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Use of Low Cost and Easily Regenerated Prussian Blue Cathodes for Efficient Electrical Energy Recovery in a Microbial Battery†

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Microbial fuel cells can directly convert chemical energy into electrical energy, but significant energy losses result from use of O₂ at the cathode. Microbial batteries (MBs) replace conventional O₂ cathodes with solid-state cathodes that can be re-oxidized under favorable conditions, enabling more efficient energy recovery. Previously, we demonstrated proof-of-concept for MBs with a silver oxide cathode. The high cost of silver and energy required for re-oxidation make this material impractical. In this work, we evaluate Prussian Blue (PB), a hexacyano-Fe(III) complex material, as a low-cost solid-state cathode in a single-chamber, membrane-free MB. Microbial oxidation of organic matter at the anode drives reduction of PB. Reduced PB is re-oxidized by exposure to air. MBs equipped with a PB electrode efficiently transfer charge to PB (85% charge transfer), achieving net energy recovery efficiencies of 18-33%, with no loss of capacity over 20 cycles of operation. The operating potential for the PB electrode creates a trade-off: increasing the PB electrode potential improves efficiency of energy recovery, but more material is required to maintain power output and ensure efficient oxidation of organic matter. Operation is otherwise straightforward: energy is only required to move reduced PB into the air then back to the MB. We conclude that PB is a promising cathode material for single-chamber, membrane-free MBs.

Microbial electrochemical cells (MECs) can harness the catalytic activity of microorganisms, converting the chemical energy of dilute organic matter into electricity¹⁻⁵. Microbial fuel cells (MFCs), the most studied MECs⁶⁻¹⁰, recover this energy from wastewater and marine sediment. Organic matter is oxidized at a microbial anode, as oxygen is reduced at a cathode. At present, however, advances in MFC technology are hindered by low efficiency of electrical energy recovery^{11, 12}, defined as the product of voltage efficiency (i.e., potential efficiency) and charge transfer efficiency (i.e.,

coulombic efficiency)¹³. For most MFCs, voltage losses occur at the cathode due to the high overpotentials that result when dissolved O₂ is reduced in water under conditions that are favorable for microbial activity, i.e., near neutral pH, ambient temperature, and ambient pressure^{14, 15}. Charge losses result from aerobic metabolism when dissolved oxygen diffuses from the cathode compartment into the anode compartment and when organic matter diffuses from the anode compartment into the aerobic cathode compartment^{15, 16}. To minimize these energy losses, researchers have investigated catalysts that decrease overpotentials for electrochemical oxygen reduction^{17, 18}. Researchers have also improved membranes/separators^{16, 19} and device configurations^{20, 21} that prevent transport of dissolved oxygen into the anode compartment and hinder diffusion of dissolved organics into the cathode compartment. Recently, we described a microbial battery (MB), a new type of MEC²², that uses a solid-state cathode to improve energy recovery efficiency. Charge loss is prevented because dissolved O₂ is not present. Upon reduction, the cathode stores valence electrons and remains in the solid state. After removal from the battery, it is re-oxidized then re-installed in the battery. Re-oxidation can be accomplished with oxygen under favorable conditions, decreasing voltage losses.

For proof-of-concept, we used the silver-oxide/silver (Ag₂O/Ag) redox couple as a convenient solid-state material for electron capture and release²². Because reduction of Ag₂O to Ag is highly favorable, with a half reaction reduction potential of 0.76 V vs. SHE, MBs equipped with such a cathode achieve high voltage and power output. For this electrode, we obtained a net energy recovery efficiency of ~30%, a value that exceeds those reported for most MFCs. While convenient for research, Ag₂O is not suitable for field applications: silver is costly, and oxidation of silver, while thermodynamically favorable, is not straightforward. Reaction kinetics are slow at ambient temperatures and pressure due to formation of a thin Ag₂O passivation layer (a few nanometers thick) on the electrode surface^{23, 24}. High temperature and pressure facilitate silver oxidation, but

additional energy is required²⁵. Previously, we used electrochemical oxidation to re-oxidize the cathode, but this step requires the input of significant energy²². Other concerns include the sensitivity of silver to certain anions in the electrolyte, especially chloride and sulfide. For MBs to be practical, a solid-state electrode material is needed that is robust, cost-effective, and easy to regenerate.

Prussian Blue (PB) analogues are a family of materials with a general chemical formula of $A_xPR(CN)_6 \cdot nH_2O$, where A is an alkali cation, such as K^+ , or Na^+ , P is a transition metal cation such as Cu^{2+} , Ni^{2+} , or Fe^{3+} , and $R(CN)_6$ is a hexacyanometallate anion such as $Fe(CN)_6^{3-}$, $Mn(CN)_6^{3-}$, or $Cr(CN)_6^{3-}$. The most commonly used form of PB has Fe in both P and R sites (Fig. 1a). PB analogues have an open-framework structure with wide channels, enabling rapid insertion and extraction of monovalent, divalent, and even trivalent cations (Fig. 1a). Commonly inserted cations are protons, sodium, potassium, and magnesium²⁶⁻²⁸. Both the P-site transition metal cation and the $R(CN)_6$ hexacyanometallate anion can be electrochemically active, with valence states changing during ion insertion and extraction. Researchers have investigated use of PB and its analogues as electrode materials in rechargeable batteries that cycle >1000 times without notable loss of capacity^{29,30}. In this work, we investigate use of oxidized PB as a solid-state MB cathode. PB is a low-cost material that upon reduction can be re-oxidized by O_2 in air at ambient temperature and pressure (Fig. 1b). We find that MBs equipped with PB electrodes achieve net energy recovery efficiencies of 18-33%; values comparable to those obtained with Ag_2O/Ag electrodes²².

To prepare PB electrodes, we electrochemically reduced home-made iron ferricyanide nanoparticles embedded on a carbon cloth substrate. Figure 2a shows scanning electron microscope (SEM) images of the as-prepared PB electrode. Most particles are around 500 nm in size, although some larger particles form by aggregation. The X-ray diffraction (XRD) pattern (Fig. S1) confirms formation of PB crystals. As-prepared PB electrodes

have a potential of about 0.15 V vs. Ag/AgCl in a sodium phosphate buffer solution (PBS) at pH 7. Equation 1 is the half reaction for PB reduction assuming that sodium ions enter the electrode to balance charge²⁷.



To test the PB cathode (3 cm × 3 cm), we fabricated a plate-shaped single-chamber, membrane-free MB (3 cm × 3 cm × 0.3 cm) and installed a pre-colonized carbon cloth microbial anode (3 cm × 3 cm). The anode was previously used in a traditional H-shaped MFC where it exhibited stable performance for more than 3 months³¹. The biofilm of the anode (Fig. 2b) sustained a current output of up to 2 mA (Fig. S2). The voltage profile is shown in Fig. 2c. The open circuit voltage (OCV) was ~0.60 V. As the MB began to generate current (0.5 mA), the operating voltage dropped by ~70 mV to ~0.53 V. The anode potential increased by ~70 mV as current output increased from 0 to 0.5 mA (Figure S2). This indicates that the overall voltage drop was due to voltage losses at the anode, not the cathode. The superior electrochemical performance of the PB cathode is consistent with previous reports indicating that members of the PB materials family can function as excellent fast-response battery electrodes^{29, 30}. Over ~5 h, the operating voltage gradually decreased to 0 V, as the PB was reduced (Equation 1), and the cathode potential declined to ~-0.38 V vs. Ag/AgCl. We then disassembled the device, removed the PB electrode, and exposed it to air. The O₂ in air oxidized the PB. After 100 min, the potential of the PB electrode recovered to ~0.10 V vs. Ag/AgCl. At this point, we re-installed it in the MB, and current generation resumed (Fig. 2c). The MB generated current for another 4 h before the cell voltage decreased to 0 V. This indicates that a 100-min exposure to air restored only four fifths (~80%) of the PB oxidation capacity. The average regeneration rate, defined as the charge capacity recovered per min during regeneration, was 0.073 C/min. After regeneration, the average operating voltage of MB, measured as the potential difference between the microbial anode and the PB cathode,

was 0.34 V. The electrolyte chemical oxygen demand (COD) decreased from 975 ± 7 mg/L to 690 ± 14 mg/L. The charge transfer efficiency, i.e., the electrical charge passing through the external circuit divided by the charge in the organic matter removed, was 85%; the voltage efficiency, i.e., the average operating voltage divided by the theoretical voltage of glucose oxidation coupled to O_2 reduction (~ 1.24 V)³², was 27%; and the energy recovery efficiency, i.e., the charge transfer efficiency multiplied by voltage efficiency, was 23%.

Our results establish that PB is an efficient solid-state cathode material, capturing 85% of the electrons transferred. To further improve energy recovery efficiency, we focused on voltage efficiency. Because the potential of the microbial anode stabilizes at around -0.38 V vs. Ag/AgCl with 0.5 mA current output, the average operating voltage is largely controlled by the potential of the PB electrode. This potential gradually decreases as PB is reduced. To understand how the extent of reduction of a PB electrode affects voltage losses, we chose four target potentials as cut-off potentials for reduction (0.10 V, 0 V, -0.10 V, and -0.20 V vs. Ag/AgCl) and fabricated a three-electrode setup in a bottle-shaped MB. PB electrodes were reduced to the cut-off potential, removed from the MB, exposed to air for a fixed period (5 min), reinstalled in the MB, then reduced back to the cut-off potential. Figure 3a shows potential profiles during reduction. When the cut-off potential was 0.10 V vs. Ag/AgCl, the average potential of the PB electrode during MB operation remained at 0.10 V vs. Ag/AgCl; the average operating voltage of the MB was 0.48 V; and the voltage efficiency was 39%. For the charge transfer efficiency of 85% (reported above), the energy recovery efficiency would be 33%. When the cut-off potentials decreased (0 V, -0.10 V, -0.20 V vs. Ag/AgCl), the average operational potentials decreased, voltage efficiencies decreased (31%, 25%, and 21%, respectively), and energy recovery efficiencies decreased (27%, 21%, and 18%, respectively). When the PB electrode was reduced to lower potentials, the driving force for re-oxidation

increased, and recovery of oxidative capacity was more rapid. As shown in Fig. 3a and 3b, a cut-off potential of -0.20 V vs. Ag/AgCl is attained in 15.8 min of operation, giving a regeneration rate of 0.095 C/min (MB voltage of 0.18 V). By contrast, a cut-off of 0.10 V vs. Ag/AgCl is attained in just 0.4 min of operation, and the average rate of regeneration is only 0.002 C/min (MB voltage of 0.48 V).

To understand how the extent of oxidation of a PB electrode affects voltage losses, we evaluated the effects of air exposure time. After reducing a PB electrode to 0 V vs. Ag/AgCl, we exposed it to air for 5, 10, 20, 50, 200, and 1000 min. The potential of the oxidized PB electrode increased from 0.02 V vs. Ag/AgCl after 5 min of air exposure to 0.11 V vs. Ag/AgCl after 1000 minutes of air exposure (Fig. 3c). The more positive initial potential resulting after oxidation led to a higher average potential during reduction: for the electrode oxidized for 5 minutes, the average potential was 0.01 V vs. Ag/AgCl; for an electrode oxidized for 1000 minutes, the average potential is 0.06 V vs. Ag/AgCl (Fig. 3d). A more oxidized cathode increases the average operating voltage of the MB from 0.39 to 0.44 V, voltage efficiency increases from 31 to 35%, and energy recovery efficiency increases from 27 to 30%. Increasing the length of the oxidation period also increases total charge recovery. For an oxidation period of 5 min, the total charge recovery is 0.23 C (reduction for 7.7 min at 0.5 mA). When this period is extended to 1000 min, the total charge recovery is 2.20 C (reduction for 73.5 min at 0.5 mA) (Fig. 3c). As the potential of the PB electrode approaches the potential for oxygen reduction, the driving force for electron transfer to O_2 decreases, and the rate of cathode oxidation decreases. For a 5 min exposure to air, the average rate of oxidation was 0.046 C/min; for a 1000 min exposure, this value decreased to 0.002 C/min (Fig. 3d).

We note that there is a trade-off between energy recovery efficiency and regeneration rate. High cut-off potentials and long regeneration periods result in high energy recovery efficiencies, but regeneration rates are slow, and more electrode material

is thus required for operation at the same current output or, equivalently, for maintenance of the same rate of organic matter removal. If cut-off potentials are decreased or regeneration periods are shortened, re-oxidation is more rapid, less material is required, but energy recovery efficiencies decrease. Future systems should be designed and operated to optimize the cut-off potential and regeneration time based upon specific operational requirements and conditions.

Stable cycling is essential for long-term operation of MBs. In this work, we demonstrated 20 cycles of stable operation in a bottle-shaped MB with a reduction current of 0.5 mA, a cut-off potential of 0V vs. Ag/AgCl, and a 5-min oxidation period. Figure 4a illustrates the MB voltage profile. Operating voltages immediately after installation of the regenerated PB electrode were ~ 0.40 V. Cut-off voltages were set to 0.38 V. Consequently, average voltages were ~ 0.39 V. The charge capacities recovered during the 5-min periods of oxidation, as calculated from the subsequent current outputs, stabilized at 0.21 ± 0.01 C within the 20 cycles (Fig. 4b). SEM images of the PB electrode (Fig. 4b, inset and Fig. S3) did not show obvious differences compared to as-prepared samples (Fig. 2a), and the XRD patterns remained the same (Fig. S1).

Use of PB enables energy recovery efficiencies (18-33%) that are comparable to those observed in our initial proof-of-concept study with $\text{Ag}_2\text{O}/\text{Ag}$ ²², but PB has a much greater potential for large-scale practical applications. PB is a low-cost material commonly used as a pigment and antidote for metal poisoning. It contains only earth abundant elements, and is readily synthesized by a solution-based chemical reaction at room temperature and normal pressure. Annual production is in the millions of tons, with material cost of $< \$1/\text{kg}$, more than two orders of magnitude lower than silver ($> \$600/\text{kg}$). Given that the energy recovery is similar, the energy cost advantage for PB electrodes is of the same order of magnitude. When oxidized by O_2 (0.61 V vs. Ag/AgCl), PB has a lower potential (< 0.2 V vs. Ag/AgCl) than Ag_2O (0.56 V vs. Ag/AgCl), and thus a larger

driving force for oxidation in air. Not surprisingly, reduced PB is oxidized much more rapidly than Ag. Consequently, the time scale for switching a PB electrode from reduction to re-oxidation is reasonable for practical applications: after 5 min of oxidation at 0 V vs. Ag/AgCl, the PB electrode can function as a cathode for 7 min with a 0.5 mA reduction current (Fig. 3a and c). A further advantage of PB is its lack of reactivity with chloride and sulfide species.

Longer-term studies with PB are needed to assess the possibility of microbial growth upon PB electrodes³³. Microbial biofilms could potentially release electrons from organic matter directly to the PB electrode thus bypassing the external circuit and resulting in charge loss. Antimicrobial additives or coatings may be needed or sterilization during PB re-oxidation. For sustainable operation of the PB electrode in large-scale MBs, we envision a design in which the PB electrodes are embedded on a rotating disk. Part of the disk would be immersed in the electrolyte solution while the rest is exposed to air. Regeneration would be accomplished by adjusting the rotation speed of the disk. Our data indicate that the rotation speed can be low, e.g. 0.1 rpm for 5-min charge and 5-min discharge cycle. The energy needed to drive this slow rotation is low^{32, 34, 35}, and can potentially be supplied by hydropower or other sustainable energy sources, such as wind and solar.

We conclude that PB is a promising cathode material for single-chamber, membrane-free MBs. The energy recovery efficiencies of a MB operating with a PB cathode were 18-33% and varied with operational conditions. Two key design trade-offs are energy recovery efficiency and the amount of material required. These variables can be manipulated by changing the cut-off voltage or the oxidation period for the PB electrode.

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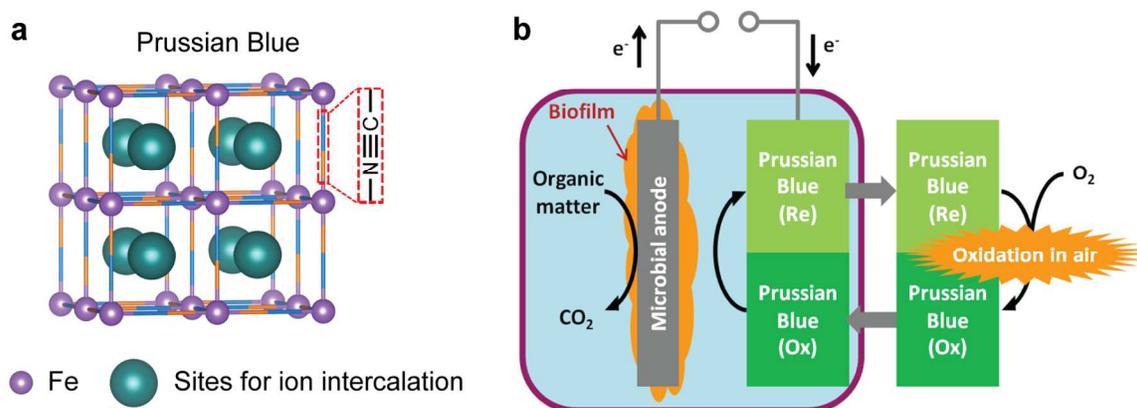


Fig. 1 Schematic of the crystal structure of Prussian Blue (PB) (a) and a microbial battery (MB) (b) equipped with a PB cathode. PB has an open framework with empty spaces where ions can be inserted (a). The PB electrode is reduced when it functions as a cathode in the MB. When exposed to oxygen in air, the reduced PB is re-oxidized (b).

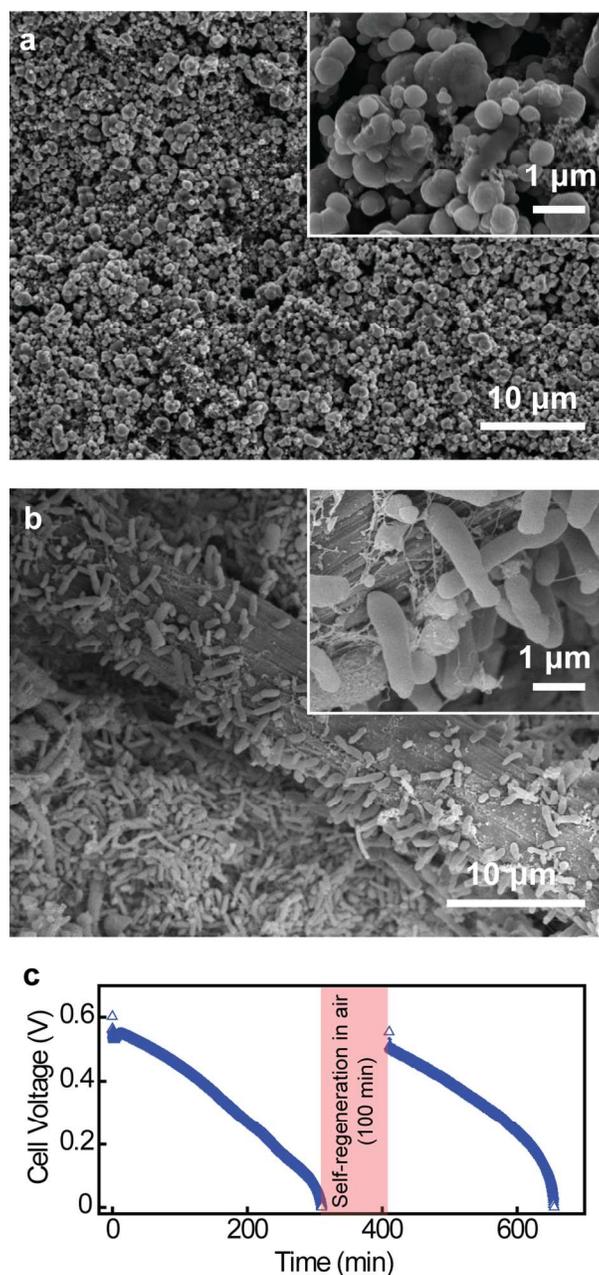


Fig. 2 MB with a PB cathode and a carbon cloth microbial anode. (a) and (b) Scanning microscope images of the as-prepared PB cathode **(a)** and the carbon cloth anode colonized by microorganisms **(b)**. **(c)** Voltage profile of the MB during operation. Glucose (1 g/L) was added to the MB anode compartment, and the MB was operated at a constant current output of 0.5 mA. The cell voltage decreased to 0 V after about 310 min. The PB electrode was then removed from the MB and exposed to air for 100 min. The oxidized PB electrode was then reinstalled, and current generation resumed.

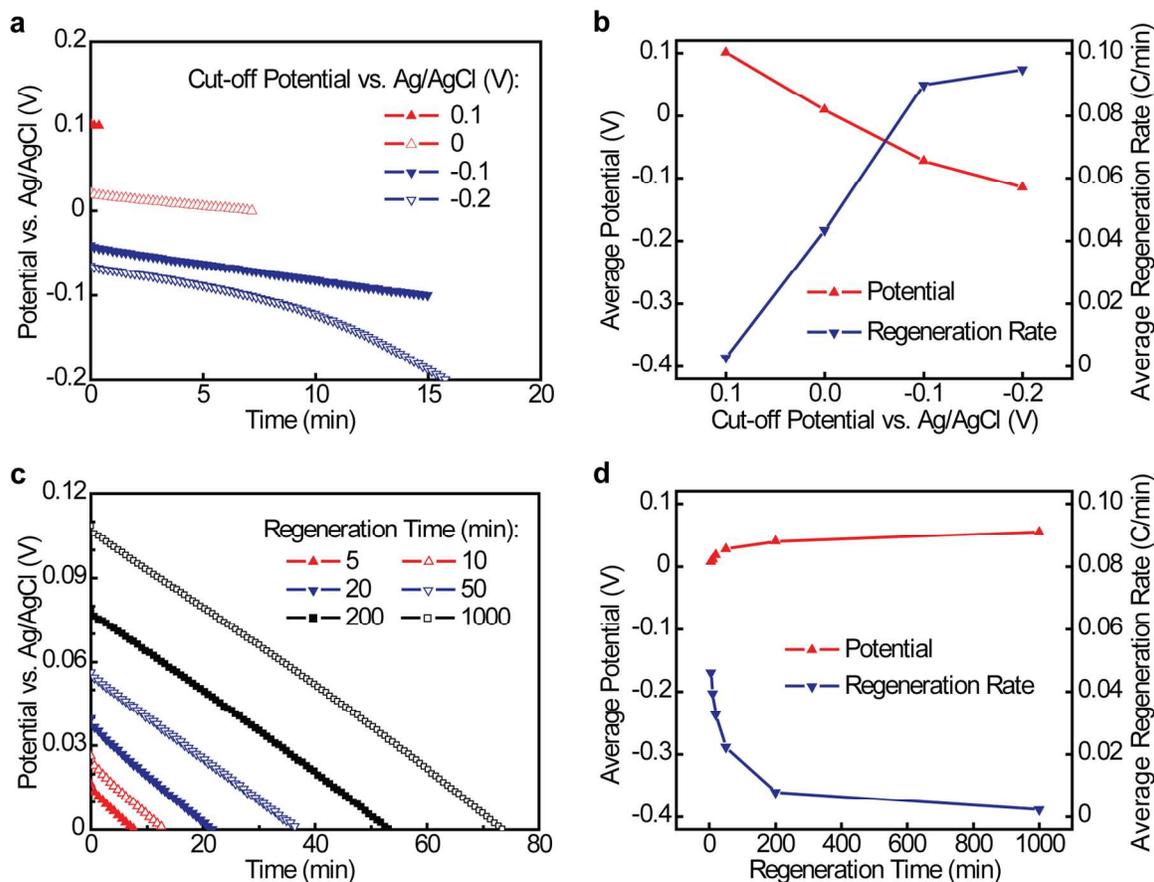


Fig. 3 PB cathodes operated with different cut-off potentials and regeneration times. (a) Potential profiles of PB electrodes after 5 min of oxidation in air followed by reduction to different cut-off potentials. Reduction currents were set at 0.5 mA. (b) Average potentials of PB electrodes during reduction and average regeneration rates during regeneration when the electrodes were operated at different cut-off potentials. (c) Potential profiles for PB electrodes reduced to 0 V vs. Ag/AgCl after different regeneration times. Reduction currents were set at 0.5 mA. (d) Average potentials of PB electrodes during reduction and average regeneration rates during regeneration when the electrodes were operated at different regeneration times.

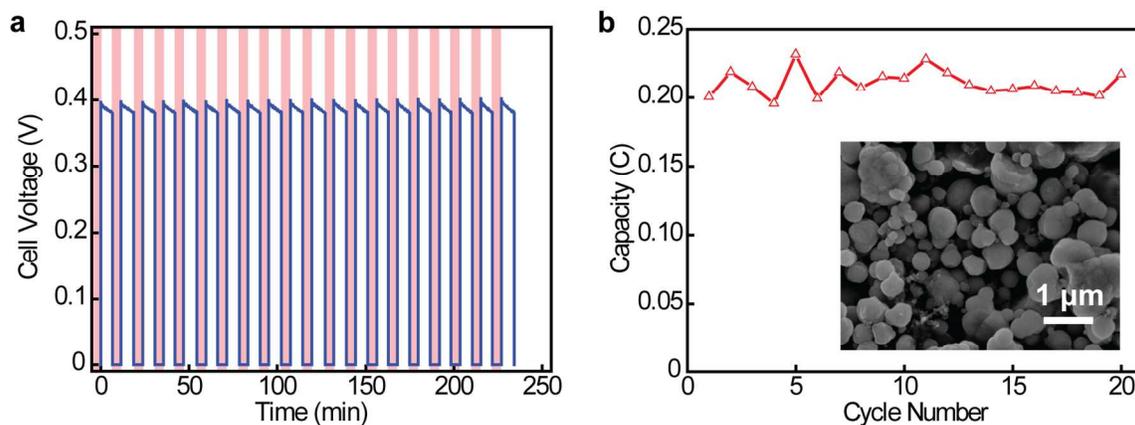
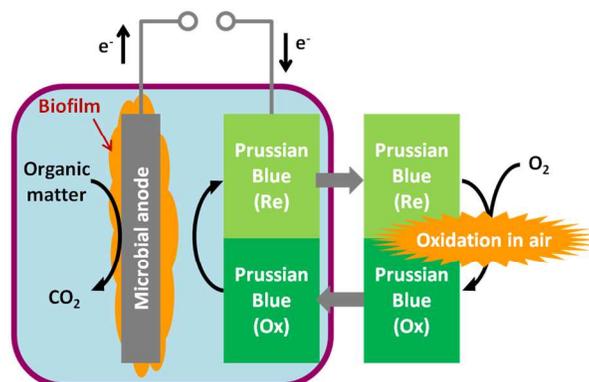


Fig. 4 Cycling performance of PB electrodes in MBs. (a) Voltage profile of the MB when the PB electrode was successively removed and reinstalled for 20 cycles. The background highlighted in pink indicates periods of regeneration. (b) Capacities recovered during the 5 min regeneration for the 20 operation cycles. Inset shows the SEM image of the PB electrode after cycling.

Broader context

Microbial batteries (MBs) recover electrical energy from waste organic matter with high efficiencies. For practical application, a robust, cost-effective, and easy to regenerate solid-state cathode is desired. This work introduces Prussian Blue (PB) as a low-cost cathode material for single-chamber, membrane-free MBs. After reduction in a MB, the PB electrode is re-oxidized by exposure to air. Use of PB electrodes enables high energy recovery efficiencies and stable performance over multiple cycles of reduction and re-oxidation. PB electrodes show great potential for practical application.

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A microbial battery (MB) equipped with a Prussian Blue (PB) cathode.

