The dissemination of the authors’ inappropriate claim may risk the research community losing the sights and de-emphasizing the thermodynamic issue when finding "green and sustainable way to mitigate climate change in the future".

A2: These comments are greatly appreciated. We respond to your comments in turn as follows:

(1) In the fields of microbial metabolic engineering and synthetic biology, regulating the level of microbial metabolic energy (ATP/NAD(P)H) is an important strategy to improve the efficiency

of bioproduction⁸. For the study of microbial utilization of one-carbon substrates, the supply of metabolic energy is an important limiting factor and has been extensively explored^{9, 10}, such as an increase in ATP/NADH contents significantly improved carbon yield¹¹.

For carbon footprint, we think it is necessary to estimate the negative carbon footprint¹ of the iPBRC strategy, because we had achieved chemicals biosynthesis (resting catalytic stage) by utilizing the mixed one-carbon substrates CO₂ and methanol, and measured the corresponding carbon emissions (photosynthetic building stage) using exhaust gas analysis equipment. Although this is not a very rigorous and comprehensive calculation, this parameter is still widely reported in related studies^{1, 12, 13}.

- (2) It should be clarified here that we did not make comparisons with photosynthetic bacteria on quantum yields/titers in the previous revision of the manuscript (According to your previous comments, we had deleted the related comparisons).
- (3) Thanks for your detailed comments. As mentioned in (1) and (2), according to your previous comments, we had deleted the related comparisons with photosynthetic bacteria, and we have revised the claim “green and sustainable way to mitigate climate change in the future” and related sentence (Please see **page 2, lines 38-39; page 22, lines 454-455** in the revised manuscript).

Q3: While the authors have avoided doing so far, the authors must provide the overall energy efficiency of the system, i.e. the ratio between the net free energy gain of the proposed reaction, assuming this is a uphill reaction (but I doubt so, it may be downhill or largely thermal neutral), and the overall energy input from sunlight and C1 substrate (methanol/formate). The authors also need to clearly calculate the percentage of solar energy’s contribution towards such a chemical transformation, and hence calculate the energy efficiency of sunlight (a component of the overall energy efficiency). For example, I hope the authors agree that in the grand scheme of things solar energy may not matter much if the 99% of free energy is provided by methanal and only 1% from sunlight. Such values are needed if the authors want a fair comparison and evaluation with photosynthetic bacteria such as algae that possess 5% energy efficiency of photosynthesis.

A3: Thanks for your constructive suggestions. We respond to your comments from the following three aspects:

- (1) As mentioned in **A2** (1) and **A2** (2), according to your previous comments, we had deleted the related comparisons with photosynthetic bacteria, and we have revised the claim “green and sustainable way to mitigate climate change in the future” and related sentence (Please see **page 2, lines 38-39; page 22, lines 454-455** in the revised manuscript).
- (2) For the light energy efficiency of the system, we think that this is a very important parameter in the study of natural and artificial photosynthetic systems. In our study, we have demonstrated to a qualitative extent that light energy is indispensable for this system, because in the absence of blue light illumination, methanol cannot provide electrons to the system through the photocatalysis of MgP (please see Supplementary Fig. 11, 34-35 in the revised manuscript). It is noted that at present, in the study of material-microbe hybrid photosynthetic system, it is very difficult to obtain an accurate value of the light energy efficiency parameter in the quantitative extent (e.g., the sample was a shaken culture medium so it was difficult to determine the irradiated sample area parameters). For example, the light energy efficiency parameters of AuNCs-*Moorella thermoacetica*¹⁴, InP-*Saccharomyces cerevisiae*¹⁵, and photocatalyst sheets-*Sporomusa ovata*¹⁶ have not yet been measured. Therefore, this is a goal that we need to actively explore and achieve in our future work.
- (3) In our study, we prefer to emphasize the use of synthetic biology approaches to develop artificial photosynthetic systems to utilize one-carbon substrates, rather than developing strategies to optimize the light energy efficiency of artificial photosynthetic systems to exceed natural photosynthetic bacteria. To avoid misleading readers, we have supplemented the relevant discussions (as follows), please see **page 18, lines 368-371** in the revised manuscript.

Although a promising proof of concept remains incomparable to natural photosynthetic bacteria, this system provides new insights into the development of fully photoautotrophic *E. coli* cell factories capable of producing value-added bioproducts in the future.

Thanks very much for your constructive comments again.

Replies to Reviewer #4:

All my earlier comments were somewhat taken care of and additionally the authors improved the readability and made it easier to follow the text in some cases.

Q: Still I feel that parts of the text have protocol character and could be more specific at some points making it easier for the broad readership of Nature Communications to follow the logic of the authors.

A: Thanks for your constructive suggestions. Following your suggestions, we have carefully checked the full text and further supplemented the following three points in more specific to make it easier for the broad readership.

- (1) We have explained in detail “connect the native porphyrin metabolism” and have revised the relevant sentence to “MgP molecules were then successfully synthesized *in vivo* by introducing the heterologous MgP biosynthesis pathway, thereby connect to the native porphyrin metabolism (protoporphyrin IX as a precursor for MgP biosynthesis) in *E. coli*”, please see **pages 6-7, lines 127-130** in the revised manuscript.
- (2) We have explained in detail “The signal of ACMA indicated a 12.9% decrease” and have revised the relevant sentence to “Under blue light illumination, the oxonol VI signal indicated an 11.9% increase (suggesting the generation of membrane potential $\Delta\Psi$, inside positive), while the ACMA signal showed a 12.9% decrease (suggesting the formation of proton gradient ΔpH , inside acidic)”, please see **pages 7-8, lines 149-152** in the revised manuscript.
- (3) We have explained in detail “the model analysis of max-min driving force” and have revised the relevant sentence to “The max-min driving force (MDF) value of the ADRP was approximately 2.76 kJ/mol, indicating thermodynamic feasibility within physiological metabolite concentration ranges”, please see **pages 10-11, lines 215-217** in the revised manuscript.

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2. Guan, X., Xie, Y. & Liu, C. Performance evaluation and multidisciplinary analysis of catalytic fixation reactions by material-microbe hybrids. *Nat Catal* **7**, 475-482 (2024).
3. Cestellos-Blanco, S., Zhang, H., Kim, J.M., Shen, Y.-x. & Yang, P. Photosynthetic semiconductor biohybrids for solar-driven biocatalysis. *Nat Catal* **3**, 245-255 (2020).
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8. Tong, T. et al. Engineering microbial metabolic energy homeostasis for improved bioproduction. *Biotechnol Adv* **53**, 107841 (2021).
9. Jiang, W. et al. Metabolic engineering strategies to enable microbial utilization of C1 feedstocks. *Nat Chem Biol* **17**, 845-855 (2021).
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11. Hu, G. et al. Light-driven CO₂ sequestration in *Escherichia coli* to achieve theoretical yield of chemicals. *Nat Catal* **4**, 395-406 (2021).
12. Liew, F.E. et al. Carbon-negative production of acetone and isopropanol by gas fermentation at industrial pilot scale. *Nat Biotechnol* **40**, 335-344 (2022).
13. Si, X. et al. Catalytic production of low-carbon footprint sustainable natural gas. *Nat Commun* **13**, 1-9 (2022).
14. Zhang, H. et al. Bacteria photosensitized by intracellular gold nanoclusters for solar fuel production. *Nat Nanotechnol* **13**, 900-905 (2018).

15. Guo, J. et al. Light-driven fine chemical production in yeast biohybrids. *Science* **362**, 813-816 (2018).
16. Wang, Q., Kalathil, S., Pornrungroj, C., Sahm, C.D. & Reisner, E. Bacteria-photocatalyst sheet for sustainable carbon dioxide utilization. *Nat Catal* **5**, 633-641 (2022).

Reply to reviewers

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Replies to Reviewer #2:	2
Replies to Reviewer #4:	4

For your convenience, the itemized answers to the reviewers' comments are presented below (our responses are marked in blue).

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The authors have made a great effort to address all reviewers' comments, which have improved the manuscript significantly. This new-to-nature photosynthesis system notably widens the metabolic boundaries of sugar heterotrophs, which may boost low-carbon biomanufacturing for a more sustainable future. Thus, I believe that this paper should be of interest to a broad readership of Nature Communications. I would like to congratulate the authors on their achievements.

A: We greatly appreciate your recognition regarding our revision on the Manuscript.

Replies to Reviewer #2:

While I appreciate the reviewers' responses towards my prior comments, I remain unsatisfied with the reviewer's claim that "it is difficult to obtain an accurate value of the light energy efficiency parameter in the quantitative extent". True, it is hard to be exactly accurate, same. But it remains valuable to provide an estimate so that researchers in the community won't be misled and can better appreciate the performance of the reported system.

A: Thanks very much for your kind comments and constructive suggestions.

Q1: As Nature Communication is towards the general research community instead of a specific field, the authors two claims, (1) focusing on the synthetic biology innovation and (2) existing reports did not report such estimate, should not constitute a valid argument of NOT reporting an estimated energy efficiency. Ref. 14~16 are the literature that the authors purported the absence of a reported energy efficiency. This is incorrect, ref. 16 indeed reports the solar-to-chemical energy efficiency of 0.7%. I don't see a technical difficulty for the authors to provide a semi-quantitative if not quantitative value of the energy efficiency.

A1: Thanks for your constructive suggestions. Following your suggestions, we have provided an estimated value of the energy efficiency for this system (as follows), please see **page 16, lines 331-333 and Supplementary Note 7** in the revised manuscript.

In this case, the solar-to-chemical energy efficiencies of this system to produce acetone, malate, and α -KG were estimated to be 2.10%, 0.83%, and 1.39%, respectively (Supplementary Note 7).

Supplementary Note 7

To semi-quantitatively estimate the solar-to-chemical energy efficiency (SCEE) of this system, we performed calculations based on the formula reported in relevant literature¹. The SCEE of this system was calculated using

$$\text{SCEE (\%)} = (R(\text{chemical}) \times \Delta G_r) / (P \times S) \times 100$$

where $R(\text{chemical})$, ΔG_r , P , and S represent the rate of chemical production ($R(\text{acetone}) = 0.0181$

mol/72 h, $R(\text{malate}) = 0.0184$ mol/72 h, $R(\alpha\text{-KG}) = 0.0104$ mol/72 h) during the resting catalytic stage in a 200 mL serum bottle, the estimated Gibbs free energy change (298K, 1atm) of the reaction (ΔG_r ($3\text{CO}_2 \rightarrow \text{acetone}$) = 1005.1 kJ/mol, ΔG_r ($4\text{CO}_2 \rightarrow \text{malate}$) = 392.2 kJ/mol, ΔG_r ($5\text{CO}_2 \rightarrow \alpha\text{-KG}$) = 1151.8 kJ/mol), the energy intensity of blue light illumination (25 mW/cm²), and the irradiated sample area (approximately 133.45 cm², the surface area of the cylinder (the diameter and height are about 5 cm and 6 cm) formed by the resting culture medium in a 200 mL serum bottle is used as the irradiated sample area), respectively.

Based on this, we put the relevant data into the formula and calculated that SCEE(acetone), SCEE(malate), and SCEE(α -KG) are 2.10%, 0.83%, and 1.39%, respectively.

Thanks very much for your constructive comments again.

Replies to Reviewer #4:

I acknowledge the affords of the authors to further improve the readability by adding specific details to follow the procedures applied.

A: Thank you very much for your comments.

References

1. Wang, Q., Kalathil, S., Pornrungroj, C., Sahm, C.D. & Reisner, E. Bacteria-photocatalyst sheet for sustainable carbon dioxide utilization. *Nat Catal* **5**, 633-641 (2022).

Reply to reviewers

Replies to Reviewer #2:	2
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For your convenience, the itemized answers to the reviewers' comments are presented below (our responses are marked in blue).

Replies to Reviewer #2:

Q: I appreciate the authors' efforts that provide a semi-quantitative estimate of their energy efficiency. However, the way that the authors estimated is incorrect. The manuscript reports a light-driven process that converts formic acid and methanol into value-added process. Therefore, the Gibbs free energy difference that is gained with the provision of light is the difference of Gibbs formation energy between the product (acetone/malate/alpha-KG) and the substrate (methanol/formic acid). However, in the authors' calculation, the Gibbs energy change from CO₂ to the product is used. As the authors use the incident photon energy in their experiments as the denominator for energy efficiency calculation, the reported value of energy efficiency in their responses over-claimed the Gibbs free energy gain not from their light-driven process. The reported values are misleading to other researchers and set an inappropriate example. The authors need to recalculate and provide an updated estimate of their energy efficiency.

A: Thanks very much for your encouragement and constructive suggestions. According to your constructive suggestions, we have recalculate and provide an updated estimate of the solar-to-chemical energy efficiency (SCEE), and the results show that SCEE (acetone), SCEE (malate), and SCEE (α -KG) are about 1.14%, 0.36%, and 0.13%, respectively.

In our study, mixed one-carbon substrates CO₂ (replaced by NaHCO₃ in the liquid culture medium) and methanol were used to produce acetone (C₃H₆O)/malate (C₄H₆O₅)/ α -KG (C₅H₆O₅), please see **Fig. 7a** and **Supplementary Fig. 43, 45, 47**. In addition, ¹³C isotope tracing experiments (please see **Fig. 5b**, NaH¹³CO₃ and CH₃OH as mixed one-carbon substrates) showed that the utilization ratio of NaHCO₃ and methanol was 39.5%/60.5%. Based on this, we re-estimated the difference of Gibbs formation energy gain from their light-driven process between the product and the substrate. The estimated Gibbs free energy change (298K, 1atm) of the reaction: ΔG_r (1.185 NaHCO₃ + 1.815 CH₃OH $\rightarrow$ C₃H₆O + 1.185 NaOH + 0.63 H₂O) = 543.7 kJ/mol, ΔG_r (1.58

$\text{NaHCO}_3 + 2.42 \text{ CH}_3\text{OH} \rightarrow \text{C}_4\text{H}_6\text{O}_5 + 1.58 \text{ NaOH} + 1.84 \text{ H}_2\text{O} = 168.5 \text{ kJ/mol}$, ΔG_r (1.975 $\text{NaHCO}_3 + 3.025 \text{ CH}_3\text{OH} \rightarrow \text{C}_5\text{H}_6\text{O}_5 + 1.975 \text{ NaOH} + 3.05 \text{ H}_2\text{O}$) = 111.2 kJ/mol.

Based on the above, according to your constructive suggestions, we have put the updated relevant data into the formula and recalculated that SCEE (acetone), SCEE (malate), and SCEE (α -KG) are about 1.14%, 0.36%, and 0.13%, respectively. Please see **page 16, lines 331-333 and Supplementary Note 7** in the revised manuscript.

The calculation method and related data are as follows:

We performed calculations based on the formula reported in the relevant literature¹ recommended by you. The SCEE of this system was calculated using

$$\text{SCEE (\%)} = (R(\text{chemical}) \times \Delta G_r) / (P \times S) \times 100$$

where $R(\text{chemical})$, ΔG_r , P , and S represent the rate of chemical production during the resting catalytic stage in a 200 mL serum bottle, the estimated Gibbs free energy change (298K, 1atm) of the reaction, the energy intensity of blue light illumination, and the irradiated sample area, respectively.

$R(\text{acetone}) = 0.0181 \text{ mol/72 h}$, $R(\text{malate}) = 0.0184 \text{ mol/72 h}$, $R(\alpha\text{-KG}) = 0.0104 \text{ mol/72 h}$.

ΔG_r (1.185 $\text{NaHCO}_3 + 1.815 \text{ CH}_3\text{OH} \rightarrow \text{C}_3\text{H}_6\text{O} + 1.185 \text{ NaOH} + 0.63 \text{ H}_2\text{O}$) = 543.7 kJ/mol, ΔG_r (1.58 $\text{NaHCO}_3 + 2.42 \text{ CH}_3\text{OH} \rightarrow \text{C}_4\text{H}_6\text{O}_5 + 1.58 \text{ NaOH} + 1.84 \text{ H}_2\text{O}$) = 168.5 kJ/mol, ΔG_r (1.975 $\text{NaHCO}_3 + 3.025 \text{ CH}_3\text{OH} \rightarrow \text{C}_5\text{H}_6\text{O}_5 + 1.975 \text{ NaOH} + 3.05 \text{ H}_2\text{O}$) = 111.2 kJ/mol.

P , 25 mW/cm².

S , approximately 133.45 cm², the surface area of culture medium in a 200 mL serum bottle cylinder. The shape of the culture medium is cylindrical, and the diameter and height are about 5 cm and 6 cm, respectively.

It is worth noting that the P and S parameters can only be estimated, especially S . Here, we take the maximum value of the liquid surface of the culture medium in the cylinder for estimation, but this cannot accurately reflect the actual light energy utilized by the cells. One possibility is that the actual utilization may be smaller than this estimated value. Another possibility is to estimate the amount of light energy absorbed from the cell surface by calculating the surface area of the cell (the surface area of all cells). The number of cells in the culture medium can be estimated, but it is difficult to determine whether all cells have absorbed light energy on their surfaces, it is also

difficult to estimate. Therefore, the estimates of P , S , and SCEE are only for readers' reference.

Thanks very much for your constructive comments again.

References

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