Electronic Supplementary Material

Co anchored on porphyrinic triazine-based frameworks with excellent biocompatibility for conversion of CO_2 in H_2-mediated microbial electrosynthesis

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1 Electrode preparation

The catalysts were dispersed in a mixture containing ethanol and 0.5 wt% Nafion (v:v = 9:1) at the concentration of 10 mg·mL^{-1}. The mixture was uniformly dispersed by 2 h of ultrasound then 12 h of vigorous stirring. The as-obtained homogenously dispersed solution known as catalyst ink was dropped onto a pretreated carbon paper (2 × 2 cm^2) with the loading amount of 1.0 mg·cm^{-2}, which was prepared to be assembled with a platinum electrode clip as the working electrode.

All the electrochemical measurements were performed in a single-chamber three-electrode system via an electrochemical workstation (BioLogic VSP300), where the as-prepared electrode, carbon rod and saturated Ag/AgCl electrode functioned as the counter electrode and reference electrode, respectively. 150 mL of minimal medium (MM, pH=7.28) containing 9 g·L^{-1} $Na_2HPO_4·12H_2O$, 1.5 g·L^{-1} KH_2PO_4, 0.2 g·L^{-1} $(NH_4)_2SO4$, 0.2 g·L^{-1} $NaHCO_3$, 80 mg·L^{-1} $MgSO_4·7H_2O$, 1 mg·L^{-1} $CaSO_4·2H_2O$, 0.52 mg·L^{-1} $NiSO_4·6H_2O$ and 0.4 mg·L^{-1} ferric citrate was used as the electrolyte as well as bacterial culture buffer. By the way, a slight modification was made on the composition of MM that the concentration of ferric citrate in MM was increased to 50 mg·L^{-1} in order to shorten the lag phase during the beginning of culturing and make full use of external electricity [1].

2 Preparation of the MES reactor

The MES system was performed in a single-chamber reactor. As shown in Fig. S1, the reactor was equipped with a working electrode, a counter electrode, an inlet pipe, an outlet pipe and a sampling tube. The as-prepared carbon paper or SS electrode and Pt mesh (1×1 cm^2) were employed as the working electrode and the counter electrode respectively. The inlet pipe was composed of a sand core aerator in order to enhance gas-liquid mass transfer. The gas inlet and outlet pipe were indirectly connected to the atmosphere through 0.20 μm PVDF filters. Current densities were applied to MES system using an electrochemical workstation mentioned above.

Pre-culture of strain. *Cupriavidus necator* H16 (ATCC 17699) was used as the biocatalyst to convert CO_2 into poly-β-hydroxybutyrate (PHB). It was stored at −80ºC with 25% glycerol. The strain was recovered in a Luria-Bertani broth at 30ºC for 48 h to get enough microbe density. The as-prepared strain was used for plate planting and pre-culture. The pre-culture was carried on MM with 3% as-prepared strain, 10% D-fructose and 10 $mg \cdot L^{-1}$ of gentamicin for 12 h to ensure the logarithmic phase. Pre-cultured strain was washed twice with MM and centrifuged at 7000 rpm for 7 min in order to prevent the introduction of organic carbon sources in the following experimental procedures. Then it was re-suspended in the MM for inoculation.

3 Quantification of PHB titers

The cell pellets stored in −20ºC were dried thoroughly at 60ºC for at least 24 hours. 1 mL of concentrated sulfuric acid was added into each tube then it was heated at 90ºC for 90 min in order to fully convert PHB into monomer followed by crotonic acid (Fig. S6).

High performance liquid chromatography (HPLC, Agilent HPLC 1260) equipped with an Aminex HPX-87H column was employed to quantify the titers of crotonic acid. 100 μL of diluted and filtered samples were injected into the column system. 2.5 $mmol \cdot L^{-1}$ H_2SO_4 aqueous solution was the solo mobile phase and the flow rate was set to 0.5 $mL \cdot min^{-1}$ for at least 40 min. UV absorption detector was used to quantify the concentration of crotonic acid at the wavelength of 220 nm with the help of the calibration curve shown in Fig. S1. The concentration of PHB can be calculated with some simple mathematical operations.

4 Calculations of proportion of PHB in Chemicals (PHB/Chemicals) and energy conversion efficiency (ECE)

PHB/Chemicals

$$\mathrm{PHB/Chemicals}\ (\%) = \frac{c_{\mathrm{PHB}}}{c_{\mathrm{Chemicals}}} \times 100 \tag{1}$$

$$c_{\mathrm{Chemicals}} = c_{\mathrm{PHB}} + c_{\mathrm{Biomass}} \tag{2}$$

ECE

$$\mathrm{ECE}(\%) = \frac{\Delta_r G^{\theta}_{\mathrm{Chemicals}}}{\Delta W_{\mathrm{Electricity}}} \times 100 \tag{3}$$

$$\Delta_r G^{\theta}_{\mathrm{Chemicals}} = \Delta_r G^{\theta}_{\mathrm{PHB}} + \Delta_r G^{\theta}_{\mathrm{Biomass}} \tag{4}$$

$$\Delta W_{\mathrm{Electricity}} = \left| \int_0^t \mathrm{current} \times \mathrm{Voltage} \times \mathrm{t}\,\mathrm{d}t \right| \tag{5}$$

The current value was maintained to be −24 $mA \cdot cm^{-2}$ under chronopotentiometry mode. Furthermore, the $\Delta W_{\mathrm{Electricity}}$ can be accurately calculated by integral of the applied voltage between anode and cathode

($E_{applied}$)-time curve (Fig S5). $\Delta_rG^{\theta}_{Chemicals}$ stands for the Gibbs free energy of total chemicals produced by bacteria, which is equivalent to the total output energy and can be divided into two parts furtherly. $\Delta_rG^{\theta}_{PHB}$ and $\Delta_rG^{\theta}_{Biomass}$ can be calculated by corresponding chemical reactions as follows：

PHB

$$4n\ CO_2(g) + 3n\ H_2O(l) \rightarrow (C_4H_6O_2)_n(s) + 4.5\ O_2(g)$$
$$\Delta_rG^{\theta} \sim \Delta_rH^{\theta} = 1903\ kJ \cdot mol^{-1}\ \text{per monomer} \quad (6)$$

Biomass

$$CO_2(g) + 0.24\ NH_3(aq) + 0.525\ H_2O \rightarrow C_{1.77}O_{0.49}N_{0.24}(s) + 1.02\ O_2(g) \quad \Delta_rG^{\theta} = 479\ kJ \cdot mol^{-1} \quad (7)$$

The molar concentration of PHB can be measured as mentioned before. The biomass can be expressed as the chemical formula $CH_{1.77}O_{0.49}N_{0.24}$ [2] and its concentration can be defined based on an empirical relationship: 1.0 OD_{600} = 448 mg·L^{-1} biomass [3]. However, the OD_{600} brought about by the accumulation of PHB (ΔOD_{600}) should be subtracted from the experimental OD_{600} value. The relationship between the concentration of PHB and ΔOD_{600} is defined as 1.0 ΔOD_{600} = 831 mg·L^{-1} PHB[4]. Above all, the mass concentration of biomass can be calculated using the following equation:

$$c_{Biomass}(mg \cdot L^{-1}) = \left\{ OD_{600} - \frac{c_{PHB}(mg \cdot L^{-1})}{831} \right\} \times 448 \quad (8)$$

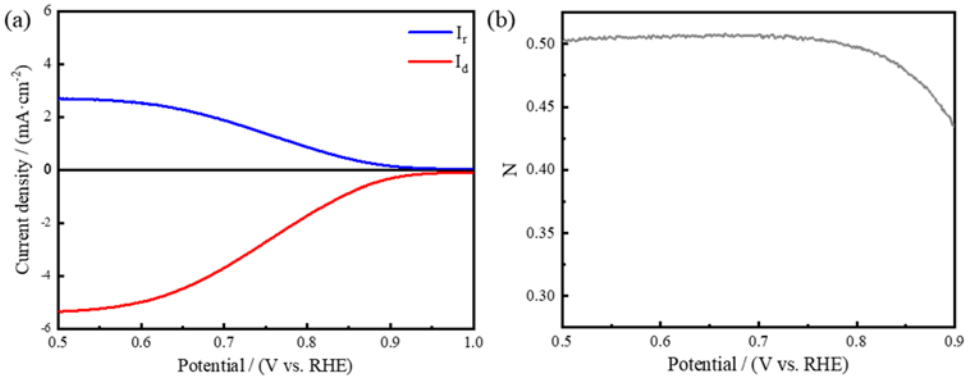

Fig. S1 Calibration for current collection efficiency (N) of RRDE. (a) The ring current densities (I_r), disk current densities (I_d) and (b) N plots measured by RRDE in the electrolyte containing 10 mmol·L^{-1} $K_3Fe(CN)_6$ and 100 mmol·L^{-1} KCl.

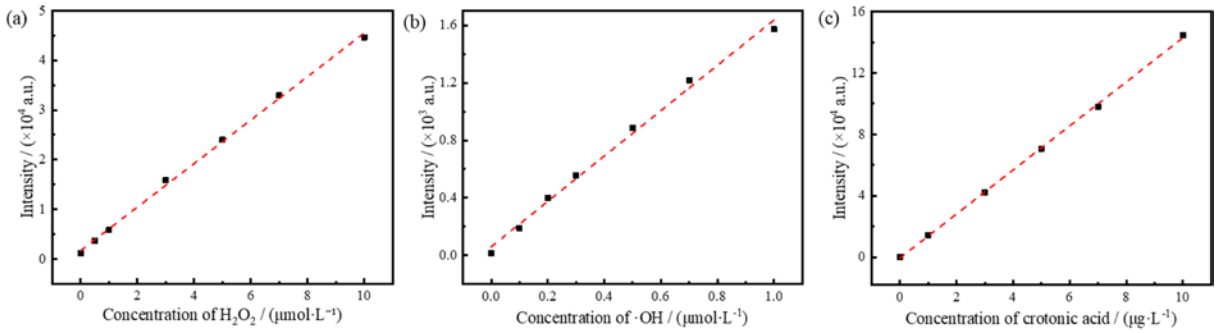

Fig. S2 Calibration curve of (a) H_2O_2 measured by fluorimetric hydrogen peroxide assay kit, (b) ·OH using terephthalic acid method and (c) crotonic acid detected by UV absorption detector at the wavelength of 220 nm.

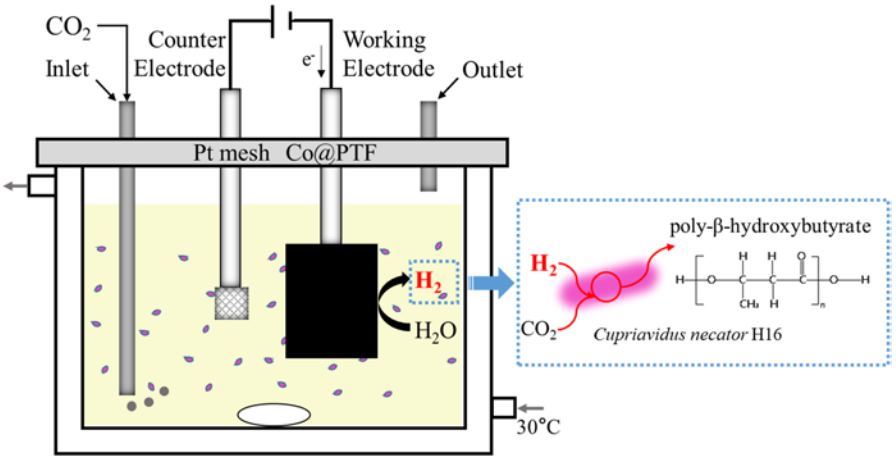

Fig. S3 Schematic diagram of MES reactor.

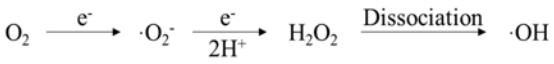

Fig. S4 The diagram for reaction pathway of ROS.

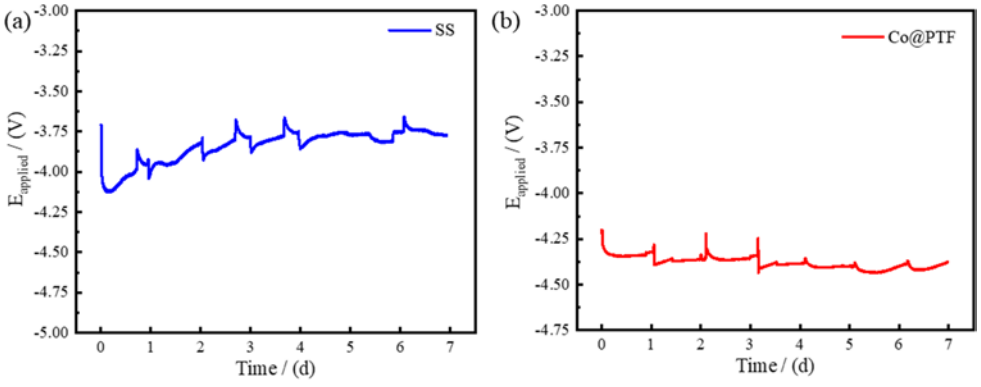

Fig. S5 $E_{applied}$-time curves of (a) SS and (b) Co@PTF at the current density of −6 mA·cm^{-2}.

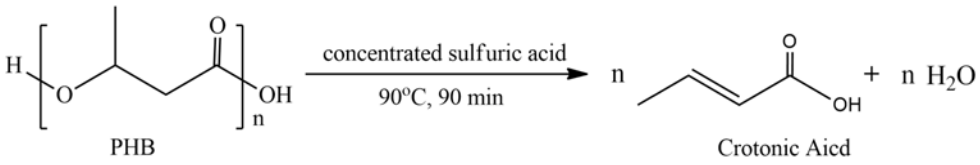

Fig. S6 Schematic diagram of PHB digestion reaction.

Table S1 The content of each element in Co@PTF measured by XPS.

Element	Atomic %	wt%
C	88.33	84.51
O	6.18	7.88
N	5.06	5.65
Co	0.42	1.97

References

1. Sydow A, Krieg T, Ulber R, Holtmann D. Growth medium and electrolyte—How to combine the different requirements on the reaction solution in bioelectrochemical systems using *Cupriavidus necator*. Engineering in Life Sciences, 2017, 17(7): 781−791

2. Abel A J, Clark D S. A comprehensive modeling analysis of formate-mediated microbial electrosynthesis. ChemSusChem, 2021, 14(1): 344−355

3. Park J M, Kim T Y, Lee S Y. Genome-scale reconstruction and in silico analysis of the *Ralstonia eutropha* H16 for polyhydroxyalkanoate synthesis, lithoautotrophic growth, and 2-methyl citric acid production. BMC Systems Biology, 2011, 5(1): 101

4. Liu C, Colón B C, Ziesack M, Silver P A, Nocera D G. Water splitting-biosynthetic system with CO_2 reduction efficiencies exceeding photosynthesis. Science, 2016, 352(6290): 1210−1213