Chem-Bio interface design for rapid conversion of CO₂ to bioplastics in an integrated system

The Chem-Bio interface design integrated electrocatalytic reduction of CO₂ to C₂ intermediates with bioconversion to valuable bioproducts. The chemical and biochemical advantages of C₂ intermediate and unique system design delivered a completely electrotrophic route for rapid CO₂ conversion to bioplastics, addressing a broad range of sustainability challenges.

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Highlights
CO₂ conversion via EMC2 intermediate
Soluble C₂ intermediates play critical roles in the efficient integration
Systematic design enables continuous and rapid conversion of CO₂ to bioproducts
EMC2 produces PHA with substantially improved productivity and product chain length

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Chem-Bio interface design for rapid conversion of CO\textsubscript{2} to bioplastics in an integrated system

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SUMMARY
Integrating catalytic CO\textsubscript{2} reduction with bioconversion could substantially advance carbon capture and utilization and mitigate climate change. However, the state-of-the-arts are limited by inefficient electron and mass transfers, unfavorable metabolic kinetics, and inadequate molecular building blocks. We overcome these barriers with the systematic design of electrocatalysis, chemical-biological interface, and microorganisms to enable efficient electro-microbial conversion with C\textsubscript{2} (EMC\textsubscript{2}) intermediates. The soluble C\textsubscript{2} intermediates can facilitate rapid mass transfer, readily enter primary metabolism, have less toxicity, carry more energy and electrons, and serve as better molecular building blocks for many microorganisms. The multi-tier Chem-Bio interface design delivered the EMC\textsubscript{2} system to achieve 6 and 8 times increase of microbial biomass productivity compared to C\textsubscript{1} intermediate and hydrogen-driven routes, respectively. The multi-module synthetic biology design produced medium-chain-length polyhydroxyalkanoates (PHAs), biodegradable polymers, representing much higher productivity and molecular chain length than the platforms based on C\textsubscript{1} intermediates, hydrogen, or electrons.

INTRODUCTION
The synthesis of fuels, chemicals, and materials from CO\textsubscript{2} is fundamental to human society.\textsuperscript{1} In the biosphere, plants, and algae convert CO\textsubscript{2} through photosynthesis to macromolecules and diverse chemicals to sustain their own growth and feed other heterotrophic organisms. However, facing the increasing CO\textsubscript{2} concentration caused by human activities, alternative CO\textsubscript{2} conversion platforms were developed to mitigate the global climate change and manufacture valuable industrial products.\textsuperscript{2} Even though chemical synthesis has successfully converted CO\textsubscript{2} into selective compounds (e.g., urea) at the commercial scale, the scope of diverse chemical compounds is still limited. Such challenge can be overcome via coupling electrochemical CO\textsubscript{2} reduction with novel biological routes to produce macromolecules, polymers, and multi-carbon commodity chemicals, which leverages the diverse biosynthesis pathways while circumventing the low efficiencies in photosynthesis.\textsuperscript{3–7} The fundamental scientific barriers are therefore the identification and utilization of optimal electron carriers and carbon building blocks to enable microbial carbon fixation and bioproduction.\textsuperscript{6}

Earlier work has established that CO\textsubscript{2} can be reduced to inorganic one-carbon (C\textsubscript{1}) intermediates such as CO or syngas at high Faradaic efficiency (FE) (Route 1 in
Figure 1A), which can be further fed to microorganisms for bioconversion. Although the anaerobic CO-utilizers are energy-efficient, the route is limited by the low gas-to-liquid transfer rate, the inadequate electron-carrying capacity of CO, limited strain choices and product profiles, as well as the often slower biomass growth and bio-production rates as compared to heterotrophic industrial microorganisms such as Pseudomonas putida (Figure 1A). To overcome some of these challenges, prior work has established that CO2 can be electrocatalytically converted to soluble C1 electron carriers like formate for bioconversions (Route 2A in Figure 1A). However, formate contains low energy content as compared to methanol and ethanol due to its chemical structure. C1 assimilation pathways like serine cycle can improve the formate utilization efficiency. The recently proposed synthetic reductive glycine pathway (rGlyP) could further improve ATP generation efficiency and empower formate to be used as a substrate for bioproduction (Figure 1; Table S1). Nevertheless, due to the inherent low energy content, formate’s energy and electron-carrying capacity remains much less as compared to methanol and ethanol according to previous analysis, regardless which pathways to go through (Table S1). In particular, the relatively low energy content and limited electron capacity (Figure 1) make formate a less optimized substrate for aerobic fermentation. As compared to formate, methanol carries more electrons and energy content per carbon (Route 2B in Figure 1A). However, the use of methanol as the electron carrier remains challenging. On one hand, the kinetics using NAD+ as the electron acceptor is often low due to the high redox potential of methanol oxidation. On the hand, even though using quinone-dependent methanol dehydrogenase (MDH) can improve the kinetics, it lowers the overall energy efficiency due to the energy dissipation by quinones. To overcome these challenges, an alternative Route 2 is developed leveraging the cell-free system, where chemical catalysis is coupled with enzymatic catalysis to covert CO2 to starch. The chemo-enzymatic catalytic system advances the novel enzyme pathway design yet fails to achieve an integrated and continuous production. Moreover, the high temperature and harsh conditions in chemical catalysis are incompatible with biocatalysis, which leads to disjointed chemical and biological syntheses. The enzyme catalysis steps by themselves are also separated, and the enzyme activity can only last for a few hours. Besides the catalytic CO2 reduction, another route relies on the direct or indirect usage of electrons from an electrode by microbes to reduce CO2 as the substrate in bioconversion (Route 5 in Figure 1A). Even though studies have been carried to improve the energy efficiency in Route 5, the process typically suffers from a limited electron transfer rate between the electrode and microorganism, and the products are often short-chain small molecules of limited applications, as the biochemical process often relies on the Wood-Ljungdahl pathway (W-L pathway). The system thus requires large surface area with low volumetric productivity and is limited to selective microorganisms such as chemolithotrophic, exoelectrogenic, and acetogenic microbes. Furthermore, another route (Route 6, Figure 1A) to bypass the catalytic CO2 reduction utilizes electrochemically generated hydrogen as electron carrier to drive CO2 conversion in Ralstonia eutropha (also known as Cupriavidus necator). However, the carbon fixation rate, titer and production efficiency of hydrogen-driven fermentation are fundamentally limited by gas-to-liquid transfer rate, the slow hydrogen oxidation, Calvin-Benson cycle intermediate regeneration, and RuBisCo carbon fixation. The choice of microbial species in Route 6 is also limited due to the hydrogenotrophic growth. Recent advances in electrochemical CO2 reduction to C2 products using copper-based catalysts have opened new avenues to address the challenges in the previous
Chem-Bio routes for CO$_2$ fixation. Even though some C$_2$ products like ethylene (Route 3 in Figure 1A) are not amenable to bioconversion, others like ethanol (Route 4A in Figure 1A) and acetate (Route 4B in Figure 1A) can serve as substrates for broader bioconversion application. Both compounds carry more energy and have better electron donation capacity than CO and formate. The acetate and ethanol both can be converted to acetyl-CoA with two to three steps and enter the metabolic pathway consisting of carbon source assimilation and primary metabolic pathways of the microorganisms. Acetyl-CoA was generated from the assimilation of electrochemically reduced intermediates, then enters downstream primary metabolic pathways. Route 3 produces ethylene. Even though renewable ethylene is a valuable commodity product with broad chemical applications, it is not compatible with biological systems. The calculation of net ATP molecules or net reducing equivalents was based on the synthesis of one acetyl-CoA and the carbon loss during that process. The details were illustrated in Table S1 and Schemes S1–S9. The net ATP generated per carbon is calculated by dividing the total net ATP utilized/generated in acetyl-CoA synthesis by the carbon numbers in the substrates. The total net ATP is the sum of the net ATP and the net reducing equivalents, assuming that one molecule of NAD(P)H is equivalent to three ATP. The details were illustrated in Table S1 and Schemes S1–S9.

Figure 1. The schematic illustration of different routes for electro-microbial CO$_2$ conversion and the systemic design of EMC2 system

(A) Schematic of electro-microbial CO$_2$ conversion routes and their comparisons. Arrows indicate the conversion process, a red cross mark $\times$ means the electrochemically reduced product cannot be utilized as the carbon source for bioconversion. Intermediates (examples) were listed in the parentheses with each route. W-L pathway, Wood-Ljungdahl pathway, rGly pathway, reductive glycine pathway, RuMP cycle, ribulose monophosphate cycle, CBB cycle, Calvin-Benson-Bassham cycle. $\ddagger$ The metabolic pathway consists of carbon source assimilation and primary metabolic pathways of the microorganisms. Acetyl-CoA was generated from the assimilation of electrochemically reduced intermediates, then enters downstream primary metabolic pathways. $\ddagger\ddagger$ Route 3 produces ethylene. Even though renewable ethylene is a valuable commodity product with broad chemical applications, it is not compatible with biological systems. $\ddagger\ddagger\ddagger$ The calculation of net ATP molecules or net reducing equivalents was based on the synthesis of one acetyl-CoA and the carbon loss during that process. The details were illustrated in Table S1 and Schemes S1–S9. * The net ATP generated per carbon is calculated by dividing the total net ATP utilized/generated in acetyl-CoA synthesis by the carbon numbers in the substrates. The total net ATP is the sum of the net ATP and the net reducing equivalents, assuming that one molecule of NAD(P)H is equivalent to three ATP. The details were illustrated in Table S1 and Schemes S1–S9. $\ddagger\ddagger\ddagger\ddagger$ Data are from the NIST webbook. $\ddagger\ddagger\ddagger\ddagger$ The scoring of mass transfer is a comprehensive evaluation of gas-to-liquid transfer, substrates’ solubility and accessibility to microbes. $\ddagger\ddagger\ddagger\ddagger\ddagger$ The scoring of biocompatibility considered the phylogenetic distribution of the assimilation pathways in each route. $/ \ddagger\ddagger\ddagger\ddagger\ddagger$ The item is not applicable.

(B) Four-tier design in EMC2. Arrows indicate the reaction and mass transfer from CO$_2$ to C$_2$ intermediates. The CO$_2$-derived intermediates enter either the primary metabolism to support the cell growth or the biosynthetic pathways to generate bioproducts (polyhydroxyalkanoates [PHAs], in this study.)
primary metabolism rapidly to serve as energy sources and metabolic building blocks (Route 4, Figure 1A). The fewer steps and favorable kinetics to convert C2 intermediates to acetyl-CoA empower these molecules to be better molecular building blocks for the synthesis of bioproducts with longer carbon chain. The metabolic kinetics of C2 intermediates could enable a new electro-microbial system with much faster conversion, more rapid cell growth, higher bioproduct yield and titer, broader species adaptation, and better biocompatibility than the current systems (Routes 1, 2, 5, and 6 in Figure 1A; Table S2). Even though previous studies achieved multi-step microbial systems using C2 intermediates produced from bioconversion,25–27 the direct electrocatalytic CO2 reduction to C2+ products allows us to maximize the benefits of electrocatalytic step for electron carrying, improve the system efficiency, and drastically increase bioproduction rate. Despite the potential, such integration between electrocatalysis and microbial conversion has not been achieved before. No study has established the scientific concept of such a new system, demonstrated feasibility, or overcame the challenges in Chem-Bio interface to achieve integrated and continuous production. In this study, we have designed and implemented a novel route (Route 4 in Figure 1A), namely electro-microbial conversion with C2 (EMC2) intermediates. EMC2 achieved seamless and systematic integration of electrocatalysis and bioconversion at chemical, cellular, and process levels, enabling continuous production. With this system, we have demonstrated the efficient electro-microbial CO2 conversion using a broadly adopted industrial microorganism, Pseudomonas putida. The innovative EMC2 platform achieved significantly improved cell biomass growth and bioproduct productivity as compared to other state-of-the-art routes (Routes 1, 2, 5, and 6) and photosynthetic systems. The study highlighted the broad applicability of the EMC2 system in carbon fixation and conversion to macromolecule products.

Electrocatalytic system design to empower EMC2

Even though acetate and ethanol are much better electron carriers and carbon intermediates than C1 compounds (Figure 1A), an efficient EMC2 system requires a four-tier design to achieve the integration of electrochemical CO2 reduction reaction (CO2RR) and the microbial conversion (Figure 1B). The first tier focuses on electrocatalysis, where the design and selection of electrolyzer, catalysts, and electrolytes need to ensure the efficient electrocatalytic CO2RR to C2 products under biocompatible conditions. The second tier is the design of Chem-Bio interface to make sure that microbial conversion does not interfere with CO2RR (Figure 1B). The third tier is the design of an integrated EMC2 system to achieve efficient mass transfer of C2 products between electrocatalysis and fermentation (Figure 1B). The fourth tier is the microbial design to efficiently channel the C2 compounds to the tricarboxylic acid (TCA) cycle and to enhance the carbon flux toward target bioproducts (Figure 1B). The four-tier design will achieve complete and efficient electrotrophic microbial conversion of CO2, where ethanol and acetate will carry the electrons and carbon from electrocatalytic CO2RR to drive bioconversion.

As the starting point to convert CO2 to C2 intermediates, electrocatalysis design is critical to generate sufficient C2 products for microbial conversion under bioamicable conditions. These constraints require the integrated design and selection of electrolyzer, electrolytes, and catalysts (Figure 2A). First, a flow electrolyzer equipped with gas diffusion electrodes (GDEs) was selected for C2 production, considering the potentially high yield of C2 products (Figure 2A). We found that flow cells with GDE setting can achieve much higher current density and C2 product content than the conventional electrocatalysis with H-cell, as the CO2 feeding to GDE is not limited.21,28
Figure 2. GDE catalysts morphology and CO$_2$RR performances under different conditions

(A) Schematic illustration of CO$_2$ flow electrolyzer and gas diffusion electrodes.

(B) *P. putida* growth comparison in basal solution and bicarbonate solution. Error bars represent the deviation of three replicate experiments.

(C) SEM image of the Cu/PTFE GDE (scale bars, 2 μm).

(D) SEM image of the Cu/28BC GDE (scale bars, 2 μm).

(E) XRD patterns of Cu/PTFE and Cu/28BC. The peak at 55° on Cu/28BC is the diffraction peak from carbon paper.

(F) CO$_2$RR performances of Cu catalytic GDEs in the flow electrolyzer using different solutions. The products are sorted in three categories: other gaseous products (H$_2$, CO, and CH$_4$), main gaseous product (C$_2$H$_4$), and liquid products (formate, ethanol, acetate, and propanol). Left group: Cu/PTFE in bicarbonate solution. Middle group: Cu/PTFE in basal solution. Right group: Cu/28BC in basal solution. Left Y scale: Faradaic efficiencies of various products. Right Y scale: potentials referred to the reversible hydrogen electrode (RHE) at the current densities ranging from 100 to 200 mA cm$^{-2}$. Error bars represent the standard deviation of potential readings during the constant-current electrolysis.
Second, the selection of electrolytes is critical. When the flow electrolyzer is used for CO$_2$RR, the whole Chem-Bio system will operate in a cascade mode with salt solution flows between the electrocatalysis and bioconversion. The same salt solution will serve as the catholyte for electrocatalysis and the media to support microbial growth. The electrolyte thus needs to be suited for both electrocatalysis and cell cultivation. Frequently used CO$_2$RR electrolytes such as extreme alkaline solutions are not amenable to microorganism growth. Considering that the microbes need a neutral and stable pH environment, we investigated the CO$_2$RR reaction in two solutions. The first one is the bicarbonate solution that contained mixed NaHCO$_3$ and KHCO$_3$ with Na/K ratio suitable for microbial growth and also was proven effective for CO$_2$RR. The second one is a “basal” solution mainly composed of phosphate buffer (NaH$_2$PO$_4$ + K$_2$HPO$_4$ + NaCl), which is widely used in fermentation, but not electrocatalysis. We have maintained both solutions at pH close to 7 to allow bioconversion integration, and the total ion concentrations in both solutions are kept the same to minimize the differences of resistance. To evaluate bioconversion compatibility, we first compared the microbial growth in the two electrolytes using ethanol as the primary carbon source. Although the pH of the bicarbonate solution was 7.2 (with CO$_2$ and air bubbling), the cell growth of *P. putida* remained to be minimal after 72 h. While in the basal solution, the *P. putida* had shown significant growth within 24 h (Figure 2B). We therefore prioritized the basal solution as the electrolyte and compared the catalytic performances in both bicarbonate solution and basal solution to identify the best catalysts.

Third, the design and configuration of catalysts are critical for C$_2$ productivity, especially in biologically compatible conditions. We first investigated several typical CO$_2$RR catalysts including CuO, Cu$_2$O, and Cu–B (copper reduced by NaBH$_4$), yet none of them achieved appreciable ethanol or C$_2$ productivity when the basal solution was used as the electrolyte. These catalysts showed higher production of hydrogen, formate, and other C$_1$ compounds (Figure S1). It is likely that these catalysts will undergo reduction and surface reconstruction in the electrocatalysis process. The strongly coordinating phosphate anions in the electrolyte could interact substantially with copper species in the catalysts and alternate their surface properties, which favor the generation of C$_1$ products such as formate as well as hydrogen. Based on these discoveries, we hypothesized that the metallic copper-based catalysts could maintain better catalytic properties under phosphate electrolyte, as metallic copper has more stable surface properties than the oxide-based or chemically pre-reduced copper catalysts.

Besides the catalyst, another important consideration is the suitable configuration of the cathode in a flow cell to achieve sufficient gas diffusion. The sputtering method thus was adopted to direct deposit Cu catalysts on gas diffusion materials, as the method allows direct coating of a thin layer of metals on the substrates, forming a continuous and conductive layer. Moreover, the sputtering method enables the selection of various porous substrates as gas diffusion layers (GDLs), including both conductive and non-conductive ones, for building the GDE. Two Cu-based GDEs were designed by sputtering Cu on porous polytetrafluoroethylene film (PTFE film, the derived GDE catalyst is denoted as Cu/PTFE) and carbon paper (Sigracet 28 BC, the derived GDE catalyst is denoted as Cu/28BC), respectively. Scanning electronic microscope (SEM) images confirmed that the Cu layers were uniformly coated around the GDLs (Figures 2C and 2D). Although their morphologies vary due to the original substrates, powder X-ray diffraction (XRD) analysis showed that the ratios of Cu (111) to Cu (100) facets were the same on the two copper GDEs.
(Figure 2E), proving that the sputtering method is reliable and repeatable for fabricating catalytic electrodes.

We then evaluated the CO2RR performances of these cathode designs in biocompatible phosphate electrolytes (basal solution) and electrocatalysis-favorable bicarbonate electrolytes. Figure 2F shows the FE for CO2RR products in the two electrolytes at the current densities ranging from 100 to 200 mA cm\(^{-2}\), which are comparable to industrial relevant current densities. In both electrolytes, Cu-based catalytic GDEs showed significant CO\(_2\) conversion to various C2 products. The main soluble C2 product was ethanol, while a small amount of acetate and 1-propanol was co-produced. Compared to the bicarbonate solution (left group, Figure 2F), the reaction in phosphate-based solution showed a higher FE of ethylene production but a lower concentration of the soluble C2 (middle group, Figure 2F). However, the total FE for soluble C2 products still reached around 15\%, indicating that CO\(_2\)RR in phosphate electrolytes could produce sufficient C2 intermediates for subsequent fermentation. Considering that \textit{P. putida} grows much better in the basal solution (Figure 2B), the basal solution was selected as the primary electrolyte for CO\(_2\)RR.

With the selection of phosphate-containing basal solution as the electrolyte, we then compared the catalytic performances of the two types of Cu electrodes with different GDLs. Although the electrodes were fabricated in the same manner, the Cu/PTFE electrode showed much better CO\(_2\)RR properties than the Cu/28BC. As shown in Figure 2F, the total FE for CO\(_2\) reduction products and the FEAs for C2 products of Cu/PTFE electrode are both higher than those of Cu/28BC. On the contrary, the hydrogen generated on Cu/28BC is almost twice of Cu/PTFE (right group, Figure 2F). The hydrogen production is not a significant challenge in the previous systems utilizing hydrogenotrophic species (i.e., \textit{Ralstonia}), where hydrogenotrophic CO\(_2\) fixation and formate electron donation probably both contributed to the cell growth.\(^7\) However, for EMC2, the hydrogen production would reduce the overall efficiency of CO\(_2\)RR and C2 intermediate concentration for bioconversion, leading to reduced cell growth rate, bioconversion productivity, and overall system efficiency. Moreover, excessive hydrogen evolution reaction (HER) could lead to the decay of GDE performance, which affects the long-term catalyst stability.\(^{32}\)

The Cu/PTFE thus represents a better electrode than Cu/28BC, and the design addresses the challenges of phosphate buffer utilization to empower the possible integration of electrocatalysis and bioconversion. As aforementioned, phosphate-based basal solution has been shown to reduce the local pH at the electrode/electrolyte interface, creating an environment favorable for hydrogen production but unfavorable for CO\(_2\)RR.\(^{33}\) Nevertheless, we showed that the Cu/PTFE electrode, GDE configuration, and phosphate-based electrolyte could together achieve efficient CO\(_2\) reduction to multi-carbon products, especially soluble C2 products (Figure 2F). The strength of Cu/PTFE electrode lies in the constant hydrophobicity achieved by design. We investigated the contact angles of phosphate-based basal solution on Cu/PTFE and Cu/28BC before and after the CO\(_2\)RR (Figure S2). Both electrodes appeared hydrophobic with contact angles >110\(^\circ\) before reaction. After the reaction, the Cu/PTFE remained hydrophobic with a slightly reduced contact angle. However, the Cu/28BC turned totally hydrophilic, as the basal solution could easily spread on the surface and soak the whole electrode. This difference is attributed to the variation of structures between the two GDEs. In the Cu/PTFE, the electroconductivity totally relied on the continuous copper layer. The intrinsic hydrophobicity of PTFE could keep the GDL hydrophobic throughout the reaction process and provide paths for CO\(_2\) to transport to the copper layer. On the contrary, carbon-based
GDL (e.g., 28BC in this case) reduced the hydrophobicity under electricity and thus could be flooded. In turn, the phosphate anions in the basal solution will increase the hydrophilicity of the copper layer, causing a more severe flooding issue, which was particularly detrimental to the GDE setup.

Overall, the systemic design and integration of catalysts, electrolyzer, and electrolyte have enabled the Cu/PTFE GDE to perform electrochemical CO₂RR in biocompatible conditions with efficient C₂ productivity, which will empower the new EMC2 system to integrate electrocatalysis and microbial conversion.

Chem-Bio interface design to achieve compatibility between electrocatalysis and bioconversion

While the phosphate electrolyte serves as the basal solution for microbial growth, the efficient microbial growth and fermentation will require nitrogen source (ammonia), minerals, vitamins, and trace elements known as Goodies (see supplemental information for the detailed formula). These elements inevitably impact electrocatalysis. It is thus critical to evaluate and minimize the impact of bioconversion media on electrocatalysis. In fact, compared with the CO₂RR on Cu/PTFE with the basal solution, the CO₂ reduction was drastically suppressed when the whole medium (basal + nitrogen source + Mg/Ca/B₁/Goodies) was used as the electrolyte. Meanwhile, the HER became the primary reaction (Figure 3A). The significant change indicated that the minor species in the solution had a significant impact on the electrocatalytic performances.

The chemical mechanism behind such changes could be the competing electrocatalytic reaction by the minor cation species in the whole media. In the CO₂RR electron consumption process, a corresponding number of hydroxides (OH⁻) would be generated at the interface between electrode and electrolyte as the result of CO₂ and water reduction. When the whole medium was used as the electrolyte, the mineral cation species could react with the generated OH⁻ and form precipitates on the electrode surface (Figure 3B), which would limit the CO₂RR efficiency and cause HER. The SEM image of the electrode after the reaction highlighted multiple insoluble species precipitated on the electrode surface (Figure 3B). The energy dispersive spectroscopy (EDS) analysis further confirmed that most of the metal elements in the Mg/Ca/B₁/Goodies mixture presented in the insoluble precipitates and deposits on the electrode surface (Figure S3). Although these elements were “trace” elements in the culture medium, the amount turned to be significant as compared to copper catalyst when they precipitated on the electrode surface. The precipitated chemical species thus changed the copper electrode property and blocked the CO₂ reduction. Moreover, the species reduced the electrode hydrophobicity, which was an important consideration for GDEs to conduct CO₂RR.

To overcome this challenge, a membrane-integrated cathode was designed by installing an anion exchange membrane (AEM) on the Cu/PTFE catalyst (Figure 3C). The AEM masking served two purposes. First and foremost, AEM would effectively prevent the mineral cations in the whole medium from contacting the catalyst surface and deposition, which in turn maintained the CO₂RR efficiency at the electrode. The SEM image confirmed the effectiveness of the design, as no deposition/precipitation occurred on the electrode when AEM was applied (Figure 3C). In the same way, EDS analysis showed no mineral elements on the Cu surface (Figure S4). Second, the AEM membrane allowed the soluble C₂ products to efficiently cross over the membrane and enter the flowing whole medium as a continuous carbon source for microbial fermentation. Among the products, anionic species, such as
acetate, can actively transport to the medium side based on the applied potential. Other neutral organic molecules (i.e., ethanol and 1-propanol), are predominantly transported by the electroosmotic potential. The thin AEM (24.50 μm) with full hydration also facilitated the transport (Figure 3C). When investigated the CO2RR performance on the AEM-integrated Cu/PTFE electrode. The results highlighted that the membrane-integrated electrode effectively protected the catalyst (Figures 3A and 3D). The CO2RR performance was restored in the integrated electrode when using whole medium (basal solution + nitrogen source + Mg/Ca/B1/Goodies) as the electrolyte. The FE values of ethanol and total soluble C2 reached back to 16.1% and 24.9% at 100 mA cm⁻², respectively, which were comparable to the simple phosphate solution under the same conditions (Figure 3A, right). In addition, the AEM-integrated cathode demonstrated remarkably high stability and even better performance in soluble C2 production than the original Cu/PTFE without AEM. At the total current density of 100 mA cm⁻², the cathode could remain the constant.

Figure 3. Effect of AEM masking on cathode for CO2RR using whole culture medium as electrolyte
(A) CO2RR performances of Cu/PTFE without (left, clear background) and with (right, colored background) AEM masking. Basal solution and whole culture medium are used as electrolytes for comparison.
(B) Illustration of insoluble species deposition on cathode surface when the whole culture medium (blue) directly contacts the electrode. SEM image shows the electrode surface after the reaction (scale bars, 2 μm). Arrows pointed to the insoluble species.
(C) Illustration of AEM (yellow) masking of the cathode. SEM image shows the electrode surface after the reaction (scale bars, 2 μm).
(D) Long-term stability at total current −400 mA (electrode area: 4 cm², current density −100 mA cm⁻²) with AEM masking. The whole culture medium is used as the catholyte. Left Y scale: whole-cell voltage of the two-electrode cell. Right Y scale: Faradaic efficiencies of ethanol (blue dot) and total soluble C2 (pink). The whole-cell voltage was illustrated without iR compensation.
performance for over 72 h. The FE for ethanol remained higher than 15%, and the FE for soluble C2 was higher than 20% (Figures 3D and S5). The excellent stability could be attributed to the blocking of mineral species by the AEM, the stable electrode-membrane interface of such a “zero-gap” design, and the hydrophobicity of PTFE layer. Most importantly, high stability is essential for biological systems integration since industrial-scale bioconversion usually lasts for long hours or days. Overall, the unique design of membrane-integrated electrodes paved the path for integrated and continuous electro-microbial conversion.

**Design of an integrated EMC2 system**

The successful electrocatalytic system design lead off the assembly of an integrated EMC2. However, the integration of the electrolyzer and fermenter needs to avoid the interference of microbial cells on catalysis while maintaining efficient mass transfer. We designed a tandem system consisting of the CO2 electroreduction unit (flow electrolyzer) and the microbial fermentation unit (Figure 4A). The cell culture medium circulated between the two units to deliver the soluble C2 intermediates generated from GDE for microbial fermentation. The detailed electrolyzer configuration is illustrated in Figure S6. Different from previous works based on solid
electrolyte and pure water, the EMC2 route requires the liquid electrolytes to serve as microbial media. Therefore, a flow-through design was implemented to allow the cultivation medium to fill the middle chamber of the dual membrane electrolyzer. Thus, the cell culture medium not only provided ion conductivity and electrolyte but also maintained the whole system in a closed circuit. On the cathode side, C2 products from CO2RR crossed the cathode AEM membrane to enter the culture medium in the middle chamber. On the anode side, water oxidation is conducted, where protons are also transported to the middle chamber. Ni foam was selected as the catalyst for water oxidation under basic conditions and a bipolar membrane (BPM) is employed to separate the anode and the middle chamber. Compared to the water oxidation under neutral or acidic conditions, multiple transition-metal-based catalysts could be used for water oxidation under alkaline conditions. The use of nickel catalyst significantly reduced the catalyst cost as compared to iridium- or ruthenium-based catalyst for acidic and neutral water splitting, which improved the system economic feasibility. The bipolar membrane effectively blocked the transmission of the liquid product from the middle chamber to the anode side, therefore maximizing the utilization of the CO2RR product.

The electrolyzer and the fermentation containers were connected by plumbing a pump to circulate the whole cultivation medium between the two compartments. At the initial stage, we discovered that the microbes do not grow when they are circulated together with the medium, probably due to the shear force in the circulation and the harsh environment in the middle chamber of electrolyzer. A two-chamber design with a filter membrane was implemented to keep the microbes in the fermentation container (Figure 4A). The size of the fermentation container needs to match the electrolyzer and catalytic performance, as too low concentration of substrate could limit the cell. Based on the electrocatalysis performance (Figure 3D, FE ethanol = 15%, Jtotal = 100 mA cm−2, electrode area = 4 cm2), we set the volume of fermentation as 30 mL with an additional 15 mL for reservoir and circulation. The integrated EMC2 system allows the concentration of carbon source to maintain above 2.0 g/L (Figure 4C), enabling rapid cell growth.

**Integrated EMC2 system achieved record cell growth rate**

A common industrial microorganism, *Pseudomonas putida* KT2440 was selected to demonstrate the effectiveness of C2 molecules as intermediate for electro-microbial conversion of CO2. As one of the first industrial strains in microbial production, *P. putida* has been engineered to utilize various substrates to produce a variety of commercial products. The use of an established industrial strain demonstrated the potentially broad applicability of the EMC2 system for biomanufacturing. Even though *P. putida* KT2440 can tolerate various harsh environmental factors, the wild-type strain did not grow well on the whole media containing CO2RR products as carbon sources (Figure 4B). The slow growth could be due to two reasons. First, to compensate the ohmic loss in the electrolyzer, we used a higher concentration of phosphate salt in the whole medium than that in the normal fermentation conditions (Table S3). Second, the short-chain organic substrates, such as ethanol, acetate, and propanol are still not optimal for bioconversion.

To overcome these barriers, we carried out adaptive evolution to improve the growth of *P. putida* KT2440 on C2 intermediates generated from CO2RR. The whole medium containing soluble electrocatalysis products (ethanol, acetate, propanol, and formate) (Figure 2F) were used to grow *P. putida* KT2440 for multiple generations. As shown in Figure 4B, the adaptive evolution significantly improved microbial growth on the CO2RR product mixture in the phosphate-based whole medium and
reduced the lag phase. Compared to the wild-type strain, the adapted strains reached the plateau phase within 24 h (Figure 4B), similar to the growth curves on other substrates (i.e., glucose, xylose, lignin, and aromatics).\(^{39}\) Besides ethanol and acetate, other soluble compounds such as formate and 1-propanol were also generated in CO\(_2\)RR (Figure 3A). We investigated their impact on the microbial cell growth and fermentation. Both formate and 1-propanol could be consumed by \textit{P. putida} as sole carbon source. Formate cannot support efficient cell growth alone due to the lack of assimilatory metabolism in the \textit{P. putida} KT2440. However, formate in CO\(_2\)RR product mixture could be rapidly consumed, and thus could contribute to the growth as an electron donor in the presence of other C2+ products through co-catabolism (Figure S7).\(^{42}\) Propanol can support cell growth as the sole substrate in a similar way as ethanol and acetate. It is expected that propanol, ethanol, and acetate can serve as both electron donor and molecular building blocks, enabling rapid cell growth and bioproduct accumulation. The adapted strains enabled the EMC2 system to achieve appreciable microbial growth and rapid carbon fixation using the C2 intermediates produced from CO\(_2\)RR.

The adapted \textit{P. putida} KT2440 was then introduced into the integrated EMC2 system to achieve continuous CO\(_2\) fixation and microbial biomass accumulation. We followed a semi-continuous cultivation strategy to achieve relatively constant C2 product consumption (Figure 4C).\(^{43}\) The semi-continuous growth cycles were repeated for four rounds to demonstrate the stability of the integrated EMC2 system. Both the electrocatalysis and cell growth showed outstanding stability throughout the fermentation, as indicated by the stable C2 concentration and OD\(_{600}\) increase (Figure 4C). The results suggested the great potential for continuous fermentation over an even longer period, as the C2 intermediates production and bacterial consumption rates reached equilibrium. The system equilibration again highlighted the importance of achieving optimal fermentation volume and electrocatalytic reaction rate in designing an integrated EMC2 system.

The EMC2 design thus showed significant advantages over the other state-of-the-art. In particular, EMC2 achieved a record microbial growth rate of 0.105 OD\(_{600}\)/h (Figure 4C). As compared to similar studies in Route 2 and Route 6, the EMC2 achieved a significantly higher growth rate (Figure 4D; Table S4).\(^{7,18}\) For instance, it is 6 times of the previous studies using formic acid as the electron donor and is eight times faster than the hydrogen-driven \textit{Ralstonia} CO\(_2\) fixation.\(^{7,18}\) Since there is no other carbon source in the original culture medium, the growth of heterotrophic \textit{P. putida} fully depends on the products generated from electrochemical CO\(_2\)RR. EMC2 thus represents a completely eletrotrophic microbial growth system, where the electrons are provided by electricity, and the CO\(_2\)RR products serve as both electron carriers and carbon sources for cellular growth. The excellent cell growth benefited from using soluble C2 intermediates that are much more amenable to bioconversion, not toxic to the cell, and not limited by gas-to-liquid transfer, which helped to overcome the metabolic, mass and electron transfer barriers in the current state-of-the-art.

**Microbial design to achieve efficient production of value-added products from CO\(_2\) in EMC2**

The superior cell growth of EMC2 (Route 4, Figure 1) opened new avenues for advancing innovative biomanning platforms with CO\(_2\) as the feedstock to produce diversified commodity chemicals. We demonstrated such electrons-to-molecules (E2M) route by producing biodegradable plastics from CO\(_2\) to maximize carbon footago reduction and mitigate the global plastics pollution as
approximately 300 million tons of environmentally persistent petrochemical plastics waste were produced annually with about 80% disposed into the environment.\textsuperscript{44} CO\textsubscript{2} to biodegradable plastics represents an ideal solution for a sustainable economy, addressing multi-facet challenges. Polyhydroxyalkanoates (PHAs) serve as an ideal commodity polymer to produce with the EMC2 system due to its favorable biodegradability and mechanical properties. In particular, the medium-chain length (mcl-) PHA has the unique advantage of being less brittle as compared to the short-chain length (scl-) products such as polyhydroxybutyrate (PHB). Even though previous research has shown the possibility of producing PHB from CO\textsubscript{2}, the production rate, product structure and properties are less optimal for petrochemical plastics replacement.\textsuperscript{18} We hereby demonstrated a viable CO\textsubscript{2}-to-mcl-PHA production route using the EMC2 system, leading to far-reaching environmental impact for carbon emission reduction and plastic pollution mitigation.\textsuperscript{45}

Despite the significance, the short-chain C2 intermediates produced by CO\textsubscript{2}RR remained to be less optimal substrates for PHA production in the wild-type \textit{P. putida}.\textsuperscript{40} As compared to longer chain organic acids, \textit{P. putida} accumulates...
much lower PHA content with acetate as the substrate (Figure S8). Similarly, PHA content only achieved 6.5% of dry cell weight (DCW) (Figure 5B), when the wild-type P. putida grows on the ethanol substrate. We, therefore, addressed the metabolic barriers for low PHA productivity with synthetic pathway design (Figure 5A). The mcl-PHA in bacteria can be synthesized in three pathways: the de novo fatty acid synthesis, the fatty acid β-oxidation-dependent pathway, the acetoacetyl-CoA pathway. Ethanol or acetate can be metabolized into acetyl-CoA with a few steps and enter the de novo PHA synthesis pathways. Furthermore, when growing on less optimal organic substrates, P. putida cells could activate the fatty acid β-oxidation pathway. The channeling of carbon flux from fatty acid β-oxidation to PHA synthesis has been proven to be effective in increasing PHA yield from less optimal substrates like lignin. Moreover, PHA can be readily degraded to support cell growth on less optimal substrates, leading to reduced PHA productivity. The synthetic pathway design thus needs to consider all four aspects, including ethanol and acetate conversion to acetyl-CoA, PHA de novo synthesis, PHA synthesis from fatty acid β-oxidation products, and the prevention of PHA degradation (Figure 5A).

A four-module design was therefore carried out to achieve efficient conversion of C2 products to PHA. We first knocked out the phaZ gene encoding the PHA depolymerase to minimize the PHA degradation, considering that acetate and ethanol are not optimal substrates for PHA accumulation. As a result, the phaZ deficiency strain ΔphaZ reduced the PHA degradation and facilitated PHA accumulation when growing on ethanol substrate in an electrocatalysis compatible medium (Figure 5B). More than 2 times increase of PHA accumulation was achieved in the ΔphaZ strain, proving the effectiveness of phaZ knockout. Second, in order to promote the ethanol and acetate conversion into acetyl-CoA, the gene encoding the acetyl-CoA synthetase (i.e., the acs gene) was overexpressed to enhance C2 product assimilation and conversion. Third, a key enzyme in PHA de novo synthesis, phaG (acyl-transferase), was overexpressed together with acs to enhance the acetyl-CoA conversion to PHA precursor (R) 3-hydroxyacyl-CoA (Figure 5A). Even though we hypothesized that the integration of the second and third design steps will couple the ethanol and acetate conversion to the PHA de novo synthesis, the over-expression of both of acs and phaG in the phaZ knockout strain (i.e., the ΔphaZ-acs-phaG strain) did not increase the PHA accumulation as expected (Figure 5B). The phenomena could be due to the reduction in storage compound synthesis when substrates are not optimal. Fourth, based on the discovery, we therefore leveraged our previous knowledge in channeling the fatty acid β-oxidation products into PHA synthesis under less optimal growth conditions to overexpress phaJ4 and phaC1. In order to achieve the carbon flux balance, we still overexpressed acs and phaG genes and used phaZ knockout as the background strain to engineer the ΔphaZ-acs-phaG-phaC1-phaJ strain. The ΔphaZ-acs-phaG-phaC1-phaJ4 indeed achieved a 31.6% increase in PHA productivity as compared to ΔphaZ strain (Figure 5B). Furthermore, the strain also achieved a 3-fold increase of PHA content on ethanol substrate compared to the wild type (Figure 5B). More importantly, the ΔphaZ-acs-phaG-phaC1-phaJ4 strain not only increased PHA content but also optimized the PHA composition in the cell. The strain accumulated slightly more uniform mcl-PHA, with C10 accounting for 75.1% of all PHA content (Figure 5B). The higher mcl-PHA content and more uniform PHA composition could enable high quality bioplastics toward petrochemical product replacement.

The engineered ΔphaZ-acs-phaG-phaC1-phaJ4 strain enabled the integration of electrocatalysis and bioconversion for highly efficient bioplastics synthesis from
The principle can be broadly applied to engineer other product manufacturing from C2 intermediate generated from CO2RR. We further evaluated the EMC2 system capacity for bioproduction using the designed ΔphaZ-acs-phaG-phaC1-phaJ4 strain. We first optimized the nitrogen-to-carbon ratio and found 1:100 as an ideal condition for PHA production (Figure S9). We then evaluated different fermentation modes. Considering that C2 intermediates were produced by CO2RR continuously, the fermentation is similar to a fed-batch mode. Our study showed that the semi-continuous fermentation mode had no negative impact on PHA productivity as compared to the traditional batch mode (Figure S10).

Therefore, we utilized the ΔphaZ-acs-phaG-phaC1-phaJ4 strain to carry out three rounds of fermentation in the integrated EMC2 system, which has been illustrated in Figure 4A. The whole electrolyzer voltage was constant during the fermentation. Since the soluble products are continuously consumed by bacteria, we used the FEs of gaseous products as the indicator for the stability of the electrolyzer. As shown in Figure S11, the CO2RR performance of the integrated EMC2 system is highly stable. The results highlighted that the EMC2 system can provide sufficient C2 carbon source from CO2RR to support the relatively high cell density at 2.75 g/L DCW. The PHA accumulation can achieve 556.2 mg/L within 24 h (Figure 5C). The EMC2 system has superior performance as compared to the current state-of-the-art, as the productivity is 4.7-fold of the PHB productivity driven by hydrogen produced from water splitting and 10.3-fold of the PHB productivity of the latest electrochemical microbial CO2 conversion (EMC) system (Figure 5D; Table S5). Furthermore, the study leads to the production of an mcl-PHA polymer with a longer unit (C8–C12, Figure S12) length, representing a much more broadly applicable product than the brittle PHB in previous studies. Moreover, the PHA production rate is also 6.4 times higher than the highest reported photosynthetic PHA production, thus representing the highest autotrophic PHA production driven by either light or electricity (Figure S13; Table S5). The results highlighted that the systemic design of catalyst, electrolyte, electrode, electro-microbial interface, and microbial strains has enabled the soluble C2 intermediates from CO2RR to be used for microbial fermentation, which delivers record-level biomass and bioproduct accumulation from CO2.

The strength and broad applicability of the EMC2 route

The utilization of soluble C2 intermediates for CO2 conversion to various products has significant potential to transform the carbon capture and utilization and bio-manufacturing. The high biomass and bioproduct productivity demonstrated several strengths of the EMC2 system (Route 4, Figure 1) as compared to the state-of-the-art.7,8,18,52 First, the C2 intermediates carry much more reducing equivalent as compared to the C1 intermediates such as CO, methanol, and formate in Routes 1 and 2 (Figure 1A). Second, acetate and ethanol’s high energy contents benefit the TCA cycle enabling higher PHA titer for better productivity (Figure 1A).53 Third, ethanol and acetate can be converted to acetyl-CoA to serve as building blocks or energy sources for PHA biosynthesis more efficiently than Routes 1 and 2 (Figure 1A). The electron and hydrogen in Routes 5 and 6 do not provide carbon for the molecular building block. They will rely on CO2 fixation by microorganisms, limiting the bioproduction rate. Fourth, the soluble C2 intermediates facilitate more rapid mass transfer as compared to CO, hydrogen, and even electron in Routes 1, 5, and 6, which again improves productivity. Fifth, EMC2 also has a biochemical advantage due to its reliance on high-flux primary metabolic pathways. Routes 1 and 5 both utilize the W-L pathway, limiting the product profile, pathway kinetics, and the type of microorganisms used. Routes 2 and 6 relies on Calvin-Benson cycle to fix CO2 yet is limited by gas-to-liquid transfer and the slow kinetics of RuBisCo.
The C2 products like ethanol and acetate go through two to three steps and produce acetyl-CoA to enter TCA, allowing the pathway to be more kinetically favorable and efficient compared to other pathways (Figure 1A; Table S1). More importantly, the integration with TCA allows the C2 intermediates to be engineered for converting into a much broader range of products as compared to the other routes. Sixth, acetate and ethanol serve as preferred substrates with less toxicity for *P. putida* and many other strains, allowing higher cell biomass and titer. EMC2 thus has the potential to be broadly applied in a very large range of microorganisms. The potentially broad adaptability also empowers product diversity and higher yield. Seventh, as compared to the more conventional microbial electrosynthesis (MES) system (Figure 1, Route 5), EMC2 does not rely on any organic substrates and is completely electrotrophic, achieving efficient E2M transformation. All the carbon source that supports cell growth and bioproduction are generated from CO₂. Furthermore, EMC2 can produce the longer chain polymer products, at higher rate due to the pathway adaptability (Figure 1). Overall, with all the strengths, EMC2 could become a game changer for utilizing CO₂ as feedstock to produce a variety of commodity chemicals and products.

The study proved the concept of EMC2 as a potentially transformative route for CO₂ conversion and implemented the platform for efficient mcl-PHA production with chemical and biological designs. The multi-tier design could also have a broad impact on the future design of chemical-biological hybrid systems for CO₂ conversion. First, the study overcame the challenges of catalyst selection in biocompatible electrolytes. The integration of GDE and Cu/PTFE electrodes allows the efficient CO₂RR to produce sufficient C2 products in the phosphate-based electrolyte, which is amenable to microbial cell growth. The combined design of electrolyte, electrode, and electrolyzer can be broadly applied to future electro-microbial conversion system design (Tier 1, Figure 1B). Second, the membrane-integrated electrode prevents the mineral cations in the growth media from interfering with electrocatalysis. The principle can be applied to future catalytic process design (Tier 2, Figure 1B). Third, the tandem design to integrate electrocatalysis with fermentation can also be broadly applied to future CO₂ conversion system design, especially for those based on soluble intermediates, to facilitate efficient mass transfer (Tier 3, Figure 1B). Fourth, the microbial design enables a higher yield of PHA from acetate and ethanol, and the principle can be applied to engineering other short-chain organic substrates to convert to PHA and lipid (Tier 4, Figure 1B). Current advancements allow electrocatalysis to potentially produce C3 products.

The metabolic engineering principle will enable higher bioproduct yield from diverse short-chain organic substrates derived from CO₂RR. Future research could further broaden the application and impact of the EMC2 platform. Electrocatalysis conditions can be optimized to produce more acetate, which could further improve the adaptability of the EMC2 system. Furthermore, *P. putida* capability for substrate co-utilization without catabolite repressions provides a unique advantage, when the electrocatalysis cannot yield a pure substrate (often containing acetate, ethanol, and trace formate), alleviating the ultra-high selectivity requirement in catalyst design. The EMC2 also overcame the challenge of distilling the low concentration ethanol or purification of complex mixtures from CO₂RR, funneling all the C1, C2, and C3 compounds to target bioproducts like PHA. Moreover, the EMC2 system allows the co-production of PHA and renewable ethylene, which enables a rapid renewable production system for different commodity products. The overall energy efficiency from sunlight to all products from our current EMC2 system reached 9.61% (Figure S14). Considering the gas phase products like ethylene and hydrogen are all valuable commodity products, the focused analysis of liquid products conversion to biomass renders the solar-to-biomass conversion efficiency at 4.50%, which is higher than the recent study...
calculated with similar approach. The results highlighted that the EMC2 system can achieve high energy efficiency, besides the production efficiency, due to its unique design in system integration and Chem-Bio interface. The further catalyst design will allow us to improve energy efficiency for biopolymer and adjust the yields between bioplastics and renewable ethylene to achieve maximized economic output.

Overall, the EMC2 exploits a set of unique chemical and biological designs to overcome the challenges for Chem-Bio interface and implements a novel CO$_2$-to-Chemical/Macromolecule route. This new route takes advantage of the C2 intermediate as more efficient electron doners, energy carriers, and molecular building blocks. The chemical and biochemical strengths of this route have enabled a record-level CO$_2$ to bioproduct conversion rate, as well as continuous and integrated production over a long period of time, all of which could transform the CO$_2$ capture and utilization and electron-to-molecule. The work represented the first research to exploit electrocatalytic system for producing mcl-PHA, a product with superior properties as compared to the shorter chain length PHB. It should be noted that bioconversion of substrates to PHA has less energy conversion efficiency than the PHB in general, as the hydrocarbon side chain of PHA are more reduced and require more energy and reductant to produce. However, the longer hydrocarbon side chain improves mechanical properties of bioplastics toward broader applications for petrochemical plastics replacement.

Future efforts in both synthetic biology and catalyst design for CO$_2$RR to C3 intermediate will improve the energy efficiency and yield of the system.

**EXPERIMENTAL PROCEDURES**

**Resource availability**

**Lead contact**
Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Susie Y. Dai (sydai@tamu.edu).

**Materials availability**
All materials generated in this study are available from the lead contact on request.

**Data and code availability**
Experimental datasets in this paper are provided in the manuscript and supplemental information.

All details of the experimental procedures can be found in the supplemental information.

**SUPPLEMENTAL INFORMATION**

Supplemental information can be found online at https://doi.org/10.1016/j.chempr.2022.09.005.

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AUTHOR CONTRIBUTIONS
J.S.Y., S.Y.D., and P.Z. conceived the original idea. P.Z. performed the material synthesis and electrochemical experiments. P.Z. and K.C. designed the EMC2 system and performed integration experiments. K.C. and B.X. designed and constructed the engineered strains, performed the cultivation and condition optimization. P.Z., J.L., and C.H. performed the materials characterization. P.Z. and K.C. analyzed the electrochemical and biological data. P.Z., K.C., J.S.Y., S.Y.D., and B.X. wrote the manuscript. All authors contributed to the scientific discussions.

DECLARATION OF INTERESTS
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REFERENCES
1. Ort, D. R., Merchant, S. S., Alric, J., Barkan, A., Blankenship, R. E., Bock, R., Croce, R., Hanson, M. R., Hibberd, J. M., Long, S. P., et al. (2015). Redesigning photosynthesis to sustainably meet global food and bioenergy demand. Proc. Natl. Acad. Sci. USA 112, 8529–8536. https://doi.org/10.1073/pnas.1424031112.

2. Hepburn, C., Adlen, E., Beddington, J., Carter, E. A., Fuss, S., Mac Dowell, N., Minx, J. C., Smith, P., and Williams, C. K. (2019). The technological and economic prospects for CO2 utilization and removal. Nature 575, 87–97.

3. Zhang, P., Dai, S. Y., and Yuan, J. S. (2021). Producing the “molecules of life” from CO2 through hybrid catalytic relay. Chem 7, 3200–3202. https://doi.org/10.1016/j.chempr.2021.11.018.

4. Overa, S., Ferric, T. G., Park, A.-H. A., and Jiao, F. (2021). Tandem and hybrid processes for carbon dioxide utilization. Joule 5, 8–13. https://doi.org/10.1016/j.joule.2020.12.004.

5. Cai, T., Sun, H., Qiao, J., Zhu, L., Zhang, F., Zhang, J., Tang, Z., Wei, X., Yang, J., Yuan, Q., et al. (2021). Cell-free chemoenzymatic starch synthesis from carbon dioxide. Science 373, 1523–1527. https://doi.org/10.1126/science.abh4049.

6. Claassens, N. J., Sánchez-Andrea, I., Sousa, D. Z., and Bar-Even, A. (2018). Towards sustainable feedstocks: A guide to electron donors for microbial carbon fixation. Curr. Opin. Biotechnol. 50, 195–205. https://doi.org/10.1016/j.copbiot.2018.01.019.

7. Li, H., Oppenorth, P. H., Wernick, D. G., Rogers, S., Wu, T. Y., Higashide, W., Malati, P., Hua, Y. X., Cho, K. M., and Liao, J. C. (2012). Integrated electromicrobial conversion of CO2 to higher alcohols. Science 335, 1596. https://doi.org/10.1126/science.1217643.

8. Haas, T., Krause, R., Weber, R., Demler, M., and Schmid, G. (2018). Technical photosynthesis involving CO2 electrolysis and fermentation. Nat. Catal. 1, 32–39. https://doi.org/10.1038/s41929-017-0050-1.

9. Abubakar, H. N., Veiga, M. C., and Kennes, C. (2011). Biological conversion of carbon monoxide: rich syngas or waste gases to bioethanol. Biofuels Bioenergy Bioprod. Biorefin. 5, 93–114. https://doi.org/10.1002/bbb.256.

10. Bar-Even, A. (2016). Formate assimilation: the metabolic architecture of natural and synthetic pathways. Biochemistry 55, 3851–3863. https://doi.org/10.1021/acs.biochem.6b00495.

11. Claassens, N. J., Bordanaba-Florit, G., Cotton, C. A. R., De Maria, A., Finger-Bou, M., Frieheime, L., Giner-Laguarda, N., Munar-Palmer, M., Newell, W., Scainici, G., et al. (2020). Replacing the Calvin cycle with the reductive glycine pathway in Cupriavidus necator. Metab. Eng. 62, 30–41. https://doi.org/10.1016/j.ymben.2020.08.004.

12. Sánchez-Andrea, I., Guedes, I. A., Hornung, B., Boeren, S., Lawson, C. E., Sousa, D. Z., Bar-Even, A., Claassens, N. J., and Stams, A. J. M. (2020). The reductive glycine pathway allows autotrophic growth of Desulfovibrio desulfuricans. Nat. Commun. 11, 5090. https://doi.org/10.1038/s41467-020-18906-7.

13. Yishai, O., Bouzon, M., Döring, V., and Bar-Even, A. (2018). In vivo assimilation of one-carbon via a synthetic reductive glycine pathway in Escherichia coli. ACS Synth. Biol. 7, 2023–2028. https://doi.org/10.1021/acssynbio.8b00137.

14. Claassens, N. J., Cotton, C. A. R., Kopljar, D., and Bar-Even, A. (2019). Making quantitative sense of electromicrobial production. Nat. Catal. 2, 437–447. https://doi.org/10.1038/s41929-019-0272-z.

15. Satawonsi, A., and Bar-Even, A. (2020). A one-carbon path for fixing CO2: C1 compounds, produced by chemical catalysis and upgraded via microbial fermentation, could become key intermediates in the valorization of CO2 into commodity chemicals. EMBO Rep. 21, e50273. https://doi.org/10.15252/embr.202050273.

16. Anwer, A. H., Khan, N., Umar, M. F., Rafatullah, M., and Khan, M. Z. (2021). Electrodeposited hybrid biocathode-based CO2 reduction via microbial electro-catalysis to biofuels. Membranes 11, 223. https://doi.org/10.3390/membranes11030223.

17. Prévot, A., Carvajal-Arroyo, J. M., Ganigué, R., and Rabaei, K. (2020). Microbial electro-synthesis from CO2: forever a promise? Curr. Opin. Biotechnol. 62, 48–57.

18. Liu, C., Colón, B. C., Ziesack, M., Silver, P. A., and Nocera, D. G. (2016). Water-splitting-biosynthetic system with CO2 reduction efficiencies exceeding photosynthesis. Science 352, 1210–1213. https://doi.org/10.1126/science.aaf5039.

19. Dinh, C. T., Burdym, T., Kibria, M. G., Seifitokalani, A., Gabardo, C. M., García de Arquer, F. P., Kiani, A., Edwards, J. P., De Luna, P., Bushuyev, O. S., et al. (2018). CO2 electroreduction to ethylene via hydroxide-mediated copper catalysis at an abrupt interface. Science 360, 783–787. https://doi.org/10.1126/science.aat9100.

20. Wang, X., Wang, Z., García de Arquer, F. P., Dinh, C. T., Ozden, A., Li, Y. C., Nam, D.-H., Li, J., Liu, Y.-S., Wicks, J., et al. (2020). Efficient electrically powered CO2-to-ethanol via suppression of deoxygenation. Nat. Energy 5, 478–486. https://doi.org/10.1038/s41560-020-00607-5.

21. Overa, S., Ko, B. H., Zhao, Y., and Jiao, F. (2021). Electrochemical approaches for CO2 conversion to chemicals: a journey toward practical applications. Acc. Chem. Res. 55, 638–648. https://doi.org/10.1021/acs.accounts.1c00674.

22. Kuhl, K. P., Cave, E. R., Abram, D. N., and Jaramillo, T. F. (2012). New insights into the electrochemical reduction of carbon dioxide on metallic copper surfaces. Energy Environ. Sci. 5, 7050–7059. https://doi.org/10.1039/c2ee21234j.

23. Gu, Z., Shen, H., Chen, Z., Yang, Y., Yang, C., Ji, Y., Wang, Y., Zhu, C., Liu, J., Li, J., et al. (2021). Efficient electrocatalytic CO2 reduction to C2+ alcohols at defect-site-rich Cu Surface. Joule 5,
429–440. https://doi.org/10.1016/j.joule.2020.12.011.

24. Zhu, Q., Sun, X., Yang, D., Ma, J., Kang, X., Zheng, L., Zhang, J., Wu, Z., and Han, B. (2019). Carbon dioxide electroreduction to C2 products over copper-cuprous oxide derived from electrocatalysed copper complex. Nat. Commun. 10, 3851. https://doi.org/10.1038/s41467-019-11599-7.

25. Hu, P., Chakraborty, S., Kumar, A., Woolston, B., Liu, H., Emerson, D., and Stephanopoulos, G. (2016). Integrated bioprocess for conversion of gaseous substrates to liquids. Proc. Natl. Acad. Sci. USA 113, 3773–3778. https://doi.org/10.1073/pnas.1516867113.

26. Lehntinen, T., Effenova, E., Tremblay, P.-L., Santala, S., Zhang, T., and Santala, V. (2017). Production of long chain alkyl esters from carbon dioxide and electricity by a two-stage bacterial process. Bioresour. Technol. 243, 30–36. https://doi.org/10.1016/j.biortech.2017.06.073.

27. Al Rowaihi, I.S., Kick, B., Grotzinger, S.W., Lehtinen, T., Efimova, E., Torm, P.-L., Lees, E.W., Mowbray, B.A.W., Parlane, F.G.L., Ager, J.W., III, et al. (2017). Phosphate science.aax4608. 11916. https://doi.org/10.1039/C7TA01871A.

28. Lees, E.W., Mowbray, B.A.W., Parlane, F.G.L., Torm, P.-L., Lees, E.W., Mowbray, B.A.W., Parlane, F.G.L., Ager, J.W., III, et al. (2017). Phosphate science.aax4608. 11916. https://doi.org/10.1039/C7TA01871A.

29. Ren, S., Joulie, D., Salvatore, D., Torbensen, K., Wang, M., Robert, M., and Berlinguette, C.P. (2022). Gas diffusion electrodes and membranes for CO2 reduction electrolyzers. Nat. Rev. Mater. 7, 55–64. https://doi.org/10.1038/s41578-021-00356-2.

30. Zhao, J., Sun, L., Canepa, S., Sun, H., Yesibolati, M.N., Sherrume, M., Xu, R., Sritharan, T., Hoo, J.S.C., Ager, J.W., III, et al. (2017). Phosphate science.aax4608. 11916. https://doi.org/10.1039/C7TA01871A.

31. Nitopi, S., Berheusenn, E., Scott, S.B., Liu, X., English, A.K., Horch, S., Seger, B., Stephens, I.E.L., Chan, K., Hahn, C., et al. (2019). Progress and perspectives of electrochemical CO2 reduction on copper in aqueous electrolyte. Chem. Rev. 119, 7610–7672. https://doi.org/10.1021/acs.chemrev.8b00705.

32. Kong, Y., Hu, H., Liu, M., Hou, Y., Koliviska, V., Vesztergombi, S., and Broekmann, P. (2022). Visualisation and quantification of flooding phenomena in gas diffusion electrodes used for electrochemical CO2 reduction: a combined EDX/CP-MS approach. J. Catal. 408, 1–8. https://doi.org/10.1016/j.jcat.2022.02.014.

33. Ma, M., Djanashvili, K., and Smith, W.A. (2016). Controllable hydrocarbon formation from the electrochemical reduction of CO2 over Cu nanoparticles. Angew. Chem. Int. Ed Engl. 55, 6680–6684. https://doi.org/10.1002/anie.201601282.

34. Zhang, S., Jiang, G., Gao, S., Jin, H., Zhu, Y., Zhang, F., and Jin, J. (2018). Cupric phosphate nanosheets-wrapped inorganic membranes with superhydrophobic and outstanding anticorrosion oil-fouling property for oil/water separation. ACS Nano 12, 795–803. https://doi.org/10.1021/acsnano.7b08121.

35. Xing, Z., Hu, L., Ripatti, D.S., Hu, X., and Feng, X. (2021). Enhancing carbon dioxide gas-diffusion electrolysis by creating a hydrophobic catalyst microenvironment. Nat. Commun. 12, 136. https://doi.org/10.1038/s41467-020-20397-5.

36. Zhao, J., Sun, L., Canepa, S., Sun, H., Yesibolati, M.N., Sherburne, M., Xu, R., Sritharan, T., Loo, J.S.C., Ager, J.W., III, et al. (2017). Phosphate science.aax4608. 11916. https://doi.org/10.1039/C7TA01871A.

37. Al Rowaihi, I.S., Kick, B., Grotzinger, S.W., Lehtinen, T., Efimova, E., Torm, P.-L., Lees, E.W., Mowbray, B.A.W., Parlane, F.G.L., Ager, J.W., III, et al. (2017). Phosphate science.aax4608. 11916. https://doi.org/10.1039/C7TA01871A.

38. Lees, E.W., Mowbray, B.A.W., Parlane, F.G.L., Torm, P.-L., Lees, E.W., Mowbray, B.A.W., Parlane, F.G.L., Ager, J.W., III, et al. (2017). Phosphate science.aax4608. 11916. https://doi.org/10.1039/C7TA01871A.

39. Nitopi, S., Berheusenn, E., Scott, S.B., Liu, X., English, A.K., Horch, S., Seger, B., Stephens, I.E.L., Chan, K., Hahn, C., et al. (2019). Progress and perspectives of electrochemical CO2 reduction on copper in aqueous electrolyte. Chem. Rev. 119, 7610–7672. https://doi.org/10.1021/acs.chemrev.8b00705.

40. Kong, Y., Hu, H., Liu, M., Hou, Y., Koliviska, V., Vesztergombi, S., and Broekmann, P. (2022). Visualisation and quantification of flooding phenomena in gas diffusion electrodes used for electrochemical CO2 reduction: a combined EDX/CP-MS approach. J. Catal. 408, 1–8. https://doi.org/10.1016/j.jcat.2022.02.014.

41. Ma, M., Djanashvili, K., and Smith, W.A. (2016). Controllable hydrocarbon formation from the electrochemical reduction of CO2 over Cu nanoparticles. Angew. Chem. Int. Ed Engl. 55, 6680–6684. https://doi.org/10.1002/anie.201601282.

42. Zhang, S., Jiang, G., Gao, S., Jin, H., Zhu, Y., Zhang, F., and Jin, J. (2018). Cupric phosphate nanosheets-wrapped inorganic membranes with superhydrophobic and outstanding anticorrosion oil-fouling property for oil/water separation. ACS Nano 12, 795–803. https://doi.org/10.1021/acsnano.7b08121.

43. Xing, Z., Hu, L., Ripatti, D.S., Hu, X., and Feng, X. (2021). Enhancing carbon dioxide gas-diffusion electrolysis by creating a hydrophobic catalyst microenvironment. Nat. Commun. 12, 136. https://doi.org/10.1038/s41467-020-20397-5.

44. Zhao, J., Sun, L., Canepa, S., Sun, H., Yesibolati, M.N., Sherburne, M., Xu, R., Sritharan, T., Loo, J.S.C., Ager, J.W., III, et al. (2017). Phosphate science.aax4608. 11916. https://doi.org/10.1039/C7TA01871A.

45. Al Rowaihi, I.S., Kick, B., Grotzinger, S.W., Lehtinen, T., Efimova, E., Torm, P.-L., Lees, E.W., Mowbray, B.A.W., Parlane, F.G.L., Ager, J.W., III, et al. (2017). Phosphate science.aax4608. 11916. https://doi.org/10.1039/C7TA01871A.

46. Lees, E.W., Mowbray, B.A.W., Parlane, F.G.L., Torm, P.-L., Lees, E.W., Mowbray, B.A.W., Parlane, F.G.L., Ager, J.W., III, et al. (2017). Phosphate science.aax4608. 11916. https://doi.org/10.1039/C7TA01871A.
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Supplemental information

Chem-Bio interface design for rapid conversion of CO$_2$ to bioplastics in an integrated system

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SUPPLEMENTAL FIGURES AND TABLES

Scheme S1. Wood-Ljungdahl pathway for CO assimilation (Route 1 in Table S1, also Route 1 in Figure 1), two CO molecules generate one acetyl-CoA, which then enters acetogenesis.

Scheme S2. Serine cycle for formate assimilation (Route 2-1 in Table S1), one formate and one bicarbonate enter serine cycle to generate one acetyl-CoA, which then can enter the TCA cycle.
**Scheme S3.** Reductive glycine cycle for formate assimilation (Route 2-2 in Table S1, also Route 2A in Figure 1), two formates enter the reductive glycine pathway (rGlyP) to generate one acetyl-CoA, which then can enter the TCA cycle.

**Scheme S4.** RuMP cycle for methanol assimilation (Route 3-1 in Table S1, also Route 2B in Figure 1), three methanol molecules enter the ribulose monophosphate pathway (RuMP) to generate one acetyl-CoA with one carbon loss. The acetyl-CoA then can be assimilated or enter the TCA cycle.
**Scheme S5.** Serine cycle for methanol assimilation (Route 3-2 in Table S1), one methanol and one bicarbonate enter the serine cycle to generate one acetyl-CoA, which then can enter the TCA cycle.

**Scheme S6.** Ethanol assimilation in this research (Route 4-1 in Table S1, also Route 4A in Figure 1), one ethanol is assimilated to generate one acetyl-CoA, which can enter the TCA cycle.
Scheme S7. Acetate assimilation in this research (Route 4-2 in Table S1, also Route 4B in Figure 1), one acetate is assimilated to generate one acetyl-CoA, which can enter the TCA cycle.

Scheme S8. Wood-Ljungdahl pathway for CO₂ assimilation (Route 5 in Table S1, also Route 5 in Figure 1), two CO₂ molecules enter the Wood-Ljungdahl Pathway to generate one acetyl-CoA, which then enters acetogenesis.
**Scheme S9.** CBB cycle for CO$_2$ assimilation in hydrogenotrophic strains (Route 6 in Table S1, also Route 6 in Figure 1), three CO$_2$ molecules enter the CBB cycle to generate one acetyl-CoA with one carbon loss. The acetyl-CoA then enters the TCA cycle. Red arrows indicate the pathway direction of the carbon source assimilation. The number of red arrows indicates the number of steps taken to generate one acetyl-CoA. Numbers in circle indicate the reactions for acetyl-CoA generation. [H] indicates reducing equivalents. Two [H] equals to one NAD(P)H or one FADH$_2$.
Figure S1. Electrocatalytic performances of other copper catalyst investigated.

A) PXRD of the other three Cu catalysts investigated. CuO was prepared by co-precipitation of Cu(NO₃)₂ and NaOH, followed by calcination in air at 500 °C for 2 hours. Cubic Cu₂O was prepared following the procedures reported by Chang et al. Cub-B represents copper catalysts reduced by NaBH₄, following the synthesis procedure by Zhou et al.

B) Faradaic efficiencies of various products (left Y scale) and potentials referred to the reversible hydrogen electrode (RHE, right Y scale).
Figure S2. The contact angle of Cu-based gas diffusion electrodes. A) Cu/PTFE before reaction. B) Cu/PTFE after reaction. C) Cu/28BC before reaction. D) Cu/28BC after reaction.
| Element | Weight% | Atomic% |
|---------|---------|---------|
| C       | 27.30   | 44.81   |
| O       | 29.13   | 35.89   |
| Na      | 6.71    | 5.75    |
| Mg      | 0.87    | 0.70    |
| P       | 1.60    | 1.02    |
| Cl      | 0.23    | 0.13    |
| K       | 4.59    | 2.31    |
| Ca      | 0.33    | 0.16    |
| Fe      | 3.44    | 1.21    |
| Cu      | 25.16   | 7.81    |
| Zn      | 0.65    | 0.20    |
| Totals  | 100.00  |         |

**Figure S3.** SEM and EDS elemental mapping of Cu/PTFE electrode without AEM masking after reaction. A) low-magnification SEM image of the after-use electrode. B) overall scan of EDS mapping. C) images by elements. Au is from the coating. Bottom: table of element composition of the selected area.
Figure S4. SEM and EDS elemental images of Cu/PTFE electrode with AEM masking after reaction. A) low-magnification SEM image of the after-use electrode. B) overall scan of EDS mapping. C) images by elements. Au is from the coating. Bottom: table of element composition of the selected area.
Figure S5. The long-term stability at total current -400 mA (electrode geometric area: 4 cm², current density -100 mA cm⁻²) of AEM-integrated cathode without changing the electrolyte. Left Y scale: whole cell voltage of the two-electrode cell. Right Y scale: Faradaic efficiencies of ethanol (blue dot) and total soluble C2+ (pink dot). The whole cell voltage was illustrated without iR compensation.
Figure S6. Evolution from conventional flow electrolyzer (A) to dual membrane electrolyzer (B). AEM stands for...
for anion exchange membrane, and BPM stands for bipolar membrane.
| Carbon source type      | Carbon source concentrations |
|-------------------------|------------------------------|
|                         | 0 hour          | 36 hours       |
| Formic acid             | 2 g/L           | 0.72 g/L       |
| Acetic acid             | 2 g/L           | 0 g/L          |
| Ethanol                 | 2 g/L           | 0 g/L          |
| Propanol                | 2 g/L           | 0 g/L          |
|                         | formate 2 g/L   | formate 0 g/L  |
|                         | ethanol 2 g/L   | ethanol 0 g/L  |
|                         | acetate 0.3 g/L | acetate 0 g/L  |
|                         | propanol 0.15 g/L | propanol 0 g/L |

**Figure S7.** Bacteria cell growth with different types of carbon sources as sole substrates and combined substrates. The error bars represent the deviation from three replicate experiments. The concentrations of carbon source at 0-hour and 36-hour cultivation were listed in the table.
Figure S8. The PHA accumulation of wild type *P. putida* KT2440 under carbon substrates with different chain lengths. The error bars represent the deviation from three replicate experiments.
Figure S9. Effect of C/N ratio in PHA accumulation.
Figure S10. Effect of EtOH carbon source feeding types on PHA accumulation. The error bars represent the deviation from three replicate experiments.
Figure S11. The performance of CO₂ electrolyzer in the EMC2 integrated system. Each round of fermentation for PHA production is 24 hours. The whole cell voltage was illustrated without iR compensation.

A) The whole cell voltage profile of the CO₂ electrolyzer during the integrated PHA production. B) The FEs of gaseous products from CO₂ electroreduction and their comparison with the electrolyzer operated without integration. The gas product was analyzed every 6 hours.
Figure S12. Gas chromatograph of PHA monomers and their corresponding MS data. Among the compounds, the derivative of C8 monomer is methyl-3-hydroxyoctanoate, the derivative of C10 monomer is methyl-3-hydroxydecanoate, and the derivative of C12 monomer is methyl-3-hydroxydodecanoate, the internal standard is methyl benzoate.
Figure S13. Comparison of productivity of the EMC2 system with currently reported electro-microbial systems with different routes that produce bioproducts with short carbon chains (C1-2), medium carbon chains (C3-6), and long carbon chains (C6+). The detailed literature list and description of their CO₂ utilization routes could be found in Table S4.
Figure S14. Staged diagram of energy flow from sunlight to various products. In the calculation, we fixed the amount of solar energy input, which is set as 100%. The energy contents of all products in the Chem-Bio system, including gases and cell biomass, are coming from the initial sunlight input. The details could be found in the energy efficiency calculation section in the supplemental experimental procedures.
Table S1. The net ATP calculations in major C1 and C2 assimilation pathways

| Route | Pathways | Substrates utilized to synthesize one acetyl-CoA | ATP/NAD(P)H generated in synthesizing one acetyl-CoA | ATP/NAD(P)H generated in oxidizing one acetyl-CoA* | Carbon loss in synthesizing one acetyl-CoA | ATP/NAD(P)H required for fixing lost CO₂ (Based on photosynthesis) | Net ATP/NAD(P)H generated in one acetyl-CoA synthesis† | Net ATP generated per carbon utilized (1NADH=3ATP)‖ | Steps to generate one acetyl-CoA |
|-------|----------|-----------------------------------------------|-----------------------------------------------------|-----------------------------------------------|-----------------------------------------------|------------------------------------------------------------------|----------------------------------------------------------|----------------------------------------------------------|--------------------------------|
| 1     | Wood-Ljungdahl Pathway (CO) | 2                                              | -1/-2                                               | 1/0                                           | 0                                             | 0/0                                                              | -1/-2                                                               | -3.5                                                     | 8                                      |
| 2-1   | Serine cycle (formate) | 1                                              | -3/-3                                               | 1/4                                           | 0                                             | 0/0                                                              | -3/-3                                                               | -12                                                      | 14                                 |
| 2-2   | rGlyP cycle (formate) | 2                                              | -2/-2                                               | 1/4                                           | 0                                             | 0/0                                                              | -2/-2                                                               | -4                                                       | 9                                      |
| 3-1   | RuMP cycle (methanol) | 3                                              | 0/5                                                  | 1/4                                           | 1                                             | 3/2                                                              | -3/3                                                                | 2                                                        | 18                                 |
| 3-2   | Serine cycle (methanol) | 1                                              | -3/-1                                               | 1/4                                           | 0                                             | 0/0                                                              | -3/-1                                                               | -6                                                       | 14                                 |
| 4-1   | Ethanol assimilation in this work | 1                                              | -2/2                                               | 1/4                                           | 0                                             | 0/0                                                              | -2/2                                                               | 2                                                        | 3                                      |
| 4-2   | Acetate assimilation in this work | 1                                              | -2/0                                               | 1/4                                           | 0                                             | 0/0                                                              | -2/0                                                               | -1                                                       | 1                                      |
| 5     | Wood-Ljungdahl Pathway (CO₂+e⁻) | 2                                              | -1/-4                                               | 1/0                                           | 0                                             | 0/0                                                              | -1/-4                                                               | -6.5                                                    | 8                                      |
* The amounts of ATP and reducing equivalents generated when one acetyl-CoA is oxidized in the primary metabolism. The acetogenesis oxidizes one acetyl-CoA and generates one ATP. The TCA cycle oxidizes one acetyl-CoA and generates one ATP and four NAD(P)H.

† The amounts of ATP and NAD(P)H required for fixing the lost carbon in acetyl-CoA synthesis were based on photosynthesis, which takes three ATP and two NAD(P)H to fix one CO₂.

# The net ATP generated in one acetyl-CoA synthesis equals the ATP generated in synthesizing one acetyl-CoA subtracted by the ATP required for fixing lost carbon. Similarly, the net NAD(P)H generated in one acetyl-CoA synthesis equals the NAD(P)H generated in synthesizing one acetyl-CoA subtracted by the NAD(P)H required for fixing lost carbon.

‖ The net ATP generated per carbon is calculated by dividing the total net ATP utilized/generated in acetyl-CoA synthesis by the carbon numbers in the substrates. The total net ATP is the sum of the net ATP and the net reducing equivalents, assuming that one molecule of NAD(P)H is equivalent to three ATP.
Table S2. The criteria for scoring in Figure 1

| Scoring Item                  | Criteria                                                                 |
|-------------------------------|--------------------------------------------------------------------------|
| Mass transfer to microorganisms | • Substrates’ accessibility to microbes                                   |
|                               | • The utilization efficiency of the assimilation pathway                  |
| Electron transfer to microorganisms | • Number of electrons carried by the intermediates                        |
|                               | • Utilization mode and possible constrains induced by mass transfer       |
| Non-toxicity                  | • Constrains in assimilation and metabolism                              |
|                               | • Energy limitations in utilization                                       |
| Bio-compatibility             | • Phylogenetic distribution of the assimilation pathways in each route    |
Table S3. Major salt components of the biocompatible electrolyte for CO₂ RR and integrated bioconversion, and the regular fermentation medium.

|                      | Biocompatible electrolyte | Normal medium |
|----------------------|---------------------------|---------------|
|                      | component | concentration | component | concentration |
| Buffer               | KH₂PO₄     | 6.0 g/L       | KH₂PO₄     | 3.0 g/L       |
|                      | Na₂HPO₄   | 12.0 g/L      | Na₂HPO₄    | 6.0 g/L       |
|                      | NaCl       | 1.0 g/L       | NaCl       | 0.5 g/L       |
| Salt mix             | MgSO₄      | 0.32 g/L      | MgSO₄      | 0.32 g/L      |
|                      | MgCl₂      | 0.28 g/L      | MgCl₂      | 0.28 g/L      |
|                      | CaCl₂      | 0.11 g/L      | CaCl₂      | 0.11 g/L      |
Table S4. Summary of literatures in Figure 4D. Details of the CO₂ utilization routes, microorganisms, and cell growth rates are illustrated.

| Number in Figure 4D | General CO₂ conversion route | Microorganism | Electro-bio integration | Continuous production | Cell growth rate (OD/h) | Ref. |
|---------------------|-------------------------------|---------------|-------------------------|-----------------------|------------------------|------|
| 1                   | CO₂ to acetate                | *Sporomusa ovata* | ✓                       | ✓                     | 0.0024                 | ³    |
| 2                   | H₂+CO₂ to PHB                 | *Ralstonia eutropha* | ✓                       | ✓                     | 0.012                  | ⁴    |
| 3                   | H₂+CO₂ to syngas (CO), then to short-chain fatty acids | *Clostridium autoethanogenum* & *Clostridium kluyveri* | ✓                       | ✗                     | 0.025                  | ⁵    |
| 4                   | CO₂ to formate, then to isobutanol 3MB(C5) | *Ralstonia eutropha* | ✓                       | ✓                     | 0.017                  | ⁶    |
| 5                   | CO₂ to formate, then to PHB    | *Cupriavidus necator* (*Ralstonia eutropha*) | ✗                       | ✗                     | 0.039                  | ⁷    |
| This work           | CO₂ to C2+ (ethanol, acetate, ...) then cell growth and PHA production | *Pseudomonas putida* | ✓                       | ✓                     | 0.105                  |      |
Table S5. Summary of literatures in Figure S13. Details of the chemo-bio route, microorganism, products, and titers are illustrated.

| Product chain length | Number in Figure S13 | Route by microorganism | Route | Microorganism | Titer (mg/L/day) | Ref. |
|----------------------|-----------------------|-------------------------|-------|---------------|-----------------|------|
| C1-2                 | 1                     | CO₂ → acetate           | Route5 | Sporomusa ovata | 300 mg/L/day     | 3    |
| C1-2                 | 6                     | CO₂ → formate, acetate  | Route5 | Moorella thermoautotrophica | formic acid: 356 mg/L/day; acetate: 420.6 mg/L/day | 8    |
| C1-2                 | 7                     | CO₂ → Acetate           | Route5 | Acetate-producing community, dominated by Clostridiales | 314 mg/L/day | 9    |
| C1-2                 | 8                     | CO₂ → acetate           | Route5 | Mixed culture | 257.7 mg/L/day | 10   |
| C1-2                 | 9                     | CO₂ → ethanol           | Route5 | Mixed culture obtained from corroded metal surface | ethanol: 170 mg/L/day; butanol: 60.4 mg/L/day | 11   |
| C3-6                 | 2                     | CO₂ → formate → C5 (mesaconate, 2S-methylsuccinate) | Route2 | Methylobacterium extorquens AM-1 | Mesaconate: 151.6 mg/L/day; 2S-methylsuccinate: 220.2 mg/L/day. | 12   |
| C3-6                 | 3                     | CO₂ → formate → isobutanol, 3-methyl-butanol | Route2 | Ralstonia. eutropha | 28 mg/L/day | 6    |
| C3-6                 | 10                    | H₂O+CO₂ → syngas → C2-C6 fatty acids | Route1 | Clostridium autoethanogenum & Clostridium kluveri | Butyrate: 444 mg/L/day; butanol: 249 mg/L/day; hexanoate: 139.4 mg/L/day; hexanol: 98 mg/L/day | 5    |
| C3-6                 | 11                    | CO₂ → butyrate, isopropanol | Route5 | Mixed microbial culture | Butyrate: 63.3 mg/L/day; Isopropanol: 39 mg/L/day | 13   |
| C6+                  | 4                     | H₂+CO₂ → PHB            | Route6 | Ralstonia eutropha | 117 mg/L/day | 4    |
| C6+                  | 5                     | CO₂ → formate // PHB    | Route2 | Cupriavidus necator (Ralstonia eutropha) | 53.7 mg/L/day | 7    |
| C6+ | 12 | CO₂ → acetate // PHB | Route1 | Mixed culture + *Ralstonia* | 153.3 mg/L/day |
|-----|----|---------------------|--------|-----------------------------|----------------|
|     |     |                     |        | *Acetobacterium woodii* (CO₂:H₂ (15:85 v/v) under elevated pressure (≥2.0 bar) + *Ralstonia eutropha* H16 | acetate: 119 mg/L/day; PHB: 118 mg/L/day |
| C6+ | 13 | CO₂ → acetate // PHB | Route1 | *Acetobacterium woodii* (CO₂:H₂ (15:85 v/v) under elevated pressure (≥2.0 bar) + *Ralstonia eutropha* H16 | acetate: 119 mg/L/day; PHB: 118 mg/L/day |
| C6+ | 14 | CO₂ → acetate // PHB | Route5 | *Sporomusa ovata* // *E.coli* | PHB: 98 mg/L/day; n-butanol: 40 mg/L/day; |
| C6+ | This work | CO₂ → soluble C2+ → bioproducts | Route4 (EMC2) | *Pseudomonas putida* | PHA: 556.2 mg/L/day |

* the reactions were carried out in one batch or continuous

* the reactions were not continuous or conducted separately (separation steps may apply).
SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Materials and Methods

Catalytic gas diffusion electrode (GDE) preparation and characterization

The copper based GDEs were fabricated using the direct current (DC) sputtering method using Kurt J. Lesker PRO Line PVD 75. The deposition rate was controlled at ~2 Å s⁻¹. The deposition time was 1500 s, the catalyst layer thickness was about 300 nm. Two categories of gas-diffusion layers (GDLs) were selected and investigated in this study: porous PTFE membrane (0.45 μm, Tisch Scientific) and carbon paper Sigracet® 28 BC (Fuel Cell Store). After deposition, the as-made GDE was weighted on the balance to determine the final copper loading (~0.1 mg/cm²). The conductivity was also tested by pinning random two points on the GDE surface using a multimeter. The resistance readings of both GDEs were less than 1 Ω, the continuous and conductive copper layers were successfully formed. The SEM images and the corresponding EDS mapping of GDEs, both before and after reaction, were taken using Tescan LYRA-3 FIB-SEM. The X-ray diffraction spectroscopy was taken on a Bruker D8 ADVANCE ECO diffractometer.

Electrochemical CO₂ reduction

All electrochemical CO₂ reduction reactions were carried out using a flow electrolyzer equipped with prepared GDEs. The GDEs were directly used for CO₂RR without further modifications.

For the normal flow-cell tests, the reactions were conducted in a customized flow cell that was composed of a gas diffusion chamber, a catholyte chamber, and an anolyte chamber (Figure S6A). On the cathode side, the CO₂RR GDE (geometric area: 1 cm²) was placed between the gas chamber and the catholyte chamber. CO₂ (Research grade, Airgas, flow rate 30 sccm) flowed through the gas chamber and fed the gas to GDE from the back side. Bicarbonate solution (NaHCO₃ + KHCO₃, total concentration 0.2 M), phosphate basal solution, or whole culture medium (see below sections for details) were used as the catholyte and circulated by a peristaltic pump at the flow rate of 3 mL/min. An anion-exchange membrane (Fumasep PK-130, Fuel Cell Store) separated the catholyte and anolyte chambers. On the anode side, Ni foam was used as the anode for water oxidation under basic conditions. 1 M KOH was used as the anolyte with the circulation speed at 10 ml/min. The Ag/AgCl (with saturated KCl) was used as the reference electrode. The electrochemical experiments were conducted on an Autolab PGSTAT302N system. The potentials were then converted to the values versus reversible hydrogen electrode (RHE) after iR compensation. The resistances (R) were determined by Electrochemical impedance spectroscopy (EIS) in the frequency ranging from 10⁵ to 10⁻¹ Hz and the amplitude was set at 10 mV.

For the dual membrane electrolyzer tests, the cell was customized based on the conventional flow cell with modifications (Figure S6B). The geometric area of electrodes was increased to 4 cm² to increase the production of soluble C₂+ intermediates. On the cathode side, Cu/PTFE GDE was used for CO₂ reduction. 30 sccm humidified CO₂ was supplied on the back side of GDE for gas diffusion. An anion-exchange membrane (AEM, Sustainion X37-50 RT, Dioxide Materials) was placed on top of the copper side on the Cu/PTFE to prevent the cations in the medium from forming deposition on the cathode. On the anode side, Ni foam was used for water oxidation and 1 M KOH was used as the electrolyte. As the anode reaction was conducted in basic condition, a bipolar membrane (BPM, Fumasep FBM, Fuel Cell Store) was placed on top of the Ni foam for cation exchange. Between the AEM and the BPM, a middle chamber was placed to allow the salt solution (basal solution or whole culture medium) flow through the chamber, which provided conductivity to close the circuit and brought the water-soluble products to the bioconversion unit. The dual membrane experiments were conducted on the Autolab PGSTAT302N system in the two-electrode mode. The whole cell voltage was recorded without any correction.

The gaseous products from CO₂RR experiments were collected at the end of the gas chamber and analyzed by gas chromatography (HP 5890) equipped with ShinCarbon ST packed column and a thermal conductive detector (TCD). Argon was used as the carrier gas. The liquid products were analyzed by ¹H NMR (Bruker AVANCE NEO 400) using D₂O as the solvent and DMSO as the internal standard.
Growth media of *Pseudomonas putida* strains

LB (DifcoTM LB broth, Lennox) medium was used for the initial cultivation of *Pseudomonas putida* strains before which were inoculated into *Pseudomonas* growth medium. Specifically, *Pseudomonas putida* was taken from the single colony on an LB agar plate and then inoculated into a 10 mL LB medium and incubated overnight at 30 °C and 180 rpm. One mL of the bacteria culture was taken out and inoculated into another flask containing 100 mL *Pseudomonas* growth medium. The flask was incubated at 30 °C and 180 rpm. Before any growing test or PHA fermentation test, the bacteria culture was taken out, centrifuged, and washed twice with 100 mM phosphate buffer before being inoculated into a new *Pseudomonas* growth medium.

The *Pseudomonas* growth medium contains (per liter of deionized water): three g potassium dihydrogen phosphate (KH₂PO₄), six g sodium phosphate (Na₂HPO₄), 0.5 g sodium chloride (NaCl). This basal buffer is added with mineral salt mix of 0.32 g magnesium sulfate (MgSO₄), 0.11 g calcium dichloride (CaCl₂), and 0.28 g magnesium chloride hexahydrate (MgCl₂·6H₂O). The solution was then adjusted to an initial pH of 7.0 using hydrogen chloride (HCl) and sodium hydroxide (NaOH) solutions and then autoclaved. Before the medium was used, 10 mL 100× Goodies solution was added per liter of the medium. 1 g/L ammonium sulfate ((NH₄)₂SO₄) was added for the nitrogen-rich condition, and ethanol was added as the primary carbon source, of which the concentration is noted in every experiment.

In the Chem-Bio integration experiments, the growth medium will also serve as the electrolyte for CO₂RR. In order to reduce the ohmic loss due to the low concentration of salt solution, while at the same time creating acceptable environment for microbial growth, we doubled the concentration of phosphate basal salt in the medium. The detailed information of major salt components in the biocompatible electrolyte, as well as their comparison with the regular fermentation medium, were illustrated in Table S2.

The 100× Goodies solution contains (per liter): 26.5 mg thiamine, 0.625 g iron (II) sulfate (FeSO₄), 0.25g calcium carbonate (CaCO₃), 0.25 g iron (II) sulfate heptahydrate (FeSO₄·7H₂O), 0.56 g zinc sulfate heptahydrate (ZnSO₄·7H₂O), 0.1 g manganese sulfate monohydrate (MnSO₄·H₂O), 0.03 g copper sulfate pentahydrate (CuSO₄·5H₂O), 0.03 g cobalt chloride hexahydrate (CoCl₂·6H₂O), 7.5 mg boric acid (H₃BO₃), and 6.4 mL hydrochloric acid (HCl).

*P. putida* strains growth at different basal salts

To test the cell growth of *P. putida* strains in different medium conditions, *P. putida* culture was inoculated into new *Pseudomonas* growth media with different modifications. 0.2 mL of bacteria culture was taken out and inoculated into 20 mL modified and unmodified *Pseudomonas* growth medium for comparison. The OD₆₀₀ was monitored every four hours since inoculation to determine the cell growth. Every test has 3 biological replicates.

To evaluate the basal salt impacts on the cell growth, the modified *Pseudomonas* growth media contained 200 mM bicarbonate (NaHCO₃ + KHCO₃, the Na:K was kept at 4:1, consistent with the phosphate basal solution) as its basal buffer.

Adaptation and cell growth test on the CO₂RR products solution

*P. putida* KT2440 from the *Pseudomonas* growth medium was inoculated into the CO₂RR products solution and several generations of adaptive evolution were carried out to adapt the strain to the salt concentration as well as the products generated from CO₂ reduction. Specifically, 0.2 mL of *P. putida* KT2440 culture was inoculated into 20 mL CO₂RR products solution (Figure 3 in the main text). OD₆₀₀ was monitored every 6 hours after inoculation to determine cell growth. When the OD₆₀₀ reached the plateau, 0.2 mL of the bacteria culture was taken out and inoculated into a new CO₂RR products solution. The process was repeated until a significantly decreased lag phase was observed during the growth.

To test the cell growth in the CO₂RR products, 0.1 initial OD₆₀₀ of the adapted and non-adapted *P. putida* KT2440 were separately inoculated in a CO₂RR products solution that contained 1.5 g/L ethanol (EtOH), 1 g/L (NH₄)₂SO₄, 1×Goodies. OD₆₀₀ was monitored at 3, 6, 9, 12, 24, 48, 60 h after inoculation to determine the cell growth. Every test has three biological replicates.
Engineer the *Pseudomonas putida* strain for higher PHA production

To efficiently convert CO₂RR C₂ products to PHA, three plasmids were constructed to genetically modify the *P. putida* KT2440 wild type strain. The *P. putida* engineering followed a previous protocol with some modifications.¹⁸ The plasmids, constructs, and strains used in the study are listed in the Table S6 and Table S7.

The first plasmid is a gene deletion plasmid pk18mobsacB-phaz to knock out the gene phaz coding PHA depolymerase from the *P. putida* KT2440 genome. Briefly, one kb upstream and one kb downstream of phaz was amplified by PCR from *P. putida* KT2440 genomic DNA. The two 1 kb fragments were then inserted into pk18mobsacB via Gibson assembly to create pk18mobsacB-phaz using the primers listed in Table S6. One µg suicide plasmid pk18mobsacB-phaz was introduced into *P. putida* KT2440 via electroporation at 2.4 kV and cells were added with 1 mL LB medium and incubated at 30 °C for two hours. The entire transformant was plated on the *Pseudomonas* isolation agar plate with 50 µg/mL kanamycin and incubated at 30 °C overnight. The colonies were streaked on the LB plate containing 20 % sucrose and incubated at 30 °C overnight. 100 sucrose resistant colonies were picked up for colony PCR using the primers amZ-F and amZ-R¹⁹ listed in Table S6. The mutants were subject to sequencing.

The second plasmid is Ptac-phaC1phaJ4 to overexpress the phaC1 (gene coding poly(3-hydroxyalkanoic acid) synthase from *P. putida* KT2440) and the phaJ4 (gene coding (R)-specific enoyl-CoA hydratase from *P. putida* KT2440) in the strain. Specifically, phaC1, phaJ4 containing ribosomal binding site (RBS) was amplified by PCR from *P. putida* KT2440 genomic DNA. phaC1 and phaJ4 were fused by overlapping PCR to produce a 2.2 kb PCR fragment. The 2.2 kb fragment was then digested by BamHI and HindIII and ligated into pkT2Ptac::lacZ vector (Table S5) to generate plasmid Ptac-phaC1phaJ4 plasmid.

The third plasmid is pBBR1k-J23107-acs-phag. Specifically, the acs (gene coding Acetyl-CoA synthetase from *E.coli* S17-1) is cloned from *E. coli* S17-1 with codon optimization. The phag (gene coding (R)-3-hydroxydecanoyl-ACP:CoA transacylase from *P. putida* KT2440) containing RBS is cloned from *Pseudomonas putida* KT2440. The two genes were fused together and inserted into pBBR1k-J23107-GFPuv vector to generate pBBR1k-J23107-acs-phag plasmid.

The plasmids were introduced into the mutant strain ΔphaZ via electroporation method to generate the engineered strains ΔphaZ-acs-phag and ΔphaZ-acs-phag-phaC1phaJ4.
| Table S6. Plasmids and strains used in this study |
|-----------------------------------------------|
| Plasmids and strains | Genotype or description | Reference |
|----------------------|-------------------------|-----------|
| **Plasmids**         |                         |           |
| pk18mobsacB          | Gene-deletion plasmid,  |           |
|                      | sacB, lacZα, kan′       | 20        |
| pk18mobsacB-phaZ     | phaZ knockout           |           |
| Ptac-phaC1phaJ4      | kan′, PVS1 replicon,    |           |
|                      | overexpression phaC1 and|           |
|                      | phaJ4                   |           |
| pkT2Ptac::lacZ       | kan′, PVS1 replicon,    |           |
|                      | overexpress lacZ        | 21        |
| pBBR1k-J23107-GFPuv  | kan′, PBBR1 replicon,   |           |
|                      | IPTG-inducible promoter,|           |
|                      | overexpress GFPuv      | 22        |
| pBBR1k-J23107-acs-phaG | kan′, PBBR1 replicon, |           |
|                      | IPTG-inducible promoter,|           |
|                      | overexpress acs and phaG|         |
| **Strains**          |                         |           |
| E. coli S17-1        | thi, pro, hsdR, recA,   |           |
|                      | mobilizer strain        | 23        |
| P. putida KT2440     | mt-2, hsdR1(r^-m^),     |           |
|                      | without TOL plasmid     | 19        |
| ΔphaZ                | PHA depolymerase        |           |
|                      | knockout                | This study|
| ΔphaZ-acs-phaG       | PHA depolymerase        |           |
|                      | knockout, overexpressing|           |
|                      | acs and phaG            | This study|
| ΔphaZ-acs-phaG-phaC1phaJ4 | PHA depolymerase |           |
|                      | knockout, overexpressing|           |
|                      | phaC1 and phaJ4 and phaG|           |
|                      | and acs together        | This study|

*kan′* kanamycin resistance.
| Primers          | Sequence                        | Purpose                                      |
|------------------|---------------------------------|----------------------------------------------|
| phaC1_oe_fwd     | CCCGGATCCtactcgtctcaggacaac     | Amplicy phaC1 for overlapping PCR            |
| phaC1_oe_rev     | agttgttagctcaacgctgtgaacgtag     | Amplicy phaC1 for overlapping PCR            |
| phaJ4_oe_fwd     | cgacgcgtgacgtaactgacaaccc       | Amplicy phaJ4 for overlapping PCR            |
| phaJ4_oe_rev     | CCCAAGCTTcagacaaaaacagagcagcag  | Amplicy phaJ4 for overlapping PCR            |
| acsphaG-gib1_fwd | gttccataacacttgcaagatcctctctctc | Gibson assemble for pBBR1k-J23107-acsphaG    |
| acsphaG-gib1_rev | tggctggggcatatgtatatctctcttttaaaagatcttttg | Gibson assemble for pBBR1k-J23107-acsphaG |
| acsphaG-gib2_fwd | aggagatatatactgccccagccaaagctac | amplify acsphaG                             |
| acsphaG-gib2_rev | cgagtttggctcctagatggcataatgctgt | amplify acsphaG                             |
| acsphaG-gib3_fwd | ttcctctgagcatccaaactgagtaaggatctc | Gibson assemble for pBBR1k-J23107-acsphaG    |
| acsphaG-gib3_rev | taccgcccacctagcgaggactctggtgctgag | Gibson assemble for pBBR1k-J23107-acsphaG    |
| acsphaG-gib4_fwd | ccagagtcctctcagatggcagttgctgag  | Gibson assemble for pBBR1k-J23107-acsphaG    |
| acsphaG-gib4_rev | agtgccttgcctgatctgctctttaggcagctgtgag | pBBR1k-J23107-acsphaG                      |
| acsphaG-oe1_fwd  | atgcccccagccaaagctacac           | amplify acs for overlapping                  |
| acsphaG-oe1_rev  | aagagttttcctatacttggtgcaagcag   | amplify acs for overlapping                  |
| acsphaG-oe2_fwd  | cagagtttgaatacccttttgcgcagccg   | Amplicy phaG for overlapping                |
| acsphaG-oe2_rev  | tcaagatggcaatctgctgtagctg       | Amplicy phaG for overlapping                |
| pk18-phaZ-gib1_fwd | tgctaccaagggcagaaatctttttaacaaagcatac | Primers used in pk18mobsacB-phaZ           |
| pk18-phaZ-gib1_rev | tgaaggtcttgctgagctgaaaattgtaatc | Primers used in pk18mobsacB-phaZ           |
| Primer Name                      | Sequence                        | Description                                   |
|---------------------------------|---------------------------------|-----------------------------------------------|
| phaZ_up-gib_fwd                 | cgaattcgagctcagcagaccttcatcatcagc | Primers used in pk18mobsacB-phaZ              |
| phaZ_up-gib_rev                 | gccgcagctgttgtgactttgggtg         | Primers used in pk18mobsacB-phaZ              |
| PhaZ_down-gib_fwd               | aagagtcacgtcaacagctgccctgtgaca   | Primers used in pk18mobsacB-phaZ              |
| PhaZ_down-gib_rev               | atccccgggtaccccggcgaattcttttg    | Primers used in pk18mobsacB-phaZ              |
| pk18-phaZ-gib2_fwd              | caattgcgccgggtaccggggctctctctcg  | Primers used in pk18mobsacB-phaZ              |
| pk18-phaZ-gib2_rev              | taaagattctcgccttgtagccatctctcg   | Primers used in pk18mobsacB-phaZ              |
| amZ-F                           | cctgaattcaaaatgcctcaagcatccagactc| ΔphaZ verification                            |
| amZ-R                           | tgggaattcttgcttgccagccagatag     | ΔphaZ verification                            |
**PHA production of engineered *P. putida* strains in flasks**

50 mL pre-cultured *P. putida* KT2440 WT or engineered strains was collated by centrifugation, washed three times with 100 mM phosphate buffer, and inoculated into 50 mL *Pseudomonas* growth medium containing 5 g/L EtOH as carbon source and 0.05 g/L (NH₄)₂SO₄ for limited-nitrogen condition. The initial OD₆₀₀ values of all the bacteria solutions were adjusted to 1.0. 0.1 mM IPTG was added for the strains harboring pBBR1k-J23107-acs-phaG plasmid to induce the overexpression of *acs* and *phaG* genes. After 3 days of fermentation, the cell was collected for PHA measurement. The studied mutants include *acs-phaG*, ΔphaZ, ΔphaZ-acs-phaG, and ΔphaZ-acs-phaG-phaC-phaJ). The results were shown in Figure 5B in the main text.

5 g/L of acetate, propionate, and butyrate were evaluated to identify the chain length impacts individually. The nitrogen source was 0.25 g/L (NH₄)₂SO₄, and the inoculation OD₆₀₀ is 0.3. The results are shown in Figure S8.

Different C/N ratios in the growth medium were investigated for PHA fermentation with the ΔphaZ-acs-phaG-phaC-phaJ strain. The carbon source EtOH was fixed at 2 g/L and a series of different (NH₄)₂SO₄ concentrations (2 g/L, 0.1 g/L, 0.02 g/L, and 0 g/L) were used to identify the optimal nitrogen concentration for PHA accumulation. The results were shown in Figure S9.

Effect of carbon source feeding on PHA accumulation was investigated by evaluating the time to add EtOH. 12 g/L EtOH was added to the medium at one time in the beginning of fermentation or was added every 12 hours for 6 times. In both cases, the (NH₄)₂SO₄ concentrations was 0.02 g/L. The influences of feeding mode to PHA accumulation are shown in Figure S10.

**EMC2 integrated system**

The integrated system was composed of the electrocatalysis unit and the fermentation unit. Whole culture medium (basal + nitrogen source + Mg/Ca/B1/Goodies) was used as the catholyte and cultural medium. In the electrocatalysis unit, the dual-membrane cell with 4 cm² geometric area electrodes was adopted for CO₂RR, of which the configuration had been demonstrated above (Figure S14). The fermentation unit was a dual chamber membrane bioreactor, designed with the smaller chamber (Chamber A, Figure 4A in the main text) as a reservoir and the bigger chamber (Chamber B, Figure 4A in the main text) as the fermenter. The culture medium was circulated between the reservoir and the electrolyzer to accumulate the C2 carbon source. The reservoir and the main chamber were separated by a filter membrane (Whatman grade 1 filter paper) to block the microbe from entering the circulation while allowing efficient mass transfer for C2 intermediates and nutrients. The bioreactor was put in a 30 °C incubator to keep constant fermentation temperature. Air was bubbled to chamber B to sustain the aerobic cell growth. Prior to the inoculation of strains, the electrolyzer pre-ran at a constant current 400 mA (100 mA cm⁻²) to accumulate the C2 to about 2 g/L.

**Cell Growth and PHA Production in EMC2 System**

For cell growth analyses, the adapted *P. putida* KT 2440 with a short lag phase in CO₂RR products solution was inoculated into chamber B (initial OD₆₀₀=0.2, Figure 4 in the main text). The cell-culture compartment was agitated at 300 rpm for a specific time length. After each round of cell growth, the media in chamber B was sampled to read the OD₆₀₀ value (SpectraMax iD5). The product profiles were quantified using ¹H NMR. The cells in chamber B were collected by centrifuge (2,800 × g, 10 min), washed, and lyophilized to record the cell dry weight (CDW). The supernatant was transferred back to chamber B to inoculate new strains for the next round of culture.

For conversion of CO₂ to PHA, the CO₂ reduction and fermentation conditions were identical to those described in the cell growth test except for the inoculum. Engineered *P. putida* KT 2440 strain ΔphaZ-acs-phaG-phaC1-phaJ4 was utilized as a biocatalyst to convert C2+ intermediates to PHA and an initial OD₆₀₀ of 3.0. A limited nitrogen source (0.02 g/L (NH₄)₂SO₄) was used to facilitate the PHA accumulation. A sequential three rounds of fermentation with each round lasting 24 hours were conducted to test the system's conversion rate. After each round of fermentation, the cells were collected and the PHA production in the cells was measured via GC-MS (Shimadzu GCMS-QP2010) as previously described.
PHA extraction and quantification

The cells were harvested by centrifugation at 4000 rpm for 10 min. The cells were then washed twice with ddH2O and lyophilized. About 5~10 mg lyophilized cells were dissolved in 2 mL of methanol-sulfuric acid (v/v=85:15) mixture and 2 mL chloroform. The resulted solution was incubated at 100 °C for four hours. This acid-catalyzed methanolysis step converted the polyesters (PHA) into corresponding monomer derivatives, which were dissolved in the chloroform. After cooling, the samples were washed with 2 mL of demineralized water several times until no acid residual existed. After each wash, the upper layer (water) was removed. Methyl benzoate was added as the internal standard. The organic layer was then diluted with chloroform to proper concentration and filtered by 0.2 μm filter. The final concentration of internal standard methyl benzoate was 21 µg/mL. The diluted samples were analyzed by GC-MS (QP2010SE, Shimadzu) with a Zebron ZB-35HT Inferno column (30 m × 250 µm ID × 0.25 µm df). Helium was used as the carrier gas with flow rate 1.0 mL/min. Column temperature program: initial temperature 50 °C for 3 min and ramped to 300 °C at the rate 10 °C/min. Injector temperature: 250 °C. Mass spectra were recorded by a 70 eV electron beam at an ionization current of 40 μA.

Calculation of energy efficiency for the Chem-Bio integrated system

To calculate the energy efficiency from sunlight to final products, we analyzed the whole process and divided it into three stages (Figure S14), which are solar to electricity via solar panel (Stage 1, blue), CO2 electrocatalysis forming gas products and water-soluble products (Stage 2, green), and the fermentation of the soluble intermediates to biomass and bioproducts (Stage 3, pink).

It should be noticed that there is no consensus for the energy conversion efficiency calculation, and the procedures in the literatures are largely based on various assumptions and different product profiles. Herein, we chose two methods that are most relevant to our study. In both studies, the solar energy was regarded as the total input (100%) for an artificial photosynthesis process. The energy stored in every product, including the gaseous products from electrocatalysis (stage 2) and the products after fermentation (stage 3, biomass and PHA) were all calculated and presented as percentage of energy remaining in the products from the initial solar energy input.

Calculating solar-to-chemical efficiency (Stages 1 and 2)

For the solar to electricity, the energy conversion efficiency for the best available solar panel from the market is currently at ~24.0% efficiency. In the electrocatalysis unit, all the products are sorted into three categories: ethylene as a major gas product, various gas products including hydrogen, carbon monoxide, and methane, as well as the soluble intermediates for fermentation (formate, ethanol, acetate, and propanol). It should be noted that each of the three categories has its market values. Ethylene is a commodity chemical, serving as building blocks for various polymers and chemical products. Other gas products as a group are similar to natural gas and syngas. The recent focus on hydrogen economy makes this faction particularly valuable. The liquid products have their values, but separation cost could be higher. The strength of EMC2 lies in converting them together into valuable commodity products like bioplastics. Based on these value proposition, we calculated the energy efficiency in Stages 1 and 2 for three categories, including ethylene, other gases, and liquid products. Based on the calculation from Stages 1 and 2, we further carried out experiments and energy efficiency analysis for the conversion of CO2RR-derived intermediates into biomass and PHA. The integration of Stages 1 to 3 data will allow us to calculate how much energy retained in biomass and PHA, along with what is the solar energy conversion efficiency for biomass conversion.

For the energy conversion efficiency in the electrocatalysis, we use the energy content of the products (energy output) divided by the total energy input following the methods established by Liu et al., which is shown as follows:

$$ EE = \frac{\sum(\Delta H_{\text{product}} \times N_{\text{product}})}{\text{Total charge (C) \times Applied voltage (V)}} $$

where the $\Delta H_{\text{product}}$ is the combustion heat of the electrocatalysis product, $N_{\text{product}}$ is the moles of products produced in specific period of time, which could be calculated as:
\[ N_{\text{product}} = \frac{\text{Total Charge (C) \times Faradaic efficiency (FE)}}{n_{\text{product}} \times F} \]

where the \( n_{\text{product}} \) is the number of electrons used for generating one product molecule (or moles of electrons used per mole of product,) and F is Faradaic coefficient, which is 96,485 C mol\(^{-1}\).

Therefore, the energy conversion efficiency could be calculated as:

\[ EE = \sum \frac{\Delta H_{\text{product}} \times \text{FE}_{\text{product}}}{n_{\text{product}} \times F \times \text{Applied voltage (V)}} \]

In the electrocatalysis step, the faradaic efficiency (FE) to each product, number of electrons used \((n_{\text{product}})\), and their combustion heats \((\Delta H_{\text{product}})\) are listed as follows:

| Product      | FE    | \( n_{\text{product}} \) | \( \Delta H_{\text{product}} \) (kJ mol\(^{-1}\)) |
|--------------|-------|--------------------------|-----------------------------------------------|
| Hydrogen     | 11.6% | 2                        | -286.0                                       |
| Carbon monoxide | 13.6% | 2                        | -283.0                                       |
| Methane      | 1.9%  | 8                        | -890.7                                       |
| Ethylene     | 39.2% | 12                       | -1411.2                                      |
| Formate      | 4.2%  | 2                        | -254.6                                       |
| Ethanol      | 16.3% | 12                       | -1367.6                                      |
| Acetate      | 5.0%  | 8                        | -875.1                                       |
| Propanol     | 3.3%  | 18                       | -2021.3                                      |

The energy conversion efficiency to three categories of products were:

\[ EE_{\text{Electricity to H2/CO/CH4}} = 15.6\% \]
\[ EE_{\text{Electricity to ethylene}} = 19.0\% \]
\[ EE_{\text{Electricity to solubles}} = 13.6\% \]

When integrating stage 1 and 2, the solar-to-product efficiencies for three categories are:

\[ EE_{\text{solar to H2/CO/CH4}} = 24.0\% \times 15.6\% = 3.75\% \]
\[ EE_{\text{solar to ethylene}} = 24.0\% \times 19.0\% = 4.56\% \]
\[ EE_{\text{solar to solubles}} = 24.0\% \times 13.6\% = 3.26\% \]

And the solar-to-all chemicals efficiency is the combination of above three, which is:

\[ EE_{\text{solar to chemicals}} = 11.57\% \]

**Calculating the energy conversion efficiency for bioconversion (Stage 3)**

In the fermentation stage, the soluble intermediates were used as the carbon source, which were transformed to cell biomass and/or PHA. The efficiency of bioproduction was calculated as:

\[ EE_{\text{bioproduction}} = \frac{\text{energy content of generated bioproducts}}{\text{total energy content of consumed soluble products}} \]

To achieve accurate evaluation of energy conversion using \( P. \ putida \), we first investigated the cell growth using the CO\textsubscript{2}RR effluent (electrolyte with soluble products) from the electrocatalysis with nitrogen
source. The experiments derived accurate energy conversion for biomass at Stage 3. We monitored the concentration of carbon source in the solution and stopped the fermentation one hour after all the carbon sources are consumed. The carbon source consumption, biomass increase, and the combustion heats ($\Delta H$) of each component are listed as follows:

| Carbon sources or Products | Amount of consumption/generation | Combustion heat ($\Delta H$) |
|----------------------------|---------------------------------|-----------------------------|
| **Consumption**            |                                 |                             |
| Ethanol                    | 0.69 mmol                       | -1367.6 kJ/mol              |
| Acetate                    | 0.46 mmol                       | -875.1 kJ/mol               |
| Propanol                   | 0.04 mmol                       | -2021.3 kJ/mol              |
| Formate                    | 0.56 mmol                       | -254.6 kJ/mol               |
| **Generation**             |                                 |                             |
| Cell biomass               | 31.2 mg                         | -20 kJ/g                   |

Therefore, the energy conversion efficiency from soluble intermediates to cell biomass is:

$$EE_{solubles to biomass} = \frac{-20 \text{ kJ/g} \times 31.2 \text{ mg}}{-1367.6 \frac{\text{kJ}}{\text{mol}} \times 0.69 \text{ mmol} - 875.1 \frac{\text{kJ}}{\text{mol}} \times 0.46 \text{ mmol} - 2021.3 \frac{\text{kJ}}{\text{mol}} \times 0.04 \text{ mmol} - 254.6 \frac{\text{kJ}}{\text{mol}} \times 0.56 \text{ mmol}} \times 100\% = 39.7\%$$

The 39.7% energy conversion efficiency is consistent with what has been previously reported, highlighting that ethanol and acetate are suitable intermediates and the Chem-Bio interface design for EMC2 is effective. When integrating the Stage 1 to 3, the solar-to-biomass efficiency is:

$$EE_{solar to biomass} = 24.0\% \times 13.6\% \times 39.7\% = 1.30\%$$

The energy efficiency for all products conversion therefore is:

$$EE_{solar to all products} = EE_{solar to H2/CO/CH4} + EE_{solar to ethylene} + EE_{solar to biomass} = 9.61\%$$

The staged diagram and energy efficiency for each category of products were presented in Figure S14.

Besides, we also calculated the energy efficiency for PHA production using engineered strain in the integrated system. The consumption of carbon source is calculated by subtracting the remaining carbon source from the total carbon source produced from the CO$_2$RR. The total amount of consumed soluble intermediates, generated PHA and biomass in one typical fermentation cycle, as well as their combustion heats ($\Delta H$) of each component are listed as follows:

| Carbon sources or Products | Amount of consumption/generation | Combustion heat ($\Delta H$) |
|----------------------------|---------------------------------|-----------------------------|
| **Consumption**            |                                 |                             |
| Ethanol                    | 2.26 mmol                       | -1367.6 kJ/mol              |
| Acetate                    | 1.60 mmol                       | -875.1 kJ/mol               |
| Propanol                   | 0.31 mmol                       | -2021.3 kJ/mol              |
| Formate                    | 0.74 mmol                       | -254.6 kJ/mol               |
| **Generation**             |                                 |                             |
| PHA                        | 16.7 mg                         | -49 MJ/kg                   |
| Cell biomass               | 17.3 mg                         | -20 kJ/g                    |
Therefore, the energy conversion efficiency from soluble intermediates to PHA and biomass is:

\[
EE_{\text{soluble to PHA & biomass}} = \frac{-49 \, \text{MJ/kg} \times 16.7 \, \text{mg} - 20 \, \text{kJ/g} \times 17.3 \, \text{mg}}{-1367.6 \, \frac{\text{kJ}}{\text{mol}} \times 2.26 \, \text{mmol} - 875.1 \, \frac{\text{kJ}}{\text{mol}} \times 1.60 \, \text{mmol} - 2021.3 \, \frac{\text{kJ}}{\text{mol}} \times 0.31 \, \text{mmol} - 254.6 \, \frac{\text{kJ}}{\text{mol}} \times 0.74 \, \text{mmol}} \times 100\% = 21.9\%
\]

Considering that PHA contains much higher energy content than biomass, a lower energy conversion efficiency for PHA production is expected. The solar to PHA and biomass efficiency is therefore about 0.71%, and the efficiency for all products when PHA is produced is 9.02%.

**Solar energy conversion efficiency for biomass**

As aforementioned, all three categories of products have their values and applications, yet only soluble products (formate, ethanol, acetate, and propanol) contributed to the cell growth and fermentation. A more relevant calculation of solar energy-to-biomass conversion efficiency was recently presented by Hann et al.\textsuperscript{26} Based on this method, we calculated the energy efficiency in stage 2 by only considering the production of the soluble products, while ethylene and all other gaseous products are excluded. The production rates \((R)\), number of electron \((n_{\text{product}})\) and the combustion energy \((\Delta H)\) of the soluble products are listed as follows:

| Soluble product | Production rate \((R, \text{mmol/h})\) | Number of electron \((n_{\text{product}})\) | Combustion energy \((\Delta H, \text{kJ/mol})\) |
|-----------------|-------------------------------------|---------------------------------|--------------------------------|
| Formate         | 0.31                                | 2                               | -254.6                          |
| Ethanol         | 0.20                                | 12                              | -1367.6                         |
| Acetate         | 0.09                                | 8                               | -875.1                          |
| Propanol        | 0.03                                | 18                              | -2021.3                         |

In a specific amount of time \((t)\), the total theoretical energy content of soluble products is:

\[
\text{Total theoretical energy content} = \sum (\Delta H_{\text{product}} \times R_{\text{product}} \times t)
\]

And the actual energy input in the CO\textsubscript{2} electrolyzer is:

\[
\text{Actual energy input} = \sum (R_{\text{product}} \times t \times n_{\text{product}} \times F) \times \text{Applied voltage (V)}
\]

The electricity-to-soluble products efficiency is:

\[
EE_{\text{soluble}} = \frac{\sum (\Delta H_{\text{product}} \times R_{\text{product}})}{\sum (R_{\text{product}} \times n_{\text{product}} \times F) \times \text{Applied voltage (V)}} = 47.2\%
\]

Therefore, the solar-to-biomass efficiency is:

\[
EE_{\text{solar to biomass}} = 24.0\% \times 47.2\% \times 39.7\% = 4.50\%
\]

In a similar way, the solar-to-PHA/Biomass conversion efficiency is 2.48%.

Among the 2.48% conversion, approximately 70% are contributed by PHA, and 30% are contributed by biomass, considering that PHA has a much higher energy content as compared to biomass (-49 MJ/kg, or -49 kJ/g\textsuperscript{33} Vs. -20 kJ/g\textsuperscript{24,32}).
SUPPLEMENTAL REFERENCES

1. Chang, I.C., Chen, P.-C., Tsai, M.-C., Chen, T.-T., Yang, M.-H., Chiu, H.-T., and Lee, C.-Y. (2013). Large-scale synthesis of uniform Cu2O nanocubes with tunable sizes by in-situ nucleation. CrystEngComm 15, 2363-2366. 10.1039/C3CE26932A.
2. Zhou, Y., Che, F., Liu, M., Zou, C., Liang, Z., De Luna, P., Yuan, H., Li, J., Wang, Z., Xie, H., et al. (2018). Dopant-induced electron localization drives CO2 reduction to C2 hydrocarbons. Nat. Chem. 10, 974-980. 10.1038/s41557-018-0092-x.
3. Su, Y., Cestellos-Blanco, S., Kim, J.M., Shen, Y.-X., Kong, Q., Lu, D., Liu, C., Zhang, H., Cao, Y., and Yang, P. (2020). Close-Packed Nanowire-Bacteria Hybrids for Efficient Solar-Driven CO2 Fixation. Joule 4, 800-811. 10.1016/j.joule.2020.03.001.
4. Liu, C., Colón, B.C., Ziesack, M., Silver, P.A., and Nocera, D.G. (2016). Water splitting–biosynthetic system with CO2 reduction efficiencies exceeding photosynthesis. Science 352, 1210-1213. 10.1126/science.aaf5039.
5. Haas, T., Krause, R., Weber, R., Demler, M., and Schmidt, G. (2018). Technical photosynthesis involving CO2 electrolysis and fermentation. Nat. Catal. 1, 32-39. 10.1038/s41929-017-0005-1.
6. Li, H., Opgenorth, P.H., Wernick, D.G., Rogers, S., Wu, T.Y., Higashide, W., Malati, P., Huo, Y.X., Cho, K.M., and Liao, J.C. (2012). Integrated electromicrobial conversion of CO2 to higher alcohols. Science 335, 1596. 10.1126/science.1217643.
7. Stöckl, M., Harms, S., Dinges, I., Dimitrova, S., and Holtmann, D. (2020). From CO2 to Bioplastic – Coupling the Electrochemical CO2 Reduction with a Microbial Product Generation by Drop-in Electrolysis. ChemSusChem 13, 4086-4093. 10.1002/cssc.202001235.
8. Yu, L., Yuan, Y., Tang, J., and Zhou, S. (2017). Thermophilic Moorella thermotrophica-immobilized cathode enhanced microbial electrosynthesis of acetate and formate from CO2. Bioelectrochemistry 117, 23-28. 10.1016/j.bioelechem.2017.05.001.
9. Gildemyn, S., Verbeek, K., Slabbinck, R., Andersen, S.J., Prévot, A., and Rabaey, K. (2015). Integrated Production, Extraction, and Concentration of Acetic Acid from CO2 through Microbial Electrosynthesis. Environ. Sci. Technol. Lett. 2, 325-328. 10.1021/acs.estlett.5b00212.
10. Jiang, Y., Li, Q., Chu, N., Hao, W., Zhang, L., Zhan, G., Li, D., and Zeng, R.J. (2020). A slurry electrode integrated with membrane electrolysis for high-performance acetate production in microbial electrosynthesis. Sci. Total Environ. 741, 140198. 10.1016/j.scitotenv.2020.140198.
11. Sikanth, S., Singh, D., Vanbroekhoven, K., Pant, D., Kumar, M., Puri, S.K., and Ramakumar, S.S.V. (2018). Electro-biocatalytic conversion of carbon dioxide to alcohols using gas diffusion electrode. Bioresour. Technol. 265, 45-51. 10.1016/j.biortech.2018.02.058.
12. Hegner, R., Neubert, K., Kroner, C., Holtmann, D., and Harnisch, F. (2020). Coupled Electrochemical and Microbial Catalysis for the Production of Polymer Bricks. ChemSusChem 13, 5295-5300. 10.1002/cssc.202001272.
13. Arends, J.B.A., Patil, S.A., Roume, H., and Rabaey, K. (2017). Continuous long-term electricity-driven bioproduction of carboxylates and isopropanol from CO2 with a mixed microbial community. J. CO2 Util. 20, 141-149. 10.1016/j.jcou.2017.04.014.
14. Zhang, K., Zhou, Y., Song, T., and Xie, J. (2021). Bioplastic Production from the Microbial Electrosynthesis of Acetate through CO2 Reduction. Energy Fuels 35, 15978-15986. 10.1021/acs.energyfuels.1c02594.
15. Al Rowaihi, I.S., Kick, B., Grötzinger, S.W., Burger, C., Karan, R., Weuster-Botz, D., Eppinger, J., and Arold, S.T. (2018). A two-stage biological gas to liquid transfer process to convert carbon dioxide into bioplastic. Bioresour. Technol. Rep. 1, 61-68. 10.1016/j.biortech.2018.02.007.
16. Liu, C., Gallagher, J.J., Sakimoto, K.K., Nichols, E.M., Chang, C.J., Chang, M.C.Y., and Yang, P. (2015). Nanowire–Bacteria Hybrids for Unassisted Solar Carbon Dioxide Fixation to Value-Added Chemicals. Nano Lett. 15, 3634-3639. 10.1021/nl503058n.
17. Liu, Z.-H., Shinde, S., Xie, S., Hao, N., Lin, F., Li, M., Yoo, C.G., Ragauskas, A.J., and Yuan, J.S. (2019). Cooperative valorization of lignin and residual sugar to polyhydroxyalkanoate (PHA) for
enhanced yield and carbon utilization in biorefineries. Sustain. Energy Fuels 3, 2024-2037. 10.1039/c9se00021f.

18. Franden, M.A., Jayakody, L.N., Li, W.J., Wagner, N.J., Cleveland, N.S., Michener, W.E., Hauer, B., Blank, L.M., Wierckx, N., Klebensberger, J., and Beckham, G.T. (2018). Engineering Pseudomonas putida KT2440 for efficient ethylene glycol utilization. Metab. Eng. 48, 197-207. 10.1016/j.men.2018.06.003.

19. Vo, M.T., Ko, K., and Ramsay, B. (2015). Carbon-limited fed-batch production of medium-chain-length polyhydroxyalkanoates by a phaZ-knockout strain of Pseudomonas putida KT2440. J. Ind. Microbiol. Biotechnol. 42, 637-646. 10.1007/s10295-014-1575-4.

20. Schägger A., Tauch A., Jäger W., Kalinowski J., Thierbach G., and Pühler A. (1994). Small mobilizable multi-purpose cloning vectors derived from the Escherichia coli plasmids pK18 and pK19: selection of defined deletions in the chromosome of Corynebacterium glutamicum. Gene 145, 69-73.

21. de Boer H.A., Comstock L.J., and Vasser M. (1983). The tac promoter A functional hybrid derived from the trp and lac promoters. Proc. Natl. Acad. Sci. U. S. A. 50, 21-25.

22. Cook, T.B., Rand, J.M., Nurani, W., Courtney, D.K., Liu, S.A., and Pfleger, B.F. (2018). Genetic tools for reliable gene expression and recombineering in Pseudomonas putida. J. Ind. Microbiol. Biotechnol. 45, 517-527. 10.1007/s10295-017-2001-5.

23. Simon R., Priefer U., Pühler A. (1983). A Broad Host Range Mobilization System for In Vivo Genetic Engineering Transposon: Mutagenesis in Gram Negative Bacteria. Nat. Biotechnol. 1, 784-791.

24. Claassens, N.J., Cotton, C.A.R., Koplij, D., and Bar-Even, A. (2019). Making quantitative sense of electromicrobial production. Nat. Catal. 2, 437-447. 10.1038/s41929-019-0272-0.

25. Zheng, T., Zhang, M., Wu, L., Guo, S., Liu, X., Zhao, J., Xue, W., Li, J., Liu, C., Li, X., et al. (2022). Upcycling CO2 into energy-rich long-chain compounds via electrochemical and metabolic engineering. Nat. Catal. 5, 388-396. 10.1038/s41929-022-00775-6.

26. Hann, E.C., Ovra, S., Harland-Dunaway, M., Narvaez, A.F., Le, D.N., Orozco-Cárdenas, M.L., Jiao, F., and Jinkerson, R.E. (2022). A hybrid inorganic-biological artificial photosynthesis system for energy-efficient food production. Nat. Food 3, 461-471. 10.1038/s43016-022-00530-x.

27. IEA (2019). The Future of Hydrogen, Report Prepared by the IEA for the G20, Japan. IEA Paris, France.

28. Staffell, I., Scamman, D., Velazquez Abad, A., Balcombe, P., Dodds, P.E., Ekins, P., Shah, N., and Ward, K.R. (2019). The role of hydrogen and fuel cells in the global energy system. Energy Environ. Sci. 12, 463-491. 10.1039/C8EE01157E.

29. Wiswann, S.T., Engbaek, J.S., Vendelbo, S.B., Bendixen, F.B., Eriksen, W.L., Aasberg-Petersen, K., Frandsen, C., Chorkendorff, I., and Mortensen, P.M. (2019). Electrified methane reforming: A compact approach to greener industrial hydrogen production. Science 364, 756-759. 10.1126/science.aaw8775.

30. Song, Y., Ozdemir, E., Ramesh, S., Adishev, A., Subramanian, S., Harale, A., Albuali, M., Fadhel, B.A., Jamal, A., Moon, D., et al. (2020). Dry reforming of methane by stable Ni-Mo nanocatalysts on single-crystalline MgO. Science 367, 777-781. 10.1126/science.aav2412.

31. Clark, D., Malered-Fjeld, H., Budd, M., Yuste-Tirados, I., Beeaff, D., Aamodt, S., Nguyen, K., Ansaloni, L., Peters, T., Vestre, P.K., et al. (2022). Single-step hydrogen production from NH3, CH4, and biogas in stacked proton ceramic reactors. Science 376, 390-393. 10.1126/science.abj3951.

32. Cordier, J.-L., Butsch, B.M., Biou, B., and von Stockar, U. (1987). The relationship between elemental composition and heat of combustion of microbial biomass. Appl. Microbiol. Biotechnol. 25, 305-312. 10.1007/BF00252538.

33. Biron, M. (2020). Environmental and Engineering Data to Support Eco-Design for Plastics. In A Practical Guide to Plastics Sustainability, M. Biron, ed. (William Andrew Publishing), pp. 209-307. 10.1016/B978-0-12-821539-5.00006-9.