

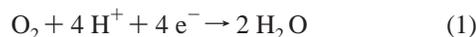
## Oxygen Is Electroreduced to Water on a “Wired” Enzyme Electrode at a Lesser Overpotential than on Platinum

Nicolas Mano,<sup>\*,†</sup> Jose L. Fernandez,<sup>‡</sup> Yousung Kim,<sup>§</sup> Woonsup Shin,<sup>§</sup> Allen J. Bard,<sup>‡</sup> and Adam Heller<sup>\*,†</sup>

Department of Chemical Engineering, Texas Materials Institute, and Department of Chemistry and Biochemistry, University of Texas, Austin, Texas 78712, and Department of Chemistry, Sogang University, Seoul, 121-742, Korea

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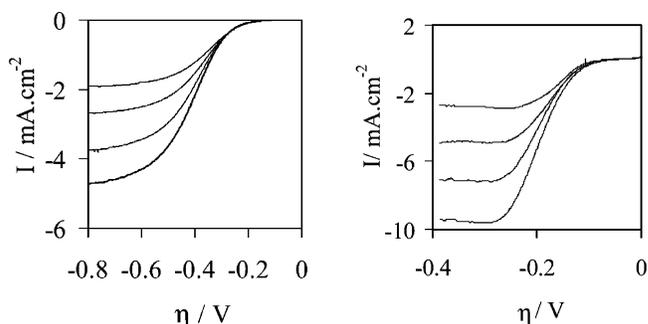
One of the most extensively studied electrochemical reactions has been the four-electron electroreduction of oxygen to water (eq 1):



This is the reaction of most fuel cell cathodes as well as of electrochemical oxygen sensors. For more than a century, the reference catalyst of the reaction has been platinum in 0.5 M H<sub>2</sub>SO<sub>4</sub>. Comparison of the overpotential of the polished platinum cathode in 0.5 M H<sub>2</sub>SO<sub>4</sub> with that of a polished vitreous carbon cathode modified with a film of bilirubin oxidase (BOD), which was electrically connected (“wired”) to the electrode by a redox polymer, showed that the overpotential of the “wired” BOD cathode was much lower than that of the Pt cathode. The “wired” BOD cathode operated in a physiological pH 7.2 (20 mM phosphate, 0.15 M NaCl) buffer solution (PBS), where the Pt cathode would have rapidly corroded. Because the solubility and diffusivity of O<sub>2</sub> in PBS at 37 °C are higher than in 0.5 M H<sub>2</sub>SO<sub>4</sub>,<sup>1,2</sup> the absolute current densities of the “wired” enzyme electrode were much higher than those of platinum at any overpotential. Thus, for example, at 1 atm O<sub>2</sub> pressure, O<sub>2</sub> was electroreduced to water at a current density of 9.5 mA cm<sup>-2</sup> at an overpotential of -0.31 V on the “wired” BOD cathode. At this overpotential, the current density of the polished platinum cathode in 0.5 M H<sub>2</sub>SO<sub>4</sub> was 16-fold lower, only 0.6 mA cm<sup>-2</sup>.

There are four electron-transfer steps in the “wired” BOD-catalyzed electroreduction of O<sub>2</sub>. At steady state, the currents associated with each are necessarily the same. Thus, the currents flowing from the carbon electrode to the redox polymer “wiring” the enzyme **I**, through the redox polymer **II**, from the polymer to the enzyme molecules **III**, and from the enzyme molecules to O<sub>2</sub> molecules **IV** are all identical. The limit of each of these currents is defined by the polymer/enzyme ratio. The limits of **I** and **II** increase upon increasing the polymer/enzyme ratio, while the limit of **IV** decreases upon increasing the ratio. By increasing the specific activity of the enzyme, not only were we able to increase the limit of **IV** but, through the associated increase in the wt % of the electron-conducting redox polymer, we were also able to push further up the limits of **I** and **II**.

The novel bioelectrocatalyst was made, as earlier,<sup>3</sup> of BOD from *Trachyderma tsunodae* and the copolymer of polyacrylamide and poly(*N*-vinylimidazole), complexed with [Os-(4,4'-dichloro-2,2'-bipyridine)<sub>2</sub>Cl]<sup>+2+</sup>, cross-linked with poly(ethylene glycol) diglycidyl ether (PEGDGE). However, the BOD from *T. tsunodae*, which was used earlier as received, was now purified,<sup>4</sup> whereby its specific



**Figure 1.** Dependence of the current density on the overvoltage for electrodes rotating at 500 rpm (top curve) and 1000, 2000, and 4000 rpm (bottom curve) under 1 atm O<sub>2</sub> at 37 °C. Scan rate: 5 mV s<sup>-1</sup>. Left: polished Pt in 0.5 M H<sub>2</sub>SO<sub>4</sub>. Right: “wired” BOD on polished vitreous carbon in a pH 7.2 saline (0.15 M NaCl) phosphate (0.02 M) buffer. The “wired” BOD film, loaded at 0.17 mg cm<sup>-2</sup>, comprised 32.3 wt % BOD, 60.2 wt % redox polymer, and 7.5 wt % PEGDGE.

activity increased 5-fold. Because less enzyme was required to provide the current **IV**, we were able to reduce the optimal enzyme weight % from 44 wt % to 32 wt % and to increase the optimal polymer weight % from 49 wt % to 60 wt %.<sup>3,5–8</sup> The resulting increase in the conductive polymer/insulating enzyme weight ratio from 1.11 to 1.88 increased the diffusivity of electrons.<sup>3,7–9</sup> An increase in the wt % of the cross-linker<sup>8,10</sup> mechanically toughened the film, which was not sheared off the 3-mm diameter disk electrode when it rotated at 4000 rpm.

While chloride, at concentrations as low as 4 ppm, affects the electroreduction of O<sub>2</sub> on Pt by reducing the operating voltage of fuel cells by 50 mV or more,<sup>11</sup> the “wired” BOD electrocatalyst functions even at a chloride concentration as high as 1 M.<sup>3</sup>

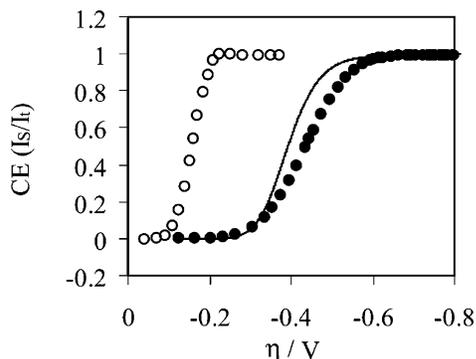
Figure 1 shows the dependence of the current density on the overpotential for the Pt cathode in 0.5 M H<sub>2</sub>SO<sub>4</sub> and for the “wired” BOD cathode in PBS. For Pt, the dependence of the current density on the overvoltage agreed with that of earlier studies. The Levich equation,  $I = 0.62nFAcD^{2/3}\nu^{-1/6}\omega^{1/2}$ , was rigorously obeyed up to 4000 rpm, where the current density reached 9.5 mA cm<sup>-2</sup>.<sup>12</sup> The slope, 0.44 mA cm<sup>-2</sup> rad<sup>-1/2</sup> s<sup>1/2</sup>, agreed with the theoretical value of 0.46 mA cm<sup>-2</sup> rad<sup>-1/2</sup> s<sup>1/2</sup>, calculated for a four-electron process ( $n = 4$ ), with O<sub>2</sub> diffusivity  $D = 2.4 \times 10^{-5}$  cm<sup>2</sup> s<sup>-1</sup>, O<sub>2</sub> solubility  $c = 1.07 \times 10^{-6}$  mol cm<sup>-3</sup>, kinematic viscosity (of the 0.15 M NaCl solution at 37.5 °C)  $\nu = 0.091$  cm<sup>2</sup> s<sup>-1</sup>, and rotating disk area  $A = 0.0707$  cm<sup>2</sup>.<sup>1,2</sup> Above 10 mA cm<sup>-2</sup>, reached at angular velocities higher than 4000 rpm, the current was controlled not by diffusion, but by the electrocatalytic reaction kinetics.

The diffusion-limited currents reached with the “wired” BOD cathodes were about twice as high as those reached with the Pt cathode. The difference is a result of the higher solubility and diffusivity of oxygen in PBS relative to 0.5 M H<sub>2</sub>SO<sub>4</sub>.<sup>1,2</sup> The product

<sup>†</sup> Department of Chemical Engineering and Texas Materials Institute, University of Texas.

<sup>‡</sup> Department of Chemistry and Biochemistry, University of Texas.

<sup>§</sup> Department of Chemistry, Sogang University.



**Figure 2.** Dependence of the collection efficiency ( $I_s/I_T$ ) on the overpotential ( $\eta$ ) in tip generation–substrate collection mode SECM for Pt in 0.5 M  $\text{H}_2\text{SO}_4$  (solid line), for Pt in pH 7.2, 0.2 M phosphate ( $\bullet$ ), and for the “wired” BOD cathode in pH 7.2 PBS ( $\circ$ ). 25  $\mu\text{m}$  Pt tip; tip current ( $I_T$ ), 81 nA; distance between the tip and the substrate, 5  $\mu\text{m}$ .

of the ratios of  $\text{O}_2$  solubility and  $\text{O}_2$  diffusivity in the two solutions is  $\sim 1.8$ , consistent with the  $\sim 1.8$ -fold higher limiting currents in PBS.

$\text{O}_2$  was electroreduced exclusively by four-electron reduction to water, not by two-electron reduction to  $\text{H}_2\text{O}_2$ . Experiments with rotating Pt ring/“wired” BOD disk electrodes showed no oxidation on the Pt ring at potentials where  $\text{H}_2\text{O}_2$ , if it were formed on the disk, would have been oxidized. Colorimetry with ABTS-horseradish peroxidase confirmed the absence of  $\text{H}_2\text{O}_2$  from the solution in which the wired BOD cathode operated.<sup>3,7</sup>

Mass transport-corrected Tafel plots in the mixed kinetic diffusion-controlled region were obtained from Koutecky–Levich plots for the Pt and for “wired” BOD cathodes. The slopes for the Pt cathode were 60 mV/dec at low current density ( $\eta < |0.5 \text{ V}|$ ) and 122 mV/dec at high current density ( $\eta > |0.5 \text{ V}|$ ), in agreement with the reported values.<sup>13,14</sup> The lower Tafel slope corresponds to  $\text{O}_2$  electroreduction on the oxidized (Pt–OH) surface. The higher slope, of relevance to polymer–electrolyte fuel cells, is characteristic of clean Pt.<sup>15</sup> For the “wired” BOD cathode, only a 120 mV/dec Tafel slope was observed.

That “wired” BOD was a more active electrocatalyst than Pt in the four-electron electroreduction of  $\text{O}_2$  to water was confirmed by scanning electrochemical microscopy (SECM), operating in a modified tip generation–substrate collection mode (TG–SC).<sup>16</sup> A current of 81 nA ( $I_t$ ) was applied to a 25  $\mu\text{m}$  Pt tip positioned 5  $\mu\text{m}$  from the substrate (the 127  $\mu\text{m}$  Pt disk or the “wired” enzyme–solution interface) to generate a constant flow of  $\text{O}_2$  by oxidizing water. The  $\text{O}_2$  diffusing to the scanned cathode surface was electroreduced at a current ( $I_s$ ) defined by the catalyst-dependent overpotential. The potential dependence of the collection efficiency CE ( $I_s/I_t$ ) is seen in Figure 2 for the “wired” BOD cathode in PBS (open circles), for the Pt cathode in 0.5 M acid sulfuric (solid line), and for the Pt cathode in pH 7.2, 0.2 M phosphate buffer (solid

circles). Consistent with the RDE experiments, the  $\text{O}_2$  diffusion-defined collection efficiency was reached already at  $-0.3 \text{ V}$  overpotential with “wired” BOD cathode. With Pt, it was reached only at an overpotential of  $-0.5 \text{ V}$  in 0.5 M sulfuric acid and at an overpotential of  $-0.65 \text{ V}$  in pH 7.2, 0.2 M phosphate.

In summary, the “wired” enzyme electrocatalyst is superior to platinum. The smooth “wired” enzyme cathode exhibits a higher current density of oxygen electroreduction than the classical smooth Pt electrode. The overpotential, for any current density, is much lower for the enzyme-based cathode. By using rotating porous “wired” enzyme cathodes, the area of which could be at least 5-fold larger than that of smooth electrodes, we are likely to reach current densities of 50  $\text{mA cm}^{-2}$ . Yet higher current densities are likely to be reached with air-diffusing cathodes of the type used in fuel cells, where Pt-loaded carbon particle cathodes reach 1  $\text{A cm}^{-2}$ . While it is likely that with adequate engineering current densities of  $\sim 1 \text{ A cm}^{-2}$  will be reached also with “wired” enzyme cathodes, these cathodes will have to be made at a cost low enough to be discarded after their shorter operational life, most likely of a few weeks.

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