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Supplementary Information for:

Microbial electrosynthesis system with dual biocathode arrangement for simultaneous acetogenesis, solventogenesis and carbon chain elongation

Igor Vassilev,[†,*] Frauke Kracke,[‡] Stefano Freguia,[†] Jürg Keller, [†] Jens O. Krömer,[§] Pablo Ledezma[†] and Bernardino Virdis[†,*]

[†]Advanced Water Management Centre, The University of Queensland, Gehrmann Laboratories Building (60), Brisbane, QLD 4072, Australia

[‡]Department of Civil and Environmental Engineering, Stanford University, 318 Campus Drive, Stanford, CA 94305, USA

[§]Department for Solar Materials, Helmholtz Centre for Environmental Research (UFZ), Permoserstraße 15, Leipzig 04318, Germany

*Correspondence to: igor.vassilev@uqconnect.edu.au (I. Vassilev), b.virdis@uq.edu.au (B. Virdis)

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Text S1. Materials and Methods.

Medium composition. The minimal growth medium contained per liter: 6 g Na_2HPO4, 3 g KH_2PO_4, 0.2 g NH_4Cl, 15 mg $CaCl_2$, 4 mg $MgCl_2 \cdot 6H_2O$ and 1 ml trace element solution (10 g L^{-1} EDTA, 1.5 g L^{-1} $FeCl_3 \cdot 6H_2O$, 0.18 g L^{-1} KI, 0.15 g L^{-1} $CoCl_2 \cdot 6H_2O$, 0.15 g L^{-1} H_3BO_3, 0.12 g L^{-1} $MnCl_2 \cdot 4H_2O$, 0.12 g L^{-1} $ZnSO_4 \cdot 7H_2O$, 0.06 g L^{-1} $Na_2MoO_4 \cdot 7H_2O$, 0.03 g L^{-1} $CuSO_4 \cdot 5H_2O$, 23 mg L^{-1} $NiCl_2 \cdot 7H_2O$) (pH = 7.4, electrical conductivity = 4.7 mS cm^{-1}).[1]

The anodic chamber of the three-chamber electrochemical system was filled with a potassium phosphate buffer, consisting of 15 g L^{-1} KH_2PO_4 and 30 g L^{-1} K_2HPO_4 (pH = 6.8, electrical conductivity = 24 mS cm^{-1}).

Operation of abiotic batch A. Each chamber including its buffer vessel (see Figure 1) was filled with 300 mL of a 100 mM HCl solution, and recirculated overnight to sterilize the reactor system and to clean the carbon granules. The HCl solution in the anodic chamber (AC1) was replaced by the potassium phosphate buffer solution (450 mL), while the acidic solution in cathodic chamber 1 (CC1) and cathodic chamber 2 (CC2) was replaced by 350 and 450 mL minimal growth medium, respectively (composition see above).[1] The medium in the cathodic compartments was sparged with N_2 until anoxic conditions were achieved. The cathode CC1 was operated in chronoamperometric mode in repeated intervals of applying -0.8 V for 20 min, while the cathode CC2 was kept at open circuit, and sequentially, after a 30 sec pause, the cathode CC2 was poised at -0.8 V for 10 min followed by a 30 sec pause, while this time the cathode CC1 was kept at open circuit. The purpose of the abiotic batch A was to analyze the migration of acetate across the membranes in the three-chamber reactor, therefore, 210 mM-C sodium acetate was added into CC2 (Figure 2A, main text). This concentration has been chosen based on previous achieved microbial

electrosynthesized acetate titers in a bioelectrochemical system with one biocathade and two-chamber setup.[1]

Operation of abiotic batch B. The medium from the batch A was replaced by fresh medium as detailed above. Operation of batch B was identical to batch A with the additional supplementation of 72 mM-C ethanol in CC2, to analyze the simultaneous diffusion of ethanol and migration of acetate across the membranes (Figure 2B, main text).[1]

Operation of batch C with biotic CC2. After the batch B was finished, the chambers were drained to ca. 95% and filled with fresh medium as detailed in the previous batch, except that the medium included 1 g L^{-1} yeast extract and 2.09 g L^{-1} sodium 2-bromoethanesulfonate to enhance bacterial growth and to inhibit methanogenesis, respectively.[1] After achieving anaerobic conditions, CC2 was inoculated with 50 mL of broth (containing planktonic cells) and 12 mL of graphite granules (containing biofilm on the surface of the granules) from a microbial electrosynthesis reactor, which was enriched with an electroactive microbiome dominated by *Clostridium* spp. capable of converting CO_2 to C2-C6 carboxylates and their corresponding alcohols via acetogenesis, solventogenesis and carbon-chain elongation.[1] Neither acetate nor ethanol was added externally, and CO_2 was not only used to regulate the pH in CC2, but also acted as the sole carbon source for the microorganisms. Further, the potential in CC1 was decreased to -0.85 V to enable a faster raise of pH and to be able to maintain it more stable at 4.9 in CC1. However, over time a trend of continuous decreasing pH in CC1 was observed, and required the addition of ca. 1 mL of 2.5 M NaOH every three to four days to raise the pH. The batch was run for 54 days to test the suitability of the three-chamber reactor to convert CO_2 into acetate via

acetogenesis and to transfer the produced acetate from CC2 into CC1 (Figure 2C, main text).

Operation of batch D with biotic CC1 and CC2. The medium in each chamber was replaced with fresh medium as described in batch C. Furthermore, CC1 was inoculated in the same manner as CC2 in batch C to evaluate the ability of the reactor system to simultaneously produce carboxylates in CC2 and alcohols in CC1. In addition, the gas outlet of CC1 was connected to the medium recirculation loop of CC2 to recycle the produced gas in CC1 (*i.e.* H_2 and CO_2) for acetogenesis in CC2. Further improvement included the adjustment of the applied voltage by changing its value and applied period in CC1 between -0.85 and -0.90 V, and 20 and 25 min, respectively, while in CC2 those parameters were varied between -0.80 and -0.85 V, and 5 and 10 min, respectively. These adjustments resulted in a stable mildly acidic pH in CC1 and neutral pH in CC2 without the need of external addition of an acid or base. The batch was run for the same period as the batch C (Figure 2D, main text).

GC method for broth samples. Liquid phase samples were taken 1-2 times per day in the abiotic batches (batch A and B) and every three to four days in the biotic batches (batch C and D) from each chamber and filtered immediately through a 0.22 µm pore filter for analysis of volatile fatty acids (VFA, C_2 to C_6) and their corresponding alcohols via Gas Chromatography (GC) using an Agilent Technologies 7890A GC System (Agilent, USA) equipped with a polar capillary column (DB-FFAP 30 m × 0.53 mm × 1.0 µm) and flame ionisation detector (make-up flow: 10 mL min^{-1} N_2; 250 ^oC). 0.2 µL of each sample containing an internal standard (formic acid and 2-ethylbutyric acid) was injected in pulsed splitless at 220 ^oC. The carrier gas consisted of high purity helium gas, while following temperature programme was used to perform the analyses: 2 min at 60 ^oC, 20 ^oC min^{-1} to 240 ^oC, hold for 2 min.[1]

GC method for gas samples. The availability of CO_2, the methanogenesis inhibition and the anaerobic condition in the reactor was confirmed by qualitative analysis of gas samples from the headspace of CC2 in batch D via gas chromatography (GC). A GC-2014 gas chromatograph (Shimadzu, Japan) equipped with a Valco GC valve (1 mL sample loop), a HAYESEP Q 80/100 packed column (2.4 m length; 1/8" outside diameter, 2 mm inner diameter) and a thermal conductivity detector were employed. The carrier gas (high purity argon) flow was set to 28 mL min^{-1} under a pressure of 135.7 kPa. The chromatograph injection port, oven and detector were operated at 75, 45 and 100 °C, respectively.[2]

Calculations. The percentage of electrons from the cathode recovered in organic carbon products is provided as Charge Efficiency ($\mathcal{E}_C$) calculated per *Eq. (S1)* below:

$$\mathcal{E}_C = \frac{F \times \sum_i (m_i \times DOR_i)_t}{\int I\, dt} \times 100 \qquad \textit{Eq. (S1)}$$

Where *F* is the Faraday's constant (96485.3365 C/mol), m_i is the absolute quantity of product *i* in mol at a specific time *t* (see Table S1), *DOR* the degree of reduction of the product *i* and $\int I\, dt$ is the integration of produced current over time *t,* which yields the overall charge transfer in the electrochemical system.

Figure S1. Photograph of the dual-cathode microbial electrosynthesis reactor and its detailed assembly schematic.

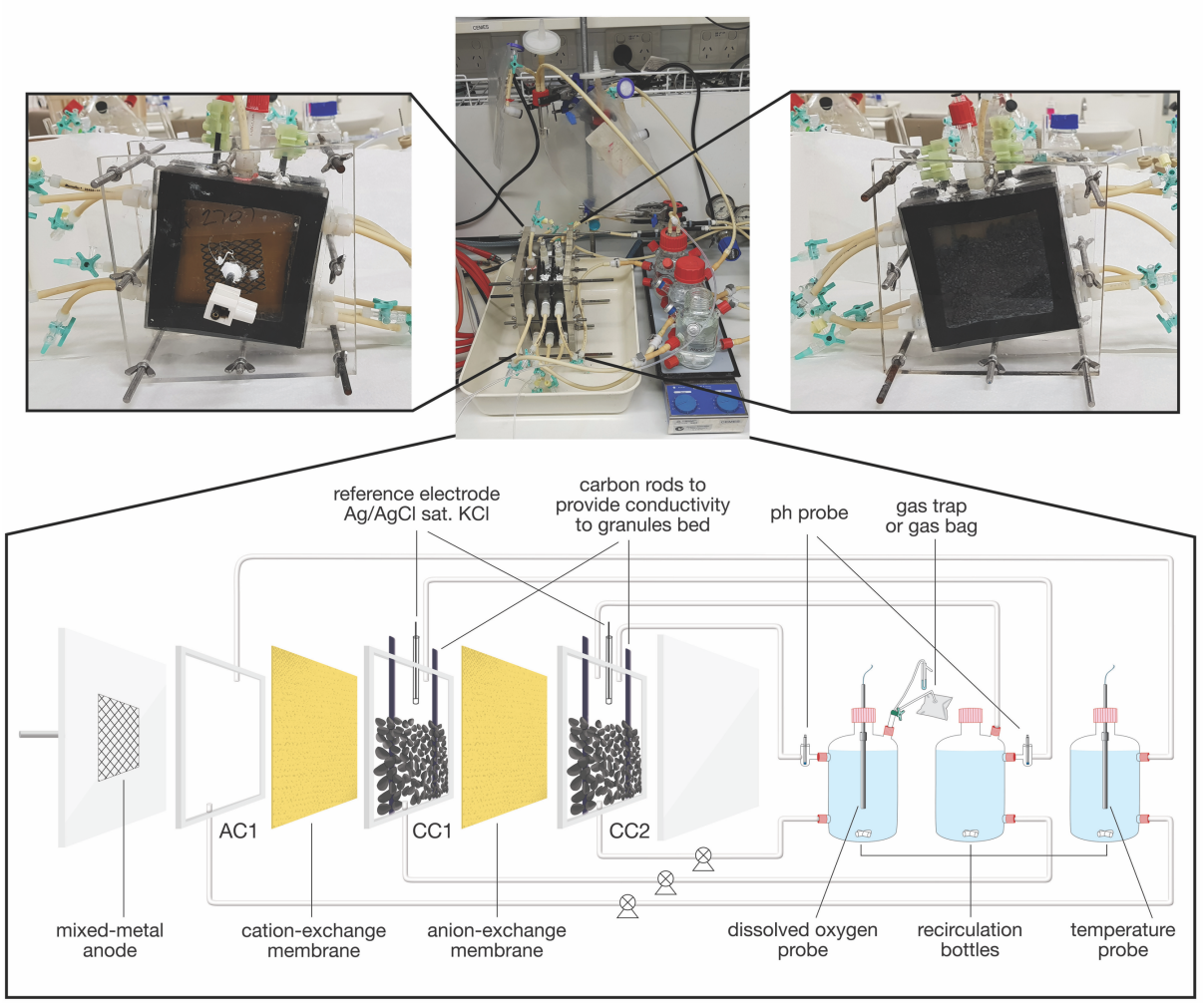

Figure S1. Three-chamber reactor set-up used in this study. Three aligned acrylic frames [10 cm (width) × 10 cm (height) × 2 cm (depth)] mounted between two acrylic plates (20 cm × 20 cm × 1 cm) represented the novel flat plate-type reactor. Three chambers were constructed (anodic chamber, AC1; cathodic chamber 1, CC1; cathodic chamber 2, CC2) by separation of the middle frame from the outer frames through ion-exchange membranes. AC1 contained a titanium mesh electrode coated with 12 g m^{-2} Ti/$Ru_{0.7}Ir_{0.3}O_2$, which functioned as the anode to catalyse efficiently the oxygen evaluation reaction.[3, 4] Each of the cathodic chambers was filled with ca. 110

cm^3 (176.2 g) graphite granules, which served as the cathode electrodes (working electrodes). Such cathode material has been demonstrated to be a suitable catalyst in several bioelectrocatalytical applications showing a high biocompatibility and corrosion-resistance.[5] External electric connection of the granular bed was achieved by inserting two graphite rods into the cathode compartments. An Ag/AgCl reference electrode in saturated KCl was placed in each cathodic chamber in close proximity to the working electrode. A peristaltic pump recirculated separately the medium in each compartment through recirculation bottles (500 mL modified Schott bottles) to ensure medium mixing. Further, the gas outlet of CC1 was connected to the medium recirculation loop of CC2 to recycle the CC1 gas in CC2 (only in batch D).

Figure S2. Cathodic current density profiles of the two abiotic and biotic microbial electrosynthesis batches in the dual-cathode microbial electrosynthesis system.

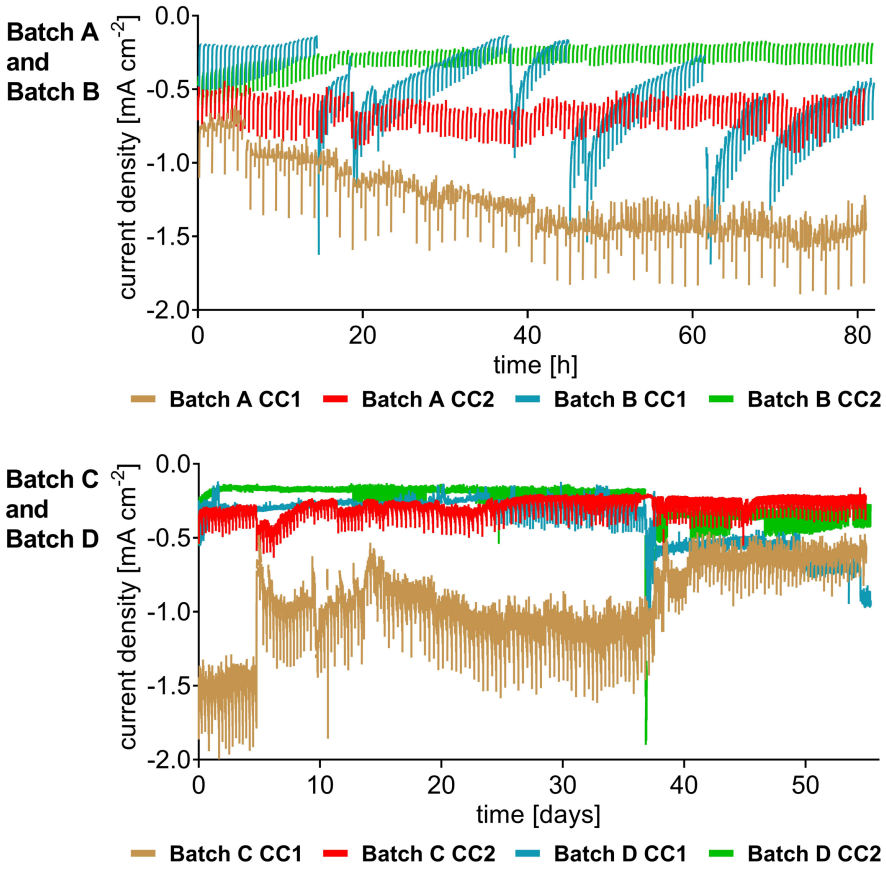

Figure S2. Cathodic current density profiles. Values of electric current are converted into current density by normalizing the measured current to the projected surface area of the membranes (*i.e.* 100 cm^2). Each cathode in the abiotic **batch A** (externally added acetate in CC2) and abiotic **batch B** (externally added ethanol in CC1 and acetate in CC2) was operated in chronoamperometric mode in repeated intervals of applying -0.80 V in CC1 for 20 min, a 30 sec pause, applying -0.80 V in CC2 for 10 min and a 30 sec pause to analyse migration of only acetate (Figure S3A) and simultaneous migration of ethanol and acetate (Figure S3B), respectively, across the ion-exchange membranes. In **batch C** (biotic CC2) the voltage in CC1 was decreased

to -0.85 V to enable a better regulation of the pH in CC1. The poised cathode in CC2 enabled microbial electrosynthesis of acetate from CO_2 and product migration from CC2 into CC1 (Figure S3C). The charge efficiency (CE) for carboxylate production was greater than 54%, taking into account the current of the CC2 cathode only. If both cathodes were considered as electron sources for microbial production, a combined CE of 22% is calculated (Table S1). The decrease in CE reflects the usage of the applied voltage not only for MES but additionally for pH regulation in the reactor system. Additionally, a loss in electron recovery is likely present by unused H_2 that was probably vented out of the system (Figure S4).[1] In **batch D** (biotic CC1 and CC2) the applied voltage was changed in value and applied time in CC1 between -0.85 and -0.90 V, and 20 and 25 min, respectively, while in CC2 those parameters were varied between -0.80 and -0.85 V, and 5 and 10 min, respectively. These adjustments resulted in a stable mildly acidic pH in CC1 for microbial catalyzed alcohol production and neutral pH in CC2 for microbial catalyzed carboxylates production with zero-chemical addition except for CO_2 (Figure S3D). The overall CE for carboxylate production in batch D was 43% taking both cathodes into account (Table S1). It is important to mention that the supply of reducing equivalents to the electroactive microorganisms was interrupted when the the cathode in CC2 was kept at open circuit. In that time period, the microbial reduction of CO_2 into multi-carbon products probably stagnated due to the lack of reducing equivalents or the produced organic acids could even have been partly consumed for energy maintenance during this phase of starvation. However, the period of current interruption was relatively short (5-25 min) and the microbial electrosynthesis activity was probably quickly recovered after resuming the supply of reducing equivalents. In fact, Rojas et al. proved that electroactive microbiomes are resilient to even long electric power supply interruption

(4-64 hours) and acetogenesis activity was relatively fast restored depending on the interruption period.[6]

Figure S3. Production of carboxylates and alcohols and their migration in the dual-cathode microbial electrosynthesis reactor versus time trace during the reactor operation

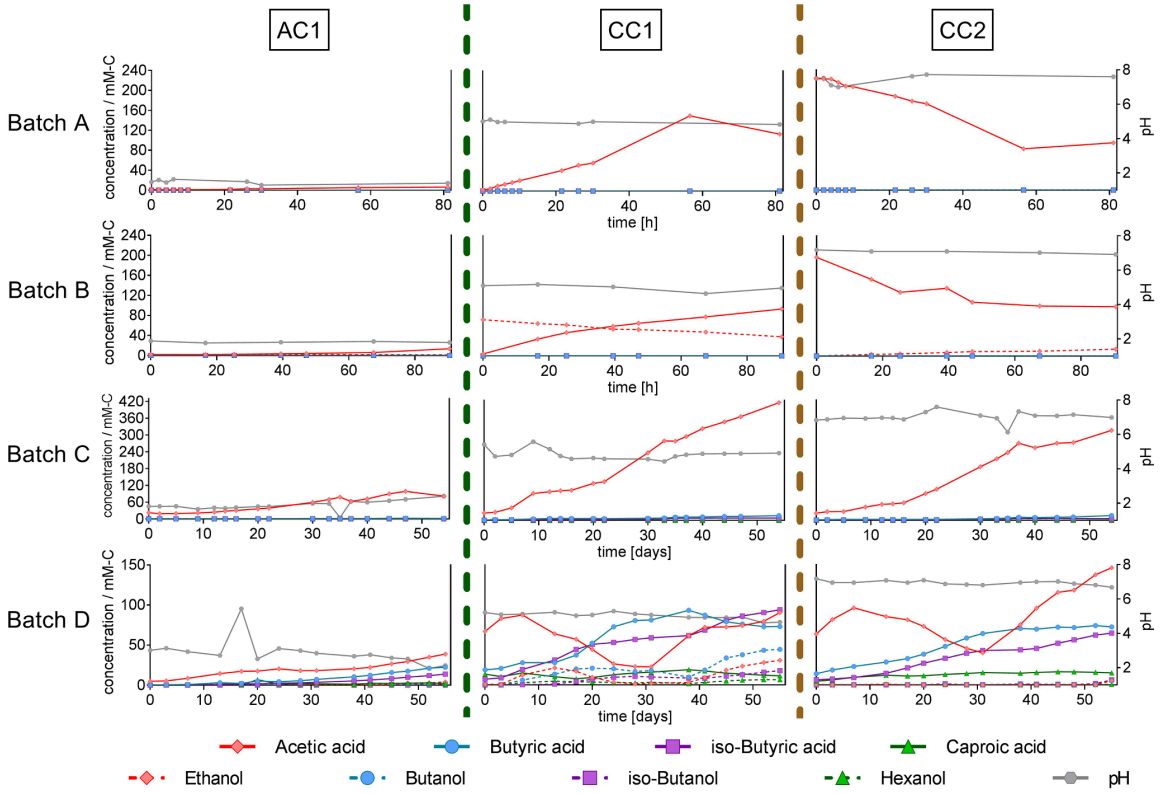

Figure S3. Migration profile and product spectrum. **Abiotic batch A:** Added acetate in CC2 to analyze the transfer of acetate from CC1 into CC2. **Abiotic batch B:** Added ethanol in CC1 and acetate in CC2 to analyze the simultaneous migration of ethanol and acetate across the membranes. **Batch C with biotic CC2:** Biotic CC2, no addition of acetate or ethanol to analyze microbial electrosynthesis of acetate and product extraction. **Batch D with biotic CC1 and CC2:** Biotic CC1 and CC2, no external addition of acetate or ethanol to analyse microbial electrosynthesis of acetate and carbon-chain elongation for the synthesis of C4 and C6 carboxylates in CC2 at neutral pH, and solventogenesis for alcohol production in CC1 at mildly acidic pH, and

migration of all products across the membranes. The microbial production stagnated between the 24th and 31st day, most likely due to the limited supply of CO_2 and H_2 as shown in Figure S4. After re-inoculation and restoring the availability of CO_2 and H_2 on day 31, the carboxylate and alcohol production activity was stabilized again.

Figure S4. Availability of CO_2 and H_2 in the reactor headspace of the dual-cathode microbial electrosynthesis reactor in batch D.

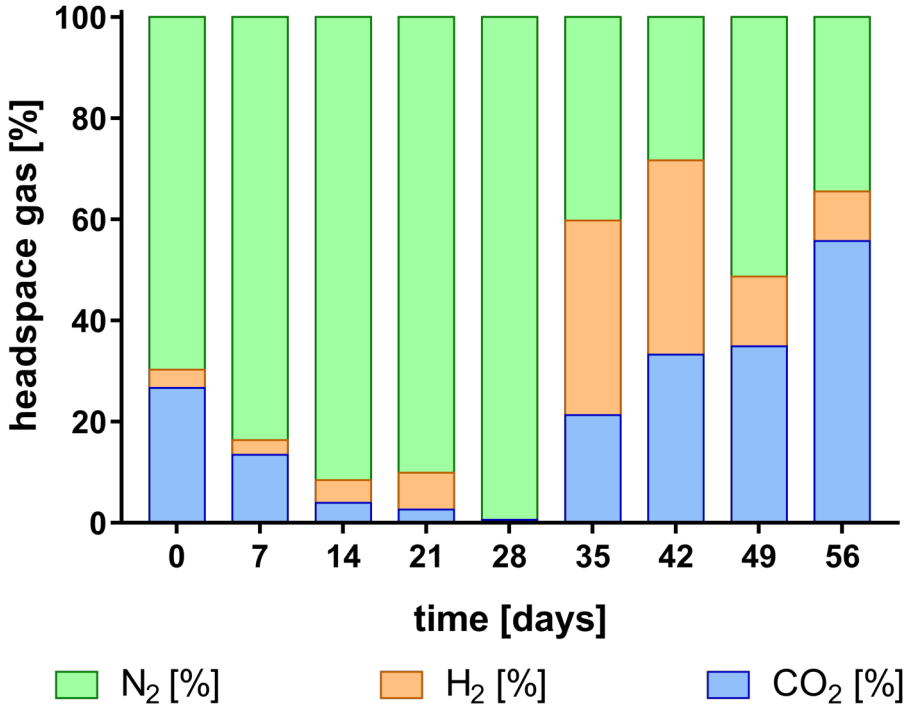

Figure S4. Qualitative gas composition analysis of the reactor headspace in batch D. Weekly gas samples from the headspace of CC2 were analyzed via GC (Text S1) to monitor H_2 production, CO_2 supply and CH_4 inhibition. At the beginning of the experiment, the medium and the reactor system were sparged with N_2 to achieve anaerobic conditions. The gas outlet of CC2 was connected to a gasbag filled with N_2 (Figure S1). H_2 evolution was catalyzed electrochemically or bioelectrochemically at the cathode in CC1 and CC2 to regulate the pH and supply the microorganisms with reducing equivalents for microbial electrosynthesis.[1] CO_2 was not only the sole carbon feedstock, but was also used as a pH regulation agent. The CO_2 supply in CC1 was regulated by the BIOSTAT® B depending on the pH in CC1 (see main manuscript). CO_2 dissolved in water as carbonic acid (H_2CO_3) and dissociated predominantly into

bicarbonate (HCO_3^-) at neutral pH (CC2), which was transferred via electro-migration into CC1. The low pH in CC1 shifted the equilibrium of the bicarbonate buffer system towards CO_2, which escaped the chamber in gaseous form and entered the medium recirculation-loop of CC2 to be recycled (Figure 1 and S1). The total provided CO_2 and the yield of the total produced multi-carbon compounds from CO_2 are given in Table S1.

It was confirmed that CO_2 and H_2 were continuously available during Batch D, except on day 28. Due to a technical issue, the supply of CO_2 and H_2 stagnated. This lack of a carbon and a reducing power source was reflected in the decrease of microbial production (Figure S3). However, the supply of both gases was restored in the following week, and thereby also microbial electrosynthesis activity.

Table S1. Main parameters of the dual-cathode microbial electrosynthesis reactor. Summary of consumed CO_2 and charge in proportion to biosynthesized products in batch C and D.

	Batch C			Batch D		
	CC1	**CC2**	**CC1+2**	**CC1**	**CC2**	**CC1+2**
Supplied CO_2 [M-C]	-	-	374.88	-	-	142.40
Total organic carbon produced [mM-C]*	447.86	346.30	794.16	368.49	294.44	662.93
Yield [mM-$C_{produced\ organic\ carbon}$/M-$C_{CO2}$]*	-	-	2.12	-	-	4.66
Average current density [mA cm^{-2}]	-0.92 ± 0.28	-0.28 ± 0.06	-	-0.40 ± 0.18	-0.24 ± 0.15	-
Consumed charge [kC]	430	130	560	190	110	300
Charge Efficiency [%]	12.71	54.21	22.34	28.57	66.69	42.54

*excluding produced biomass

Text S2. Supplementary References

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