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A Miniature Biofuel Cell Operating in A Physiological Buffer

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We describe a biofuel cell operating at 37 °C in a glucose-containing, aerated, pH 7.2, physiological buffer solution (0.14 M NaCl, 20 mM phosphate). It consists of two 7-μm diameter, 2-cm long, 0.44 mm^2, electrocatalyst-coated, carbon fibers. Glucose is electrooxidized to gluconolactone on the anode fiber, and dissolved O_2 is electroreduced to water on the cathode fiber. When the cell operates continuously for one week at 37 °C, its 1.9 μW power output (50 nW per mm of fiber) at 0.52 V declines to 1.0 μW. In its weeklong operation the cell generates 0.9 J of electrical energy while passing 1.7 C charge. The 1.7 C charge is 100 times higher than the 0.016 C charge that would have been generated at 100% current efficiency through the oxidation of a 7-μm diameter, 2-cm long zinc fiber in a battery.

The enabling chemical components of the cell are the electrocatalyic "wired" enzyme films of its electrodes, comprising immobilized redox enzymes and redox hydrogels "wiring" their reaction centers to electrodes.[1] Phase separation of the enzyme and the hydrogel-forming cross-linked redox polymer is avoided by forming electrostatic adducts of the enzymes, which are polyanions at pH 7.2, and their "wires", which are polycations. The anode electrocatalyst film comprises polymer ***I*** (redox potential −0.19 V vs Ag/AgCl, Figure 1) and glucose oxidase (GOx) from *Aspergillus niger*, while the cathode electrocatalyst consists of bilirubin oxidase (BOD) from *Trachyderma tsunodae* and the copolymer of polyacrylamide and poly (*N*-vinylimidazole) complexed with [Os (4,4′-dichloro-2,2′-bipyridine)$_2$Cl]$^{+/2+}$ ***II*** (redox potential +0.36 V vs Ag/AgCl). [2,3] The electron transport kinetics in the hydrogel formed upon cross-linking and hydration of the anodic polymer is unusually fast, the apparent electron diffusion coefficient being 5×10^{-7} cm^2 s^{-1}, 10 times faster than in other redox hydrogels. The activation energy for electron diffusion is only 28.3 kJ/mol, half of that in other redox hydrogels.[4] The fast electron diffusion is a result of the 13-atom long flexible spacer arm tethering the polymer's redox centers to its backbone (Figure 1). Redox centers at termini of proximal spacer arms, which are charged and mobile in the hydrated polymer, wipe electrons from overlapping volume elements. The faster electron transfer to and from of the spacer-tethered redox functions allows the reduction of the excess potential required to drive electrons from the enzyme to the redox polymer. By replacing the 2,2′-bipyridine complexes of Os$^{2+/3+}$ by the novel [Os (*N*,*N*′-dialkylated-2,2′-bis-imidazole)$_3$]$^{2+/+3}$ complex (Figure 1), the redox potential of the anodic wire is downshifted to −0.19 V vs Ag/AgCl, allowing the poising of the operating anode at − 0.1 V, just 0.26 V more than the redox potential of the FAD/FADH$_2$ cofactor in GOx at pH 7.2.[5]

The unique characteristic of the *T. tsunodae* BOD−polymer ***II*** cathode film is the absence of inhibition of its copper enzyme at

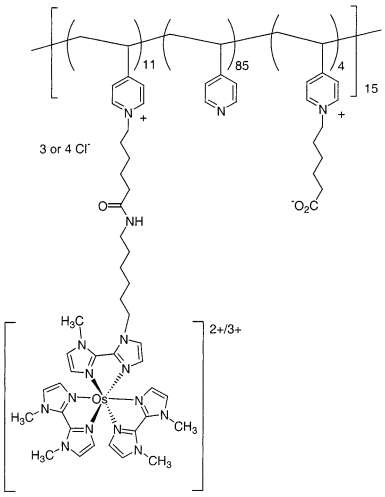

Figure 1. Structure of the redox polymer ***I***. The polymer electrically connects the reaction centers of glucose oxidase to the carbon fiber of the anode.

pH 7.2 or by chloride, differentiating it from both laccase and dissolved BOD, which are inhibited, and the stabilization of the BOD in its electrostatic adduct with the redox polymer, which increases its half-life from 2 h to 1 week.[2,3] Both laccase and BOD have four Cu$^{+/2+}$ redox centers. One of the laccase centers (the type-2 center) is inactive at neutral pH and is inhibited by Cl$^-$.[6] Although dissolved BOD is also inhibited by chloride,[7,8] it is not inhibited in its cross-linked electrostatic adduct with redox polymer,[2,3] even at Cl concentrations as high as 1 M.

The reported cell follows four decades of work on small methanol/O_2 fuel cells and relatively large methanol/O_2 and glucose/O_2 biofuel cells. The smallest reported nonbiological fuel cell is a recent 12 mm^2 on-chip methanol/O_2 cell. It produces at 70 °C and at 0.6 V 17 μW/mm^2, exclusive of the area of its methanol container.[9] Much earlier Karube, Suzuki et al. developed whole organism-based fuel cells comprising hydrogen- or methane-generating bacteria, such as *Clostridium butyricum*, delivering the generated gas to a phosphoric acid fuel cell.[10,11] In the alternative enzyme-based cells, methanol or glucose was electrooxidized in a reaction catalyzed by a dehydrogenase or an oxidase, and oxygen was electroreduced at a platinum-activated cathode. Yahiro, Lee, and Kimble reported the first enzyme-based glucose/O_2 biofuel cell.[12] Turner, Higgins, Hill, and their colleagues built a series of two-compartment cells, in which redox couples mediated the transport of electrons from oxidases and dehydrogenases to the anodes.[13,14] With a methanol/O_2 (Pt) cell they reached a power density of 0.2 μW mm^{-2} at 0.3 V at pH 9.5 and at 20 °C. Palmore and her colleagues increased the power density of the methanol/O_2(Pt) cell to 6.7 μW mm^{-2} at 0.49 V.[15] In these cells the anode

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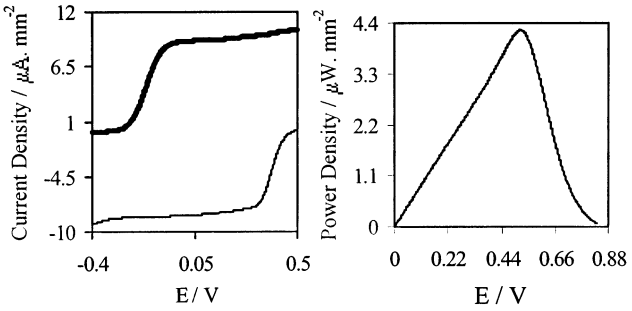

Figure 2. (Left) Polarization of the anode (heavy line) and of the cathode (fine line). (Right) Dependence of the power density on the cell voltage. Quiescent solution under air, 37 °C, pH 7.2, 0.14 M NaCl, 20 mM phosphate, 15 mM glucose solution.

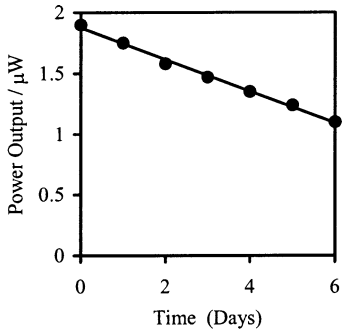

Figure 3. Stability of the power output of the cell operating at +0.52 V in a quiescent solution under air at 37 °C in a pH 7.2, 0.14 M NaCl, 20 mM phosphate, 15 mM glucose solution.

and cathode compartments had to be separated by an ion-exchange membrane. In the absence of separation the methanol and the glucose diffused to the cathode, where they were oxidized to products, which poisoned the platinum catalyst. The compartments had to be separated also because the more successful cells contained dissolved redox mediators, which shuttled electrons between the dehydrogenase or the oxidase and the anode. In the absence of a membrane the enzyme-reduced mediator would have been electrooxidized at the higher potential cathode, not at the anode, and the cell would have been electrochemically shorted. Because miniature ion-exchange membranes are difficult to make and even more difficult to seal, the enzyme-based biofuel cells were not miniaturized. When made with laccase-based cathodes instead of platinum cathodes, the cells could not be operated in a physiological buffer solution because laccase, although highly active at pH 5 in the absence of chloride, was nearly totally inhibited at 0.14 M NaCl and at pH 7.2.

Advance toward a compartment-less biofuel cell anode was made by Persson and Gorton,[16] who electrooxidized glucose on a carbon anode on which a redox mediator was chemisorbed and glucose dehydrogenase was cross-linked. The first compartment-less glucose−O_2 biofuel cell was reported by Katz et al.[17] The cell, built with 0.8 cm^2 porous gold electrodes, operated in a physiological buffer solution but produced only 0.35 μW mm^{-2} and only at 0.06 V. Chen et al.[18] reported a miniature compartment-less glucose−O_2 biofuel cell, based on the "wiring" of glucose oxidase and laccase to 7-μm diameter, 2-cm long carbon fiber electrodes. Because the cell was made with laccase, it operated only in a chloride-free pH 5 citrate buffer.[19] At 0.38 V the output of the cell at 37 °C was 0.6 μW.

In the miniature cell of this report the immobilized electrocatalyst of the anode contained 35 wt % of GOx, 60 wt % of the polycationic redox polymer ***I*** (Figure 1), and 5 wt % of the cross-linker poly(ethylene glycol) (400) diglycidyl ether (PEGDGE). The electrodes were specific enough to obviate the need for a membrane. The redox potential of the [Os (*N*,*N*′-dialkylated-2,2′-bis-imidazole)3]$^{2+/3+}$ centers of redox polymer ***I*** is highly reducing, −190 mV vs Ag/AgCl, unprecedented for immobilized polymeric mediators accepting electrons at a high rate from FAD/FADH2 centers of GOx. The polymer enables the electrooxidation of glucose at a current density of 1.1 mA cm^{-2} at a potential as low as −100 mV vs Ag/AgCl.[5] Even though the operating anode is poised at − 0.1 V vs Ag/AgCl, the loss of current by electroreduction of O_2 at the anode is not excessive, because polymer ***I*** effectively competes with O_2 for the electrons of reduced glucose oxidase. The electrocatalyst of the cathode contained 44.6 wt % BOD, 48.5 wt % polymer ***II***, and 6.9 wt % PEDGE.[2]

Figure 2 (left) shows the polarizations of the microanode and the microcathode at 37 °C. The glucose electrooxidation current density reached its plateau of 10 μA mm^{-2} near −0.1 V vs Ag/AgCl, and the O_2 electroreduction current density near +0.35 V vs Ag/AgCl (9.5 μA mm^{-2}). The power output of the cell operating at 0.52 V in a quiescent solution was 1.9 μW, corresponding to a power density of 4.3 μW mm^{-2} (Figure 2, right). When the cell operated continuously at 0.52 V and at 37 °C for one week, it lost ∼6% of its power per day (Figure 3).

We hope that when fully engineered the simple miniature biofuel cells will be of value in powering small autonomous sensor−transmitter systems in animals and in plants.

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