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COMMUNICATION

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

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A microbial electrosynthesis cell comprising of two biological cathode chambers sharing the same anode compartment is used to promote the production of C2-C4 carboxylic acids and alcohols from carbon dioxide. Each cathode chamber provides ideal pH conditions to favor acetogenesis/carbon chain elongation (pH = 6.9), and solventogenesis (pH = 4.9), respectively, without the requirement of external acid/base dosing.

The development of technologies for the production of commodity chemicals and fuels from renewable feedstock that do not compete with food production and promote a circular, carbon-neutral economy is one of the greatest challenges that society and industry will face in the near future.[1, 2] In this context, higher alcohols (*i.e.* alcohols containing more than two carbon) produced from renewable carbon are regarded as promising future biofuels due to their high energy density and low hygroscopicity.[3, 4] Typically, they are obtained through the reduction of short- and medium-chain carboxylates (SCCs and MCCs) via inorganic catalytic hydrogenation,[5] which requires high temperature and pressure, the use of expensive catalysts and/or toxic reagents.[6, 7] A sustainable alternative route is the microbial fermentation of synthesis gas (syngas), whereby organisms such as *Clostridium* spp. (*e.g.*, *C. ljungdahlii* and *C. autoethanogenum*) metabolize CO_2 and/or CO into acetate and ethanol using H_2 and/or CO as the electron donor for CO_2 reduction.[8] When gas streams rich in CO_2 but poor in H_2 and CO are used, reducing equivalents can be provided by the cathode electrode of an electrochemical system, either directly in the form of electrons, or indirectly as H_2 produced through water electrolysis.[9, 10] This technology, referred to as microbial electrosynthesis, is capable of converting waste gas streams into valuable organic molecules.[9, 11] Depending on the culturing conditions, notably the pH of the medium, a desired acetate-to-ethanol ratio can be achieved.[12, 13] For example, while acetate is the preferred fermentation product at circumneutral pH (>6), mildly acidic pH (<5) conditions can steer the production toward ethanol.[12, 13] Further, in the presence of both acetate and ethanol and under neutral pH, organisms such as *C. kluyveri* can produce SCCs and MCCs through the reverse *β*-oxidation carbon chain elongation pathway.[14] Finally, higher alcohols can be obtained through solventogenesis of the SCCs and MCCs under mildly acidic pH.[6, 13, 15] While the production of higher alcohols can be approached by culturing different *Clostridium* spp. capable of all production steps in a single reactor system operated at a relatively low pH,[15, 16] the application of a single pH value that is a compromise to allow for all three production phases to occur simultaneously, generally results in a less efficient production compared to systems operated under ideal pH conditions for each production phase.[13]

In this communication, we address the issue of competing pH requirements for acetogenesis, solventogenesis, and chain elongation by introducing a three-chamber electrochemical system design comprising of two biological cathode chambers and one abiotic anode compartment. This original configuration achieves the physical separation of acetogenesis/chain elongation from solventogenesis, and allows their operation under optimal pH conditions without using acid/base dosing, but through a combination of electrochemical control of the cathodes potential and CO_2 gas sparging.

Figure 1 provides a schematic representation of the three-chamber electrochemical system and its operating principles. Additional details are provided in the Supplementary Information, Figure S1. The flat plate reactor consisted of three

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† Electronic Supplementary Information (ESI) available: Details of materials and methods including a detailed assembly schematic of the reactor, main parameters, production profiles and cathodic current density profiles of batches A-D, gas analysis of the reactor head space in batch D. See DOI: 10.1039/x0xx00000x

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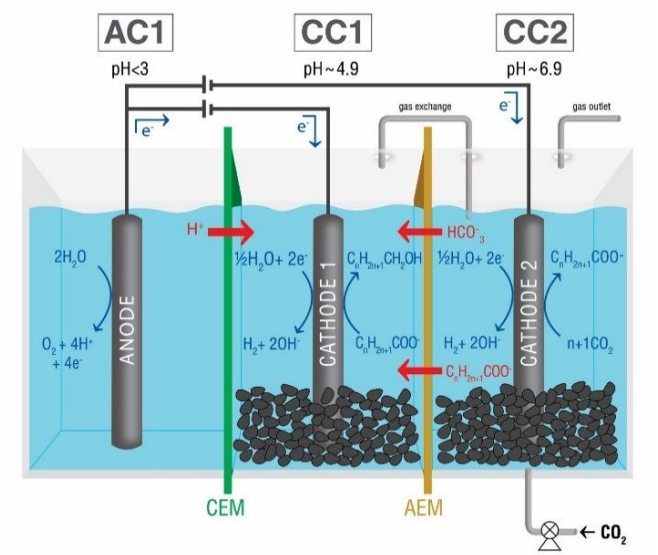

Figure 1. Schematic representation of the microbial electrosynthesis reactor with dual biocathode for the production of organic acids at neutral pH and simultaneous reduction of the produced organic acids to the corresponding alcohols at mildly acidic pH. CEM: cation-exchange membrane; AEM: anion exchange membrane.

aligned acrylic frames [10 cm (width) × 10 cm (height) × 2 cm (depth)] mounted between two acrylic plates (20 cm × 20 cm × 1 cm) to yield three reaction chambers with internal volume of 200 cm^3. The anode chamber, herein indicated as AC1 (Figure 1, left side) was separated from the middle chamber (cathodic chamber 1, CC1) by a 100 cm^2 cation-exchange membrane (CEM, CMI-7000, Membranes International Inc., U.S.A.), while an anion-exchange membrane (AEM, AMI-7001, Membranes International Inc., U.S.A.) separated CC1 from a second cathodic compartment CC2, Figure 1, right side). A titanium mesh electrode coated with 12 g m^{-2} Ti/$Ru_{0.7}Ir_{0.3}O_2$ (5 cm × 5 cm × 0.1 cm; Magneto Special Anodes, Netherlands), was placed in the anode chamber and functioned as the anode. Each cathodic chamber was filled with ca. 110 cm^3 (176.2 g) graphite granules (average diameter: 6 mm; El Carb 100, Graphite Sales Inc., U.S.A.), serving as cathode electrodes (working electrodes). Ag/AgCl reference electrodes in saturated KCl (RC-1CP, Japan) were placed in each cathodic chamber in close proximity to the electrodes. All potentials herein are reported with respect to the standard hydrogen electrode. Electrochemical control was provided using a multichannel potentiostat (potentiostat/galvanostat VSP, BioLogic Science Instruments, France). To assure adequate mixing conditions, the electrolytes (for composition see Supporting Information, Text S1) were recirculated through the chambers with a peristaltic pump at a flow rate of 55.7 ± 1.2 mL min^{-1}. Temperature (set to 35 °C), pH, and dissolved oxygen were monitored using a BIOSTAT® B (Sartorius, Germany).

To perform the biotic batch tests (see below), the cathode chambers were inoculated with 50 mL of broth (containing planktonic cells) and 12 mL of graphite granules (containing biofilm) from an electrochemical reactor operating in our laboratories, enriched with an electroactive microbiome dominated by *Clostridium* spp. and converting CO_2 to C2-C6 carboxylates and alcohols.[11]

pH control in the two cathode chambers to values optimal for acetogenesis/chain elongation, and solventogenesis, was achieved by using a combination of CO_2 sparging in CC2, and alternating control of the electrochemical potential of cathodes CC1 and CC2. Two phases can be distinguished, indicated herein as Phase 1 and Phase 2. During Phase 1, cathode CC1 is kept at open circuit while cathode CC2 is poised at values between -0.80 and -0.85 V for 5 to 10 minutes, depending on the particular experiment (see below for details), resulting in the following abiotic processes influencing the pH: (*i*) *water electrolysis*, producing oxygen (O_2) and protons (H^+) at anode AC1, and hydrogen (H_2) and hydroxyl ions (OH^-) at cathode CC2. (*ii*) *Electromigration*, driving the transfer of protons from anode AC1 to cathode CC2 (and the migration of hydroxyls from cathode CC2 to anode AC1) to maintain electroneutrality. Importantly, the particular membrane arrangement (Figure 1), and the inefficient selectivity toward protons and hydroxyls displayed by ion exchange membranes such as those used in this study,[17] typically result in a pH decrease in AC1 and in CC1, and a pH increase in CC2. Hence, to maintain the pH in CC2 around the set point of ca. 7, considered optimal for carboxylates production,[13] the medium was gassed with CO_2 to buffer the excess of hydroxyl ions with protons deriving from the dissociation of carbonic acid (H_2CO_3). Further, as the continuous operation of Phase 1 would cause the pH in the middle chamber (CC1) to become increasingly acidic, a second operational phase (Phase 2) is included, whereby the potential of cathode CC1 is controlled at values between -0.8 and -0.9 V for 20 to 25 minutes (see below for additional experimental details) while cathode CC2 is kept at open circuit. The purpose of Phase 2 is to assure a proton consuming cathodic reaction in CC1 to balance excess proton accumulation during Phase 1, which helps maintaining the pH at values around the set point of 4.9 in CC1, considered optimal for solventogenesis.[13]

In the presence of microorganisms in CC1 and CC2, the following additional processes can be expected: (*i*) *homoacetogenesis* in CC2, promoting the microbial conversion of CO_2 into acetate using reducing equivalents provided by the cathode during Phase 1;[18] (*ii*) *electromigration* of negatively charged carboxylates across the AEM from CC2 to CC1; (*iii*) *solventogenesis*, *i.e.,* the microbial conversion of acetate (and later also other SCCs and MCCs,) into ethanol (and higher alcohols) in CC1;[6] (*iv*) *solvents transfer* to CC2 using H_2 produced in CC1 during Phase 2 as stripping gas; (*v*) *carbon chain elongation*, converting acetate into carboxylates with higher C content (*e.g.*, SCCs and MCCs) using ethanol as electron, energy, and carbon donor.

The microbial electrosynthesis system was evaluated through two abiotic batch tests (tests A and B) and two biotic batch tests (tests C and D). For abiotic batch A, in order to characterize the ability of the system to transfer carboxylates from chamber CC2 to CC1, 210 mM-C acetate were added to CC2. In abiotic batch B, to analyze the electromigration of acetate and the potential diffusion of ethanol across the membranes, in addition to acetate in CC2, 72 mM-C ethanol were added to CC1. In biotic batch C, CC2 was inoculated with active biomass (enriched with *Clostridium* spp.)[11] to test the ability of the system to promote microbial acetogenesis from CO_2, while CC1 was kept abiotic. Finally, in batch D, to test the ability of the system to simultaneously produce carboxylates and alcohols, both CC1 and CC2 were inoculated with active microbiome.

During the batch tests, cathodes CC1 and CC2 were operated alternatively in potentiostatic mode according to Phase 1 and 2 described above. In batch A and B, cathode CC1 was operated at a potential of -0.8 V for 20 min, while cathode CC2 was kept at open circuit (Phase 2). Successively, cathode CC2 was poised at -0.8 V for 10 min, while cathode CC1 was kept at open circuit (Phase 1). A 30 sec pause was applied between the phases. In biotic batch C, the applied potential in CC1 was reduced from -0.8 to -0.85 V to allow a faster pH increase in CC1. A minor addition of NaOH was required during this test to fix the pH at the set point of 4.9 (Text S1). A stable pH without base dosing was achieved in batch D by further reducing the potential of CC1 to values between -0.85 and -0.9 V for intervals between 20 and 25 min during Phase 2, while the potential of cathode CC2 during Phase 1 ranged between -0.80 and -0.85 V for periods of 5 and 10 min. During this test, a tube connecting the headspace of chamber CC1 and the recirculation loop of CC2 was included to allow for ethanol transfer from CC1 to CC2 using H_2 as stripping gas and facilitate chain elongation in CC2. Additional details regarding the operation of each batch test is provided in the Supporting Information (Text S1).

Organic acids and alcohols were quantified via gas chromatography (GC) as described in the Supporting Information (Text S1).[11] During batch D, gas samples from the headspace of CC2 were analyzed via GC to monitor H_2 production, CO_2 availability and methanogenesis inhibition (for method, see Text S1; for results, see Figure S4).[11] All calculations used in this study are detailed in the Supporting Information (Text S1).

In batch A (abiotic test, with acetate added to CC2), of the 210 mM-C acetate initially added to CC2, 50% migrated to CC1, and 2% to AC1 by the end of the batch, respectively (Figure 2A). Similarly, during batch B (abiotic test, with acetate in CC2 and ethanol in CC1), about 46% of the initial acetate concentration in CC2 had migrated to CC1 and 6% to AC1 (Figure 2B), respectively, proving that the dual cathode electrochemical system and the particular operations were effective at transferring acetate from CC2 to CC1 through the AEM to make it available for solventogenesis once a suitable microbiome is used (refer to batch D below). It is important to note that it is likely that acetate migration to CC1 competed with the transport of other ions, including HCO_3^-, which was present in

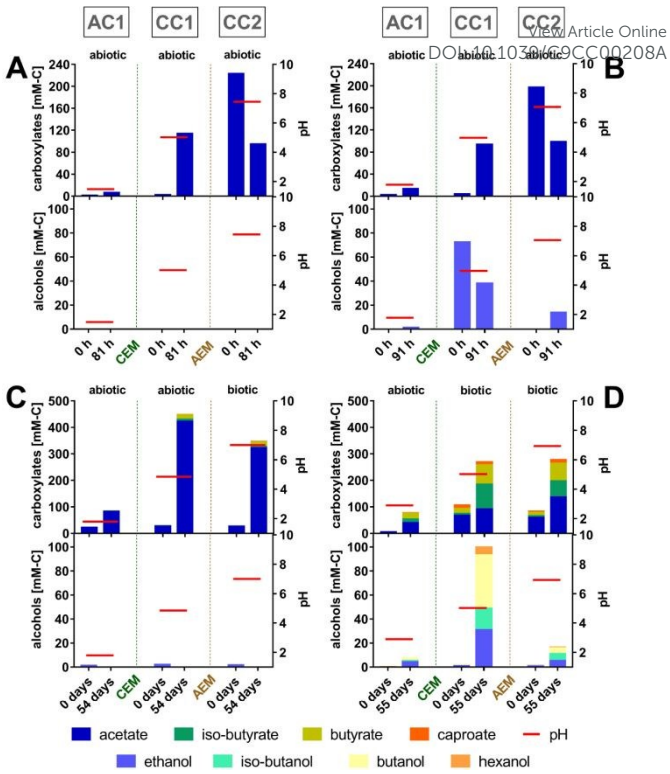

Figure 2. Production and migration of carboxylates and alcohols in the microbial electrosynthesis reactor.

CC2 due to the use of CO_2 for pH control.[19] During batch B, 72 mM-C of ethanol were added to CC1 to simulate a scenario where ethanol is produced through biological solventogenesis at the lower pH of 4.9 in CC1. By the end of the batch after 91 h, of the ethanol initially added, only 53% was left in CC1, whereas 13.8 mM-C were observed in CC2, accounting for 19% of the initial total. Small titers were observed in AC1 (<2% of the total), leaving about 26% of the added ethanol unaccounted for. Since the connection between CC1 headspace and CC2 medium-recirculation loop was only included in batch D, we conclude that some ethanol was removed from CC1 through H_2 stripping, while the rest reached the adjacent chambers likely due to diffusion driven by a concentration gradient between the electrolytes.[20]

It is important to remark that the absence of measurable levels of carboxylates or alcohols other than those injected in the two abiotic batches, indicates that under the given experimental conditions the process of chain elongation was not promoted by electrocatalysis alone (that is, in the absence of a suitable microbial catalyst). Accordingly, in batch C, neither acetate or ethanol were added to the cathode chambers, and CC2 was instead inoculated with an active microbiome catalyzing the reduction of CO_2 into acetate via acetogenesis, putatively using reducing equivalents provided by the cathode in form of H_2. The presence of H_2 in the reactor gas headspace was confirmed by GC measurements (Figure S4).[11] The results (reported in Figure 2C and Figure S3) show that acetate reached levels of up to 322.1 mM-C (9.50 g L^{-1}) at the end of the batch, with a maximum production rate of 15.6 mM-C day^{-1} (0.46 g L^{-1} day^{-1}). As observed in previous batches A and B, acetate migration across the AEM was responsible for

transferring more than 55% of the acetate produced in CC2 to CC1 at a migration rate of 8.8 mM-C day^{-1} (0.26 g L^{-1} day^{-1}), resulting in levels of acetate up to 421.7 mM-C (12.4 g L^{-1}) in CC1 at the end of the batch. While small titers of C4 carboxylates (<7 mM-C) were also observed in CC2 and in CC1 (Figure 2C), the absence of significant levels of C4-C6 carboxylates and alcohols implies that microorganisms did not performed carbon chain elongation nor solventogenesis in CC2. This is not surprising, since only CC2 was inoculated with active biomass while maintained at neutral pH to favor acetogenesis activity alone. Accordingly, during batch test D, cathode CC1 was also inoculated with the same active microbiome used to seed CC2 in batch C. Figure 2D shows that a larger product diversification was observed during this test. In addition to the acetate observed in CC2 and CC1, confirming the same acetogenic functionality observed previously in batch C, titers of ethanol were observed in CC1 and are likely linked to the microbial solventogenesis of the acetate that had migrated from CC2, favored by the acidic conditions at which CC1 was operated. Importantly, under the hypothesis that the simultaneous presence of ethanol and acetate in CC2, and under the neutral pH conditions at which CC2 was operated will trigger the microbial synthesis of carboxylates with higher C content via the reverse *β*-oxidation chain elongation pathway, a connection between the headspace of CC1 and the electrolyte in CC2 was included during this batch test to promote ethanol transfer from CC1 to CC2 via gas stripping, using H_2 electrochemically produced in CC1 (Figure 1). Indeed, levels up to 60.3 mM-C (1.33 g L^{-1}) isobutyrate, 67.4 mM-C (1.49 g L^{-1}) butyrate, and 13.9 mM-C (0.27 g L^{-1}) caproate were observed in CC2 (Figure 2D). Similar levels of C4-C6 carboxylates were also observed in CC1, and are likely ascribed to their electromigration from CC2, and additionally from microbial chain elongation activity occurring under sub-optimal pH conditions in CC1, as observed previously.[13] Importantly, acidic pH conditions in CC1 resulted in the biosynthesis of C4-C6 alcohols in addition to ethanol (Figure 2D). Levels up to 17.8 mM-C (0.33 g L^{-1}) isobutanol, 44.5 mM-C (0.82 g L^{-1}) butanol, and 6.6 mM-C (0.11 g L^{-1}) hexanol were observed, proving that the specific arrangement and operation of the electrochemical system could successfully promote the production of higher alcohols. Amongst the options for alcohols recovery, gas stripping and multistage pervaporation was proposed by Xue *et al.* as an efficient approach for the extraction of butanol (the main solvent produced in their microbial electrochemical system), and could possibly be applied in this work as well.[21] Extraction of the target products would indeed yield several advantages to the bioprocess, including an increase in their economic value (due to the increased purity), as well as an increase in the production rates (since in-line extraction would guarantee that products titers are kept below inhibition levels).[21] Further improvements in productivity could potentially be achieved by increasing the availability of supplied CO_2 through implementation of a gas diffusion electrode as demonstrated by Srikanth and co-workers for butanol production.[22]

In conclusion, this research introduces a novel bioelectrochemical approach that enables the application of optimal pH conditions to a dual biocathode electrochemical cell to simultaneously favor acetogenesis, solventogenesis and carbon chain elongation, thereby enabling the production of valuable carboxylates and higher alcohols from CO_2 and electricity. Chemical-free pH control of the cathode compartments is achieved by CO_2 sparging of the outermost cathode chamber, and by fine-tuning of the electrochemical potential of the two cathodes. While production rates obtained in this study warrant further optimization to achieve levels required for practical implementation of the technology, the possibility to operate an electrochemical system with multiple, electrically independent compartments to suit the different requirements of particular biocatalytic processes, will certainly expand the current horizon of applications of microbial electrochemical technologies.

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Conflicts of interest

There are no conflicts to declare.

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