

RESEARCH ARTICLE Hot Paper

Bio-Photocatalytic Synergy Enables Valorization of CH₄ and CO₂ Into Hydrogen and 4-Hydroxybenzoate in Engineered Methanotrophic Bacteria

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Received: 26 November 2025 | **Revised:** 10 February 2026 | **Accepted:** 9 March 2026

Keywords: biohybrid systems | carbon dioxide | metal complexes | methane | methanotrophic bacteria

ABSTRACT

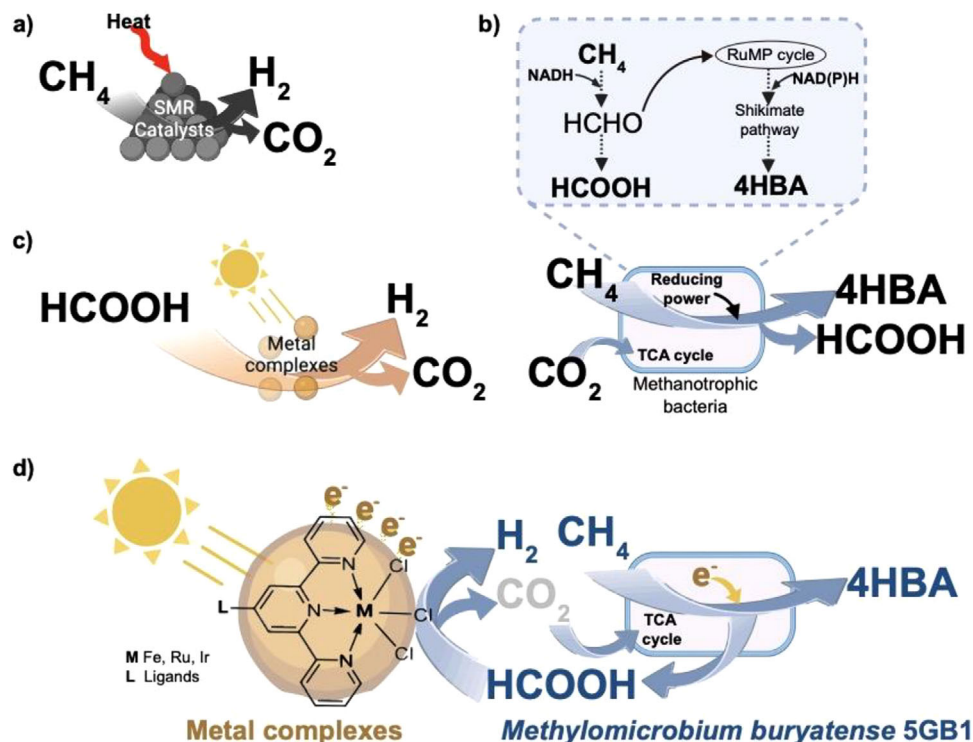
Valorization of decentralized one-carbon chain (C₁) resources into value-added products has garnered significant attention, owing to its dual benefits in resource recovery and carbon mitigation. To address the challenge of co-converting methane (CH₄) and carbon dioxide (CO₂), we developed a solar-driven biohybrid system that synergistically integrates biosynthesis and photocatalysis. The core of this system relies on a genetically engineered methanotrophic cell factory and newly designed biocompatible photocatalytic metal complexes. The methanotrophic bacteria utilize CH₄ as the primary feedstock to biosynthesize 4-hydroxybenzoate (4HBA) while realizing CO₂ sequestration. The photocatalytic metal complexes, meanwhile, capture formate (a metabolic byproduct of cells) to generate hydrogen (H₂) and photoelectrons—with the latter directly supplying reducing power to support cellular activities in genetically tailored cells. Under optimal conditions, this solar-driven system achieved a 4HBA titer of 472.36 µg/L, a H₂ yield of 0.59 mmol H₂/mol CH₄, and over 50% reduction in carbon emissions. The study not only establishes a feasible biomanufacturing strategy for the upcycling of C₁ gaseous feedstocks but also highlights the potential of integrating biological and photochemical technologies to advance sustainable energy and environmental solutions.

1 | Introduction

Methane (CH₄) is a primary global fossil fuel with extensive centralized reserves. However, a substantial portion of methane exists in spatially decentralized, sparse point sources (e.g., biogas, mine gas, and flared shale gas). These sources are inherently characterized by small-scale distribution, low flow rates, and low pressure [1, 2], which frequently preclude economical pipeline transport. Such inherent characteristics pose a distinct challenge

for conventional thermocatalytic processes (e.g., steam methane reforming, SMR; Scheme 1a), which rely on large-scale facilities and intensive energy input [3–5]. Furthermore, these CH₄ streams often coexist with carbon dioxide (CO₂), another critical one-carbon chain (C₁) resource. Consequently, there is an urgent need for alternative approaches to valorize CH₄ and CO₂ into more desired products, enabling on-site, small-scale conversion with improved energy efficiency and reduced environmental impact.

Ziyue Jiao, Yujing Gao, and Wenxin Wei contributed equally to this work.



SCHEME 1 | Schematic illustration of greenhouse gas valorization in a biohybrid system. a) H_2 production from CH_4 by SMR. b) Bioconversion of CH_4 and CO_2 for 4HBA biosynthesis by methanotrophic bacteria. c) Solar-driven formate dehydrogenation by metal complexes. d) In situ H_2 and 4HBA co-synthesis by a biohybrid system. The yellow arrows represent the flow of photo-generated electrons, while the blue arrows indicate the metabolic mass transfer of substrates.

Recent advancements in methane utilization via photocatalysis and electrocatalysis pursue high selectivity but require harsh operating conditions: a $\text{Pd}/\text{Co}_3\text{O}_4$ Schottky heterojunction catalyst achieves high ethane selectivity for CH_4 conversion at 320°C via full-solar-spectrum utilization [6], while an iron-embedded liquid metal catalyst realizes 99.9% acetic acid selectivity under 2 MPa with magnetic field modulation and diluted H_2O_2 [7]. In contrast, biomanufacturing commonly operates under ambient conditions (30°C , 0.1 MPa) and relies on the intrinsic specificity of biocatalysts, offering a complementary strategy for the in situ one-step conversion of dispersed C_1 substrates into diverse multi-carbon products [8–10].

Among them, methanotrophic cell factories have emerged as potential platforms, leveraging their ability to assimilate CH_4 for growth and biosynthesize diverse value-added products [11–18]. Specifically, the shikimate pathway in Type I methanotrophic bacteria has garnered attention as a promising carbon sink for producing aromatic/phenolic products, which can be used in pharmaceuticals, cosmetic preservatives, and polymer precursors [19, 20] (Scheme 1b). Worth mentioning, methanotrophic cells rely on reducing power to oxidate CH_4 and synthesize central metabolites [21]. Meanwhile, this process generates formate as a byproduct, leading to carbon loss. Nevertheless, formate can be utilized as an efficient carrier for hydrogen (H_2) production via solar-driven metal complexes, simultaneously producing photoelectrons along with CO_2 (Scheme 1c) [22]. These outputs directly supply reducing power and supplementary carbon substrate for the cell factory, closing the carbon and energy loop.

Thus, a biohybrid system integrating a methanotrophic cell factory for shikimate-pathway-derived 4-hydroxybenzoate (4HBA) biosynthesis with solar-driven formate dehydrogenation for the reducing power and H_2 production offers a viable avenue for CH_4 and CO_2 valorization (Scheme 1d). This framework synergistically harnesses renewable energy to drive biological carbon conversion and photocatalytic power production in a single system. The capture of solar energy provides the activation energy required for rapid formate dehydrogenation under mild conditions. Meanwhile, the photogenerated electrons are shuttled to the intracellular environment to regenerate reducing power, thereby fueling the reductive biosynthesis of 4HBA and sustaining energy-consuming metabolic pathways. While merging photocatalysts with methanotrophic cells presents an eco-friendly paradigm for C_1 -to-multicarbon conversion, several critical challenges remain to be addressed to unlock its full potential. These include: 1) manipulating CH_4 assimilation to optimally distribute carbon flux between the shikimate pathway and formate accumulation; 2) ensuring compatibility between metal-based photocatalytic complexes and the biological culture system to prevent toxicity or metabolic disruption; 3) facilitating CO_2 biological sequestration within the cell factory to maximize carbon migration efficiency; and 4) harnessing the intracellular reducing power generated via photocatalysis to enhance co-synthesis of 4HBA and H_2 .

To overcome the aforementioned difficulties, our study first genetically manipulated *Methylobacterium buryatense* 5GB1 (hereafter 5GB1) to enhance carbon flux and inhibit formate oxidation to CO_2 . Concurrently, we designed different metal

complexes with improved photocatalytic efficiency, water solubility, and biocompatibility to drive formate conversion into H_2 under solar lights. Photoelectrons generated by these complexes were supplied to 5GB1, enabling solar-driven regeneration of reducing power that synergistically boosted 4HBA biosynthesis from CH_4 . To minimize carbon emissions during the photochemical process, an artificial serine cycle pathway was assembled in the engineered 5GB1 to channel CO_2 into the carbon flux. Our integrated system establishes a pioneering approach for upcycling small-scale C_1 resources into valuable chemicals and fuels, demonstrating the potential of merging biological and photochemical systems to create a sustainable, carbon-neutral technology framework.

2 | Results and Discussion

2.1 | Metabolic Engineering for Formate Generation

In the biohybrid system, formate affects the efficiency of the coproduction from CH_4 by serving as a substrate for photocatalysis and acting as a sacrificial agent for generating photoelectrons. The bioconversion and excretion of formate are governed by carbon oxidation pathways, which subsequently influence the cellular energy supply. In Type I methanotrophic cells, CH_4 is initially catalyzed by particulate methane monooxygenase (pMMO), which converts C-H bonds into C-OH groups for methanol synthesis [23]. This process is subsequently followed by the stepwise formation of the key intermediate of formaldehyde to be assimilated via the ribulose monophosphate (RuMP) pathway for cellular growth and a byproduct of formate through the tetrahydromethanopterin pathway (Scheme 1b), which can be excreted from 5GB1 cells (Figure S1). However, formate will be further oxidized by formate dehydrogenase (FDH) into CO_2 for producing reducing power in the form of nicotinamide adenine dinucleotide (NADH) and maintaining energy balance. Considering the pivotal role in the aforementioned carbon oxidation and assimilation processes, variations in the concentration of formate can significantly influence cellular viability. Therefore, we modulated carbon flux between the shikimate pathway and formate excretion to effectively produce 4HBA and H_2 within the biohybrid systems.

Maximizing formate accumulation requires upregulating carbon flux from CH_4 to formate while simultaneously downregulating the flux from formate to CO_2 . In the RK07 strain (Figure 1a), molybdenum-containing FDH (*EQU24_01465*) was knocked out to prevent formate oxidation, and hexulose 6-phosphate synthase (HPS), an enzyme that catalyzes the condensation of formaldehyde and ribulose 5-phosphate in the RuMP pathway, was downregulated by an arabinose-inducible promoter (pBAD). Accompanied by a suppressed induction of formaldehyde assimilation and inhibited formate oxidation, the RK07 strain demonstrated a 3.64-fold increase in cell yield compared to the WT strain (Figure 1b), corroborating the anticipated regulation of the flux from CH_4 into oxidation and assimilation pathways. Additionally, the processes of formate excretion and the elevated concentration of formaldehyde impeded cellular growth (Figure 1c). According to the redox arm model [24], in vivo electron donor for pMMO is endogenous ubiquinol,

which is reduced by NADH, thus linking formate oxidation with NADH generation to drive CH_4 oxidation in 5GB1. To investigate the severe growth defect of insufficient electron source and modifications to the RuMP pathway, we regulate the flux from formate to CO_2 in WT and generated the RE08 strain (Figure 1a). This strategy enabled the controlled expression of FDH in response to arabinose induction, resulting in an enhanced formate titer with the decreasing arabinose concentration by fine-tuning FDH activity (Figure 1b). Nonetheless, severe growth inhibition persisted (Figure 1c), resulting in lower overall formate production. These results highlight that the disturbances in key metabolic pathways disrupt the balance between formaldehyde assimilation and formate excretion, underscoring the need for carefully coordinated pathway modulation to support both cell growth and formate accumulation.

Consequently, we knocked out the FDH in WT to generate the KO12 strain (Figure 1a). This modification led to a significant increase in formate accumulation, with the concentration of formate reaching 595.75 mg/L after 120 h, which represents a more than 19-fold increase compared to the WT strain (Figure 1b). Growths under varying exogenous formate concentrations revealed a distinct toxicity profile (Figure S2). While cell growth was robust at 0.644 g/L, a cell mass reduction (~25%) was observed at concentrations of 1.38 g/L and 2.76 g/L, indicating a clear inhibition threshold. This physiological inhibition is primarily attributed to the dual role of formate as a weak acid that induces cytoplasmic acidification and a metabolic inhibitor that suppresses cytochrome c oxidase activity [25]. Such stress disrupts the proton motive force and respiratory efficiency, which are critical for the energy-intensive process of methane assimilation. The methane-to-formate yield in the KO12 strain reached 0.15 mol formate/mol methane within the first 12 h. However, this yield subsequently decreased by 25.71% over time due to the assimilation of formaldehyde during the logarithmic growth phase (Figure 1d). Compared with the RK07 and RE08 strains, the manipulation of the KO12 strain slightly impacted the cell growth, as shown in Figure 1c. However, the strategy of FDH knockout on the genome would still restrict NADH generation in formate oxidation. To solve this issue, integrating electrons converted from solar energy in the designed biohybrid system provides an effective way to regenerate NADH and supply the necessary reduction power for pMMO. Moreover, the photosensitizers employed in this system facilitate the conversion of the formate byproduct, thereby enhancing the overall efficiency of solar-driven methane upcycling.

Concurrently, the KO12 strain demonstrated the potential for producing value-added chemicals within the methanotrophic cell factory. The endogenous shikimate pathway has garnered considerable attention due to its intrinsic capacity to synthesize aromatic compounds, such as 4HBA, which serves as a valuable platform intermediate for the production of both commodity and fine chemicals [26]. Unlike petroleum-based synthesis, microbial fermentation provides an environmentally friendly and sustainable method for the production of 4HBA [27, 28]. In 5GB1 cells, the biosynthesis of 4HBA begins with the chorismate formation via the endogenous shikimate pathway, which requires reducing power for shikimate production, followed by the cleavage of chorismate to 4HBA by chorismate lyase (Figure S3). Notably, 305.42 μ g/L of 4HBA was detected in the KO12 strain, possessing

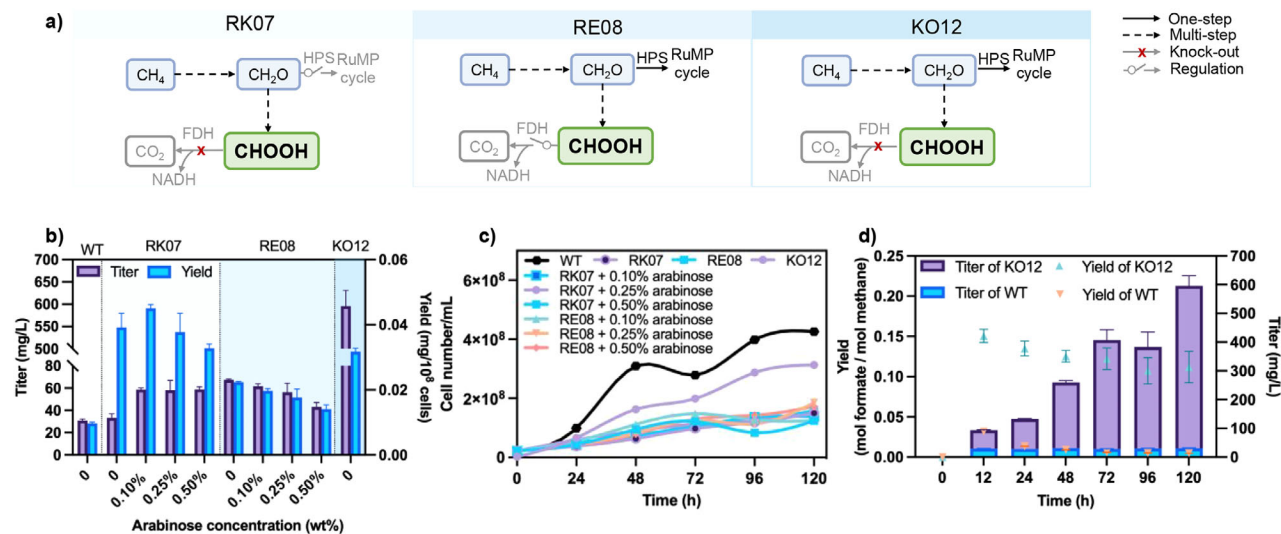


FIGURE 1 | Formate production by methanotrophic bacteria. a) Schematic illustration of formate production in engineering strains. b) Comparison of formate titer and cell yield of wild-type (WT), RK07, RE08, and KO12 strains. c) Comparison of cell numbers of WT, RK07, RE08, and KO12 strains. Arabinose was added to regulate gene expression in RK07 and RE08 strains. d) Comparison of formate titer and production yield from CH_4 of WT and KO12 strains. Data represent mean $\pm$ s.d. derived from three independent biological replicates ($n = 3$). Note that some error bars are obscured by the data symbols due to small standard deviations.

the inherent capability of the shikimate pathway in 5GB1 to support 4HBA biosynthesis.

2.2 | Photocatalysts Design and Characterization

Many metal complexes capable of dehydrogenating formate have been limited in biohybrid applications due to poor aqueous solubility and cytotoxicity [29, 30]. Photocatalytic systems typically require specific conditions, such as a non-neutral environment and sometimes the use of organic solvents, which can be detrimental to the bacteria. Their photocatalytic production of H_2 from formate solutions using an iridium complex needs a pH over 10 to achieve optimal H_2 yield. Building on this, other researchers have developed a variety of metal-based photocatalysts that achieve high turnover frequencies for H_2 production from formate [31–33], but none of them show good performance in biocompatible conditions. Consequently, to enable in situ conversion of formate for electron supply within biological environments, it is essential to overcome challenges related to water solubility and biocompatibility while maintaining high photocatalytic efficiency [34–36], facilitating more seamless integration with methanotrophic cells. The introduction of hydrophilic carboxyl groups was employed to resolve these compatibility issues. The carboxyl group acts as a hydrophilic anchor, promoting solubility via hydrogen bonding and ionization. Furthermore, its polarity creates a barrier against penetration into the hydrophobic bacterial cell membrane, thereby significantly mitigating cytotoxicity compared to nonfunctionalized counterparts. This modification significantly enhances water solubility and minimizes adverse interactions with the cellular membrane, thereby enabling stable operation within the culture medium.

In terms of photocatalyst design, our efforts focused on the systematic selection and modification of metal centers and their ligands. In this study, we synthesized 9 different complexes

by combining 3 metal centers (Fe, Ru, Ir) with 3 ligand types (unmodified, $-\text{NO}_2$, $-\text{COOH}$) (Figure S4a). These metals were selected based on their demonstrated efficacy in photocatalytic H_2 production from formate. To further enhance the performance of these complexes, we utilized ligand engineering by incorporating nitro and carboxyl groups separately, resulting in 9 complexes. These modifications significantly improved photocatalytic H_2 production by enhancing the electron density at the metal center, facilitating efficient electron transfer during the photocatalytic reaction. Additionally, these functional groups increased the solubility of the photocatalyst in aqueous environments, improving their interaction with the reaction medium and substrates.

For a clearer understanding, 3 representative complexes $\text{Ir}(\text{tpy})\text{Cl}_3$, $\text{Ru}(\text{tpyNO}_2)\text{Cl}_3$, and $\text{Fe}(\text{tpyCOOH})\text{Cl}_3$ with different metals and ligands were illustrated in Figure 2a, and the other 6 complexes were shown in Figure S4a. Firstly, spectral testing was employed to characterize the light utilization of all synthesized complexes. UV-Vis spectroscopy revealed broad absorption profiles for each complex (Figures 2b and S4c–e), while fluorescence emission spectra indicated emission wavelengths concentrated around 550 nm for the tpy ligand, which shifted to a range of 450–510 nm for the tpyNO_2 ligand (Figure S4c–e). Although the modifications to the ligand functional groups had a negligible impact on the iridium and ruthenium complexes, the spectroscopic analysis demonstrated that the visible light absorption of iron complexes was significantly improved. To verify the generation of photoelectrons, the photophysical properties of the complexes were subsequently evaluated. Photocurrent response curves confirmed the presence of photogenerated electrons (Figures 2c and S4f–h), with $\text{Fe}(\text{tpyCOOH})\text{Cl}_3$ exhibiting the highest photocurrent response.

Electrochemical impedance spectroscopy (EIS) indicated lower charge-transport resistance (R_{ct}) values under illumination, suggesting light sensitivity and enhanced charge separation in

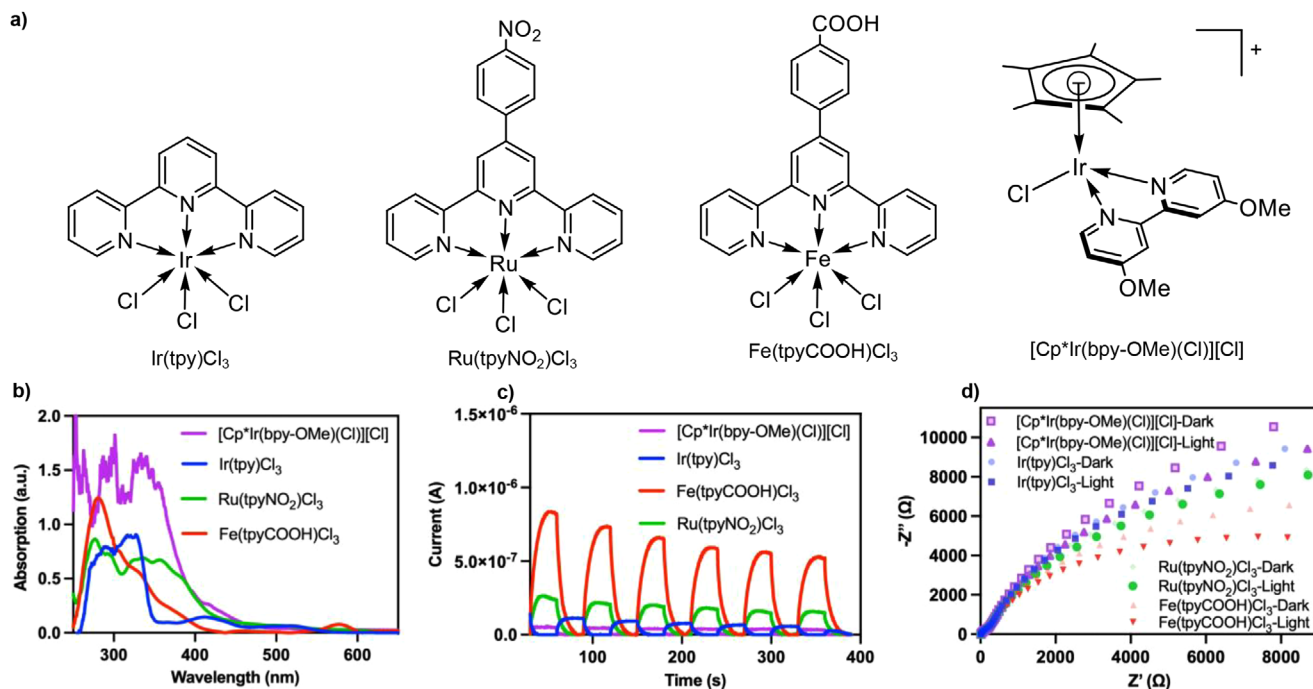


FIGURE 2 | Synthesis and characterization of photocatalysts. a) Scope of photocatalysts; b) UV-Vis spectrum; c) Photocurrent response curves; d) EIS of photocatalysts.

$\text{Ru}(\text{tpy})\text{Cl}_3$, $\text{Fe}(\text{tpyNO}_2)\text{Cl}_3$, and $\text{Fe}(\text{tpyCOOH})\text{Cl}_3$ (Figures 2d and S4i–k). The R_{ct} values characterize the kinetic barrier of interfacial electron transfer in photocatalytic systems, where a lower R_{ct} denotes faster electron transfer dynamics, a pivotal factor governing photocatalytic efficiency. $\text{Fe}(\text{tpyCOOH})\text{Cl}_3$ exhibited the lowest R_{ct} values in both dark (55) and light (49) conditions among all investigated complexes (Table S1), which was the core reason for its superior photocatalytic hydrogen evolution performance. In particular, its R_{ct} was substantially lower than those of Ru/Ir-based counterparts (e.g., $\text{Ru}(\text{tpyNO}_2)\text{Cl}_3$: 64; $\text{Ir}(\text{tpy})\text{Cl}_3$: 71; $\text{Ru}(\text{tpyCOOH})\text{Cl}_3$: 72) and even the carboxyl-modified Ir complex ($\text{Ir}(\text{tpyCOOH})\text{Cl}_3$: 62). This indicated that the $\text{Fe}(\text{tpyCOOH})\text{Cl}_3$ -based catalytic system featured an intrinsically low kinetic barrier for interfacial charge transfer in the ground state, providing a favorable thermodynamic basis for efficient electron transfer upon photoexcitation. Upon light irradiation, all complexes showed a decrease in R_{ct} due to the generation of photoexcited electron-hole pairs that facilitate interfacial charge transfer. Notably, $\text{Fe}(\text{tpyCOOH})\text{Cl}_3$ displayed the lowest light-state R_{ct} (49) and demonstrated superior visible light response and efficiency in photogenerated electron generation. In comparison with other complexes (e.g., $\text{Ru}(\text{tpyCOOH})\text{Cl}_3$: R_{ct} reduced from 72 to 65, magnitude 7; $\text{Ir}(\text{tpy})\text{Cl}_3$: from 71 to 65, magnitude 6), $\text{Fe}(\text{tpyCOOH})\text{Cl}_3$ sustained the fastest interfacial electron transfer rate in the photoexcited state, which ensured the rapid migration of photogenerated electrons from the active sites of the complex to the reaction substrate (H^+) and effectively suppressed the recombination of photogenerated electron-hole pairs.

Furthermore, the redox properties of the complexes are essential for photocatalysis, particularly regarding their electron transfer capabilities. Cyclic voltammetry (CV) analysis revealed more negative reduction potentials for the iridium complexes

and improved electron-donating characteristics in the modified iron complexes (Figure S5). These findings indicate that $\text{Fe}(\text{tpyCOOH})\text{Cl}_3$ possesses exceptional visible light absorption, photoactivity, and electron-donating properties, rendering it highly advantageous for photocatalytic applications. We further synthesized a well-developed Cp*Ir-based photochemical formic acid dehydrogenation catalyst [22] for performance comparison. As shown in Figure S6a–d, $[\text{Cp}^*\text{Ir}(\text{bpy-OMe})(\text{Cl})]\text{Cl}$ exhibited a broad absorption tail that extended to 500 nm, with notable visible light absorption and narrower energy band gaps. Its emission wavelength clustered around 430 nm, while $\text{Ir}(\text{tpy})\text{Cl}_3$ shifted to 600 nm when 2,2':6',2''-terpyridine was used as the ligand. CV analysis (Figure S6e,f) indicated that $[\text{Cp}^*\text{Ir}(\text{bpy-OMe})(\text{Cl})]\text{Cl}$ had a more positive potential than that of $\text{Ir}(\text{tpy})\text{Cl}_3$, reflecting its electron-accepting ability. From an optical perspective, compared to $\text{Fe}(\text{tpyCOOH})\text{Cl}_3$, $[\text{Cp}^*\text{Ir}(\text{bpy-OMe})(\text{Cl})]\text{Cl}$ exhibits enhanced visible light absorption capabilities but demonstrates diminished photoelectron generation and electron transfer efficiency. Consequently, further investigation is necessary to ascertain the optimal catalyst for the subsequent in situ biohybrid system.

2.3 | Performance of Solar-Driven H_2 Production From CH_4

In the assessment of feasibility for the dehydrogenation of biosynthesized formate, the aforementioned photocatalysts were incorporated into the bacterial solution with 691.81 mg/L formate. After simple integration, $\text{Fe}(\text{tpyCOOH})\text{Cl}_3$ exhibited the highest H_2 and CO_2 release rates among the 9 complexes (Figures 3a, S7). While carboxyl modification universally improved water solubility, the $\text{Fe}(\text{tpyCOOH})\text{Cl}_3$ complex outperformed its carboxyl-modified Ir and Ru complexes in terms of photocatalytic hydrogen evolution,

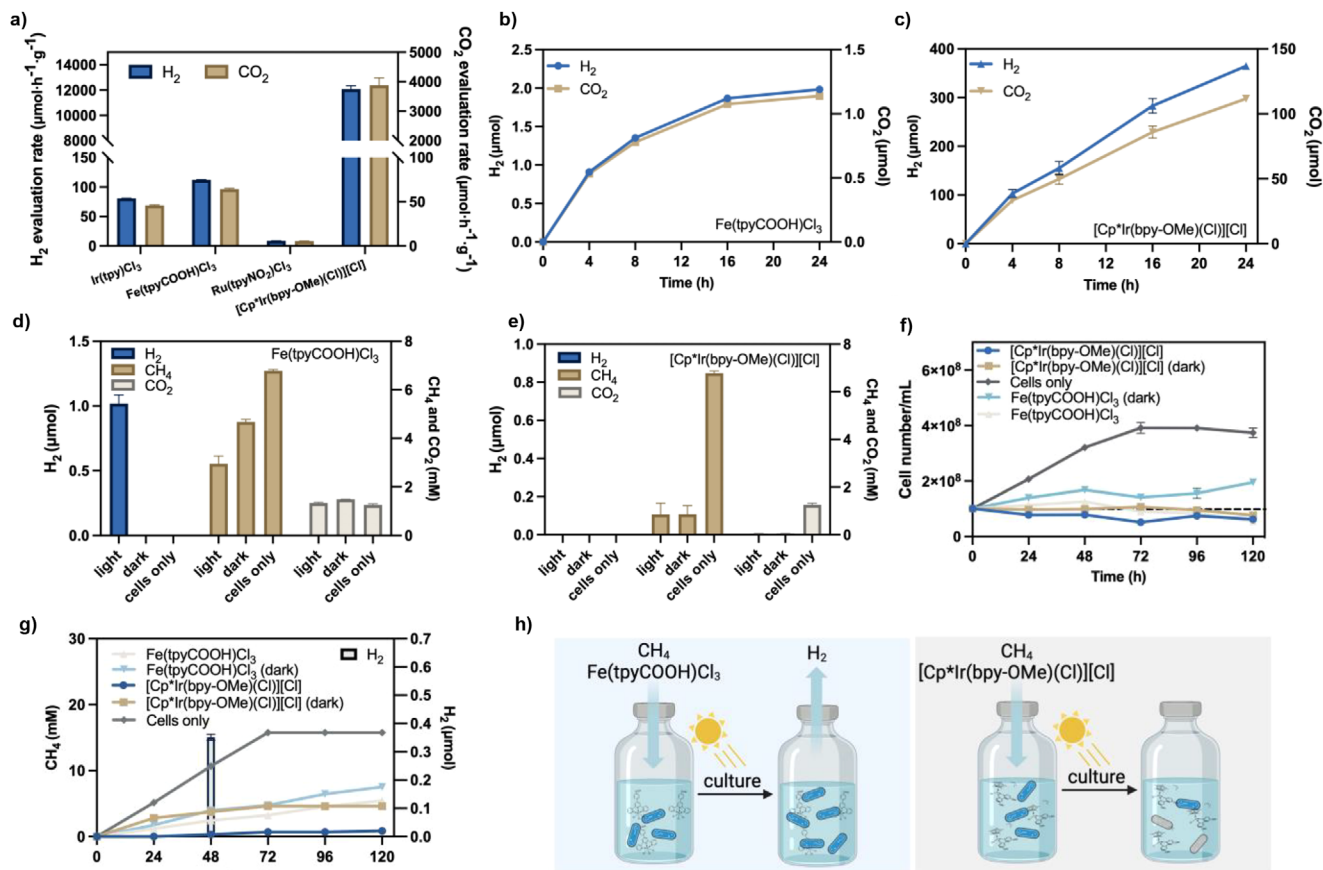


FIGURE 3 | H₂ production from CH₄ with a series connection of cell factory and photocatalysis. a) H₂ and CO₂ production rates by bacterial solution and different photocatalysts. b) The kinetic curve of H₂ and CO₂ production by bacterial solution and Fe(tpyCOOH)Cl₃. c) The kinetic curve of H₂ and CO₂ production by bacterial solution and [Cp*Ir(bpy-OMe)(Cl)]Cl. d) In situ H₂ production from CH₄ by growing cells and Fe(tpyCOOH)Cl₃ in nitrate mineral salt (NMS) medium. e) In situ H₂ production from CH₄ by growing cells and [Cp*Ir(bpy-OMe)(Cl)]Cl in NMS medium. f) Changes in cell number of the biohybrid systems after photocatalysis. g) In situ H₂ production from CH₄ by culturing cells with Fe(tpyCOOH)Cl₃ or [Cp*Ir(bpy-OMe)(Cl)]Cl in NMS medium. Data represent mean ± s.d. derived from three independent biological replicates (n = 3). Note that some error bars are obscured by the data symbols due to small standard deviations. h) Comparison of the biohybrid systems integrating Fe(tpyCOOH)Cl₃ or [Cp*Ir(bpy-OMe)(Cl)]Cl. The blue cells and gray cells represent the growing cells and dead cells, respectively.

confirming the unique and optimal synergy between the Fe metal center and carboxylate ligand for the present system. This further indicates that while ligand modification (via hydrophilicity and electronic effects) is critical, the central metal exerts a dominant role in governing the performance of the final biohybrid system. Thus, the Fe complex retained the best performance even with identical ligand modification. Among the 3 Fe-based complexes investigated, Fe(tpyCOOH)Cl₃ demonstrated the most robust photocatalytic activity toward hydrogen evolution, a phenomenon underpinned by the synergistic optimization of electronic structure, aqueous solubility, and biocompatibility. From an electronic structure perspective, Fe(tpyCOOH)Cl₃ exhibited the highest-lying LUMO levels (α : -0.09705 eV; β : -0.09993 eV), which afford a more favorable thermodynamic driving force for proton reduction by facilitating electron transfer from the excited-state complex to H⁺ (Figure S8). Concurrently, its relatively elevated HOMO levels (α : -0.23779 eV; β : -0.23684 eV) promoted efficient oxidation of the sacrificial electron donor, thereby sustaining the catalytic cycle. The carboxyl (-COOH) moiety played a dual role. It enhanced aqueous solubility via deprotonation to carboxylate anions, increasing the concentration of accessible active

sites and accelerating reaction kinetics, while also conferring superior biocompatibility, as carboxyl groups were endogenous to biological systems and mitigate cytotoxicity compared to the nitro (-NO₂) substituent in Fe(tpyNO₂)Cl₃. In contrast, the parent complex Fe(tpy)Cl₃ suffered from limited solubility, while the nitro-modified analogue Fe(tpyNO₂)Cl₃, despite improved solubility, likely faced challenges due to the strong electron-withdrawing nature of the -NO₂ group, which may adversely tune the electronic structure and interfacial charge transfer kinetics.

However, the [Cp*Ir(bpy-OMe)(Cl)]Cl system showed higher release rates for H₂ and CO₂ than Fe(tpyCOOH)Cl₃ (Figure 3b,c). Subsequently, CH₄ served as the sole carbon source for the in situ production of formate by the KO12 strain with the addition of either [Cp*Ir(bpy-OMe)(Cl)]Cl or Fe(tpyCOOH)Cl₃. Upon light irradiation for 24 h, 1.08 μmol of H₂ was generated from CH₄ in the system with Fe(tpyCOOH)Cl₃, indicating both effective biocatalytic and photocatalytic activities (Figure 3d). CH₄ utilization by the KO12 strain decreased by 56.52% under light and 31.04% in dark conditions. At the same time, formate production and dehydrogenation were observed (Figure S9), suggesting the

viability of the KO12 strain. Based on the testing cycles for H_2 and CO_2 (Figure 3b), we tested the photocurrent response of $\text{Fe}(\text{tpyCOOH})\text{Cl}_3$ after 24 h of pre-illumination (Figure S10). Upon light exposure, the pre-irradiated sample showed an immediate photocurrent spike, followed by a gradual decay to a steady state. In comparison, the as-synthesized compound responded more slowly and exhibited no decay. This difference suggests that light irradiation activates the material, improving charge-transfer kinetics. The decay in the pre-illuminated sample is likely due to gradual catalyst deactivation during cycling, which enhances recombination of photogenerated carriers. Nevertheless, the stabilized photocurrent remained higher than that of the reference compound $\text{Ru}(\text{tpyNO}_2)\text{Cl}_3$. To evaluate the structural stability of the complex under light irradiation, we compared its infrared absorption spectra before and after light exposure. As shown in Figure S11, no significant shifts or changes in intensity were observed in the major characteristic absorption peaks of the complex after illumination. Specifically, the stretching vibration peak near 1710 cm^{-1} corresponding to the carbonyl group ($\text{C}=\text{O}$) of the carboxyl group, and the stretching vibration peak near 1480 cm^{-1} associated with the carbon–nitrogen bond ($\text{C}-\text{N}$) on the pyridine ring, both remained present after irradiation without noticeable alteration in shape or position. These results indicate that the key functional groups and the main framework of the complex remained intact after 24 h of continuous light exposure, with no significant photodegradation or structural transformation observed, confirming its good photostability. However, H_2 and formate were undetectable when using the KO12 strain and $[\text{Cp}^*\text{Ir}(\text{bpy-OMe})(\text{Cl})][\text{Cl}]$ under light irradiation, which adversely affected CH_4 utilization by 87.37% (Figure 3e). Even in the absence of light, $[\text{Cp}^*\text{Ir}(\text{bpy-OMe})(\text{Cl})][\text{Cl}]$ hindered the KO12 strain's CH_4 utilization more significantly than $\text{Fe}(\text{tpyCOOH})\text{Cl}_3$, resulting in lower CO_2 generation. Although $[\text{Cp}^*\text{Ir}(\text{bpy-OMe})(\text{Cl})][\text{Cl}]$ demonstrated superior catalytic efficiency in H_2 production from formate, its pronounced inhibitory effect on microbes considerably reduced the CH_4 utilization rate. In contrast, $\text{Fe}(\text{tpyCOOH})\text{Cl}_3$ effectively alleviates this limitation.

Biocompatibility of the photocatalyst is critical for the long-term cell viability in the in situ biohybrid system. To evaluate this, we monitored cell growth by checking cell numbers within the in situ biohybrid system after 120 h of photocatalytic processing (Figure 3f). The biohybrid system with $[\text{Cp}^*\text{Ir}(\text{bpy-OMe})(\text{Cl})][\text{Cl}]$ exhibited a decrease in cell numbers compared to the initial inoculation. Conversely, an increase in cell numbers was observed in the presence of $\text{Fe}(\text{tpyCOOH})\text{Cl}_3$ under both light and dark conditions. When CH_4 was served as the carbon source for formate production, H_2 was produced in our in situ biohybrid system, which integrated a cell factory during cell growth with the photocatalytic effects of the biocompatible $\text{Fe}(\text{tpyCOOH})\text{Cl}_3$ (Figure 3g). The expected enhancement in solar-driven H_2 production was not observed when $[\text{Cp}^*\text{Ir}(\text{bpy-OMe})(\text{Cl})][\text{Cl}]$ served as the photocatalyst. Although Cp^*Ir -based materials exhibited strong photochemical formate dehydrogenation performance in bacterial solutions, the in situ H_2 production from CH_4 through the formate-centered route was not realized using $[\text{Cp}^*\text{Ir}(\text{bpy-OMe})(\text{Cl})][\text{Cl}]$ (Figure 3h). Among the tested materials, Fe-based photocatalysts stood out for their excellent biocompatibility in the biohybrid system (Figure 3h), likely attributed to their catalytic center that mimics the structure of natural enzymes as well

as the improved aqueous solubility imparted by their ligand. These findings revealed the enhanced biocompatibility and photocatalytic efficiency of the synthesized metal complex for H_2 production from CH_4 within the biohybrid system. To further elucidate the interfacial organization and physical interactions within the biohybrid assembly, surface charge analysis was performed (Figure S12). Zeta potential measurements revealed a shift from -15.93 mV to -14.47 mV upon addition of the complex, suggesting that the photocatalysts are partially adsorbed onto the bacterial surface. This proximity likely facilitated more efficient electron transfer from $\text{Fe}(\text{tpyCOOH})\text{Cl}_3$ to the KO12 strain. Under visible-light irradiation, $\text{Fe}(\text{tpyCOOH})\text{Cl}_3$ underwent intramolecular electron transfer to generate the excited-state species $[\text{Fe}(\text{tpyCOOH})\text{Cl}_3]^*$. This excited species subsequently donated an electron, producing the positively charged cation $[\text{Fe}(\text{tpyCOOH})\text{Cl}_3]^+$. Electrostatic attraction between the cationic complex and the negatively charged bacterial cells resulted in a marked positive shift in the Zeta potential of the system.

Notwithstanding this advantage, the measurable impact of $\text{Fe}(\text{tpyCOOH})\text{Cl}_3$ on cell growth and CH_4 conversion efficiency (Figure 3f,g) highlights the complexity of interactions within the biohybrid system. Two potential mechanisms may contribute to these effects: one hand, π -stacking interactions between tpy scaffolds of the catalyst and biological macromolecules (e.g., proteins, nucleic acids) could promote nonspecific binding to DNA [37], potentially disrupting cellular processes; On the other hand, the optical properties of the photocatalyst may drive the overproduction of photoelectrons, which in turn induce oxidative damage to bacterial cells. These observations underscore the need to balance the catalytic activity of photocatalysts with their biocompatibility, representing a critical consideration for optimizing biohybrid systems. The implementation of photoelectron transfer from $\text{Fe}(\text{tpyCOOH})\text{Cl}_3$ to strains in this biohybrid system is essential for bio-upcycling CH_4 into multi-carbon products.

2.4 | CO_2 Bio-Sequestration for Enhanced Carbon Utilization

Given the identification of a biocompatible metal complex, the subsequent photocatalysis to generate both photoelectrons and convert formate into H_2 is expected to be integrated for overcoming the insufficient reducing power during CH_4 conversion by 5GB1 cells. However, the process of formate dehydrogenation exhibits considerable drawbacks in terms of environmental sustainability due to the intrinsic nature of its operation, which releases CO_2 in the outlet gas. Concurrently, the increased synthesis of 4HBA via the shikimate pathway disrupts the carbon flux entering the tricarboxylic acid (TCA) cycle (Figure S3). The incorporation of CO_2 into the TCA cycle emerges as a promising strategy to recycle CO_2 within central carbon metabolism and enhance carbon flux [17]. To improve carbon utilization efficiency via CO_2 bio-capture, a carbon sequestration pathway was introduced into the KO12 strain to generate the DC03 strain (Figure 4a). The phosphoenolpyruvate carboxylase (PPC) catalyzes the HCO_3^- -dependent carboxylation of phosphoenolpyruvate (PEP) to form the four-carbon molecule oxaloacetate (OAA), which is a crucial intermediate in linking the serine cycle to the TCA cycle. It should be noted that the NMS medium used in this

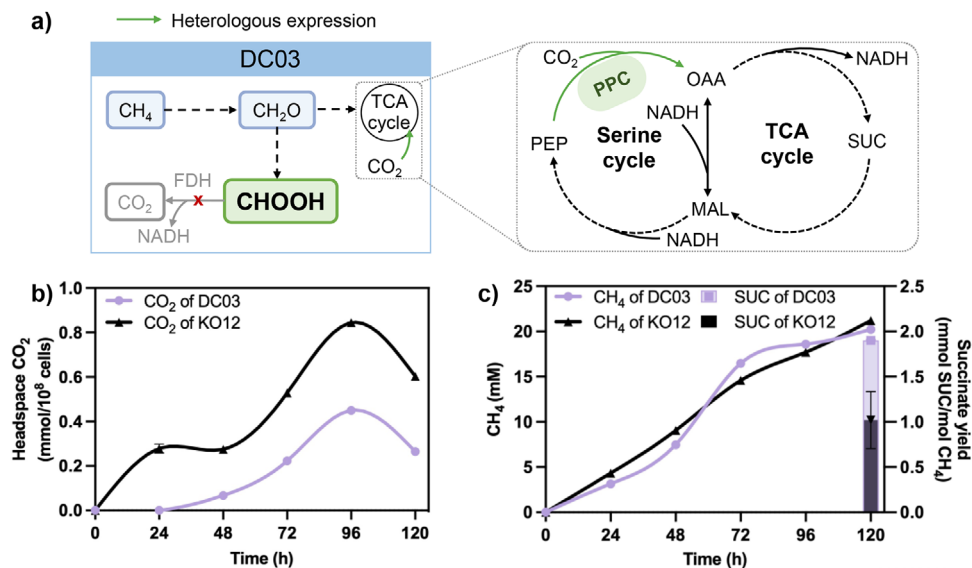


FIGURE 4 | CO₂ bio-sequestration for succinate production. a) Schematic illustration of the metabolic engineered pathway for CO₂ bio-sequestration in the DC03 strain. SUC, succinate; MAL, malate. b) CO₂ emission of KO12 and DC03 strains. c) Influence of CO₂ bio-sequestration on production in KO12 and DC03 strains. Data represent mean $\pm$ s.d. derived from three independent biological replicates ($n = 3$). Note that some error bars are obscured by the data symbols due to small standard deviations.

study contains a carbonate buffer, providing a baseline level of exogenous carbon. Thus, the observed succinate production in strain DC03 reflects the assimilation of both formate-derived CO₂ and medium-derived exogenous bicarbonate. Relative to the WT strain, the DC03 strain, genetically modified to heterotrophically overexpress the *ppc* gene, exhibited a notable decrease 48.84% in CO₂ release via metabolic reconstruction of the serine cycle, along with comparable CH₄ utilization. This resulted in an 86.44% increase in carbon flux from CH₄ to succinate (Figure 4b,c).

Although DC03 and KO12 strains exhibit inherently different CO₂ utilization capacities, headspace CO₂ levels decreased after 120 h for both strains. This phenomenon is mainly attributed to changes in microbial metabolic activity during the late culture stage. After 120 h, cells enter the decline phase, and the activity of CO₂-producing metabolic pathways (e.g., the TCA cycle) is significantly reduced. The modification in the DC03 strain mitigated CO₂ emission during the in situ photocatalytic process and boosted the accumulation of organic acids. It is important to clarify that the CO₂ fixation catalyzed by PPC in the DC03 strain operates as an enhanced carbon assimilation route. The carbon derived from CH₄ primarily enters the metabolic network via the RuMP cycle, providing essential precursors for cell growth. In contrast, the CO₂ fixation pathway introduces an extra carbon source directly into the downstream intermediates of the TCA cycle. This node primarily serves to replenish metabolic intermediates and balance the carbon flux, thereby avoiding direct competition for key upstream intermediates such as formaldehyde or ribulose 5-phosphate.

2.5 | In situ Solar-Driven 4HBA and H₂ Coproduction From CH₄

Leveraging optimized carbon flux distribution, improved biocompatibility of photocatalyst, and artificial CO₂ bio-sequestration,

we subsequently developed a biohybrid system that integrates a real-time cell factory with the solar-driven properties of photocatalysts. This system aims to capture the solar energy for photoelectron generation and facilitate the sustainable upcycling of CH₄ into both H₂ and 4HBA. Introducing electron carriers with specific redox potentials, which are capable of being photoreduced to a radical state, can effectively utilize surplus photoelectrons to provide the required reducing power [36, 38, 39]. For instance, the electron mediator, methyl viologen (MV²⁺), facilitates redox reactions of metabolic cofactors [40], thereby promoting energy-intensive pathways in our system, specifically CH₄ oxidation and the shikimate pathway (Scheme 1b). In this system, MV²⁺ was added to shuttle photoelectrons from Fe(tpyCOOH)Cl₃ to the intracellular space of DC03 strain (Figure 5a). To balance electron transfer efficiency with cellular viability, we applied MV²⁺ at a concentration of 0.1 mg/L to avoid the cytotoxicity effects based on a previous study [41]. Consequently, this concentration ensures efficient photoelectron shuttling from the photocatalyst to the microbial metabolism while preserving the catalytic activity of the DC03 strain. The addition of Fe(tpyCOOH)Cl₃ under illumination enabled DC03 strain to effectively convert CH₄ through the solar-driven regeneration of reducing power (Figure S13), leading to the coproduction of H₂ and 4HBA. Initial CH₄ oxidation kinetics (0–72 h) were nearly identical across all groups (Figure 5b, lines). However, the biohybrid system demonstrated a 2.15-fold higher oxidation capacity by 120 h. Concurrently, the inclusion of Fe(tpyCOOH)Cl₃ under illumination enabled rapid H₂ evolution, reaching 0.59 mmol H₂/mol CH₄ (Figure 5b, bars) within 24 h, which highlights the superior efficiency of the in situ formate-to-H₂ conversion. Meanwhile, 4HBA production increased with solar-driven CH₄ utilization up to 472.36 μ g/L, leading to a 133% enhancement. The presence of formate creates a significant metabolic burden that limits cellular productivity (Figure S2). By integrating the in situ photocatalysis, the formate concentration was successfully maintained at 1.28 g/L under illumination. This strategic modulation effectively shifts the

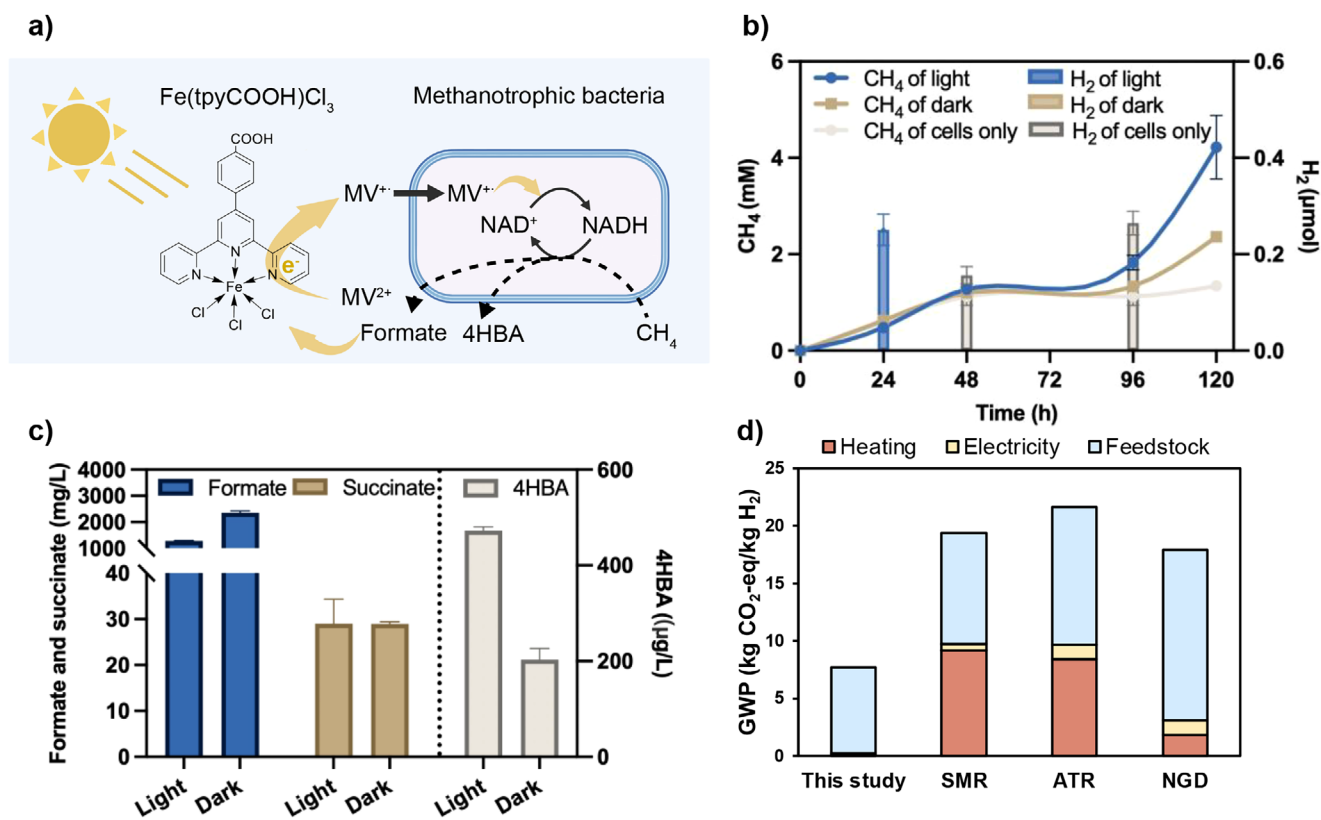


FIGURE 5 | H_2 and 4HBA coproduction from CH_4 by the biohybrid system integrating $\text{Fe}(\text{tpyCOOH})\text{Cl}_3$ and DC03 strain. a) Schematic illustration of solar-driven NADH production with the introduction of electron mediators. b) Solar-driven CH_4 oxidation and H_2 production. c) Formate dehydrogenation and solar-driven 4HBA production. Data represent mean $\pm$ s.d. derived from three independent biological replicates ($n = 3$). Note that some error bars are obscured by the data symbols due to small standard deviations. d) GWP of H_2 production from CH_4 with a series connection of cell factory, photocatalysis, and conventional pathways. ATR, autothermal reforming; NGD, natural gas decomposition.

cellular environment from a state of significant cell growth inhibition to a tolerable mild-stress regime, thereby preserving better cell viability and sustaining the regeneration of reducing power.

Methanotrophic cell factories have emerged as promising platforms, primarily due to their unique ability to convert CH_4 into valuable products. A key advancement in this field is the first reported biosynthesis of 4HBA directly from CH_4 , which is a shikimate pathway-derived compound with extensive applications in pharmaceuticals and industry. Compared to conventional glucose-based fermentation processes [27, 28], this study establishes a nonedible C_1 gas as a feedstock, with its efficiency further enhanced by integration with solar-driven photocatalysis. In our biohybrid system, the harnessing of solar energy facilitated the conversion of CH_4 , aided in the regeneration of cofactors to modulate the carbon flux within energy-related metabolic pathways [42–44], and boosted the efficiency of the shikimate pathway for 4HBA production. Notably, formate excretion is a well-documented phenomenon in the methanotrophic cell factories [45–47]. Building on this characteristic, our genetic modification of 5GB1 cells achieved two critical outcomes: first, it yielded the highest formate titer reported to date among methanotrophic bacteria; second, it introduced an innovative functional design, where formate serves simultaneously as an H_2 carrier and a sacrificial agent for photocatalysis (Figure 5c). This dual role of formate bridges the biological (CH_4 conversion)

and photochemical (solar energy utilization) components of our system, addressing a key challenge in integrating methanotrophy with photocatalysis for efficient C_1 upcycling.

To further examine the photochemical changes in $\text{Fe}(\text{tpyCOOH})\text{Cl}_3$, UV-Vis absorption spectra were recorded before and after 120 h of visible-light irradiation (Figure S14). The main peak at 300 nm remained largely unchanged, indicating that the core molecular structure was preserved. In contrast, the absorption at 409 nm decreased significantly, while a new peak appeared at 570 nm. These spectral changes are consistent with photoinduced electron transfer, leading to the formation of excited-state species and the corresponding oxidized cationic derivative. This result confirms that the complex maintains its structural integrity and catalytic activity under the specific ionic strength and pH conditions of the biological culture, providing a solid foundation for long-term biohybrid operation. In order to investigate the biosynthesis of valuable metabolites, we assessed succinate production from CH_4 and CO_2 within this system (Figures 5c and S15). Artificial CO_2 capture route resulted in a succinate concentration of 28.99 mg/L, enhancing the carbon flux into the TCA cycle. While the bio-sequestration of CO_2 for succinate production has been demonstrated, no corresponding increase in succinate yield was observed when photoelectrons were supplied to the bacteria under illumination. This finding may stem from the competitive redirection of PEP into the shikimate pathway, which is driven

by solar-powered synthesis of 4HBA (Figure S3). Conversely, the solar-driven generation of reducing power may result in the down-regulation of the carbon flux into the TCA cycle [48], likely because the TCA cycle itself produces large amounts of NADH (Figure 4a), suggesting a regulatory balance between externally supplied reducing power and endogenously generated cofactors. Strain engineering and photocatalyst optimization are pivotal, whereas the superior performance observed in this study is not merely the result of individual improvements but benefits from the synergistic integration within the in situ system. The continuous photochemical consumption of formate relieves the byproduct inhibition associated with methane oxidation, while the concurrent supply of solar-driven reducing power supports energy-intensive metabolic processes, specifically methane oxidation and the shikimate pathway. Together, these mechanisms establish the self-regulation that dynamically coordinates carbon flux distribution, redox balance, and solar energy capture. This systemic property is unique to our biohybrid platform and represents a core innovation distinct from isolated biological or photochemical systems.

To assess the sustainability of our approach, we conducted a life cycle assessment (LCA) focusing on global warming potential (GWP). Our approach achieved emission reductions of 60.14% relative to SMR, 64.26% compared to ATR, and 56.85% in comparison to natural NGD [4] (Figure 5d and Table S2). For instance, the biohybrid system demonstrated a reduction in emissions amounting to 11.65 tonnes of CO₂ relative to the SMR process while generating 1 tonne of H₂ derived from 2 tonnes of CH₄. The most significant reduction was observed in the heating-related emissions, which decreased from 9.17 kg CO₂-eq/kg H₂ in the SMR process to 0.0036 kg CO₂-eq/kg H₂ in our system. Our findings indicate a substantial reduction in CO₂ emissions compared to the conventional methods for CH₄ utilization. Notably, our in situ system exhibits substantial potential for applications in various scenarios, where CH₄, water, and solar energy are abundant, such as industrial methane-emitting sites (e.g., natural gas fields, landfill biogas facilities) and rural areas with accessible water and solar energy. By leveraging these locally available resources, our platform offers a viable route for on-site conversion of low-value C₁ gases into high-value products. This capability directly aligns with the long-term objectives of low-carbon development, including industrial emission reduction, rural clean energy supply, and circular utilization of waste carbon resources, thereby providing critical technical support for advancing green and sustainable production modes.

3 | Conclusion

With the escalating release of C₁ sources from waste management and agricultural activities, the demand for sustainable strategies to upcycle these emissions has grown increasingly urgent. Harnessing renewable energy for converting C₁ gases into chemicals and fuels has emerged as a particularly promising approach, given its environmental compatibility. In this context, we developed an in situ biohybrid platform enabling the co-production of H₂ and 4HBA from greenhouse gases. This system integrates the biocatalytic conversion of CH₄ to formate and 4HBA with solar-driven processes for formate dehydrogenation and photoelectron generation. By optimizing flux distribution between

4HBA biosynthesis and formate excretion, we achieved efficient CH₄ upcycling. For integration with the biological culture system, we designed metal complexes with photocatalytic activity under ambient conditions and tailored biocompatibility. Meanwhile, a biological sequestration pathway was employed to capture CO₂ emissions during the photocatalytic process. Consequently, formate, a byproduct excreted from cells, was converted into H₂ via photodehydrogenation and served as a sacrificial agent for the metal complexes to generate photoelectrons supporting 4HBA production. This platform thus leverages solar energy to transform CH₄ into an essential phenolic intermediate alongside a clean fuel, representing a promising strategy for the sustainable utilization of dispersed carbon sources and the advancement of solar-to-chemical conversion.

Acknowledgments

This work was supported by the National Key Research and Development Programs of China (2023YFE0106600), the National Natural Science Foundation of China (52303267), the Natural Science Basic Research Program of Shaanxi (2025JC-QYXQ-004), and the Youth Innovation Team of Shaanxi Universities.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting File 1: anie71855-sup-0001-SuppMat.Pdf.

The authors have cited additional references within the Supporting Information [1–8].