Coupled Electrochemical and Microbial Catalysis for the Production of Polymer Bricks

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References
SI 1 Materials and Methods

All experimental electrochemical potentials refer to Ag/AgCl reference electrode (saturated KCl, 0.197 V vs. standard hydrogen electrode (SHE)) unless stated otherwise.

SI 1.1 Chemicals

All chemicals were of at least analytical grade. All solutions were prepared with de-ionized water (Merck Millipore, USA). Na$_2$CO$_3$, NaHCO$_3$, CaCl$_2$, NH$_4$Cl, MgSO$_4$, Na$_2$EDTA, ZnSO$_4$, CuSO$_4$, K$_2$HPO$_4$, NaH$_2$PO$_4$ were obtained from Carl Roth GmbH (Germany). H$_2$SO$_4$, CoCl$_2$, MnCl$_2$, Na$_2$MoO$_4$, FeSO$_4$ were supplied by Merck KGaA (Germany), InCl$_3$ (anhydrous, 99.999%) from Chempur (Germany), and H$_3$BO$_3$ from Riedel-de Haen (Germany). CO$_2$ (99.5 mol-% purity) was purchased from Air Products GmbH (Germany). Compressed air (CA) was produced in-house by a compressor and used as an air-like gas mixture with 78 Vol-% N$_2$, 21 Vol-% O$_2$, 0.93 Vol-% Ar, 0.04 Vol-% CO$_2$, and other gases in traces.

SI 1.2 Electrochemical CO$_2$ reduction (ECO$_2$R)

All experiments were carried out under potentiostatic control by a potentiostat/galvanostat (SP-200, BioLogic Science Instruments, France) using a three-electrode setup consisting of a working electrode (WE), an Ag/AgCl reference electrode (RE, SE 11, 0.197 V vs. SHE, Xylem Analytics Germany Sales GmbH & Co / Sensortechnik Meinsberg, Germany) and a platinum-based counter electrode (for details on material and electrode size see below).

SI 1.2.1 1 L-electrobioreactor

The 1 L-electrobioreactor comprises an INFORS reactor vessel (INFORS HT Multifors, Switzerland) equipped with the up-grade kit described previously by Rosa et al.$^{[1]}$. The system was always operated in a two-chamber configuration except for the In-deposition. The counter electrode (platinum foil, 0.05 $\times$ 3.5 $\times$ 8 cm, Goodfellow, UK) was housed separately in a counter electrode chamber inlay interfaced via an ion exchange membrane (fumasep FKE, FuMA-Tech GmbH, Germany). Unless stated otherwise all experiments were carried out at 30 °C.
Figure S1: Detailed scheme of the 1 L-electrobioreactor.

SI 1.2.1.1 Graphite backbone pre-treatment and electrodeposition of Indium (In) on the graphite electrode backbone

For the 1 L-electrobioreactor the geometric electrode surface area, $A_{\text{WE,geom}} = 50 \text{ cm}^2$, was reached by using two graphite rods (99.5 %, CP-2200, $\varnothing 1.0 \times 25 \text{ cm}$, CP-Graphitprodukte, Germany) as electrode backbones with a final geometric catalytic surface area after In-deposition of $25 \text{ cm}^2$ each. For electrochemical backbone cleaning and In-deposition the graphite rods were treated individually. According to the workflow, the electrode surface area is defined by the immersion depth of the graphite backbone into the In-salt solution ($0.1 \text{ M InCl}_3$ in 1 M de-aerated acetate buffer, pH = 4.5). To assure precise immersion depth and geometrically fixed position of the graphite rods, a 1 L glass beaker equipped with a custom-made lid, previously described by
Gimkiewicz et al.[2] for a 50 mL electrochemical cell was used. The lid was printed from polylactic acid (Innofil3D, The Netherlands) using a 3D printer (Ultimaker 2+, Ultimaker, The Netherlands), and was fit into the 1 L glass beaker using a tailor-made 3D printed adapter (Figure S2)[3].

Figure S2: Scheme of the electrochemical cell utilized for electrochemical cleaning of the graphite backbone and the In-deposition. The lid of the 50 mL electrochemical cell was fit into a 1 L glass beaker by a tailor-made 3D printed adapter.

The graphite rods for the graphite backbone were pre-treated by smoothening and polishing with abrasive paper (P1200, 3M, USA). Subsequently, the graphite backbone was ultrasonicated at 35 kHz for 30 s in a water bath (Super RK103H, Sonorex, Germany) and after washing three times with MilliQ water it was dried under a nitrogen stream. For electrochemical cleaning 700 mL of 0.1 M H₂SO₄ was used. For In-deposition at an electrode surface area of 25 cm², the volume of InCl₃-solution was 625 mL. The respective immersion depth of the graphite backbones into the InCl₃-solution was fixed by using the WE port of the lid and a rubber stopper (Figure S2). Electrochemical parameters for backbone cleaning were set as described previously by
Gimkiewicz et al.\cite{2}, with the change that 40 cycles were used for electrochemical cleaning. In was deposited on the graphite backbone at $E_{\text{depos}} = -0.9 \text{ V}$, thereby the initial linear sweep towards $E_{\text{depos}}$ was set from $E_i = 0 \text{ V}$ to $E_1 = 0.65 \text{ V}$ at a scan rate of 20 mV s$^{-1}$. This was followed by a potential jump from $E_1$ to $E_{\text{depos}}$ to avoid the hydrogen evolution reaction (HER) in the potential range between $E_1$ and $E_{\text{depos}}$ before In-deposition. The charge for In-deposition on the graphite backbone, $Q_{\text{depos}}$, was set to 16 C for each graphite rod. $Q_{\text{depos}}$ for In-loading on each graphite backbone corresponds to an average of 1975 atomic layers of In being a total amount of 6.347 mg In on 25 cm$^2$. Consequently, 12.693 mg In was used for the electrode fabrication of one 1 L-electrobioreactor. The liquid phase was always mixed by a magnetic stir bar at 1000 rpm.

**SI 1.2.1.2 Sterilization of the 1 L-electrobioreactor**

To assure sterile working conditions in the 1 L-electrobioreactor, standard steam sterilization procedure was applied after In-deposition (see section **SI 1.2.1.1**) as previously described by Hegner et al.\cite{3}. Therefore, the In-coated graphite backbone was assembled to the WE inlet ports of the electrobioreactor lid and the lid was subsequently assembled to the reactor vessel. Notably, no counter electrode chamber inlay and RE were assembled to the lid before steam sterilization. Thus, the port of the inlay was closed with a tight-fitted cap made from PEEK, and the RE port was closed with a glass rod of the same diameter as the RE. The counter electrode chamber hosting the counter electrode was autoclaved separately by dry heat sterilization. Dry heat sterilization was performed by inserting the counter electrode chamber hosting the counter electrode into a 1 L glass vessel being subsequently tightly closed with an autoclaving bag so that no water vapor could get in contact with the counter electrode chamber hosting the ion exchange membrane and the counter electrode\cite{4}.

In the next step, the In-electrodes containing electrobioreactor and the glass vessel with the counter electrode chamber hosting the counter electrode were put into the autoclave (Laboklav 195-MSLV, SHP Steriltechnik AG, Germany) for steam sterilization at 121 °C for 20 min. After cooling down the assembling of the electrobioreactor was performed in sterile conditions using a laminar flow box. Prior to its assembly, the reference electrode was sterilized in Beckmann solution for 30 min.
**SI 1.2.1.3  ECO$_2$R to formate in 30 mM carbonate buffer solution**

The ECO$_2$R to formate was performed in a volume of 808 mL of a 30 mM carbonate buffer at $E_{WE} = -1.6$ V and 30 °C for an experimental duration of 42 h. For preparing the carbonate buffer 25 mL of sterile-filtered (0.2 µm cellulose acetate filter type 11107, Sartorius Stedim Biotech GmbH, Germany) carbonate stock solution (1.06 g Na$_2$CO$_3$ and 7.57 g NaHCO$_3$ dissolved in 100 mL deionized water) were added to 783 mL sterile deionized water. Before the start of the formate production, the electrolyte solution was purged for at least 10 min with CO$_2$ at a gassing rate of 1.2 vvm. To avoid acidification of the electrolyte solution in the counter electrode chamber (30 mM carbonate buffer) that would lead to an increase of the cell potential, it was continuously pumped with a peristaltic pump (ISMATEC Reglo Analog MS-4/8, Cole-Parmer GmbH, Germany) from a 20 L reservoir to the counter electrode chamber and from there to a 20 L waste tank. The flow rate was set between 2 mL min$^{-1}$ and 68 mL min$^{-1}$, as required to maintain the cell potential. Liquid samples of 1 mL volume of the carbonate buffer solution in the WE chamber were taken for HPLC analysis at 0 h, 1 h, 2 h, 4 h, 18 h, 24 h, and 42 h. The pH was measured (sample volume 100 µL) with a drop pH meter (LAQUAtwin B-712, Japan). Furthermore, at the end of the ECO$_2$R (42 h) the conductivity of the carbonate buffer solution in the WE chamber was measured.

**SI 1.2.1.4  Indium catalyst stripping**

A major sustainability feature of the workflow is the re-use of the graphite electrode backbone for further sets of experiments. Thus, at the end of the experiment In was stripped from the graphite electrode backbone into 1 M de-aerated acetate buffer ($p$H = 4.5) by potential-sweep. The In-stripping was conducted for one electrode at a time using the 1 L glass beaker equipped with a tailor-made 3D printed adapter (Figure S2) and a working volume of 625 mL. In was not stripped back into the In solution for deposition (SI 1.2.1.1) to avoid contamination of the deposition solution with other ions from the experiment.
Coupled electrochemical and microbial catalysis of mesaconate and 2S-methylsuccinate using *Methylobacterium extorquens* AM-1 pCM160_RBS-yciA

The *M. extorquens* AM-1 pCM160_RBS-yciA strain was constructed by Sonntag *et al.*[5].

Pre-culture cultivation of *M. extorquens* AM-1 pCM160_RBS-yciA for coupled electrochemical and microbial catalysis

A pre-culture was grown by inoculation of 300 µL of *M. extorquens* AM-1 pCM160_RBS-yciA cryo-culture stock solution in 50 mL phosphate-buffered minimal medium using an Erlenmeyer flask of 300 mL volume. The phosphate-buffered minimal medium was based on Peyraud *et al.*[6] but slightly modified: 2.4 g L\(^{-1}\) K\(_2\)HPO\(_4\), 0.956 g L\(^{-1}\) NaH\(_2\)PO\(_4\), 1.5 mg L\(^{-1}\) CaCl\(_2\), and 0.64 mg L\(^{-1}\) MnCl\(_2\). Pre-culture cultivation was carried out at 30 °C and 150 rpm (Unitron Incubator Shaker, INFORS HT, Switzerland) for 10 days. Furthermore, the minimal medium was supplemented with 50 µL kanamycin stock solution (50 mg L\(^{-1}\), i.e., final concentration 50 µg L\(^{-1}\)) and 123 mM methanol (i.e., 4 g L\(^{-1}\)) by addition of 5 mL L\(^{-1}\)) was added as carbon and energy source. The growth of the pre-culture was monitored by measuring the optical density at 600 nm (\(OD_{600\text{ nm}}\)) at the beginning and the end of the pre-culture cultivation with a UV/Vis spectrophotometer (UviLine 9400; SI Analytics; Germany). Subsequently, the pre-culture biomass was harvested by centrifugation of 35 mL pre-culture at 11,000 \(\times\) g for 10 min and subsequently washed two times with 30 mM carbonate-buffered minimal medium (section SI 1.3.2) without any carbon source to remove phosphates and methanol. Finally, the pre-culture biomass was re-suspended in 10 mL of 30 mM carbonate-buffered minimal medium without any carbon source to serve as inoculum for the electrobioreactor that is already containing formate as carbon- and e- source (i.e., Phase 2), which was electrochemically produced from CO\(_2\) (section SI 1.3.2).

Inoculation of the 1 L-electrobioreactor

After ECO\(_2\)R to formate (Phase 1) and prior to inoculation of *M. extorquens* AM-1 pCM160_RBS-yciA, the formate containing carbonate-buffer in the 1 L-electrobioreactor was supplemented with 10 mL L\(^{-1}\) chloride concentrate (0.15 g L\(^{-1}\) CaCl\(_2\) × 2 H\(_2\)O, 162 g L\(^{-1}\) NH\(_4\)Cl), 10 mL L\(^{-1}\) sulfate concentrate (20 g L\(^{-1}\) MgSO\(_4\) × 7 H\(_2\)O), 1 mL L\(^{-1}\) trace element solution (TES) (15 g L\(^{-1}\) Na\(_2\)EDTA × 2 H\(_2\)O, 4.5 g L\(^{-1}\) ZnSO\(_4\) × 7 H\(_2\)O, 3 g L\(^{-1}\) CoCl\(_2\) × 6 H\(_2\)O, 0.64 g L\(^{-1}\) MnCl\(_2\), 1 g L\(^{-1}\) H\(_3\)BO\(_3\), 0.4 g L\(^{-1}\) Na\(_2\)MoO\(_4\) × 2 H\(_2\)O, 0.3 g L\(^{-1}\) CuSO\(_4\) × 5 H\(_2\)O, 3 g L\(^{-1}\) FeSO\(_4\) × 7 H\(_2\)O) and 1 mL L\(^{-1}\) kanamycin (50 mg L\(^{-1}\)) via a sterile septum. The TES stock solution was sterile filtered before use. The chloride concentrate and the sulfate concentrate were autoclaved at 121 °C for 20 min. Subsequently, a sample of 8 mL for measuring the conductivity was taken. Furthermore, the
gassing was switched from 1.2 vvm CO₂ to a mixture of 0.3 vvm CO₂ and 0.34 vvm CA leading to a pH of the carbonate-based minimal medium of 7.3.

The electrobioreactor was inoculated with *M. extorquens* AM-1 pCM160_RBS-yciA pre-culture biomass to an *OD*₆₀₀ nm of 0.09 ± 0.01 and was run for 96 h (Phase 2). For the inoculation, the resuspended pre-culture biomass (SI 1.3.1) was injected with a sterile syringe through a sterile septum into the medium. The liquid phase was continuously purged with a gas mixture of 0.3 vvm CO₂ and 0.34 vvm CA and mixed at 150 rpm. 2 mL samples were taken at 0 h, 8 h, 24 h, 48 h, 77 h, and 96 h after inoculation. 1 mL of the respective sample was taken for the *OD*₆₀₀ nm (UviLine 9400; SI Analytics; Germany) and pH measurement (drop pH-meter, LAQUAtwin B-712; Japan). The remaining 1 mL of sample volume was used for HPLC analysis.

**SI 1.4 Liquid phase analysis by HPLC**

HPLC (Shimadzu Scientific Instruments, Japan) measurements of the aqueous liquid phase were performed with a photodiode array detector (SPD-M20A prominence, Shimadzu Scientific Instruments, Japan) using a Hi-Plex H column (300 mm × 7.7 mm ID, 8 μm pore size, Agilent Technologies, Germany) equipped with a pre-column (Carbo-H 4 mm × 3 mm ID, Security Guard, Phenomenex). Isocratic elution at 40 °C with 0.005 M H₂SO₄ was set at 0.5 mL min⁻¹ for 30 min. Peak identification and calibration of formate (0.1 mM to 22.2 mM, six-point calibration; calibration limit is 0.1 mM, *R*²=0.99), mesaconate (0.007 mM to 7.8 mM, seven-point calibration, *R*²=0.999) and 2S-methylsuccinate (0.015 mM to 0.461 mM, six-point calibration, *R*²=0.999) was carried out with external standards. The wavelengths used for measuring formate and 2S-methylsuccinate were 210 nm and for mesaconate 242 nm, respectively.

The samples of the ECO₂R were centrifuged at 15,700 × g for 2 min and 100 μL of the sample were used for HPLC measurement. The samples of Phase 2, which contained biomass of *M. extorquens* were centrifuged at 15,700 × g for 10 min, the supernatant was transferred into a new Eppendorf tube and was sterile filtered (0.2 μm PTFE filter, VWR International, Germany). 100 μL of the undiluted, sterile-filtered sample was used for the HPLC measurement and analyzed immediately. The remaining liquid sample volume was stored at -20 °C for long-term storage.
SI 1.5 Data processing and calculations

SI 1.5.1 Coulombic efficiencies (CE) and production rate (r) of the ECO2R to formate

The Coulombic efficiency (CE) for electrochemical formate production from CO₂ was calculated by setting the charge transferred to formate (Q), in relation to the total charge (Q_total). Here, Q is derived from the mass m of formate within the electrolyte solution (volume of the electrolyte solution/ microbial minimal medium was assumed to be constant over time) determined by HPLC analysis:

\[ CE = \frac{Q}{Q_{total}} \times 100 \% \]  
Eq. (SI 1)

with

\[ Q = \frac{m}{M} \times z \times F \]  
Eq. (SI 2)

where \( M = 45 \text{ g mol}^{-1} \) is the molar mass of formate, \( z = 2 \) is the number of transferred electrons per CO₂ molecule, and \( F = 96485 \text{ C mol}^{-1} \) is the Faraday constant. \( Q_{total} \) can be derived from the integrated current (i) of the ECO₂R:

\[ Q_{total} = \int_0^t i(t)dt \]  
Eq. (SI 3)

The formate production is given as rate, \( r \) (mmol h⁻¹ cm⁻²), normalized to the geometric WE surface area \( A_{WEgeom} \) (cm²) of 50 cm².

\[ r = \frac{\Delta m}{M \times A_{WEgeom} \times \Delta t} \]  
Eq. (SI 4)

with \( \Delta m \) being the difference in mass of formate produced within the electrolyte solution between two sampling points \( t = n \) and \( t = n + 1 \).

\[ \Delta m = m_{t=n+1} - m_{t=n} \]  
Eq. (SI 5)
SI 1.5.2   Electrical power input for the ECO₂R to fomate

The electrical power input for the production of 1 mol of formate, \( P_{\text{formate}} \), is the power \( P_{\text{total}} \) for the electrochemical half cell reaction at the WE integrated (cathodic half cell) over time and normalized by the amount of formate produced (\( n_{\text{formate}} \)) from ECO₂R as determined by HPLC. \( E_{\text{WE}} \) used for calculating \( P_{\text{total}} \) refers to SHE as reference electrode:

\[
P_{\text{formate}} = \frac{P_{\text{total}}}{n_{\text{formate}}} \quad \text{Eq. (SI 6)}
\]

with

\[
P_{\text{total}}(t) = -E_{\text{WE}} \text{ (vs. SHE)} \times \int_0^t i(t) \, dt\quad \text{Eq. (SI 7)}
\]

SI 1.5.3   Calculation of the formate conversion efficiency and the overall conversion efficiency of coupled electro- and biocatalysis of C5-dicarboxylates

For the calculation of the formate conversion efficiency \( \eta_{\text{C5/formate}} \), which is considering formate as the carbon source and electron donor, the amounts of formate, 2S-methylsuccinate, and mesaconate in C-mol were used. The underlying stoichiometries for the formation of mesaconate and 2S-methylsuccinate are the following:

**Mesaconate:**

Reduction:

\[
2 \text{HCOOH} + 3 \text{CO}_2 + 14 \text{H}^+ + 14 \text{e}^- \rightarrow \text{C}_5\text{H}_6\text{O}_4 + 6 \text{H}_2\text{O} \quad \text{Eq. (SI 8)}
\]

Oxidation:

\[
7 \text{HCOOH} \rightarrow 7 \text{CO}_2 + 14 \text{H}^+ + 14 \text{e}^- \quad \text{Eq. (SI 9)}
\]

Total:

\[
9 \text{HCOOH} \rightarrow \text{C}_5\text{H}_6\text{O}_4 + 4 \text{CO}_2 + 6 \text{H}_2\text{O} \quad \text{Eq. (SI 10)}
\]

**2S-Methylsuccinate:**

Reduction:
\[ 2 \text{HCOOH} + 3 \text{CO}_2 + 16 \text{H}^+ + 16 e^- \rightarrow \text{C}_5\text{H}_8\text{O}_4 + 6 \text{H}_2\text{O} \quad \text{Eq. (SI 11)} \]

Oxidation:

\[ 8 \text{HCOOH} \rightarrow 8 \text{CO}_2 + 16 \text{H}^+ + 16 e^- \quad \text{Eq. (SI 12)} \]

Total:

\[ 10 \text{HCOOH} \rightarrow \text{C}_5\text{H}_8\text{O}_4 + 5 \text{CO}_2 + 6 \text{H}_2\text{O} \quad \text{Eq. (SI 13)} \]

The C-mol of the respective compounds were calculated as follows.

\[
\Delta \text{C-mol}_{\text{formate}} = \Delta n_{\text{formate}} \times 1 
\]
\[
\Delta \text{C-mol}_{\text{mesaconate}} = \Delta n_{\text{mesaconate}} \times 5 
\]
\[
\Delta \text{C-mol}_{\text{methylsuccinate}} = \Delta n_{\text{methylsuccinate}} \times 5 
\]

Eq. (SI 14)

Eq. (SI 15)

Eq. (SI 16)

Please note that \(\Delta\) describes the difference of the respective concentration of a compound between start (i.e., inoculation of \textit{M. extorquens} AM-1 pCM160\_RBS-\textit{yciA}, \(t = 0\) h, formate already being present as produced by the previous ECO\_2R in Phase 1) and end (\(t = 96\) h, formate consumed and C5-dicarboxylates produced) of the individual reactor runs. Here, \(\Delta \text{C-mol}_{\text{formate}}\) describes the total amount of formate (C-mol) that was metabolized by \textit{M. extorquens} AM-1 pCM160\_RBS-\textit{yciA}, whereas \(\Delta \text{C-mol}_{\text{mesaconate}}\) and \(\Delta \text{C-mol}_{\text{methylsuccinate}}\) refer to the amount of products (C-mol), which were formed.

Ultimately, the efficiency of exploiting formate as a substrate for the synthesis of C5-dicarboxylates \(\eta_{\text{C5/formate}}\) is calculated as follows:

\[
\eta_{\text{C5/formate}} = \frac{\Delta \text{C-mol}_{\text{mesaconate}} + \Delta \text{C-mol}_{\text{methylsuccinate}}}{\Delta \text{C-mol}_{\text{formate}}} \times 100 \% 
\]

Eq. (SI 17)

Note that the calculation is based on comparing start (i.e., \(t = 0\) h that is the inoculation of \textit{M. extorquens} AM-1 pCM160\_RBS-\textit{yciA}, formate already being present as produced by the previous ECO\_2R) and end concentrations (\(t = 96\) h, formate consumed and C5-dicarboxylates produced) of the respective compounds in Phase 2 of the individual reactor runs. For detailed information on the time code see Figure 1 in the main manuscript.
Finally, the overall conversion efficiency ($\eta$) of coupled electrocatalysis and biocatalysis of C5-dicarboxylates is calculated as follows:

$$\eta = \frac{CE}{100} \times \frac{\eta_{C5/formate}}{100} \times 100\%$$

Eq. (SI 18)

SI 1.5.4 Calculation of the maximum formate conversion efficiency to C5-dicarboxylates

The theoretical maximum value for the efficiency of conversion of formate to the respective C5-dicarboxylates can be calculated using the stoichiometry described by Eq. (SI 8) and Eq. (SI 11).

$$\eta_{\text{theoretical}}^{\text{mesaconate/formate}} = 55.6\%$$

$$\eta_{\text{theoretical}}^{\text{methylsuccinate/formate}} = 50.0\%$$

It is important to note that the product ratio mesaconate/methylsuccinate must be also taken into account, which is 0.7 in the present study (see Figure 2A in the main manuscript):

$$1 \text{ mole}_{C5} = 0.413 \times \text{mole}_{\text{mesaconate}} + 0.588 \times \text{mole}_{\text{methylsuccinate}}$$

Eq. (SI 19)

$$\frac{n_{\text{mesaconate}}}{n_{\text{methylsuccinate}}} = 0.7$$

Eq. (SI 20)

$$\eta_{\text{theoretical}}^{\text{C5/formate}} = 0.413 \times \eta_{\text{theoretical}}^{\text{mesaconate/formate}} + 0.588 \times \eta_{\text{theoretical}}^{\text{methylsuccinate/formate}}$$

Eq. (SI 21)

$$\eta_{\text{theoretical}}^{\text{C5/formate}} = 52.3\%$$
SI 1.5.5  Stoichiometry of C5-dicarboxylate formation from methanol as a substrate

**Mesaconate:**

Reduction:

\[ 2 \text{CH}_3\text{OH} + 3 \text{CO}_2 + 6 \text{H}^+ + 6 e^- \rightarrow \text{C}_5\text{H}_6\text{O}_4 + 4 \text{H}_2\text{O} \]  
Eq. (SI 22)

Oxidation:

\[ \text{CH}_3\text{OH} + \text{H}_2\text{O} \rightarrow \text{CO}_2 + 6 \text{H}^+ + 6 e^- \]  
Eq. (SI 23)

Total:

\[ 3 \text{CH}_3\text{OH} + 2 \text{CO}_2 \rightarrow 3 \text{C}_5\text{H}_6\text{O}_4 + 3 \text{H}_2\text{O} \]  
Eq. (SI 24)

**2S-Methylsuccinate:**

Reduction:

\[ 6 \text{CH}_3\text{OH} + 9 \text{CO}_2 + 24 \text{H}^+ + 24 e^- \rightarrow 3 \text{C}_5\text{H}_8\text{O}_4 + 12 \text{H}_2\text{O} \]  
Eq. (SI 25)

Oxidation:

\[ 4 \text{CH}_3\text{OH} + 4 \text{H}_2\text{O} \rightarrow 4 \text{CO}_2 + 24 \text{H}^+ + 24 e^- \]  
Eq. (SI 26)

Total:

\[ 10 \text{CH}_3\text{OH} + 5 \text{CO}_2 \rightarrow 3 \text{C}_5\text{H}_8\text{O}_4 + 8 \text{H}_2\text{O} \]  
Eq. (SI 27)

SI 1.5.6  Statistical analysis

For significance testing, a student's t-test was performed on the respective results (*OriginPro* software (OriginLab, Northampton, USA)). In the case of unequal sample numbers, the *Welch's* t-test (unequal variance t-test) was applied for the calculation of the *p*-value.

Unless stated otherwise in the text and captions of the main manuscript, all experiments were conducted with a minimum number of three independent replicates (*n* = 3). In this regard, independent replicates mean that the procedure of electrode pre-treatment, In-deposition, subsequent formate production, *M. extorquens* AM-1 pCM160_RBS-yciA cultivation and cultivation in the 1 L-electrobioreactor as well as finally In-stripping was performed for every single replicate. All values are provided as the mean ± standard deviation (SD).
SI 2 Results

SI 2.1 Individual data of the 4 independent 1 L-electrobioreactor runs for the production of C5-dicarboxylates from CO₂

Figure S3: Top row: single electrobioreactor runs of ECO₂R to formate in Phase 1 and its subsequent conversion to mesaconate and methylsuccinate in Phase 2. Bottom row: OD₆₀₀ nm and pH during coupled electrochemical and microbial catalysis of the single electrobioreactor runs. The event of ECO₂R-stop and inoculation of *M. extorquens* AM-1 pCM160_RBS-yclA is indicated by the arrows at t = 0 h.
SI 2.2 Coulombic efficiency (CE) and rate (r) of the ECO$_2$R to formate and the conductivity (κ) of the reaction media

The conductivity (κ) of the electrolyte solution increased from initially 2.3 mS cm$^{-1}$ to 7.5 ±1.1 mS cm$^{-1}$ as a consequence of the ion transfer across the membrane of the two-chamber set-up to ensure charge balancing. Due to the proton-consuming reaction of formate production (Eq. (SI 28)) and hydrogen evolution reaction (HER, Eq. (SI 29)) also the pH increased slightly as can be anticipated from Figure S4B.

\[
CO_2 + 2 \, e^- + \, H^+ \rightarrow \, HCOO^- \quad \text{Eq. (SI 28)}
\]

\[
2 \, H^+ + 2 \, e^- \rightarrow \, H_2 \quad \text{Eq. (SI 29)}
\]
SI 2.3  Electron (e⁻) balance of long-term ECO₂R to formate in the 1 L-electrobioreactor

Figure S5: Long-term ECO₂R to formate in the 1 L-electrobioreactor using 0.05 M NaHCO₃ as electrolyte solution. Note that data shown do not originate from the set of experiments described in the main manuscript but from independent experiments. A) CE of ECO₂R to formate; B) loss of indium, which was deposited on the graphite backbone (i.e., 12.693 mg, SI 1.2.1.1) by leaching in the electrolyte solution. In was quantified by ICP-MS. ICP-MS analysis was performed as described by Hegner et al.[7]. Reported values are mean values ± standard deviation of n = 3.
SI 2.4 Pathway scheme for the microbial catalysis of mesaconate and methylsuccinate from formate by *M. extorquens* AM-1 pCM160_RBS-yciA

Figure S6: Segment of the metabolism of *M. extorquens* AM-1 including formate as C-source and e- donor for the catalysis of the C5-dicarboxylates mesaconate and 2S-methylsuccinate by the ethylmalonyl-CoA (EMC) pathway via the serine cycle. In *M. extorquens* AM-1 pCM160_RBS-yciA, the thioesterase yciA from *Escherichia coli* (marked in red) is heterologously expressed based on the plasmid pCM160_RBS-yciA. This strain was constructed by Sonntag et al.\[^{5}\]. yciA facilitates the hydrolytic cleavage of coenzyme A from 2S-methylsuccinyl-CoA and mesaconyl-CoA to yield 2S-methylsuccinate and mesaconate, which are excreted to the microbial medium\[^{8}\]. CO2 with green background indicates CO2 being produced from formate oxidation catalyzed by formate dehydrogenase (FDH). CO2 with orange background indicates that it is CO2 required for the carbon backbone formation of the C5-decarboxylates. See also the stoichiometries in section SI 1.5.3. Note that the oxidation of methanol to formate is not shown. The figure is reused from Rohde et al.\[^{9}\] and was adapted. The reuse is with permission. Copyright (2017) The American Society for Microbiology.
### SI 2.5 Product yields of the C5-dicarboxylates mesaconate and methylsuccinate

**Table S1:** Overview on achieved and theoretically achievable product yields for the C5-dicarboxylates mesaconate and methylsuccinate exploiting formate or methanol as substrate.

| Parameter | Value         | \( Y_{\text{theoretical product/substrate}} \) | \( Y_{\text{product/substrate}} \) relative to \( Y_{\text{theoretical product/substrate}} \) [%] | Reference          |
|-----------|---------------|-----------------------------------------------|-------------------------------------------------------------------------------------------------|--------------------|
| \( Y_{\text{mesaconate/formate [mol/mol]}} \) | 0.0012 ± 0.0001 | 0.111                                         | 1.1                                                                                             | this study         |
| \( Y_{\text{methylsuccinate/formate [mol/mol]}} \) | 0.0020 ± 0.0015 | 0.100                                         | 2.0                                                                                             | this study         |
| \( Y_{\text{C5/formate [mol/mol]}} \) | 0.0032 ± 0.0016 | 0.105                                         | 3.1                                                                                             | this study         |
| \( Y_{\text{mesaconate/formate [g/g]}} \) | 0.0033 ± 0.0004 | 0.310                                         | 1.1                                                                                             | this study         |
| \( Y_{\text{methylsuccinate/formate [g/g]}} \) | 0.0058 ± 0.0043 | 0.291                                         | 2.0                                                                                             | this study         |
| \( Y_{\text{C5/formate [g/g]}} \) | 0.0091 ± 0.0047 | 0.300                                         | 3.1                                                                                             | this study         |
| \( Y_{\text{C5/methanol [g/g]}} \) | 0.17           | 1.260                                         | 13.5                                                                                             | Sonntag *et al.*[8] |
SI 2.6 C5-dicarboxylate production from sodium formate – a control experiment

**Figure S7:** C5-dicarboxylate production from sodium formate with *M. extorquens* AM-1 pCM160_RBS-yciA using bottle cultivation. The pre-culture was grown for 3 days as described in S 1.3.1 and was then used to inoculate 100 mL carbonate buffered minimal media containing 25 mM formate (w/ formate) as carbon source or no carbon source (w/o formate). 1 mL L⁻¹ kanamycin was added to the media before inoculation. The experiment was carried out in 250 mL Schott bottles at 30 °C and 150 rpm stirring. The headspace of the Schott bottles was continuously gased with 20 mL min⁻¹ CO₂ and 30 mL min⁻¹ CA. Samples for OD₆₀₀ nm, pH, and HPLC measurements were taken at 0, 24, 48, 72, 96, and 120 h. The HPLC analysis was performed as described in S 1.4 with the modification that the sterile filtered samples were diluted 1:4 and used for the HPLC measurement immediately after sampling. A) Control experiment with the addition of sodium formate. After 48 h, when sodium formate was almost depleted, roughly 30 mM of formate was spiked to the medium. B) Negative control experiment without sodium formate addition. In the absence of formate, neither mesaconate nor methylsuccinate is produced. C) Course of OD₆₀₀ nm and pH of the liquid phase with (w/) and without (w/o) formate addition. Reported values are mean values ± standard deviation of n = 3.
SI 2.7 Formate produced by ECO$_2$R vs. sodium formate as a microbial substrate

Table S2: Comparison of the *M. extorquens* AM-1 pCM160_RBS-yciA conversion of formate to C5-dicarboxylates using the 1 L-electrobioreactor and bottle cultivation (SI 2.6). Reported values are mean values ± standard deviation of $n = 4$ for the electrobioreactor and $n = 3$ for the bottle cultivation.

| Parameter                             | Unit             | Electrobioreactor | Bottle cultivation |
|---------------------------------------|------------------|-------------------|--------------------|
| formate conversion efficiency $\eta_{\text{C5/formate}}$ | [%]              | 1.6 ± 0.8         | 1.6 ± 0.4          |
| C5-dicarboxylate yield $Y_{\text{C5/formate}}$          | [mol/mol]        | 0.0032 ± 0.0016   | 0.0034 ± 0.0090    |

*takes into account that formate acts as C-source and e-source for the formation of the target dicarboxylic acids
SI 2.8 Details on the quick economic evaluation of the coupled electrochemical and microbial catalysis

For this economic evaluation, the formate market price of 0.68 € kg⁻¹ (0.74 $ kg⁻¹) is based on the TEA study by Jouny et al. [10], while the mesaconate market price of 0.91 € kg⁻¹ is based on the TEA by Lundberg et al. [11]. Both reflect market prices at industrial-scale production. An evaluation for methylsuccinate is much more difficult as market prices for industrial-scale production are not available. Currently, methylsuccinate can be purchase in kg-scale for ≈45 € kg⁻¹ [12]. In this economic evaluation, we assume that methylsuccinate production in a full-scale plant would drop this price by two orders of magnitude. Consequently, our assumption for a methylsuccinate market price (at full-scale production) is ≈0.45 € kg⁻¹. This assumption might be an underestimation of the methylsuccinate market price. The current market price of mesaconate and methylsuccinate is compared to the calculated price if these compounds were produced by the coupled electrochemical and microbial catalysis. These calculations are based on 1) the costs of electrochemically produced formate from CO₂ considering only the investment for electricity (i.e., \( \text{Cost}_{\text{formate,ECO}_2} \)) and 2) the yield of the products from the microbial catalysis based on stoichiometry (i.e., \( \frac{Y_{\text{theoretical mesaconate/formate}}}{\text{total formate consumption}} \) and \( \frac{Y_{\text{theoretical methylsuccinate/formate}}}{\text{total formate consumption}} \)) or the actually achieved yields (i.e., \( \frac{Y_{\text{mesaconate/formate}}}{\text{total formate consumption}} \) and \( \frac{Y_{\text{methylsuccinate/formate}}}{\text{total formate consumption}} \)). A more profound and detailed estimation of the economic viability of the proposed coupled electrochemical and microbial catalysis (i.e., capital expenditures (capex) and operating expenses (opex)) is not possible at the current technology readiness level. This paragraph rather intends to provide a first quick and simplified insight into the economic viability of the coupled electrochemical and microbial catalysis of C5-dicarboxylates from CO₂. Costs that are not considered include investment and maintenance (reactors, electrodes, membranes, electrolyte solution, electronic steering device, pumps, microbial media, etc.), the energy consumption of devices, and human resources.
Table S3: Simplified economic evaluation of coupled electrochemical and microbial catalysis of C5-dicarboxylates from CO₂. This table is in addition to Table 3 in the main manuscript. Reported values are mean values ± standard deviation of $n = 4$.

| Parameter | Unit       | Formate       |                      |
|-----------|------------|---------------|----------------------|
|           |            |               |                      |
| Formate market price | € mol⁻¹ | 0.031         |                      |
|           | € kg⁻¹     | 0.68[10]      |                      |
| $P_{formate}$ based on cathodic half cell reaction | kWh mol⁻¹ | 0.706 ± 0.216 |                      |
|           | kWh kg⁻¹   | 13.75 ± 4.20  |                      |
| Price per kWh | € kWh⁻¹ | 0.025[13]      |                      |
| $Cost_{formate, ECO₂R}$ of this study | € mol⁻¹ | 0.015 ± 0.005 |                      |
|           | € kg⁻¹     | 0.34 ± 0.10   |                      |

Phase 1: ECO₂R to formate

Phase 2: microbial catalysis of C5-dicarboxylates

| Parameter | Unit       | Mesaconate | Methylsuccinate |
|-----------|------------|------------|-----------------|
|           |            |            |                 |
| Product market price | € mol⁻¹ | 0.12        | 0.06            |
|           | € kg⁻¹     | 0.91[11]  | 0.45            |
| Price of the product based on $Cost_{formate, ECO₂R}$ and $Y_{theoretical product/formate}$ | € mol⁻¹ | 0.14 ± 0.04 | 0.15 ± 0.04 |
|           | € kg⁻¹     | 1.07 ± 0.32 | 1.17 ± 0.31     |
| Price of the product based on $Cost_{formate, ECO₂R}$ and achieved $Y_{product/formate}$ (Table 2) | € mol⁻¹ | 12.88 ± 2.67 | 11.93 ± 8.32 |
|           | € kg⁻¹     | 99.03 ± 20.50 | 90.35 ± 62.96  |

with

$Cost_{formate, ECO₂R} = P_{formate} \times 0.025 \text{ € kWh}^{-1}$ Eq. (SI 30)

$Price of mesaconate based on stoichiometry = \frac{Cost_{formate, ECO₂R}}{Y_{theoretical mesaconate/formate}}$ Eq. (SI 31)

$Price of mesaconate based on Y_{mesaconate/formate} = \frac{Cost_{formate, ECO₂R}}{Y_{mesaconate/formate}}$ Eq. (SI 32)

$Price of methylsuccinate based on stoichiometry = \frac{Cost_{formate, ECO₂R}}{Y_{methylsuccinate/formate}}$ Eq. (SI 33)

$Price of methylsuccinate based on Y_{mesaconate/formate} = \frac{Cost_{formate, ECO₂R}}{Y_{methylsuccinate/formate}}$ Eq. (SI 34)
SI 2.9 Comparison of different process modes for the coupled electro-and biocatalytic production of C5-dicarboxylates from CO₂ via formate

Figure S8: Compilation of different process modes for the coupled electro-and biocatalytic production of C5-dicarboxylates from CO₂ via formate. A) Sequentially coupled catalysis. B) Repetitive sequentially coupled catalysis. C) Parallelly coupled catalysis. D) Spatially separated coupled catalysis. Note that in scheme A) and B) the two vessels represent the two sequential process phases that were carried out seamlessly in one pot. In scheme D), the question mark represents the fact that an electrochemical cell specifically designed and optimized for ECO₂R would have a different cell geometry than the electrobioreactor used in this study. For example, a flow cell geometry as, for example, illustrated by Kopljär et al.¹⁴, Lee and Cho et al.¹⁵, or Syntrivanis et al.¹⁶.

Sequential process mode (Figure S8A, proof-of-concept in this study):

**Advantages:**
The sequential process mode is conducted in a single-vessel setup, which reduces the number of operational units to a minimum. In Phase 1, ECO₂R takes place in base media. This avoids mixed-potential formation leading to stable electrode performance for ECO₂R. Consequently, strategies for in-situ electrode regeneration (see the main manuscript) are not required. Furthermore, the co-production of reactive oxygen species (ROS) can be avoided. ROS are cytotoxic and causing potential inhibition in Phase 2, the biocatalytic phase. The one-pot arrangement allows keeping the setup sterile and enables the use of (tailor-made) pure cultures.

**Disadvantages:**
A clear disadvantage of the sequential process mode in a one-pot arrangement is that a batch process only allows discontinuous production of C5-dicarboxylates. Furthermore, higher material input as a cost factor and maintenance effort due to repeated start-up and product harvest can be expected. Additionally, too high formate concentrations in Phase 1 can be cytotoxic and reduce the performance of Phase 2.

Repetitive sequential process mode (Figure S8B):

**Advantages:**
In the repetitive sequential process mode, the first of all ECO₂R cycles (Phase 1) can be conducted in base medium and thus includes the advantages of the sequential process mode. In Phase 2, formate, as well as nutrients and trace elements, may be completely consumed and converted into desired products or incorporated into cellular biomass, respectively. This leaves only base medium behind for another re-start of ECO₂R (Phase 1) for formate production, which is followed by Phase 2. This process sequence can then be repeated permanently. Generally, this process mode can be considered as fed-batch and takes place in a one-pot arrangement, allowing to maintain sterility and hence to use of (tailor-made) pure cultures.

**Disadvantages:**
Over time, a reduced electrode performance for ECO₂R due to electrode aging and/or mixed potential formation by trace impurities of nutrients or trace metals is to be expected. Consequently, in-situ electrode regeneration will be required. Furthermore, the co-production of cytotoxic ROS may occur due to trace impurities. Over time, product inhibition of the microbial catalysis can occur that would require (i) product harvest at the end of the repetitive
sequential process or (ii) continuous product removal (e.g., extraction) during the repetitive sequential process. Similar to the sequential process mode, too high formate concentrations by ECO₂R (Phase 1) can reduce the performance of Phase 2 due to the cytotoxicity of formate.

Parallel process mode (Figure S8C):

**Advantages:**
This process mode allows a continuous or fed-batch production process. This is also the process mode where the benefit of demand-driven formate production (ECO₂R) is emphasized the most as it can be adjusted to the formate consumption. This adjustment is needed for example, due to increasing/decreasing cell density of the biocatalyst while avoiding cytotoxic concentrations of formate. As it takes place in a one-pot arrangement, sterility can be kept to use (tailor-made) pure cultures.

**Disadvantages:**
The disadvantage of this process mode is that mixed potential formation will decrease the electrode performance for ECO₂R. Thus, strategies for in-situ electrode regeneration will be required. A co-production of ROS will most likely occur. To minimize the ROS co-production, intensive optimization of the reaction medium for parallel electrocatalytic and biocatalytic reactions is necessary.

Spatially separated process mode (Figure S8D)

**Advantages:**
Both processes, the electrocatalytic and biocatalytic process, can take place at the respective optimal conditions in optimized reactor geometries (e.g., flow cell for ECO₂R and CSTR for the biocatalytic stage). This allows high formate production rates by ECO₂R with current densities of > 500 mA cm⁻² using 3D electrode geometries such as gas diffusion electrodes. This would allow also using a small ECO₂R reactor providing formate for the biocatalytic stage. This two-pot arrangement can be run as a batch, fed-batch, and continuous process.

**Disadvantages:**
This is a cascade process comprising multiple operational units, which represent an additional cost factor. Moreover, formate downstreaming (e.g., de-salination) may be required. Additionally, it will be challenging to kept sterility between the ECO₂R stage and the biocatalytic stage to use (tailor-made) pure cultures. On top of that, O₂ intrusion between the ECO₂R and biocatalytic stage might be problematic for the use of strict anaerobic pure cultures.
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