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Ludovic Jourdin
University of Queensland

Timothy Grieger
University of Queensland

Juliette Monetti
University of Queensland

Victoria Flexer
University of Queensland, v.flexer@awmc.uq.edu.au

Stefano Freguia
University of Queensland

See next page for additional authors

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Abstract

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Keywords

obtained, rate, acid, acetic, microbial, electrosynthesis, dioxide, carbon, production, high

Disciplines

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Authors

Ludovic Jourdin, Timothy Grieger, Juliette Monetti, Victoria Flexer, Stefano Freguia, Yang Lu, Jun Chen, Mark S. Romano, Gordon G. Wallace, and Jurg Keller

High Acetic Acid Production Rate Obtained by Microbial Electrosynthesis from Carbon Dioxide

Ludovic Jourdin,^{*,†,‡,§} Timothy Grieger,[†] Juliette Monetti,[†] Victoria Flexer,^{*,†,||} Stefano Freguia,^{†,‡} Yang Lu,[†] Jun Chen,[§] Mark Romano,[§] Gordon G. Wallace,[§] and Jurg Keller[†]

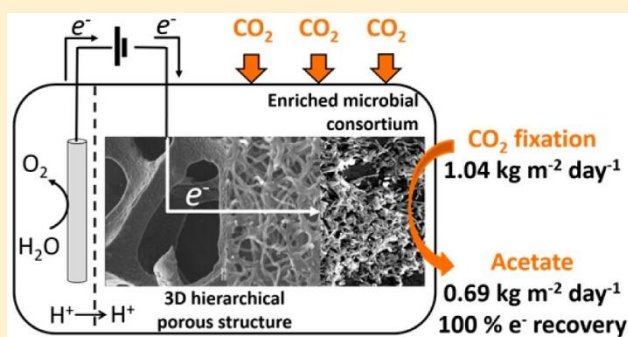
[†]Advanced Water Management Centre, The University of Queensland, Level 4, Gehrmann Building (60), Brisbane, Queensland 4072, Australia

[‡]Centre for Microbial Electrosynthesis, The University of Queensland, Level 4, Gehrmann Building (60), Brisbane, Queensland 4072, Australia

[§]RC Centre of Excellence for Electromaterials Science, Intelligent Polymer Research Institute, AIIM Facility, Innovation Campus, University of Wollongong, Wollongong, NSW 2522, Australia

^{*} Supporting Information

ABSTRACT: High product specificity and production rate are regarded as key success parameters for large-scale applicability of a (bio)chemical reaction technology. Here, we report a significant performance enhancement in acetate formation from CO₂, reaching comparable productivity levels as in industrial fermentation processes (volumetric production rate and product yield). A biocathode current density of $-102 \pm 1 \text{ A m}^{-2}$ and an acetic acid production rate of $685 \pm 30 \text{ (g m}^{-2} \text{ day}^{-1})$ have been achieved in this study. High recoveries of $94 \pm 2\%$ of the CO₂ supplied as the sole carbon source and $100 \pm 4\%$ of electrons into the final product (acetic acid) were achieved after development of a mature biofilm, reaching an elevated product titer of up to 11 g L^{-1} . This high product specificity is remarkable for mixed microbial cultures, which would make the product downstream processing easier and the technology more attractive. This performance enhancement was enabled through the combination of a well-acclimatized and enriched microbial culture (very fast start-up after culture transfer), coupled with the use of a newly synthesized electrode material, EPD-3D. The throwing power of the electrophoretic deposition technique, a method suitable for large-scale production, was harnessed to form multiwalled carbon nanotube coatings onto reticulated vitreous carbon to generate a hierarchical porous structure.



INTRODUCTION

Microbial electrosynthesis (MES) is a biocathode-driven process in which electroactive microorganisms derive electrons from solid-state electrodes to catalyze the reduction of carbon dioxide and generate valuable extracellular multicarbon reduced end-products.^{1–3} MES is a novel and promising strategy that can convert electrical energy into chemical energy that can be stored, distributed, and consumed on demand. Intermittent renewable sources such as solar and wind power can be envisioned as suitable sustainable energy sources to power MES.^{4,5} In view of the threats of global warming and diminishing fossil fuel resources, MES is among one of the very attractive technologies for the renewable production of fuels and chemicals, which our society heavily depends on.^{6,7}

Microbial electrosynthesis remains a nascent concept, with

only a few studies that have demonstrated the process at a laboratory scale using either pure cultures^{5,8–11} or mixed microbial consortia.^{6,12–20} The use of mixed microbial cultures is attractive because they are readily obtainable in large quantities, are more tolerant to environmental stress and

fluctuations,²¹ and have thus far showed higher production rates over long-term operation.^{6,12,13} To date, mainly acetate production has been achieved in a sustained fashion by electroactive microorganisms, using electricity and carbon dioxide as the sole energy and carbon sources. Recently, however, a study has also shown the simultaneous conversion of CO₂ into a mixture of products composed of acetate, butyrate, ethanol, and butanol using mixed microbial cultures.¹⁶ Acetate can be an important end-product as well as a platform for further chemical syntheses. It is also widely used as carbon substrate for industrial and environmental biological processes such as denitrification or biological phosphorus removal. In this context, microbial electrosynthesis could be an interesting option for the on-site production of organics for such processes at a wastewater treatment plant.

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However, the reported performances of bioelectrosynthesis processes are still insufficient for scaling MES to practical applications. Optimizing MES relies on the enhancement of bacterial attachment, biofilm development, electron-transfer rate at the cathode surface (microorganism-electrode interaction), and chemical production rate.⁶ Biocathode materials,^{6,22} selective microbial consortia, and efficient reactor designs are key elements to be optimized toward this objective.

To date, only a handful of studies have focused on the development of prospective electrode materials for biocathode processes and microbial electrosynthesis.^{6,9,10,22} Lovley et al.^{9,10} have recently proposed a number of treatments to modify electrode materials for the improvement of microbial electrosynthesis of acetate from CO₂ by pure cultures of *Sporomusa ovata*. They highlighted that surface-charge modification might not be a sufficiently effective strategy on its own. They reported a sevenfold-higher production rate (ca. 0.02 mM cm⁻² day⁻¹) and current density (0.0475 mA cm⁻²) on chitosan-modified carbon cloth over those of a nonmodified carbon cloth. We have recently reported on the development of a new biocompatible, highly conductive three-dimensional microbial bioelectrode, with a hierarchical porous structure by direct growth of multiwalled carbon nanotubes (MWCNT) on reticulated vitreous carbon (RVC), which is called NanoWeb-RVC and uses the chemical vapor deposition technique (CVD).^{6,23} NanoWeb-RVC showed excellent performance as the biocathode material for acetate production.⁶ We reported one of the highest current densities (37 A m⁻² normalized to projected surface area) and bioproduction rates (192 g m⁻²

day⁻¹ of acetate) reached to date on biocathodes for the bioreduction of carbon dioxide. The NanoWeb-RVC benefits from all of the advantages of both the macrostructured RVC and nanostructured surface modification. The high surface-area-to-volume ratio of the macroporous RVC maximizes the available biofilm area while ensuring effective mass transfer to and from the biocatalysts. The carbon nanostructure, in turn, putatively enhances the microbe-electrode interaction, bacterial attachment, biofilm development, and microbial extracellular electron transfer. Therefore, in addition to showing very high intrinsic performance as a biocathode material for MES, NanoWeb-RVC electrodes create an extremely efficient material from an engineering perspective as well. However, for scale-up beyond certain size of electrodes, there are some limitations with the chemical vapor deposition (CVD) technique. Indeed, the requirement for specialized and expensive equipment and the complexity of the CVD process make this method unsuitable both for the production of large-size samples and for large-scale production. Therefore, the quest for new electrode materials to be incorporated in bioelectrosynthesis reactors is still ongoing.

One of the most efficient methods of generating thin films from colloidal suspensions is electrophoretic deposition (EPD).^{24–26} EPD has been extensively used in the deposition of carbon nanotubes (CNT) to form highly porous electrodes for electrochemical applications.^{27–30} Depending on the settings used, the deposited CNT layer may be highly uniform with no agglomeration.³¹ Compared to other methods of processing CNTs, EPD is relatively easy to carry out with simple equipment requirements.^{32,33} Moreover, it is capable of producing thin films from colloidal suspensions on irregularly shaped substrates.²⁶ Furthermore, the scaling-up of this method can be easily accomplished by simply increasing the dimensions of the departing substrate to be coated. Because the synthetic

protocol only uses aqueous suspensions of MWCNT, the risk of fire hazards is minimized. All of these characteristics make the EPD method attractive for the large-size and industrial-scale production of hierarchical porous electrodes.

Here, we report on a microbial electrosynthesis process achieving a high acetic acid production rate of up to 685 g m⁻² day⁻¹ from CO₂. This was achieved through the combination of a well-acclimatized microbial culture and a newly synthesized electrode material. We harnessed the throwing power of EPD to form MWCNT coatings onto RVC to generate a new hierarchical porous structure, hereafter called EPD-3D, which we used as biocathode electrode. It is important to highlight that the simplicity of the equipment required for EPD and short deposition time make this method suitable and attractive for the industrial large-scale production of EPD-3D.³⁴

MATERIALS AND METHODS

Preparation of EPD-3D Electrophoretic Deposition Technique. RVC foam (Duocel, ERG Materials and Aerospace Corporation), with a porosity of 45 pores per inch (ppi), was activated by immersion in 2 M HNO₃ overnight. The RVC was then cleaned by washing with Milli-Q water, after which it was oven-dried for 3 h. A stainless steel wire was attached to the RVC using silver paste to ensure good electrical conductivity.

MWCNTs (NC3100, Nanocyl) were oxidized to facilitate dispersion in water. This was done by first plasma-treating the MWCNT in air for 30 s. The MWCNT were then dispersed in a 1:1 mixture of 3 M H₂SO₄ and 3 M HNO₃ using a Branson B1500R-MT bath sonicator for 2 h. The dispersion was then centrifuged at 4400 rpm for 30 min using an Eppendorf centrifuge (5415 D). The solids were collected and washed with water, after which centrifugation was repeated. This washing step was done four times; the solids were then collected and oven-dried. The MWCNT were then dispersed in Milli-Q water at a concentration of 100 mg L⁻¹ using a Branson S450-D 400 W probe sonifier. A 1/8 in. tapered microtip was used during sonication with 20% amplitude for 1 h.

EPD was carried out using a Keithley 2400 sourcemeter, wherein 10 V was applied for 10 min. The negative terminal of the sourcemeter was connected to stainless-steel mesh bent in a cylinder and placed in a vial (Scheme S1). The RVC was connected to the positive terminal of the sourcemeter and positioned in the center of the stainless-steel mesh cylinder. The MWCNT dispersion was then added, after which EPD was carried out. A uniform electric field is thus generated around the RVC substrate, which ensures uniform deposition of the MWCNT on the RVC.

Electrode Preparation. EPD-3D electrodes were pierced with a 0.5 mm thick Ti wire that acted as a current collector. The electrical connection was reinforced by means of conductive carbon paint that was left to dry for 1 day.

A total of two EPD-3D electrodes were cut in blocks of about 1.21 cm × 1.19 cm × 1.03 cm each. When it comes to using EPD-3D as an electrode in bioelectrochemical systems, it is important to understand the difference between normalizing performance to projected or to total surface area, as previously defined.^{6,23} The projected surface area is of particular interest from an engineering perspective because it determines the size of a bioelectrochemical reactor for large-scale applications. Normalization by total surface area allows for the assessment of the intrinsic material performance as a biocathode material. The value of the total area is much higher than the projected surface area of the electrode. For a 45 ppi RVC scaffold, a value of 26.2

$\text{cm}^{-2} \text{ cm}^{-3}$ is given by the RVC manufacturer using the multipoint BET method by the adsorption of Krypton gas at cryogenic temperatures and is confirmed by Friedrich et al.³⁵ The total and projected surface area of each of the EPD-3D 45 ppi electrodes used in this study was approximately 36.9 and 1.36 cm^2 , respectively.

To assess performance from an engineering perspective, we also normalized performance to the volume of the cathode electrode, taking into account its 3D nature and the total electrode surface available for biofilm development per unit volume ($2620 \text{ m}^2 \text{ m}^{-3}$).

All electrodes were pretreated in a N_2 plasma for 20 min before being introduced in the reactors to remove surface contamination and render the surface hydrophilic.³⁶

Source of Microorganisms. Planktonic cells from the microbial electrosynthesis systems using NanoWeb-RVC described by Jourdin et al.⁶ after half a year of operation were collected, centrifuged, resuspended in fresh catholyte, and used as inoculum for the MESs described in this study. Therefore, no organics were introduced in the new reactors. The enriched inoculum was added to a final concentration of about 200 mg L^{-1} as the chemical oxygen demand (COD) in both reactors on the same day. The original source of the mixed microbial consortium was from both natural environments (stormwater pond sediments located on the University of Queensland, Saint Lucia campus, Brisbane, Australia) and engineered anaerobic systems (from the Luggage Point Wastewater Treatment Plant anaerobic digester, Brisbane, Australia). The microbial community composition of the original inoculum was reported in ref 37 and can also be found in Figure S1.

Electrochemical Experiments. The reactor design, materials, experimental conditions, and medium composition were identical to those described in Jourdin et al.⁶ Each of the two EPD-3D electrodes was immersed in a different reactor to act as independent duplicate. Each reactor was filled with 250 mL of inorganic medium containing bicarbonate as the sole carbon source and polarized at -0.85 V versus SHE for 63 days. Final concentration of 1 to 4 $\text{g L}^{-1} \text{ NaHCO}_3$ was added periodically as the sole carbon source. Experiments were carried out under strict anaerobic and dark conditions at $35 \text{ }^\circ\text{C}$. The BESs were operated in fed-batch mode. A multichannel potentiostat (VMP-3, Bio-Logic SAS, France) was used for all experiments. All potentials are reported here versus standard hydrogen electrode (SHE). To suppress methanogenic activity, we added 15 mM 2-bromoethanesulfonic acid. During the experiment, the catholyte medium pH was regularly adjusted to 6.7 by dosing with 1 M HCl as needed. The anolyte contained $6 \text{ g L}^{-1} \text{ Na}_2\text{HPO}_4$ and $3 \text{ g L}^{-1} \text{ KH}_2\text{PO}_4$, and platinum wire was used as counter electrode (purity 99.95%, 0.50 mm diameter \times 50 mm length; Advent Research Materials, Oxford, England). The two chambers were separated by a cation-exchange membrane (CEM, Ultrex CM17000, Membranes International, NJ). Linear-sweep voltammetry tests were also performed by scanning potentials from 0 to -1.2 V versus SHE at 1 mV s^{-1} . LSV was also run on an abiotic EPD-3D control electrode.

The concentrations of volatile fatty acids in the liquid phase were determined by a gas chromatography method,³⁷ and bicarbonate consumption was followed by a total organic carbon analyzer method.³⁷ Methane, hydrogen, and carbon dioxide gases in the reactors' headspace were measured using a gas chromatography-thermal conductivity detection (GC-TCD) method.³⁷

Scanning Electron Microscopy and Microbial Community Analysis. Scanning electron microscope images of the bare EPD-3D electrodes were obtained using a JEOL JSM 7500FA cold-field-gun field-emission microscope (SEM images shown in Figure 1A,B). After biofilm development, at the end

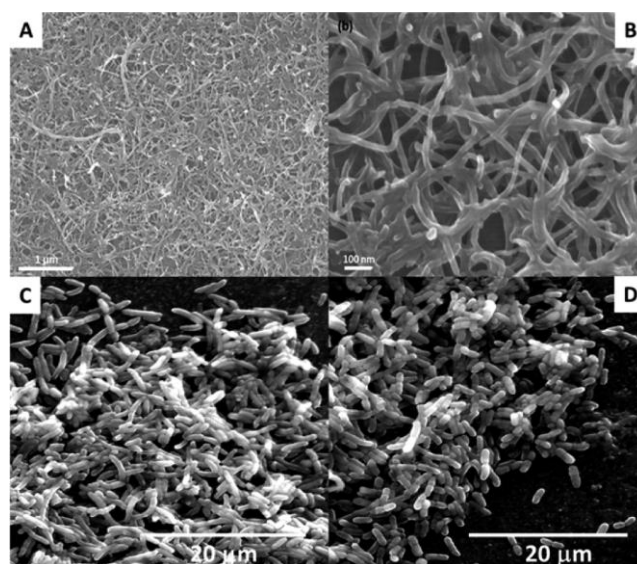


Figure 1. Scanning electron micrograph images at different magnifications of (A,B) EPD-3D and (C,D) of a biofilm developed after 63 days on EPD-3D.

of the chronoamperometry experiment, pieces of EPD-3D were cut and prepared for SEM observation (images shown in Figure 1 C,D) as described in the Supporting Information.

Biofilm and planktonic cells were collected from two replicate biocathodes under steady MES performance. Those reactors were started using the same inoculum and electrode materials as described above and reached the same performance (data not shown) as the other reactors described here. Community compositions were obtained as described in the Supporting Information.

RESULTS AND DISCUSSION

Typically, an EPD setup consists of two conductive substrates, i.e., electrodes, oriented such that they are parallel to each other³² in such a configuration that a uniform electric field is generated around the substrate to be coated. These electrodes are submerged in a solvent, wherein the colloidal nanoparticles are dispersed.³⁴ EPD occurs in two steps, initially electrophoresis followed by deposition.³⁸ In electrophoresis, the MWCNT (which are negatively charged due to the oxidation process) migrate toward the positive electrode due to the electric field being applied to the dispersion (Scheme S1). The MWCNT, due to particle coagulation then form a coherent deposit on the RVC surface (deposition step).³⁹ The layer of MWCNT deposited on the surface of the RVC samples is depicted in Figure 1A. The porous layer of MWCNT can clearly be seen in Figure 1B. The MWCNT layer does not block the pores in RVC; i.e., the original macroporous structure is maintained (Figure S2).

The simplicity of the cell needed for EPD and short deposition time make this method suitable for large-scale production as long as the substrate on which the CNTs are deposited on is highly conductive, which is the case here.³⁴ The

other advantages of EPD include the high degree of uniformity of the deposited MWCNT layer and the ability to deposit on substrates that are irregularly shaped.²⁹

Current Density Enhancement. A total of two bioelectrochemical reactors, each equipped with one EPD-3D electrode, were filled with 0.25 L of inorganic medium that contained bicarbonate as the sole carbon source. Starting right after inoculation, current consumption at a fixed cathode potential of -0.85 V versus SHE was recorded during 63 days and is plotted in Figure 2A,B for each of the duplicate reactors.

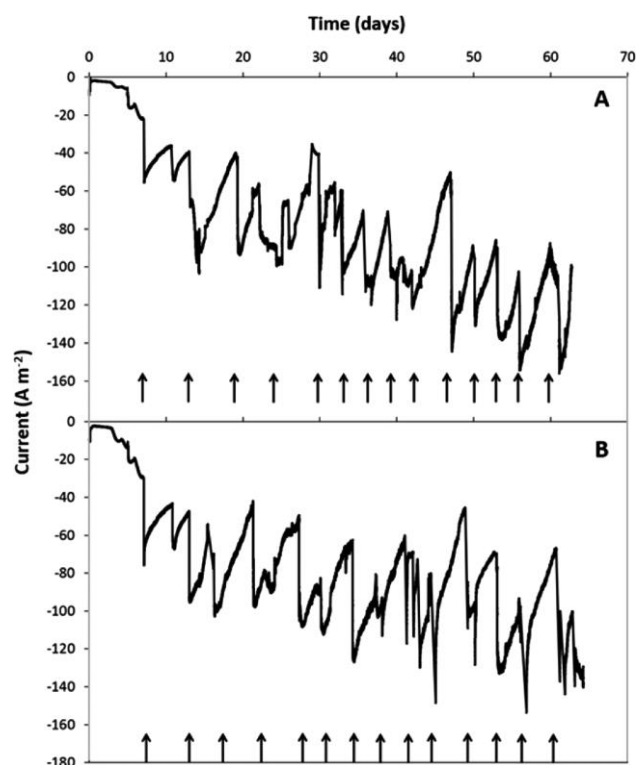


Figure 2. Current density evolution over time on EPD-3D for two duplicate reactors (a,b) at an applied cathode potential of -0.85 V vs SHE, normalized to the projected surface area. The arrows show the days that bicarbonate was added to the cathode chamber and pH adjusted to 6.7.

During this period, carbon dioxide consumption as well as volatile fatty acids production was followed for each reactor. Figure 3 below shows their averaged performance, with

standard deviations represented as error bars. All of the data points in Figures 2–4 as well as in Table S1 have been normalized to the projected surface area. Reported values in the text have also been normalized to the projected surface area unless otherwise specified.

The current density right after inoculation was fairly low, as was expected, but gradually and exponentially increased after only 2 days following inoculation. After 10 days, the current density had already reached about -50 A m⁻². The reactors were run in fed-batch mode, and bicarbonate was periodically added, while at the same time the pH was adjusted to 6.7, as represented by the arrows in Figure 2. The plot indicates that the current increased right after each carbon source addition and pH adjustment and then slowly decreased following its consumption. The pH appeared to have some influence on microbial electrosynthesis rates as previously observed as

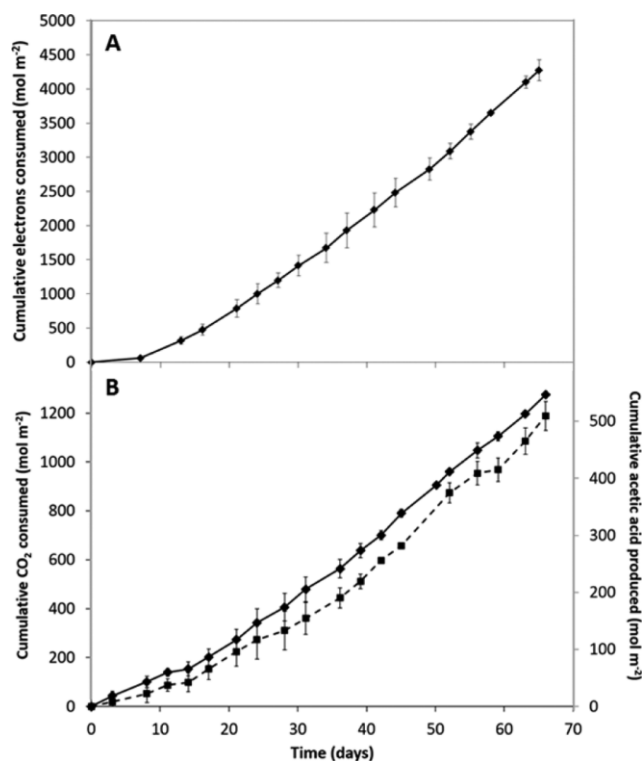


Figure 3. (A) Cumulative electron consumption and (B) carbon dioxide consumption (diamonds) and acetate production (squares) over time on EPD-3D, normalized to the projected surface area.

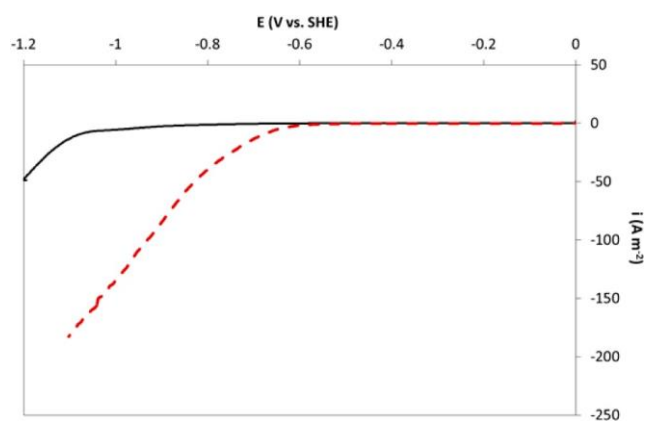


Figure 4. Linear sweep voltammetry on abiotic cathode (solid line) and biocathode (dashed line). Scan rate, 1 mV s⁻¹.

well.^{17,19} Lowering the pH from near-neutral to 5 was shown

by LaBelle et al.¹⁹ not to affect acetate production, while concomitant H₂ production significantly increased. Further decrease of the pH below 5 was found to be detrimental to acetate production, while electrohydrogenesis rates further increased.¹⁹ Keeping the pH slightly acidic (ca. 5.8) was shown to increase acetate production, and the current demand in another study was either a direct effect of the low pH or due to the indirect increase of substrate availability.¹⁷ As observed in [Figure 2](#), the general trend was an increase of the maximum current density reached after each addition and pH adjustment. The maximum current density reached at the end of the test was about -150 A m^{-2} , which represents, to the best of our knowledge, the highest current density reported to date for cathodic microbial carbon dioxide reduction to organics.

The cumulative electron consumption curve of EPD-3D is shown in Figure 3A. The average of the two duplicate electrodes is plotted. Results of duplicates were in good agreement, and the standard deviation is small.

The electron consumption rate is defined as the slope of that curve at different time intervals. A total of three phases in the electron consumption pattern could be observed on EPD-3D with increasingly higher rates, which can be correlated to biofilm growth.⁶ The start-up time was remarkably short as seen in Figure 3A. This can be explained by the successful transfer of an enriched microbial culture, which is an important characteristic for the practical implementation and scaling-up of a biological system. Within the first 7 days, a relatively slow electron consumption of $8.4 \text{ (mol m}^{-2} \text{ day}^{-1})$ was recorded. A first significant increase up to $67 \pm 2 \text{ (mol m}^{-2} \text{ day}^{-1})$ of electrons consumed was observed between day 7 and 44. Finally, from day 44 to the end of the test, $91 \pm 1 \text{ mol m}^{-2} \text{ day}^{-1}$ electrons were consumed, corresponding to a cathodic current density of $-102 \pm 1 \text{ A m}^{-2}$. This value is significantly higher than what has been reported to date (see Table S1). It is important to stress here again that this value is based on the average between both duplicates but also that significant fluctuations in actual current were observed following carbon dioxide consumption and pH increase (Figure 2). It is likely that a more constant current profile could be established under continuous carbon dioxide feeding or controlled, slightly acidic pH conditions. Under such “steady-state” conditions, an even higher current density would be expected, given that a peak current density as high as -150 A m^{-2} was reached immediately following carbon dioxide addition and pH adjustment, as seen in Figure 2. However, the optimization of the operational strategy was outside the scope of this study, and the experimental setup did not allow such a continuous operation and control of the process.

Microbial Electrosynthesis Enhancement. The carbon dioxide consumption and volatile fatty acids production on both EPD-3D reactors were followed throughout the experiments and are shown in Figure 3B. The maximum rates can be seen, compared to other studies, at the bottom row of Table S1. The sole product generated was acetate, and no other volatile fatty acids or alcohols accumulated in any of the reactors.

Consistent with the electron consumption development shown in Figure 3A, similar phases with increasing CO₂ consumption and acetate production rates were observed with fast start-up time following the culture transfer to the EPD-3D reactors. A maximum average CO₂ consumption rate of $24.8 \pm 0.5 \text{ (mol m}^{-2} \text{ day}^{-1})$ and an acetic acid production rate of $11.6 \pm 0.5 \text{ (mol m}^{-2} \text{ day}^{-1})$ were reached from 50 days onward on EPD-3D. At this point of the process, on the basis of a carbon balance, $94 \pm 2\%$ of carbon dioxide was found to be converted to acetate only, while an electron balance revealed that $100 \pm 4\%$ of the electrons consumed were recovered as acetate. It is speculated that after approximately 50 days, a mature biofilm has already been established. Consequently, at this point, the largest proportion of the CO₂ and electrons consumed are recovered into acetate. It is suggested that after the biofilm has reached a certain state of development, bacterial growth slows down, and only a very small proportion of the CO₂ and electrons fed are used to this end. The recycling of carbon and electrons from decaying biomass may also occur.

The conversion efficiencies and product purity achieved in these experiments are very high, especially for mixed cultures, which makes it interesting for potential large-scale production

applications and downstream processing. Furthermore, the achieved acetate production rate of almost $685 \pm 30 \text{ (g m}^{-2} \text{ day}^{-1})$ is about 3.6 times higher than the highest production rate reported previously (Table S1).⁶ Moreover, a fairly high acetate titer of up to 11 g L^{-1} was obtained at the end of the test, with no signs of product inhibition of the active microorganisms at that point. It is, therefore, quite conceivable that the titer would have reached even higher values had the test not been stopped. A high product titer is a critical characteristic of prospective large-scale implementation because it renders the downstream processing much easier than when the product concentration is low. Indeed, product separation and recovery is one of the main costs of established chemical production plants, such as industrial fermentation.^{40,41}

Bioelectrocatalytic Activity. Hydrogen did not accumulate in the headspace of the reactors during the chronoamperometry test at -0.85 V versus SHE. However, this fact does not exclude H₂-mediated electron transfer (abiotically or biocatalytically generated) as a possible mechanism of electron transfer from electrodes to acetate-producing microorganisms. Such an electron-transfer pathway via biotically produced hydrogen was indeed hypothesized in a previous study for methane and acetate production in bioelectrochemical systems.¹² Moreover, H₂-producing microorganisms were also shown to sustain autotrophic growth and self-regenerate under purely cathodic conditions without any external electron or organic carbon sources.³⁷ Alternatively, electrons could also be delivered directly from the cathode or via another soluble mediator than H₂ to the acetate-producing bacteria. A detailed study of the specific extracellular electron-transfer mechanism active in these experiments is currently underway but is beyond the scope of work presented here. Nevertheless, the electrochemical performance of the biocathode EPD-3D was compared to that of an abiotic control EPD-3D electrode on the basis of linear-sweep voltammetry recorded at pH 7 (Figure 4).

A clear shift of the reductive wave onset toward higher potentials (ca. -0.65 V versus SHE) compared to the onset of current in the abiotic reactor (ca. -1.1 V versus SHE) can be observed.

As reported previously,^{6,12,37,42} this may be an indication of biological catalytic activity on the surface of the electrode. In addition, a predominant biofilm development on top of the EPD-3D was clearly revealed at the end of the chronoamperometry experiment using scanning electron microscopy (Figure 1C,D).

Remarkably for a biocathode, the SEM images show a well-developed, almost continuous biofilm with entangled, multi-layered microorganisms with an approximate total thickness of $5\text{--}10 \text{ }\mu\text{m}$. This strongly suggests that the high electron consumption and microbial electrosynthesis performance reached in these experiments may be attributed to sustained bioelectrocatalytic activity through the development of a uniform biofilm covering the three-dimensional structure of the EPD-modified RVC. Indeed, previous experiments with the same inoculum on unmodified RVC showed that the surface appeared to be largely unchanged, with no biofilm development.⁶

Highly Specific Microbial Enrichment and MES and Exclusive Occurrence within the Biofilm. To further investigate the relative importance of the biofilm over the microorganisms in suspension on MES performance, we analyzed the microbial community composition of both biofilm

and solution in two duplicate reactors. Pyrosequencing recovered a total of 227 991 high-quality sequences with an average of $56\,997 \pm 3172$ (standard error) sequences per sample. Microbial communities were consistent between duplicates and distinguishable between biofilm and planktonic cells. The dominating microorganisms were either a niche including *Burkholderiales*, *Clostridiales*, *Natranaerobiales*, and *Methanobacteriales* in biofilm or *Burkholderiales* alone in planktonic cells with other operational taxonomic units (OTUs) presented as less than 1% relative abundance (Figure 5).

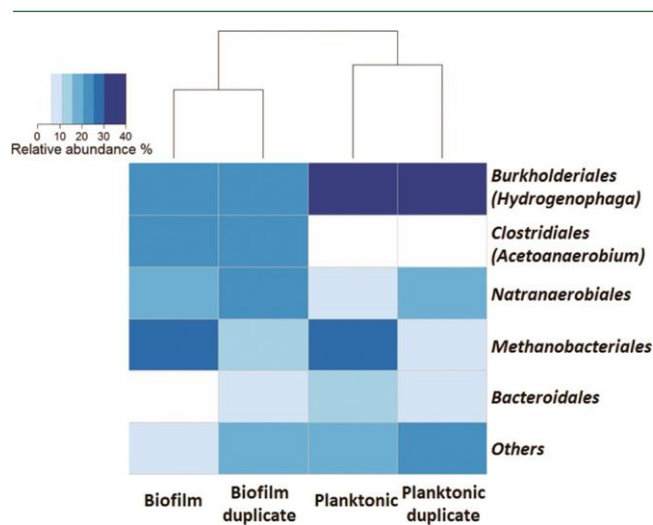


Figure 5. Heatmap of microbial community in biofilm and planktonic cells from two replicate reactors based on order-level summary from pyrosequencing analysis. Genus information is also provided in brackets if it dominates in the corresponding order. Other orders that contains OTUs with less than 1% relative abundance are summarized and presented as “Others”.

A total of three main bacterial orders accounted for up to 65% of the whole community in the biofilm: *Burkholderiales*, *Clostridiales*, and *Natranaerobiales*. This shows that the specific experimental conditions allowed for the high enrichment of a few species only, starting from a large variety of microorganisms (Figure S1).³⁷ Descending from the order *Clostridiales*, the main genus was *Acetoanaerobium*. *Acetoanaerobium* is an anaerobic bacterium that has been reported in the past for its ability to produce acetate from CO₂ and H₂.⁴³ Remarkably, *Acetoanaerobium* was mainly dominant in the biofilm and present in suspension at only a low abundance. The main genus of *Burkholderiales* detected was *Hydrogenophaga*, with more than 37% of abundance in suspension and also fairly high abundance (ca. 22%) in the biofilm. To the best of our knowledge, *Hydrogenophaga* has only been reported as a facultative autotrophic hydrogen-oxidizing bacteria.⁴⁴ It was isolated from anodic biofilms of acetate-fed microbial fuel cells⁴⁵ and speculated to work in close syntrophy with acetate-oxidizer *Geobacter* as hydrogen-utilizing exoelectrogen, hence directly transferring electrons to the electrode.⁴⁶ *Hydrogenophaga* was also reported to be the dominant species and a key player in autotrophic biofilms active in denitrification.^{47,48}

More in-depth investigation is needed to shed light on its function in these peculiar biocathode conditions.

obiales are fermentative polyextremophiles and normally grow

further classification (low identity to cultured microorganisms), the function of this group remained unclear in this study.

The relative abundance of these key biofilm microorganisms, except *Hydrogenophaga*, considerably dropped in the community in suspension, highlighting the likely major importance of the biofilm in relation to the process performance.

To confirm this hypothesis, we removed the planktonic cells by replacing the whole catholyte suspension with fresh, cell-free catholyte solution. Similar current densities were recorded before and after the planktonic cells were removed, and still about 100% of the electrons consumed were assimilated into acetate after the medium was replaced (Figure S3). This confirms that microbial electrosynthesis of acetate from carbon dioxide exclusively occurs within the biofilm. The specific extracellular electron-transfer mechanisms active in these highly efficient biocathodes remain to be investigated, but this observation could have a great impact on reactor design, potential strategies for running the reactors, and for product separation.

IMPLICATIONS TOWARD PRACTICAL IMPLEMENTATION

Microbial electrosynthesis performance can be assessed using several key parameters; these are listed in Table S1 for most MES-to-acetate studies reported to date.

The results summarized in Table S1 were obtained with both pure and mixed microbial cultures, in either fed-batch or continuous mode, using different cathode electrode materials and cathode-applied potentials, which makes the comparison between those studies somewhat difficult. Regardless, it seems clear that the performance (current density and acetate production rate) reached in this study is at least one order of magnitude higher than what has been reported to date (except our own 2014 study⁶).

Looking at the requirements for modern industrial scale bioproduction processes such as industrial fermentations, production rates in the range of 2 to 4 (g L⁻¹ h⁻¹) with a high yield of at least 99% (conversion of substrate into final product) is necessary for process viability. Usually, a high titer of above 100 g L⁻¹ is also regarded as a considerable advantage for downstream processing. In the past few years, several research groups have attempted to assess the scaling-up feasibility of bioelectrochemical systems,^{50–52} in particular of MFC and microbial electrolysis technologies.^{53–55} The engineering and economic potential of electricity-driven bioproduction processes has also been recently discussed.⁵⁶ It has become common practice within the research community to assess BES efficiency for engineering applications by normalizing current and other production-related parameters to the projected surface area as presented above. When taking into account the 3D nature of the electrode and its total surface area per volume unit (2620 m² m⁻³), the rates obtained on EPD-3D corresponds to a production rate of 2.8 ± 0.1 (g L_{electrode}⁻¹ h⁻¹) of acetate with a $100 \pm 4\%$ product yield, which are parameters well within the desired characteristics window of industrial bioproduction processes and similar to what is being achieved in bioreactors using pure cultures and pressurized with H₂/CO₂ gas,⁵⁷ where mixed cultures achieve lower rates of about 0.006 to 0.01 (g L⁻¹ h⁻¹).^{58,59} This corresponds to about 66 (kg m

⁻³ electrode day⁻¹) of acetate and 98 (kg CO₂ m⁻³ electrode

day) captured. These volumetric rates are 22 times higher under high saline and pH conditions.⁴⁹ Due to the difficulty on

than that of the MES system also driven by a mixed community at -0.8 V versus SHE developed by LaBelle et al.¹⁹, which

recorded concomitant production of hydrogen and formate and 40% electron recovery into acetate. The above rates normalized to electrode volume were calculated by extrapolating the performance normalized to the volume of a theoretical cathode chamber that would be filled with the 3D electrode of the same volume. The volumetric acetate production rate normalized to actual catholyte volume used in this study (250 mL) was obviously lower, around 0.015 (g L⁻¹ catholyte h⁻¹). However, the main aim of this study was the demonstration of high-rate and high-efficiency microbial electrosynthesis of acetate. The reactor configuration (e.g., electrode volume/catholyte volume ratio) was far from being optimized. The total electrode surface area to liquid volume ratio remains to be investigated but was out of the scope of this study. Nevertheless, one should not overlook the fact that true three-dimensional electrodes as used here that, with large pores, makes the intraporous liquid volume significant. Therefore, in continuous systems, other parameters such as the hydraulic retention time needs to be considered and could allow approaching the highest production rates mentioned above when the cathode chamber is filled with such a three-dimensional electrode. However, further research in this direction needs to be performed.

In addition of an efficient MES microbial community, we hypothesize that the high efficiency shown by the new EPD-3D electrodes originates from its MWCNT nanostructure, which enhances the microbe-electrode interaction, bacterial attachment, biofilm development, and microbial extracellular electron-transfer rate, as previously suggested for microbial anodes^{23,60–64} and cathodes.⁶ In addition, the high surface area to volume ratio of the EPD-3D scaffolds maximizes the area available for biofilm development, while the large porosity (0.56 mm diameter) ensures effective mass transfer to and from the biocatalysts. This makes EPD-3D an interesting candidate material for the practical implementation of the MES technology.

The best-performing biocathode reported to date (NanoWeb-RVC) has an acetate production rate 3.6 times lower than that obtained in this study while using the same microbial culture. Additionally, in parallel with EPD-3D, we tested other materials (graphite plate and graphite felt) using the same inoculum, on which a current density in the order of 6–8 A m⁻² was obtained (data not shown). Furthermore, with the NanoWeb-RVC, only 70% of the electrons were assimilated into acetate.⁶ Said reactors benefited from the same microbial community and electrode's macroporous structure as EPD-3D, which probably explains its good performance compared to other work listed in Table S1. However, the morphology of that electrode structure is clearly different from the new EPD-3D material. First, the MWCNT in EPD-3D are considerably thinner in diameter as those previously grown (9.5 nm now (NC3100, Nanocyl) versus a 60 nm average diameter in the previously reported work).⁶ Moreover, the MWCNT layer on the EPD-3D consists of functionalized MWCNT because the nanotubes underwent a strong chemical oxidation prior to the electrophoretic deposition step (see the Materials and Methods section).^{65–67} Finally, when comparing the high-resolution SEM images of both structures, we observe that the MWCNT layer in EPD-3D is much denser than the previously reported work (Figures 4A,B and 3A,B in ref 6). A denser structure will most probably improve the electrical conductivity of this new material, and it will also increase the probability of the CNTs being at the right position to better electrically communicate with bacteria.

The titer reached in this study, 11 g L⁻¹, although one of the highest reported to date on MES process (Table S1), is still not comparable to the product titer obtained by the fermentation processes. However, it is believed that a higher titer is achievable. Acetate concentrations as high as 44 g L⁻¹ have been reported in bioreactors pressurized with H₂ and CO₂ gas using *Acetobacterium woodii*.⁵⁷ Moreover, continuous MES product extraction at a relatively low titer is also currently being investigated. Therefore, increases in the product concentrations or alternative extraction methods will likely be possible in future. Overall, product titer and production rate have a direct impact on the capital and operation costs of a plant, while the product yield is directly linked to the substrate(s) cost, and purity influences the product separation costs. The results presented in this study advance most of these parameters in a beneficial way and therefore bring microbial electrosynthesis a step closer to its practical implementation.

AUTHOR INFORMATION

Corresponding Authors

*Phone: +31 (0)6 5396 6172; e-mail: ludovic.jourdin@wur.nl

*Phone: +54 9388 4155 240; e-mail: vflexer@unju.edu.ar.

Present Addresses

[†]Subdepartment of Environmental Technology, Wageningen University, Wageningen, The Netherlands.

[‡]Centro de Investigaciones y Transferencia-Jujuy, CONICET, Jujuy, Argentina.

Notes

The authors declare no competing financial interest.

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