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Adapting gas fermenting bacteria for light-driven domino valorization of CO₂†

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The solar-driven valorization of CO₂ to fuels and chemicals provides an exciting opportunity to develop a circular chemical industry, but the controlled production of multicarbon organics remains a major challenge. Here, we present an abiotic-biotic domino strategy that integrates a photocatalytic CO2-tosyngas conversion system with evolved syngas-fermenting bacteria to enable the upcycling of CO2 into valuable C2 products, including acetate and ethanol. To optimize microbial syngas fermentation through an accessible and chemist-friendly platform, we employ adaptive laboratory evolution (ALE) of Clostridium ljungdahlii (Cl). The adapted strain, Cladapt, exhibits a 2.5-fold increase in growth rate and a 120-fold enhancement in C_2 production compared to the wild type (Cl_{wl}). Isotopic labeling confirmed Cl_{adat} 's high conversion efficiency, yielding 6:1 and 9:1 ratios of ${}^{13}C$: ${}^{12}C$ in acetate and ethanol, respectively. Whole genome sequencing revealed mutations in Cl_{adapt} , offering initial clues to its enhanced metabolism. A scaled-up semiconductor-molecule hybrid photocatalyst, TiO₂|phosphonated Co(terpyridine) $_2$, was employed to generate sufficient syngas (CO/H $_2$ ratio: \sim 30 : 70 with 1.3 mmol of CO after 6 days) for Cl_{adapt} to demonstrate photocatalytic $CO_2 \rightarrow syngas \rightarrow C_2$ conversion (yielding 0.46 \pm 0.07 mM, or 3.2 µmol, of acetate). This study offers a streamlined approach to improving syngas fermentation in Cl, insights into microbial adaptability, and an ALE-guided pathway for solar-powered CO₂ upcycling using an inorganic-microbial domino strategy.

Introduction

Semi-biological photosynthesis merges synthetic and microbial approaches to produce sustainable fuels using sunlight, particularly excelling in selective multicarbon product formation *via* the metabolic pathways available through biocatalysis. ^{1,2} Several recent reports demonstrate the promise and feasibility of this emerging approach: A hybrid solar water splitting-biosynthetic system uses green H₂ as intermediate to fix CO₂ and efficiently produce biomass and fuels using *Cupriavidus necator*. ³ Coupling a synthetic photocatalyst with *Shewanella oneidensis* resulted in selective hydrogenation of C=C and C=O bonds. ⁴ The photosensitization of *Moorella thermoacetica* with extracellular CdS or intracellular gold nanoclusters established a pathway for bacterial CO₂ utilization, integrating microbial systems with nanomaterials to enhance photosynthetic efficiency. ^{5,6}

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Gas fermenting acetogenic bacteria, particularly Clostridium species, have emerged as versatile platforms to produce biofuels and biochemicals from syngas, a mixture of H₂, CO and CO₂.⁷⁻⁹ These Clostridium strains utilize the Wood-Ljungdahl pathway, which consists of two branches to produce the C2 compounds (Fig. 1A): the methyl branch, reducing CO₂ to formate and further to a methyl group, and the carbonyl branch, which forms an acetyl group from CO. Clostridium ljungdahlii (Cl) has shown promise in fermenting these gases into valuable products, underscoring the economic viability of this approach in biotechnology. For instance, a carbon-negative fermentation process was established using engineered and closely related Clostridium autoethanogenum to convert waste gas feedstocks into acetone and isopropanol with high efficiency.10 An Ag-catalyst based gas diffusion electrolyzer for CO2-to-syngas conversion was coupled with syngas-fermenting C. autoethanogenum and C. kluyveri, producing butanol and hexanol with high selectivity and therefore offers a sustainable pathway for industrial chemical production from CO2 and water using renewable energy.11

To further harness and optimize the metabolic capabilities of these microorganisms, adaptive laboratory evolution (ALE) emerges as a powerful tool to select and enhance beneficial traits without the need for genetic engineering. The principles and applications of ALE have demonstrated its simplicity and efficacy in tailoring microbial phenotypes for improved

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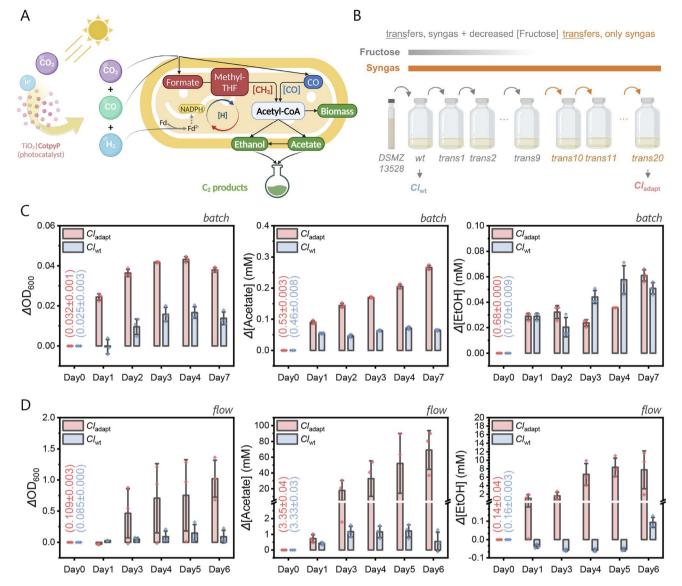


Fig. 1 Adaptive laboratory evolution (ALE) of C. ljungdahlii. (A) Schematic illustration of the syngas-fermenting metabolic pathways in C. ljungdahlii (Cl), including integration with photocatalytic syngas production. [CH₃] (red color), methyl branch; [CO] (blue color), carbonyl branch. (B) Schematic summary of the ALE process. The wild-type strain (Cl_{wt}) was adapted to a syngas environment through gradual fructose reduction and removal over 20 transfers, resulting in the adapted strain (Cl_{adapt}). (C) Comparative analysis of growth and C_2 product generation (acetate and ethanol) between Cl_{adapt} and Cl_{wt} under batch gas purging conditions (syngas purging for 30 min daily, 112 mL headspace). (D) Similar comparative analysis under continuous gas flow conditions (10 mL syngas per min). Growth conditions: ATCC Medium 1754 (pH 5.9), 37 °C with 150 rpm mixing. Numbers in brackets indicate the values at the start of the experiment.

industrial performance.12,13 Examples of successful ALE applications illustrate the transferability, flexibility and transformative potential of ALE in metabolic engineering and synthetic biology.14-19 For example, ALE of Sporomusa ovata integrated with light-harvesting silicon nanowires led to a 2.4fold increase in CO2-reducing current density, enhancing bioelectrochemical CO2 reduction. 16 Similarly, C. autoethanogenum strains developed through ALE showed superior growth and product profiles in continuous bioreactor cultures. 19 ALE using CO₂ and H₂, with other *C. autoethanogenum* lineages exposed to 2% CO, has also significantly enhanced growth rates and ethanol production, revealing extensive proteome and metabolome changes that highlight new targets for metabolic engineering.20

Here, we explore the synergistic potential of ALE-derived gasfermenting bacteria and photocatalysis for the overall conversion of CO₂ to acetate and ethanol (Fig. 1A). An adapted strain of Cl was developed and shows improved syngas-to-acetate conversion. The syngas for fermentation by Cl was subsequently sourced from a synthetic CO₂-to-syngas photocatalyst to demonstrate an overall $CO_2 \rightarrow syngas \rightarrow acetate domino-reaction.$ We therefore present a novel approach for solar energy storage in multicarbon chemicals and biomass that harnesses the symbiotic strength of synthetic and biological catalysts.

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Experimental Section

Materials and chemicals

Deuterium oxide (D₂O) solution (99.9 atom% D) containing 0.05 wt. or 0.75 wt% 3-(trimethylsilyl)propionic-2,2,3,3- d_4 acid, sodium salt, triethanolamine (TEOA, ≥99.0%) and glycerol (for molecular biology, ≥99.0%) as well as all chemicals used for bacterial growth and the isotopically labelled gases ¹³CO₂ and ¹³CO were purchased from Sigma-Aldrich. TiO₂ powder P25 (10-30 nm diameter; 50 m² g⁻¹) was obtained from Evonik. [Co(2,2':6',2"-terpyridine-4'-phosphonic acid)₂](BF₄)₂ (denoted as CotpyP) was synthesized and characterized according to reported procedures.21 Reaction gases CO2 with 2% CH4 as internal gas chromatography standard, and synthesis gas (syngas) cylinders (25% CO, 10% H₂, 65% CO₂) were purchased from Brin's Oxygen Company (BOC).

Bacteria growth, syngas adaptation, storage and handling

Clostridium ljungdahlii (Cl) DSMZ 13528 was purchased from The Leibniz Institute DSMZ (German Collection of Microorganisms and Cell Cultures GmbH). Cultures were initially grown in ATCC Medium 1754, which contained essential nutrients and vitamins, supplemented with 5.00 g L⁻¹ fructose as a carbon source to establish initial growth conditions conducive to syngas adaptation. The specific setups for feeding syngas (Fig. S1†), including batch and continuous flow modes, were designed to mimic industrial conditions and evaluate the strains' performance under varying operational parameters.

The adaptation to syngas was conducted in a two-stage process. In the first stage, freshly cultured wild-type Cl (Cl_{wt}) cells were acclimatized to a syngas environment by gradually decreasing the fructose concentration in the ATCC Medium 1754 over nine consecutive transfers, each lasting 72 hours. This gradual reduction aimed to incrementally expose the microbial population to syngas with less dependency on the fructose, thereby selecting for cells with enhanced syngas utilization capabilities. The syngas (25% CO, 10% H₂, 65% CO₂) roughly mimics the syngas ratios obtained in typical industrial processes, such as biomass gasification, and state-of-the-art electrolysis systems.22,23 In the second stage, cultures were exclusively grown on syngas without any fructose supplementation for an additional 11 transfers. This stringent selection pressure ensured the enrichment of syngas-adapted phenotypes capable of sustained growth and metabolism solely on syngas.

Post-adaptation, the adapted Cl culture, designated as Cl_{adapt} (internal strain registration name as RLM034 for strain request), and the wild-type control, $Cl_{\rm wt}$ (internal strain registration name as RLM028 for strain request), were stored for long-term preservation. Cultures were mixed with an equal volume of sterile 40% glycerol solution (in ddH₂O) to achieve a final concentration of 20% glycerol and stored at -80 °C. This method ensured the viability and genetic stability of the microbial samples for future analyses and experiments.

During bacterial cultivation and transfers, the medium bottles were continuously purged with N2 to minimize O2 exposure. Additionally, when taking optical density (OD) and

nuclear magnetic resonance (NMR) samples, the medium bottles remained under syngas purging, further preventing oxygen intrusion.

Optical density, product quantification, and isotopic labelling

The optical density was monitored at 600 nm (OD₆₀₀) to assess cell growth and adaptation progress at each transfer stage using an ultraviolet-visible (UV-vis) spectrophotometer (Agilent Cary 60).

Gaseous H₂ and CO in the headspace were analyzed by gas chromatography using a Shimadzu Tracera GC-2010 Plus with a barrier discharge ionization detector, equipped with a Shin-Carbon micro-ST Column (0.53 mm diameter) kept at 40 °C using Helium carrier gas. Typically, 20, 50 or 100 µL of headspace gas were injected using a gas-tight syringe (Hamilton). The response factors for the gases were determined by calibration with known amounts of H2 and CO.

Fructose consumption and the production of formate, acetate and other C2 compounds, indicative of the metabolic activity and efficiency of syngas conversion by the adapted and wild-type strains, were quantified by quantitative ¹H nuclear magnetic resonance (qNMR) spectroscopy using a Bruker 400 MHz Neo Prodigy Spectrometry. The chemical shift (δ) of the ¹H NMR spectrum is referenced against the internal standard 3-(trimethylsilyl)propionic-2,2,3,3- d_4 acid, sodium salt (0.05 wt%) or 0.75 wt% in D_2O). To prepare a typical NMR solution, 570 μL of a filtered aliquot (0.22 µm syringe filter) from the bacteria culture was combined with 30 µL of the internal standard solution in an NMR tube.

For isotopic labelling experiments, a sealed bottle containing a bacterial culture of Cladapt in ATCC Medium 1754 (modified) was purged with ¹³C-labelled syngas gas (65% ¹³CO₂/25% ¹³CO/ 10% H₂) using mass flow controllers (Alicat Scientific). The ATCC Medium 1754 was modified by changing the NaH12CO3 into NaH13CO3, for the purpose of 13C labelling. When indicated, 30 mM of ¹³C-formate was also added into the medium. The culture was kept in a shaker-incubator with a constant temperature of 37 °C and shaking (150 rpm) for 12 days. Subsequently, the products in the media were characterized by ¹H NMR spectroscopy as described above.

Calculation on growth rate and doubling time

The growth rate is highest and most consistent during the exponential phase. The growth rate (μ) can be calculated using the formula:

$$\mu = \frac{\Delta \ln(\mathrm{OD})}{\Delta t} = \frac{\ln(\mathrm{OD}_{t_2}) - \ln(\mathrm{OD}_{t_1})}{(t_2 - t_1)}$$

where $\Delta \ln(OD)$ is the change in the natural logarithm of OD, and Δt is the change in time (difference between time point 1

A python script was developed to analyze bacterial growth from optical density (OD) measurements over time. It automatically detects the exponential growth phase within a given time range, calculates the growth rate and doubling time, and plots the growth curve with the exponential fit. Source code is available on https://github.com/su2lin/microbiology.

Sequencing

Whole Genome Sequencing (WGS) of the adapted strain $(Cl_{\rm adapt})$ and its wild-type counterpart $(Cl_{\rm wt})$ was performed to identify genomic variations associated with enhanced syngas fermentation capabilities. Sequencing and analysis were conducted by GENEWIZ. For each strain, a single cell pellet sample was prepared by centrifuging 13 mL of liquid culture to remove the culture medium. The resulting pellets were resuspended in DNA/RNA Shield reagent (Zymo Research Corporation), flashfrozen in liquid nitrogen for stability, and shipped on dry ice to ensure sample integrity during transit.

For WGS data processing, raw sequencing reads were initially assessed for quality using FastQC. Poor quality reads and bases were then filtered and trimmed using Trimmomatic. The quality-filtered reads were mapped to the *C. ljungdahlii* reference genome using the Burrows Wheeler Aligner (BWA), which is designed for mapping low-divergent sequences against large reference genomes. The resulting SAM files were converted to BAM format and indexed using samtools to facilitate visualization of the alignments. Finally, single nucleotide variants (SNVs) were detected using beftools. This comprehensive bioinformatics pipeline—encompassing quality control, trimming, mapping, indexing, and SNV detection—ensured accurate identification of genomic variations between Cl_{adapt} and Cl_{bat} .

Photocatalytic syngas production

In a typical experiment, an aqueous suspension containing TiO₂ (P25, 365 mg) and CotpyP (20 μ mol $g_{TiO_2}^{-1}$) in 0.22 L of 0.1 M TEOA solution (pH ≈ 7) was added to a scaled-up photoreactor²⁴ (total volume = 1.52 L, Fig. S2†). Then, the photocatalytic suspension was purged under stirring (400 rpm) with CO₂, containing 2% CH₄ as gas chromatography internal standard, for 45 min. The stirred photoreactor was subsequently irradiated from the top with a solar light simulator (G2V Sunbrick LED Solar Simulator) with $\lambda = 366$ nm to 423 nm (20.4 mW cm $^{-2}$) for 24 h at ambient temperature. The irradiation provided by the solar light simulator for $\lambda = 366$ –423 nm is consistent with AM1.5 G irradiation, but wavelengths $\lambda >$ 423 nm were removed to avoid unnecessary heating of the photoreactor.

The photocatalytic process was monitored by analyzing the headspace (volume = 1.30 L) after completion (24 h, batch mode experiment) or every 24 h (flow mode experiment) to monitor H_2 and CO formation using a gas chromatograph (see above). For the batch mode experiments, after 24 h of irradiation, the photoreactor was connected for 6 days to a bioreactor containing ≈ 13 mL of ATCC Medium 1754 with the adapted bacterial strain. For the flow mode experiments, the photoreactor was connected before solar light irradiation to three bioreactors and during irradiation, CO_2 was flowed using a mass flow controller (Alicat) at a constant flow rate 30 mL min $^{-1}$ and every 24 h, a fresh batch of 20 μ mol CotpyP

 $g_{TiO_2}^{-1}$ was added into the photoreactor. Photocatalysis batch and flow experiments were performed in triplicates.

Statistical analysis

The mean (\bar{x}) with its standard deviation $(\sigma_{\bar{x}})$ expressed as $\bar{x} \pm \sigma_{\bar{x}}$ from $n \ (n \ge 3)$ independent experiments (x_i) . The standard errors were calculated using OriginPro 2024 (OriginLab).

$$\overline{x} = \frac{1}{n} \sum_{i=1}^{n} x_i$$

$$\sigma_{\overline{x}} = \sqrt{\frac{1}{(n-1)} \sum_{i=1}^{n} (x_i - \overline{x})^2}$$

Results and discussion

Microbial adaptation to syngas fermentation

The wild-type C. ljungdahlii was initially cultured in ATCC Medium 1754 with 5.0 g L $^{-1}$ fructose and preserved with 20% glycerol at -80 °C (Cl_{wt}). ALE was performed over 20 transfers (Fig. 1B): transfers 1–9 involved gradual fructose reduction under constant syngas (25% CO, 10% H $_2$, 65% CO $_2$, 112 mL headspace), followed by 11 transfers using syngas exclusively. Each 72 hour transfer was conducted at 37 °C and 150 rpm, with OD $_{600}$ monitored to confirm consistent growth (Fig. S3 and S4 \dagger). The final adapted culture was designated $Cl_{\rm adapt}$.

To validate the success of ALE, we assessed bacterial growth on syngas under batch and continuous flow conditions (Fig. S1†), simulating potential future application designs.24 Cell populations were monitored via OD600 readings, and C2 products (acetate and ethanol) were quantified using quantitative ¹H NMR (qNMR) spectroscopy (Fig. 1C and D). The adapted strain, Cl_{adapt} , exhibited faster growth (Fig. S5†) and higher final OD600 values than the wild-type Cl_{wt} in both modes. In batch mode, Cl_{adapt} reached an OD_{600} increase (ΔOD_{600}) of 0.043 \pm 0.002 by day 4, approximately 2.5 times higher than the 0.017 \pm 0.003 ΔOD_{600} of $\mathit{Cl}_{\mathrm{wt}}$. In continuous flow mode, by day 6, $\mathit{Cl}_{\mathrm{adapt}}$ and $\mathit{Cl}_{\mathrm{wt}}$ recorded OD_{600} values of 1.02 \pm 0.34 and 0.09 \pm 0.12, respectively, indicating an 11-fold increase in the adapted strain. Growth rate analysis (Table S1 \dagger) showed that $Cl_{
m adapt}$ had rates of 0.38 \pm 0.02 per day in batch mode and 0.96 \pm 0.11 per day in flow mode, compared to $Cl_{\rm wt}$'s slower rates of 0.26 \pm 0.01 and 0.23 \pm 0.22 per day, respectively (Fig. S6†). The enhanced growth of Cladapt under flow mode suggests improved syngas utilization following ALE, likely due to increased CO tolerance, as previous studies have shown high CO concentrations can impede growth rates. 25,26

In batch mode (Fig. 1C), $Cl_{\rm adapt}$ produced 0.27 \pm 0.01 mM acetate and 0.06 \pm 0.01 mM ethanol over seven days, amounting to 0.05 \pm 0.02 mM C_2 compounds per day from syngas. In contrast, $Cl_{\rm wt}$ produced 0.06 \pm 0.01 mM acetate and 0.05 \pm 0.01 mM ethanol, equivalent to 0.02 \pm 0.01 mM C_2 compounds per day, approximately three-fold less than $Cl_{\rm adapt}$, consistent with the observed growth rate differences. Under continuous flow mode (Fig. 1D), $Cl_{\rm adapt}$ generated 69.1 \pm 28.3 mM acetate and 7.7 \pm 5.2 mM ethanol in six days, translating to 12.8 \pm

5.6 mM C_2 compounds per day. In contrast, Cl_{wt} generated 0.54 \pm 0.70 mM acetate and 0.09 \pm 0.03 mM ethanol, or 0.11 \pm 0.12 mM C2 compounds per day, approximately 120-fold lower than Cl_{adapt} . This large discrepancy between these two modes of culture suggests that continuous flow conditions, providing sustained syngas availability, greatly enhance the performance of Cladapt. Notably, under batch mode, ethanol production between the two strains showed no significant differences. However, under flow mode, the Cl_{adapt} strain produced 85 times more ethanol than Clwt, which may be attributed to the higher availability of reducing substrates (i.e., CO and H₂) in continuous flow conditions, facilitating the conversion of acetate into ethanol. Additionally, negative ethanol values observed in the first five days of continuous flow mode (Fig. 1D) resulted from the initial presence of ethanol in the growth medium and its subsequent consumption by the bacteria over time. These findings confirm the successful adaptation of Cl_{adapt} to syngas, demonstrating significantly higher C₂ compound production, especially under continuous flow operation.

Metabolic insights into carbon flux

To confirm the source of carbon in the C_2 products (Fig. 1A), we performed isotopic labelling experiments using ¹³C-syngas as the sole substrate for $Cl_{\rm adapt}$ and $Cl_{\rm wt}$ over 12 days. The isotopologues were analyzed by qNMR spectroscopy (Fig. 2A, S7

and Tables S2, S3† for acetate, and Fig. S8 and Table S4, S5† for ethanol). Cladapt and Clwt showed acetate (38.2 and 5.3 mM) containing mixtures of ¹³C and ¹²C, including ¹³CH₃-¹³COO⁻, ¹³CH₃-¹²COO⁻, ¹²CH₃-¹³COO⁻, and ¹²CH₃-¹²COO⁻. However, only Cl_{adapt} produced ethanol (0.7 mM) containing mixtures of ¹³CH₃-¹³CH₂OH and ¹²CH₃-¹³CH₂OH. For further discussion, see ESI Note 1.† The ¹³C/¹²C ratios were 86:14 for acetate and 95:5 for ethanol in Cl_{adapt} , and 70:30 for acetate in Cl_{wt} , indicating that most of the carbon in the products originates from ¹³C-syngas, with a smaller portion of ¹²C likely coming from the parental culture or cellular carbon reserves. This finding confirms that both Cladapt and Clwt can produce C2 compounds by converting the carbon in syngas. The higher 13C content and the presence of ethanol as a more reduced product in Cl_{adapt} indicates better syngas uptake and conversion activity than Clwt.27

Notably, both strains produced less $^{13}\text{CH}_3-^{12}\text{COO}^-$ than $^{12}\text{CH}_3-^{13}\text{COO}^-$ (Table S3†), a metabolic preference that extended to $^{12}\text{CH}_3-^{13}\text{CH}_2\text{OH}$ (Table S5†). This suggests a preference for retaining the ^{12}C -methyl rather than ^{12}C -carboxyl group. Compared to Cl_{wt} ($^{13}\text{CH}_3-^{12}\text{COO}^-/^{12}\text{CH}_3-^{13}\text{COO}^-=0.9$), the Cl_{adapt} ($^{13}\text{CH}_3-^{12}\text{COO}^-/^{12}\text{CH}_3-^{13}\text{COO}^-=0.5$) shows a more unbalanced $^{12}\text{C}/^{13}\text{C}$ distribution. This observation may reflect a bottleneck in the Cl's Wood–Ljungdahl pathway's methyl branch, 28 which Cl_{adapt} has not yet overcome, or

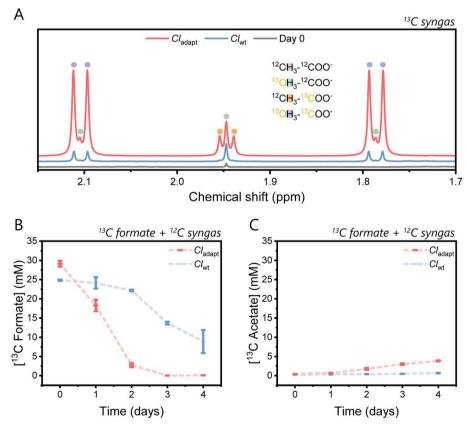


Fig. 2 Growth analysis of strains using 13 C- and 13 C/ 12 C mixed-substrates. (A) 1 H qNMR spectra showing acetate production by Cl_{adapt} and Cl_{wt} , when cultivated on 13 C-syngas (112 mL headspace) over 12 days. The isotopologues are color-coded based on the position of the 13 C atoms, as methyl or carboxyl, in acetate. (B) Consumption of 13 C-formate, and (C) production of 13 C-acetate, when cultivated on 13 C-formate (\sim 30 mM) alongside 12 C-syngas (112 mL headspace) over 4 days. Growth conditions: ATCC Medium 1754 (pH 5.9), 37 °C with 150 rpm mixing.

alternatively the carbonyl branch has been significantly enhanced during adaptation.

To investigate differences in the methyl branch of the Wood-Ljungdahl pathway between $Cl_{\rm adapt}$ and $Cl_{\rm wt}$, we performed a second isotopic labeling experiment using 13 C-formate and 12 C-syngas. $Cl_{\rm adapt}$ exhibited significantly faster growth, achieving a cell population five times greater than $Cl_{\rm wt}$ within four days (Fig. S9A†). $Cl_{\rm adapt}$ consumed \sim 25 mM of 13 C-formate within two days, whereas $Cl_{\rm wt}$ utilized only \sim 15 mM over four days (Fig. 2B), resulting in a markedly higher production of 13 C-acetate in $Cl_{\rm adapt}$ (3.86 \pm 0.11 mM vs. 0.66 \pm 0.06 mM) (Fig. 2C). This finding highlights $Cl_{\rm adapt}$'s enhanced efficiency in utilizing formate for such as growth and acetate production, reflecting optimized pathway dynamics compared to $Cl_{\rm wt}$. For further discussion, see ESI Note 2 (Fig. S9 and 10).†

Whole genome sequencing

We conducted whole genome sequencing analysis to investigate the genetic changes in $Cl_{\rm adapt}$ compared to $Cl_{\rm wt}$. Whole genome sequencing of $Cl_{\rm adapt}$ and $Cl_{\rm wt}$ revealed several mutations compared to the reference genome in the NCBI database (Table S6, S7 and Fig. S11†). Notably, some mutations were present in both strains, suggesting that the wild-type strain had already diverged from the reference genome prior to our study. Additionally, mutations identified exclusively in $Cl_{\rm adapt}$ may have emerged either through the ALE process or as random variations in the single colony selected for sequencing. Similarly, the presence of unique mutations in $Cl_{\rm wt}$ suggests possible genetic drift or reversion events. Given these observations, future studies should validate key mutations through Sanger sequencing of multiple colonies to distinguish true evolutionary changes from colony-specific variations.

Notwithstanding these limitations—including the restricted sample numbers and incomplete functional annotation of some C. ljungdahlii genes—the molecular differences observed between $Cl_{\rm adapt}$ and $Cl_{\rm wt}$ suggest that the strains are distinct at a genetic level. While these differences may partly explain the variations observed in growth and C_2 production, further studies are needed to establish a definitive link. For an in-depth analysis of the mutations differentiating $Cl_{\rm adapt}$ from $Cl_{\rm wt}$, please refer to ESI Note 3.

Photocatalytic domino valorization

To source syngas for microbial fermentation directly from solar CO_2 conversion, we scaled up a previously reported photocatalytic system composed of light-absorbing TiO_2 nanoparticles (P25) and a phosphonated cobalt(II)(terpyridine) $_2$ CO_2 reduction molecular catalyst (CotpyP, 20 μ mol $g_{TiO_2}^{-1}$; Fig. 3). 21,29 The choice of this photocatalytic semiconductor-molecule hybrid system was based on its suitability for scale-up to produce sufficient syngas quantities and coupling with aqueous microbial fermentation: it consists of Earth-abundant elements and readily available light absorber (TiO_2) and co-catalyst (CotpyP) components in the required quantities. The photocatalyst system is also robust and can be easily repaired by addition of fresh CotpyP (see Experimental Section). It is

therefore versatile and well suited for longer-term and multicycle experiments for aqueous CO₂ photoreduction under batch³⁰ and flow conditions to couple with biocatalysis (see below; Table S8†).

We readily scaled-up this hybrid TiO₂|CotpyP photocatalyst from 5 mg to 365 mg without a significant effect in its catalytic activity during photocatalysis in the presence of triethanolamine (TEOA, 0.1 M) as sacrificial electron donor in water (0.22 L) using filtered simulated solar irradiation (see Experimental Section). The photoreactor used for scaling-up is cylindrical, with a surface area of 217 cm² and a volume of 1.5 L (Fig. S2†). This photoreactor features a transparent top window that allows the simulated sunlight to photoexcite the semiconductor TiO₂ nanoparticles, leading to electron transfer from TiO₂ to the catalyst CotpyP. The reduced CotpyP reacts with aqueous CO₂ resulting in the formation and release of CO and H₂ gaseous products. Further details on the catalytic CO₂ reduction

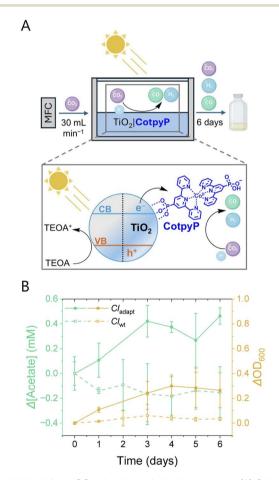


Fig. 3 Light-driven CO_2 domino valorization system. (A) Scheme of flow mode setup: the mass flow controller (MFC) purges CO_2 through the photoreactor, where $TiO_2|CotpyP$ generates photocatalytically syngas in CO_2 -saturated water containing TEOA (0.1 M). The photoreactor is irradiated by filtered simulated sunlight from the top; and the syngas output flows then through the bioreactor where bacteria are cultured. (B) Production of acetate and biomass by Cl_{adapt} (solid line) and Cl_{wt} (dotted line) in the bioreactor when connected to the photocatalytically produced syngas over 6 days, expressed as variation of acetate concentration and optical density (OD_{600}).

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mechanism of TiO₂-bound CotpyP can be found in a previous report.21 The positive charges (holes) formed in TiO2 are neutralized through the oxidation of the sacrificial electron donor TEOA present in the solution, which regenerates the original ground state of the semiconductor and allows the

Finally, we coupled the scaled-up synthetic photocatalytic CO_2 reduction system to the syngas-fermenting Cl_{adapt} , creating an inorganic-bacterial system capable of domino valorization of CO2 into acetate and biomass with solar energy (Fig. 3A). The aqueous TiO₂|CotpyP photocatalyst suspension (0.22 mL) was exposed to a flow of CO2 over six days (flow mode), mimicking the flow conditions described above for fermentation, and the photogenerated syngas was continuously flowed from the photoreactor to the bioreactor (Fig. 3A; see also ESI Note 4 for batch mode experiment details).

In flow mode, TiO₂|CotpyP produced syngas under irradiation and exhibited activities of 148 \pm 97 nmol CO $g_{TiO_2}^{-1}$ min⁻¹ and $367 \pm 606 \text{ nmol H}_2 \text{ g}_{\text{TiO}_3}^{-1} \text{ min}^{-1} \text{ (Table S9}^{\dagger}\text{)}$. The generated solar syngas had thus a CO: H_2 ratio of ~ 30 : 70 and TiO_2 | CotpyP under flow conditions produced 1.3 mmol of CO after 6 days of continuous experiment. Under dark conditions, TiO₂|CotpyP was unable to produce syngas. Compared to other state-of-the-art photocatalytic flow systems, such as ZnSe-BF4|cobalt porphyrin, which produces 19×10^{-3} mmol of CO after 17 h, 30 and Bi₂WO₆, which produces 1.2×10^{-2} mmol of CO after 4 h, ³¹ TiO₂|CotpyP exhibits a very high yield (Table S8†).

Syngas produced by $TiO_2|CotpyP$ allowed the Cl_{adapt} cultures to increase their biomass from the start of the flow experiment until day 4, reaching a ΔOD_{600} of 0.30 \pm 0.09, which remained approximately constant until day 6 (Fig. 3B). In contrast to the batch mode (Fig. S12†), the flow configuration showed a rise in acetate from day 0 to day 6, with a maximum Δ [acetate] of 0.46 \pm 0.07 mM (Fig. 3B). Under identical experimental conditions, $Cl_{\rm wt}$ exhibited significantly lower performance, with a $\Delta {\rm OD}_{600}$ of only 0.03 \pm 0.01 and no acetate production after 6 days. The lack of ethanol production, lower ΔOD_{600} , and Δ [acetate] for Cl_{adapt} can be attributed to the low syngas concentration (<0.1%) in the photocatalysis gas stream, as well as the slower formation rate by TiO₂|CotpyP compared to direct feeding with a premixed syngas cylinder. As shown in Fig. 1D, Cladapt achieves high biomass and C2 product outputs under 10 mL of syngas per minute (which equals to 102 μmol CO min⁻¹ and 41 μmol H₂ min⁻¹), highlighting the limitations of the presented photocatalytic configuration and the need for improved systems capable of delivering higher syngas formation rates to support Cl_{adapt} .

Our results demonstrate that simple photocatalytic powder systems can be readily scaled up and, in principle, be used to supply solar syngas directly to gas-fermenting adapted bacteria. This approach offers an alternative to wired devices such as electrolyzers and photoelectrochemical cells. 3,5,6,11 Furthermore, these findings highlight the need for innovative renewable energy-driven syngas production systems and device architecadvanced electrolyzers as electrochemical systems-for researchers in biohybrids and domino catalysis. Future systems should be designed to bridge the potential mismatch between syngas production and fermentation rates, maximizing the capabilities of gasfermenting adapted bacteria to sustain high levels of C2 production and cell growth over extended periods.

Conclusions

The reported proof-of-concept semi-biological system demonstrates the integration of photocatalytic CO2 reduction with adapted bacterial biosynthesis. Unlike previous nanomaterialbacteria biohybrid systems that relied on ill-defined charge transfer pathways or photogenerated H2, our approach employs direct ALE-derived adaptation to achieve high C₂ product yields solely from syngas produced from solar CO2 reduction. This work illustrates the scalability of photocatalytic powder systems and underscores the simplicity of implementing adaptive evolution in a chemistry laboratory, thereby enhancing the microorganisms' natural capabilities while seamlessly coupling them with inorganic catalytic systems. While our sequencing analysis of the ALE-derived strain has limitations—stemming from restricted sample numbers and incomplete functional annotation-it provides initial insights into the genomic changes that may underpin the observed phenotypic improvements. Future investigations will be essential to pinpoint the key genes responsible for faster growth and higher product yields, as well as to optimize these biological-inorganic hybrid systems by improving syngas formation rates, eliminating the sacrificial electron donor in the photocatalysis process, and advancing genetic and metabolic engineering strategies. Overall, this study highlights the transformative potential of combining innovations from biology, materials science, and chemistry to address critical global environmental challenges.

Data availability

Experimental data supporting the findings of this study are available from the University of Cambridge data repository (https://doi.org/10.17863/CAM.118249).

Author contributions

Lin Su conceptualization, data curation, formal analysis, funding acquisition, investigation, visualization, methodology, project administration, writing - original draft, review & editing; Santiago Rodríguez-Jiménez conceptualization, data curation, formal analysis, funding acquisition, investigation, visualization, methodology, project administration, writing - original draft, review & editing; Marion I. M. Short investigation, methodology, writing - review & editing; Erwin Reisner conceptualization, resources, formal analysis, supervision, funding acquisition, validation, investigation, visualization, project administration, writing - original draft, review & editing.

Conflicts of interest

There are no conflicts to declare.

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