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We report on the four-electron electrocatalytic reduction of oxygen to water at a current density of 5 mA cm⁻² and at +0.7 V (NHE) in pH 5 citrate buffer at 37.5°C. The electroreduction of O₂ to water near neutral pH and at ambient temperature has been one of the longest-standing and best-researched problems of electrochemistry. It has been a missing element of a miniature membrane-less biofuel cell of projected respective areal and volumetric power densities of 1 μW mm⁻² and 10 μW μL⁻¹. Unlike a primary lithium battery, the cell, in which glucose is electrooxidized at the anode and dissolved O₂ is electroreduced at the cathode, can be miniaturized to sub-millimeter dimensions because it does not require a difficult to miniaturize case or seal. The application of the cell is in powering a chip-based sensor and transmitter, operating in the human body for a week and transmitting to a distance of ~1 m. Such a system requires only a few hundred nW of power and its fuel cell will comprise only the electrocatalytic anode and a cathode strips, each of ~1 mm length, deposited on the chip.

The novel electrocatalyst was made of hydrophilic carbon cloth coated with a crosslinked electrostatic adduct of laccase from *Coriolus hirsutus* (a polyanion above pH 4) and an electron-conducting redox polymer (a polycation). The 0.78 V (NHE) redox polymer electrically connected (“wired”) the laccase reaction centers to the carbon fibers. The laccase-“wiring” redox potential polymer was formed of poly-N-vinyl imidazole by coordinating 1/5th of its rings to [Os(tpy)(dme-bpy)]^{2+/3+}, where tpy is 2,2':6',2''-terpyridine and dme-bpy is 4,4'-dimethyl-2,2'-bipyridine. In the catalytic process the copper-containing reaction centers of laccase are oxidized by O₂; the oxidized copper centers oxidize Os²⁺ centers of the redox polymer to Os³⁺; and the Os³⁺ centers of the redox polymer are electroreduced, at a potential negative of their +0.78 V (NHE) potential. The crosslinking of the redox polymer-enzyme adduct on the carbon fibers yields a mechanically tough composite, withstanding more than 0.1 N m⁻² shear stress generated when the 4 mm diameter cathode is rotated at 1000 RPM. The carbon-cloth composite electrodes were assembled on 3-mm diameter vitreous carbon rotating disk electrodes mounted in Teflon™ sleeves. The cloth (Toray TGP-030, Japan) had a 78% void fraction consisted of 10 μm diameter fibers and had a nominal thickness of 350 μm. They were made hydrophilic by plasma treatment, coated with the “wired” laccase and were cemented, using conductive carbon paint to the tips of the rotated vitreous carbon rods. At 4000 RPM, where the current density reaches 6 mA cm⁻² at 0.6 V (NHE), the current becomes limited by the kinetics of the electrocatalyst. In a 22 hour long test, a current density of > 2 mA cm⁻² was maintained when the rotating electrode was poised at +0.7 V (NHE).

Laccase cathodes on which O₂ is electroreduced to water were introduced by Tarasevich et al. who formed these by directly adsorbing laccase on Teflon™-bound high-surface area carbon-black particles.¹ The cathodes operated for days with little or no evidence of degradation, at a current density of 175 μA cm⁻² when poised at 1.10 V (NHE) at pH 5. Members of the same group later reported a current density of 10 mA cm⁻² at pH 3.5 for an electrode poised at 0.8 V (NHE), but did not provide sufficient information to allow other investigators to reproduce these results.² Publications of the past decade do not mention high current density O₂ cathodes, all reported current densities being less than 200 μA cm⁻².³⁻⁹

The principles of the design of the novel electrocatalyst were the following:

Independence of orientation of the enzyme: Use of “wired” laccase. When a redox enzyme is adsorbed on the surface of an electrode, its electroreduction/electrooxidation is orientation dependent, because its redox center must be close to the electrode. In a randomly oriented monolayer of enzyme molecules, fewer than 1% participate in the desired reaction. In contrast, when the electrostatic adduct of polyanionic enzyme and a water soluble electron-conducting redox polymer are crosslinked on an electrode to form a film that swells in water, most of the enzyme molecules electrocatalyze the reaction. The reason is that electrons are exchanged between the enzyme's reaction centers and the redox polymer through collisions with the tethered, but mobile, redox functions of the hydrated polymer.

Electroreduction of multiple layers of laccase. Electron exchange between the mobile but tethered redox functions leads to electron conduction in the hydrogel, allowing the electrical connection of multiple layers of redox enzymes to electrodes. Because unique orientation is not required and because a multiple layers of enzyme are “wired”, the current density exceeds by three orders of magnitude that for an absorbed, randomly oriented, monolayer.

Formation of an electrostatic complex of laccase and its connecting redox polymer. Because the increase of entropy is small when two macromolecules are mixed, they phase separate unless they are chemically bound to each other. Electrostatic

bonding of the polycationic redox polymer and the polyanionic laccase prevents phase separation.

Hydrophilic carbon fibers. The large ion-accessed area of the hydrophilic carbon fibers and the large open pores of the

carbon “paper” provide for rapid diffusion of ions and for prompt neutralization of the base produced in the electroreduction of O₂.

Tailoring the potential of the redox polymer. Tailoring the charge of the redox polymer to +0.78V (NHE) assures spontaneous reduction of the more oxidizing laccase centers, yet allows the poisoning of the cathode at 0.7V(NHE). This potential is oxidizing enough for operation of a future 0.5 V operating potential fuel cell in which glucose will be electrooxidized on a “wired” glucose oxidase anode poised at 0.2 V(NHE).¹¹

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References

- (1) Tarasevich, M. R.; Yaropolov, A. I.; Bogdanovskaya, V. A.; Varfolomeev, S. D. *Bioelectrochem. Bioenerg.* **1979**, *6*, 393-403.
- (2) Tarasevich, M. R.; Bogdanovskaya, V. A.; Gavrilova, E. F.; Orlov, S. B. *J. Electroanal. Chem. Interfacial Electrochem.* **1986**, *206*, 217-227.
- (3) Lee, C. W.; Gray, H. B.; Anson, F. C.; Malmstroem, B. G. *J. Electroanal. Chem. Interfacial Electrochem.* **1984**, *172*, 289-300.
- (4) Yaropolov, A. I.; Kharybin, A. N.; Emneus, J.; MarkoVarga, G.; Gorton, L. *Bioelectrochemistry and Bioenergetics* **1996**, *40*, 49-57.
- (5) Jin, W.; Wollenberger, U.; Bier, F. F.; Makower, A.; Scheller, F. W. *Bioelectrochem. Bioenerg.* **1996**, *39*, 221-225.
- (6) Santucci, R.; Ferri, T.; Morpurgo, L.; Savini, I.; Avigliano, L. *Biochem. J.* **1998**, *332*, 611-615.
- (7) Thuesen, M. H.; Farver, O.; Reinhammar, B.; Ulstrup, J. *Acta Chem. Scand.* **1998**, *52*, 555-562.
- (8) Trudeau, F.; Daigle, F.; Leech, D. *Anal. Chem.* **1997**, *69*, 882-886.
- (9) Palmore, G. T. R.; Kim, H.-H. *J. Electroanal. Chem.* **1999**, *464*, 110-117.