Microbial Battery Powered Enzymatic Electrosynthesis for Carbon Capture and Generation of Hydrogen and Formate from Dilute Organics

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ABSTRACT: A microbial battery (MB) breaks down waste organics and stores the liberated electrons in a solid-state cathode. Here, we demonstrate that these electrons can be utilized, along with the products (e.g., carbon dioxide, CO2) of the MB, to electrochemically generate higher-value products from waste. We utilize a series-stacked MB with mediated enzymatic electrosynthesis (EES) to produce either hydrogen (H2) from protons (H+) or formate (HCOO−) from CO2 with the electrons and carbon directly derived from waste organics. We also demonstrate that a reduced solid-state cathode can be used as a solid-state anode as an electron source for EES. This work shows that higher-value products can be electrochemically generated at high faradaic efficiencies (up to 91% for hydrogenase and 72% for formate dehydrogenase) using the energy, electrons, and carbon contained in the waste organic input without the need for continuous oxygen, potentiostatic control, or supplemental power.

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Power-producing microbial electrochemical technologies can convert chemical energy from dilute organics into electricity,1–3 however, there is increasing interest in generating higher-value chemical products.4 In microbial fuel cells (MFCs), a bioanode oxidizes dilute organic matter, releasing protons, electrons, and CO2; the electrons typically reduce oxygen at a cathode. Previously, we introduced the concept of a microbial battery (MB),3,5,6 which utilizes a solid-state redox cathode rather than the oxygen reduction reaction (ORR) cathode of a MFC. Prussian Blue (PB) analogues have the structure $A_xPR(CN)_6 \cdot nH_2O$, where $A$ is an alkali cation, such as K+ or Na+, $P$ is a transition metal cation such as Cu2+, Ni2+, or Fe3+, and R(CN)6 is a hexacyanometallate anion such as Fe(CN)63−, Mn(CN)63−, or Cr(CN)63−. PB analogues have been used as cathodes in rechargeable batteries due to their open-framework structure with wide channels, enabling rapid insertion and extraction of cations including protons, sodium, potassium, and magnesium.7 Both the transition metal cation and hexacyanometallate anion can be electrochemically active during ion insertion and extraction. In this study, we employed NaFeIIFeIII(CN)6, or simply PB, a low-cost material that upon reduction can be removed and regenerated by electrochemical or atmospheric oxygen reoxidation.8 Because MBs do not require oxygen delivery in the catholyte, they can use alternately stacked O2-free electrode configurations, unlike MFCs with air cathodes, and a smaller oxygen-independent footprint is enabled compared to MFCs with air cathodes. If connected in series, individual cell voltages are additive, providing sufficient voltage to drive electrochemical reactions that are otherwise thermodynamically unfavorable. A MB stack can thus provide power for microbial electrolysis (ME),9–10 microbial electrosynthesis (MES),11–15 or enzymatic electrosynthesis (EES) without supplemental voltage.

EES, an emerging field, utilizes isolated electroactive enzymes as biocatalysts and typically shows low overpotential, high faradaic efficiency, high selectivity, and high turnover at mild pH and temperatures and has been used to generate
Enzymes can be bound to an electrode where they accept electrons via direct electron transfer, or they can exist free in solution utilizing redox mediators such as viologens, which can transfer electrons to electroactive enzymes, such as hydrogenases and formate dehydrogenases. These enzymes have the potential to be low-cost and/or continuously supplied by extracellular secretion, though many are deactivated by oxygen. This is an issue because the most practical source of electrons is water, a poor electron donor, that upon oxidation releases inhibitory O2.

In a MB, however, solid-state cathodes reduced by oxidation of dilute organics can serve as EES solid-state anodes for synthesis of hydrogen or formate (from CO2) while simultaneously regenerating the solid-state cathode. Use of CO2 and electrons released by the oxidation of dilute organics as inputs for an EES cell demonstrates a synergistic process that can both treat and create value from the ∼3.3 × 1011 m3 year−1 of wastewater expected to be produced globally by 2025. This wastewater has the potential for ∼2.3 × 107 tonnes year−1 of CO2 capture, with up to ∼635 TWh year−1 available from the oxidation of dilute organics. Formate, a value-added chemical of increasing interest as a renewable energy carrier, could be synthesized with this CO2 and energy, generating up to 2.7 × 107 tonnes year−1. While formate synthesis has been accomplished in MFCs, a continuous input of O2 was required as an electron acceptor. Here we introduce a MB–EES coupled system (Figure 1A) that oxidizes dilute organic matter, produces CO2 and electrons in an O2-independent stacked MB, and stores the released electrons in a solid-state cathode. The cathode is then employed in an EES cell where it acts as an electron donor and becomes reoxidized, while a viologen-mediated EES process generates hydrogen with hydrogenase or formate with formate dehydrogenase, reducing CO2 produced by the MB. Once this solid-state anode is sufficiently reoxidized, the electrodes are...
switched again, creating a cyclic and synergistic process. Two PB solid-state cathodes are employed with two bioanodes, which together provide sufficient cell voltage ($E_{OCP}$ in acetate medium = $\sim$1.2 V, $E_{OCP}$ in real wastewater = $\sim$1.0 V) to power the process without the requirement of supplemental voltage. The result is an O2-independent MB system that oxidizes dilute organic waste while shuttling CO2 and electrons to an EES cell that generates higher-value products.

Biocatalytic Current Development. A viologen derivative, PBV (1,1′-trimethylene-2,2′-bipyridinium dibromide) was used as an enzymatic redox mediator.30 Figure 1B presents a cyclic voltammogram (CV) for the reduction of H+ in the presence of PBV•+, where a redox couple can be observed between the one-electron reduced form of PBV (PBV•+) and the two-electron reduced form of PBV (PBV0) at approximately $-0.93$ V vs Ag/AgCl ($-0.70$ V vs SHE).

In the presence of active hydrogenase and PBV•+, a reductive catalytic wave is observed that is attributed to $2\text{H}^+$ reduction to $\text{H}_2$ by hydrogenase at an onset potential of approximately $-0.75$ V vs Ag/AgCl, attributed to that of PBV2+ $\leftrightarrow$ PBV•+ (similar to $-0.51$ V vs SHE37 and $-0.55$ V18 vs SHE reported elsewhere), is seen by CV with the graphite rod in Figure 1B but is not as evident due to PBV•+ reduction to PBV0 and bulk H+ reduction and is better identified by CV with the glassy carbon electrode (Figure S2). This indicates that the appropriate range for PBV reduction by the MB stack is $-0.74$ to $-0.93$ V vs Ag/AgCl. Figure 1C presents a similar CV for formate dehydrogenase in the presence of CO2. A similar reductive catalytic wave is observed at an onset potential of approximately $-0.77$ V vs Ag/AgCl, attributed to CO2 reduction to formate by formate dehydrogenase. The reductive catalytic current density is lower in magnitude than that observed with hydrogenase, suggesting slower turnover by formate dehydrogenase than hydrogenase, as substrate limitation (CO2) is expected for the formate dehydrogenase system. Controls in the presence of PBV•+ but in the absence of enzyme in both panels B and C of Figure 1 (solid gray lines) show similar voltammetric behavior that is associated with PBV reduction, where PBV is reoxidized by the graphite rod electrode in the absence of enzyme. The controls without enzyme or PBV present seen in Figure 1B,C (dashed lines) show only background $2\text{H}^+$ reduction to $\text{H}_2$; no formate was detected.
Biocatalytic Reduction of H² and CO₂ by a Microbial Battery. After showing with cyclic voltammetry that hydrogenase and formate dehydrogenase are able to support substrate reduction mediated by PBV in the EES system, we coupled the EES systems with a two-cell MB stack to provide electrons and a suitable cathodic potential for PBV reduction; CO₂ was added from the MB cell for experiments containing formate dehydrogenase. Guest ions (e.g., protons released by the oxidation of organics) in the PB electron carrier are also released into the EES system via the solid-state electrode when the PB is reoxidized. Figure 2A shows representative data for current density and cumulative H₂ production over six 4 h cycles of the MB—hydrogenase system.

For each of the 4 h cycles, the current decreased over the cycle duration, while the PB cathode in the MB system was continually reduced and the PB anode in the EES system was continually oxidized (Figure 2C). After 4 h, the \( E_{\text{OCP}} \) of the MB system decreased from 1.34 to 1.29 V for the first cycle, and the observed decline in current was a result of a lower driving force due to ongoing reduction of the PB cathode, as observed with single-cell MBs. After switching the PB cathodes and anodes (in an anaerobic chamber to avoid oxidation), the \( E_{\text{OCP}} \) of the MB system increased to 1.33 V due to the PB anodes having been reoxidized in the EES system, and the cell current returned to its starting value.

Faradaic efficiency (the ratio of experimental to theoretical H₂ production) remained relatively constant across all six cycles at an average of 91 ± 4%; a high faradaic efficiency was expected because no reactions other than proton reduction to H₂ were anticipated. Figure 2B shows representative data for current density and cumulative formate production over six 4 h cycles of the MB—formate dehydrogenase system. The observed current density was lower with formate dehydrogenase than that with hydrogenase, possibly due to substrate (CO₂) limitation (as noted from the formate dehydrogenase—EES CV, Figure 1C) or to the fact that the formate dehydrogenase was part of a larger complex (Vhu hydrogenase) with slower kinetics. The faradaic efficiency was also lower with formate dehydrogenase than that with hydrogenase, at an average of 72 ± 11% averaged across all six cycles, and lower than the ~90% previously reported for formate dehydrogenase. We hypothesize that the lower efficiency here was likely due to proton reduction at the graphite rod (which is thermodynamically favorable) instead of PBV reduction due to a lower CO₂ concentration; further, no effort was made to regulate the potential of the graphite rod cathode. The purpose of the study was a proof-of-concept for coupling a MB system in series to an EES cell without cathode. The purpose of the study was a proof-of-concept for multiple EES systems (up to 70%) although lower than redox-polymer-immobilized formate dehydrogenase (up 99.5%). Total charge decreased by 17 ± 6 and 49 ± 4%, and the faradaic efficiency decreased by 0.5 ± 1 and 5 ± 2%

Enzyme Stability and System Efficiency. The six 4 h cycles were then repeated 5 and 10 days after initial enzyme addition to assess longer-term operation; Figure 3A shows the total charge and faradaic efficiency of the MB—hydrogenase system.

Initial faradaic efficiencies were similar to those reported elsewhere for MFC-coupled EES (up to 70%); although lower than redox-polymer-immobilized formate dehydrogenase (up 99.5%). Total charge decreased by 17 ± 6 and 49 ± 4%, and the faradaic efficiency decreased by 0.5 ± 1 and 5 ± 2%

Figure 3. (a) Total coulombs produced and faradaic efficiency over six 4 h cycles in the hydrogenase-EES cell: initial and 5 and 10 days after enzyme introduction (error bars represent the s.d.). (b) Total coulombs produced and faradaic efficiency over six 4 h cycles in the formate dehydrogenase-EES cell: initial and 5 and 10 days after enzyme introduction (error bars represent the s.d.). Total current is calculated by integrating current as a function of time over all six cycles, and faradaic efficiency is calculated using Faraday’s law with \( z = 2e^- \) for both proton reduction to H₂ and CO₂ reduction to formate. (c) Energy efficiency balance for formate generation in the formate dehydrogenase-EES cell from oxidation of dilute organics in the MB cell, determined 24 h after initial addition of formate dehydrogenase (error bars represent the s.d.). Detailed calculations are given in the Supporting Information.
with hydrogenase, although the decrease in faradaic efficiency was greater. Proton reduction to H₂ instead of CO₂ reduction was confirmed by the presence of excess H₂ in the formate dehydrogenase-EES headspace, which was not detected in the initial cycle after enzyme addition. Further, the EES cell retained a pinkish hue after 10 days, indicating slower kinetics of reoxidation by enzymes. We previously found a mediator-free formate dehydrogenase (also NAD⁺/NADH-independent) system to be stable for only 6 days, maintaining a faradaic efficiency of ∼90%;21 therefore, a drop in faradaic efficiency is not surprising in a nonpotentiostatic coupled system without a reducing agent. For longer-term use, electrons will eventually accumulate in the PB cathodes due to a lower driving force (caused by a reduction in organic substrate concentration in the MB cell) as well as a greater net reduction than oxidation due to the alternating series-parallel configuration. The maximum charge capacity in the PB cathode was determined to be ∼75 C (193 mAh g⁻¹ PB) (Figure S3), suggesting ∼12 days of continuous use with four PB electrodes with continuous oxidation and reduction at similar operating conditions. After this time, the solid-state electrodes should be reoxidized electrochemically or in air and reused. Figure 3C shows a representative energy balance of the stacked MB—formate dehydrogenase system (details are given in the Supporting Information). Approximately 36% of the energy contained in the oxidized acetate is assimilated for microbial cell synthesis (ηCoulombic = 64%); the remainder is the energy available for the MB—formate dehydrogenase system. A mixed culture exoelectrogen community (including exoelectrogenic and nonexoelectrogenic acetotrophs39) accounted for a lower efficiency, and pure cultures have been observed with ηCoulombic up to 95%.40 The relatively low current density also contributed to a lower ηCoulombic because nonexoelectrogenic acetotrophs would have more time to metabolize the substrate. Of the available energy, 59% is retained in the generation of formate, while 41% is lost to activation, concentration, and solution resistance overpotentials, as well as other cathodic reactions such as H₂ evolution. This results in an average total energy conversion efficiency from the chemical oxygen demand (COD) in dilute organics to formate of 38 ± 6%, which is comparable with that of methane fermentation and combined heat and power,32 but instead generates formate, a higher-value product and useful energy carrier. This calculation assumes eventual reoxidation of the cathode in air without energy penalty; electrochemical reoxidation would require a net energy input and would need to be included in an energy balance.

Increased Output MB Stack. The stacked MB is not limited to only 12%, despite an almost linear increase in EODEP. Four factors limited the overall maximum current output: (i) the maximum biocatalytic current for each of the anodes, (ii) concentration overpotentials associated with relatively low concentrations of voliogen and CO₂, (iii) PBV or hydrogenase (error bars represent the s.d.) for (a) 4 g L⁻¹ synthetic acetate media and (b) real wastewater from the Stanford Codiga Resource Recovery Centre (microscreened municipal wastewater).

Figure 4. EODEP and total current produced in a stacked MB—hydrogenase system with increasing numbers of MB cells. Current measured 5 min after connecting the stacked MB cell to the hydrogenase-EES cell. The control is a plain graphite rod without PBV or hydrogenase (error bars represent the s.d.) for (a) 4 g L⁻¹ synthetic acetate media and (b) real wastewater.

Anodes should thus be as balanced (with respect to maximum current density) as possible to minimize the effect of this overpotential and the attendant limitation of maximum current. Power also increases with more MB cells in the stack (Figure S6A,C) but peaks when this limiting current is reached. Voltage reversal (system failure due to unbalanced anodes31,42) was not detected in the MB stack in either high COD acetate media (3410 mg L⁻¹) or low COD real wastewater (780 mg L⁻¹), although current was observed to decrease slightly with four MB cells in the lower COD wastewater at the lowest external resistance (Rex) tested (Figure S6B,D).

A concentrated CO₂ stream is critical for practical carbon capture in wastewater treatment.31 In this work, we purposefully ran the MB with an external resistor in order to build up the CO₂ concentration prior to its reduction in the EES cell, producing energy that was not utilized. To increase CO₂ concentration and decrease EES overpotential, a high MB
stack anode surface-area-to-volume ratio is desirable. Under such conditions, more of the produced CO₂ will remain in the gas phase for transfer to the EES cell, leaving less dissolved CO₂ in the treated water. Densely stacked MBs are advantageous for this purpose because, unlike MFCs, they do not require continuous cathode exposure to air or the separator of an air cathode. A larger EES cathode surface-area-to-volume ratio in the EES cell is likewise advantageous for minimizing concentration overpotentials and allowing for higher titer outputs. Solid-state enzymatic redox electrodes could also increase electrode packing density while potentially eliminating the need for membranes separating the MB and EES cells. While still proof-of-concept, this work shows promise for the generation of higher-value products from dilute waste organics. Total energy conversion efficiency from dilute organics to formate is 38 ± 6%, although this could be optimized with higher current density bioanodes and greater electrode surface area. EES has an advantage of reaction selectivity compared to nonbiological processes or even whole-cell MES, although low current densities and costly enzyme purification could hinder the process from gaining widespread adoption. Improvements in electroactive materials for EES, as well as lower cost production of enzymes, would greatly improve the economics and sustainability of the process—potentially ushering in a new era of bioelectrocatalysis that could couple bioelectrochemical wastewater treatment to a co-located biorefinery. In summary, this work shows the first solid-state anode system for EES and the first system that oxidizes waste organics to release electrons, protons, and CO₂ with subsequent recombination into a single product.

**EXPERIMENTAL SECTION**

**Enzymatic Electrosynthesis.** The Vhu hydrogenase and formate dehydrogenase (referred to as hydrogenase and formate dehydrogenase in this study, respectively) of *Methanothermobacter maripaludis* were isolated as previously reported. Isolation details can be found in the Supporting Information. Microbial Battery Stack. The MB stack consisted of two individual hydraulically isolated MB cells (a 3 cm × 3 cm carbon cloth anode from a stable MFC and 3 cm × 3 cm PB-coated carbon foam), connected in series. Fabrication and operation details can be found in the Supporting Information.

**Enzymatic Electrosynthesis.** The EES cell consisted of two identical PB electrodes (operating as solid-state anodes) connected in parallel and a polished graphite rod cathode, separated by Nafton-117. A buffered catholyte contained an enzyme (approximately 530 μg L⁻¹ for hydrogenase and 340 μg L⁻¹ for formate dehydrogenase) and 500 μM 1,1′-trimethylene-2,2′-bipyridinium dibromide (PBV) as a redox mediator known to reduce both hydrogenase and formate dehydrogenase, which was synthesized according to the literature. Fabrication and operation details can be found in the Supporting Information.

**MB–EES System Characterization.** The MB–EES coupled system (Figure 1A) was operated in 4 h cycles, followed by switching the two PB cathodes from the MB cell with the two PB anodes from the EES cell in an anaerobic chamber to avoid any atmospheric oxidation and continuing with the next 4 h cycle to allow continuous operation of both the MB and EES cells with repeated reduction and oxidation of the PB electrodes. Operation details can be found in the Supporting Information.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsenergylett.9b02203.

Additional electron micrographs, cyclic voltammograms of mediators, charge capacity measurement of the PB electrode, potential of the graphite rod cathode, polarization curves of anodes, power curves of cells in series, energy balance calculations, and fabrication and operation of MB and EES systems (PDF)

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**Author Contributions**

K.D., R.D.M., C.S.C., Y.C., and A.S. conceived the research. K.D. prepared and characterized the microbial battery system and performed the experiments. X.S. supervised the electrochemical experiments. R.D.M. prepared the mediators and extracted the enzymes. J.D. helped with the system coupling and electrosynthesis cell fabrication. K.D., R.D.M., X.S., and C.S.C. analyzed the data. All of the authors contributed to the creation of the manuscript. C.S.C. supervised the work.

**Notes**

The authors declare no competing financial interest.

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