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**DESIGN OF CARBON NANOTUBE-BASED GAS-DIFFUSION FOR O<sub>2</sub> REDUCTION BY MULTICOPPER OXIDASES]  
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# Design of Carbon Nanotube-Based Gas-Diffusion Cathode for O<sub>2</sub> Reduction by Multicopper Oxidases

Carolin Lau, Emily R. Adkins, Ramaraja P. Ramasamy, Heather R. Luckarift, Glenn R. Johnson, and Plamen Atanassov\*

Multicopper oxidases, such as laccase or bilirubin oxidase, are known to reduce molecular oxygen at very high redox potentials, which makes them attractive biocatalysts for enzymatic cathodes in biological fuel cells. By designing an enzymatic gas-diffusion electrode, molecular oxygen can be supplied through the gaseous phase, avoiding solubility and diffusion limitations typically associated with liquid electrolytes. In doing so, the current density of enzymatic cathodes can theoretically be enhanced. This publication presents a material study of carbon/Teflon composites that aim to optimize the functionality of the gas-diffusion and catalytic layers for application in enzymatic systems. The modification of the catalytic layer with multiwalled carbon nanotubes, for example, creates the basis for stronger  $\pi$ - $\pi$  stacking interactions through tethered enzymatic linkers, such as pyrenes or perylene derivatives. Cyclic voltammograms show the effective direct electron contact of laccase with carbon nanotube-modified electrodes via tethered crosslinking molecules as a model system. The polarization behavior of laccase-modified gas-diffusion electrodes reveals open-circuit potentials of +550 mV (versus Ag/AgCl) and current densities approaching 0.5 mA cm<sup>2</sup> (at zero potential) in air-breathing mode.

## 1. Introduction

Carbon is a commonly used material for gas-diffusion electrodes (GDE) in metal-air batteries and fuel cells that use oxygen as cathode reactant (oxidant) from the ambient air.<sup>[1]</sup> In general a GDE consist of several layers of a carbon-polymer composite (consisting of carbon black or graphite powder with teflon (PTFE) or polyaniline as a binder) of varying

hydrophobicity often integrated on a metallic mesh for mechanical stability and structural support. Three constituent layers are typically defined as backing material, gas-diffusion layer (GDL) and catalytic layer. The backing material serves as mechanical support and electron conductor, together with the GDL it should be highly gas-permeable, corrosion resistant and highly electronic conductive. An ideal GDL is completely hydrophobic (water repellant) and, in many cases, has an increasing hydrophilic character towards the electrolyte. The catalytic layer contains the catalyst bound to the same polymer-carbon mixture.<sup>[2]</sup> The balance of hydrophilic and hydrophobic properties of the carbon-air and carbon-electrolyte interfaces determines the performance of such GDE.<sup>[3-5]</sup> This balance is essential to form the "tri-phase interface", an interfacial structure which is a line of contact of the gaseous, liquid, and solid phases responsible respectively for oxygen, hydroxonium ion and electron transport.

Designing GDE therefore turns into a task of fabricating functional materials that combine the hydrophilic and hydrophobic properties needed for creating and sustaining such tri-phase interface. The goal of such a design is to achieve a high-surface-area material with a tri-phase interface that maximizes exposure of the immobilized catalysts to the gas (air) and the liquid (electrolyte) phases.<sup>[2,6,7]</sup>

GDE have been widely used in alkaline and phosphoric acid fuel cells and in metal-air batteries where they are used to incorporate metal (Pt, Pd, Ag) or metal oxide (MnO<sub>2</sub>, Co<sub>2</sub>O<sub>3</sub>) catalysts that are supported on dispersed carbonaceous materials (usually activated carbon or carbon blacks). Such GDE, however, have been poorly explored for biocatalytic systems, such as oxygen reduction catalyzed by multicopper oxidases (MCO). Integration of enzymes in GDE presents most of all a materials challenge: the functional material should be electrically conductive with noncompromised electron conductivity; it should be hydrophilic enough to allow immobilization of the enzyme from an aqueous solution and its surface should be amenable to fortuitous interaction or chemical immobilization of enzymes. In addition, the material should be susceptible to treatments that yield transition to the superhydrophobicity required in the GDL of GDE. Example treatments

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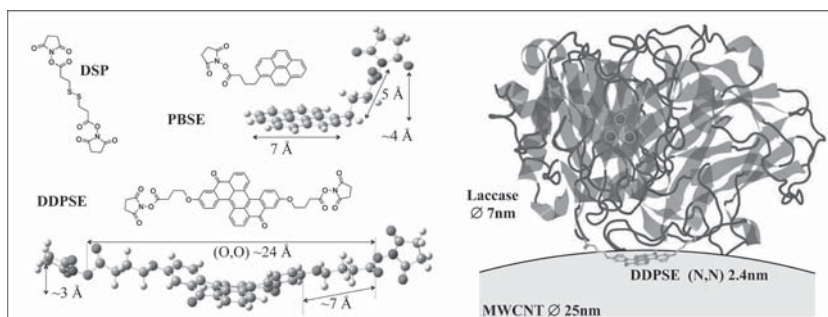
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include, chemical modification or blending with a hydrophobic composite or dispersed phase (such as PTFE or PTFE-modified carbon black). One approach is to seek a combination of all these three materials properties as a functional interface at three scales: molecular- (i.e., surface chemistry characteristics); meso- (e.g., enzyme-carbon black interactions) and macro- (e.g., materials blended in PTFE emulsions).

Multicopper oxidases (MCO), such as laccase, bilirubin oxidase, and ascorbate oxidase are known to catalyze the four-electron reduction of oxygen to water.<sup>[8,9]</sup> Mechanistic studies show that the electrons are transferred rapidly from the electrode over an exterior T1 copper center to the inner-molecular tri-nuclear (T2/T3) copper center, where the actual oxygen reduction takes place. The MCO possess very high cathodic thermodynamic redox potentials due to a direct electrical communication between the outer T1 copper center and the electrode.<sup>[8–15]</sup> By achieving direct electron transfer (DET), the maximal possible electrochemical potential range can be used for power generation in a biofuel cell. Thus, combining an oxygen reducing biocatalyst via direct electrical communication with an air-breathing gas-diffusion electrode will provide significant improvements in bio-cathode development. So far only a few enzyme based GDEs are reported, such as an air-breathing carbon black/PTFE glucose oxidase sensor<sup>[16]</sup> and a floating carbon black/fluoropolymer material, for laccase.<sup>[17,18]</sup> or multicopper oxidase,<sup>[19]</sup> catalyzed oxygen reduction. Recently, we also demonstrated the development of gas-diffusion cathodes for oxygen reduction based on adsorptive immobilization of laccase and bilirubin oxidase.<sup>[20,21]</sup>

Carbon nanotubes (CNTs) are known for their extraordinary electrical and mechanical properties,<sup>[22]</sup> high surface area, low resistivity, and chemical stability, that together makes them an interesting material for various applications in microelectronics, composite materials, and electrical applications. On the molecular scale, the CNTs walls consist of  $sp^2$  graphene carbon atoms with delocalized  $\pi$ -electrons which provide perfect conjugation sites for other highly aromatic molecules. Conjugated conductive polymers<sup>[23]</sup> as well as conjugated polyaromatic hydrocarbons,<sup>[24–26]</sup> are known for their  $\pi$ - $\pi$  stacking interaction with single walled carbon nanotubes.<sup>[27–31]</sup> The noncovalent  $\pi$ - $\pi$  interactions are stronger than simple electrostatic binding or adsorption, and as such provide an effective mechanism for associating biomolecules with CNT surfaces. **Figure 1** shows a mechanism for utilizing pyrenebased tethered linkers as used in this study to achieve a noncovalent side wall functionalization of multiwalled carbon nanotubes (MWCNTs) with application to biocathode development. The following describes the chemistry functionalization of MWCNTs with tethered crosslinkers for covalent binding of biocatalysts, the modification of carbonaceous materials towards catalytic and gas-diffusion layers and the integration of those different composites into the final device; the gas-diffusion electrode.



**Figure 1.** Left: Structure of the crosslinker DSP (dithiobis(succinimidyl) propionate), PBSE (1-pyrenebutyric acid *N*-hydroxysuccinimide ester), and DDPSE (4,4'-[(8,16-dihydro-8,16-dioxodibenzo[*a*,*j*]perylene-2,10-diyl)dioxy] dibutyric acid di(*N*-succinimidyl ester)). Right: Size comparison of laccase and the covalently bound DDPSE crosslinker on a MWCNT of approximately 25 nm diameter. The succinimide ester group reacts with the enzyme's primary amine groups, forming a new amide bond and releasing *N*-hydroxysuccinimide.

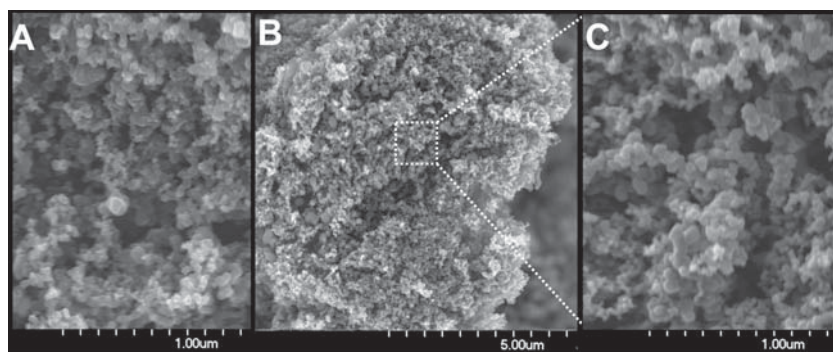
## 2. Results and Discussion

### 2.1. Fabrication of Gas-Diffusion Electrodes

#### 2.1.1. Chemistry: Molecular Tethering of CNTs for Covalent Binding of Biocatalysts

Carbon black (CB) and MWCNTs were modified with different PTFE contents and used as base material for the biocatalytic layer. Bifunctional crosslinkers were used to modify the MWCNT sidewalls with biocatalyst. All linkers share a common functionality consisting of a highly aromatic functional group which attaches through  $\pi$ - $\pi$  interactions to the MWCNT sidewalls and an *N*-hydroxysuccinimide ester group, which covalently binds to the proteins amine groups (see Figure 1). In comparison to the nonconjugated crosslinker DSP, which non-specifically binds to two primary amine groups, the tethered crosslinker PBSE and DDPSE (Figure 1) have a defined aromatic functionality for specific  $\pi$ - $\pi$  stacking interactions with MWCNTs. The two additional protein binding “spacer arms” of DDPSE should also provide a more controlled and sterically fixed binding of an enzyme.

A size comparison of a 25 nm diameter MWCNT, 7 nm diameter laccase, and 26 Å N–N distanced DDPSE is shown on the right side in Figure 1. In this configuration, the MWCNT appears as an almost planar surface with respect to the crosslinkers and enzyme. The molecular structure of PBSE (Figure 1) reveals a planar pyrene moiety of about 7 Å diameter whereas the DDPSE's heterocyclic region is significantly larger ( $\approx 11$  Å diameter) and with a slightly angled orientation that may provide both more spatially expansive, and spatially defined interaction that enhance the  $\pi$ - $\pi$ -stacking interaction with the MWCNT surface. One may speculate that the perfectly planar PBSE should theoretically attach better than the angled orientation of DDPSE. Alternatively, however, DDPSE provides a larger surface area and more delocalized electrons for  $\pi$ - $\pi$  stacking interaction with the MWCNT surface. Additionally the presence of the two juxtaposed succinimidyl moieties that act as “arms” in DDPSE (rather than the single succinimidyl ester of PBSE) can bind to two amine groups of the same protein



**Figure 2.** SEM images of (A) unmodified carbon black (Vulcan XC72R) and (B,C) a PTFE modified carbon black micro emulsion droplet (35 wt% PTFE). Image (C) shows a higher magnification image of the PTFE-carbon black surface revealing a complete surface coverage with carbon black particles.

and thereby draw the protein closer to the MWCNT surface; 3 Å (C–N) in DDPE versus 4 Å (C–N) in PBSE which favors a direct electron transfer from the laccase copper center to the carbon nanotube.

### 2.1.2. Materials: PTFE–CB and PTFE–MWCNT Composites

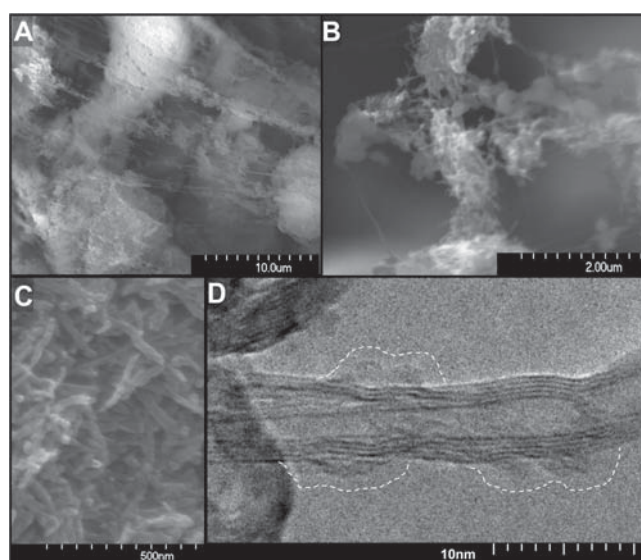
PTFE was selected as a binding material because it is a hydrophobic but oxygen-permeable polymer. The carbon black particles ( $\approx 5$  nm diameter, see **Figure 2A**), mixed well with a PTFE particles to yield stable suspensions. In aqueous solvents, the highly hydrophobic PTFE forms micro droplets that are decorated on the outside by the more hydrophilic carbon black particles (Figure 2B,C). The high magnification scanning electron microscopy (SEM) images in Figure 2B confirm the hierarchical structure of the resulting spherical, composite particles which are up to several micrometers in diameter.

Creating a bilayered gas-diffusion electrode with a carbon nanotube-rich catalytic layer should theoretically increase its catalytic performance dramatically. As such, a derivative gas-diffusion electrode was prepared of a PTFE–CNT composite that could be directly pressed onto the carbon black gas-diffusion layer. **Figure 3A–C** show SEM images of the resulting MWCNT–PTFE composites of 35, 22, and 3.5 wt% PTFE, respectively. The fiber like structure of the PTFE that binds the MWCNT together is noticeable in the resulting agglomerate. The amount of PTFE visibly decreases from Figure 3A,B to C. The present results are consistent with the previous catalytic properties for oxygen reduction reactions (ORRs) from MWCNT–carbon composite materials.<sup>[32]</sup>

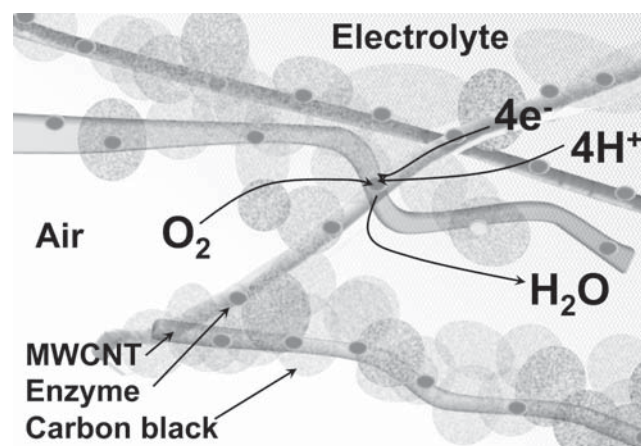
The carbon nanotube buckypaper used to support the catalytic layer of the cathode readily accommodated the immobilized laccase and provided high electronic conductivity since no binding agents were required within the material. A high-resolution TEM image reveals the change in the carbon nanotube morphology that was attributed to the immobilization and distribution of proteins on the surface (Figure 3D). Because of the difficulties in imaging proteins with TEM, we highlighted the immobilized enzymes.

The functionality and electrochemical performance of gas-diffusion electrodes is dictated by the hydrophobic and

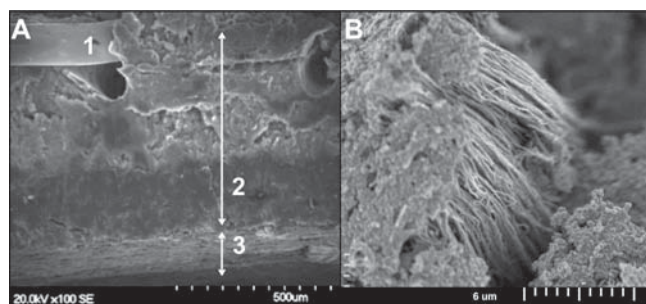
hydrophilic design properties of both layers (**Figure 4**). The gas-diffusion layer provides microstructured gas channels for an efficient oxygen supply in combination with electric conductivity, mechanical stability, and water repellency. As host for the biocatalyst, the catalytic layer must provide hydrophilic micropores as well as hydrophobic gas diffusion pathways so that a three-phase interphase can be formed. Biocatalytic oxygen reduction takes place with its highest efficiency when the tri-phase boundary of liquid electrolyte, gaseous substrate, and solid catalyst on a carbon support is optimal. The transport of oxygen through the liquid phase is significantly hindered by diffusion and its inherent



**Figure 3.** High-resolution SEM images of PTFE-modified MWCNTs with (A) 35 wt% PTFE, (B) 22 wt% PTFE, and (C) 3.5 wt% PTFE. Image (D) shows a transmission electron microscope (TEM) image of a MWCNT after immobilization of enzymes. (Due to the difficulties in protein imaging we highlighted the shape of the proteins.)



**Figure 4.** Schematic of the three-phase interphase of a gas-diffusion electrode.



**Figure 5.** Cross-section SEM images of a gas-diffusion electrode at different magnifications. (A) Cross section of GDE with (2) GDL (CB with 35 wt% PTFE) and (3) MWCNT catalytic layer (3.5 wt% PTFE) with (1) nickel mesh as the current collector. (B) Higher-magnification SEM of MWCNTs pressed into the gas-diffusion layer. Sample was broken in liquid nitrogen for imaging.

low water solubility. In the gas diffusion architecture described herein, the three phase interphase (Figure 4) is formed on the outside of the teflonized carbon or carbon nanotube particles. The aqueous electrolyte will primarily occupy the larger pore spaces, whereas the gas transport is ensured by the hydrophobic PTFE microchannels.<sup>[2,32]</sup> The accumulation of electrolyte, known as a concept of “flooded pores”, significantly hinders the oxygen transport and the electrode performance.<sup>[33]</sup> Many models have been developed for the two-phase transport in porous gas-diffusion electrodes in order to optimize those systems.<sup>[6]</sup> As a result, it is clear that the major design criteria for gas-diffusion electrodes are a well-balanced PTFE-to-carbon content and an optimal thickness for both layers. Typical PTFE contents for gas diffusion layers range from 25 to 60 weight%.<sup>[2]</sup> Herein, we optimized the gas-diffusion layer PTFE ratio to 35 wt% which, provided enough hydrophobic, water-repellant character to seal the electrolyte, yet provided an efficient gas supply and retained the high electric conductivity of the carbon black materials. Reported thicknesses for metal-catalyzed ORR are approximately 500  $\mu\text{m}$  for gas-diffusion layers and 150  $\mu\text{m}$  for catalytic layers, and these design parameters were retained within our design (Figure 5A).<sup>[2,34]</sup> Calculations for enzymatic cathodes show a 5 to 10-fold increase in catalytic performance with oxygen supplied by gas phase diffusion compared to solute diffusion of dissolved oxygen.<sup>[35]</sup> In fact, the oxygen partial pressure has been shown to affect laccase catalysis rates.<sup>[36]</sup>

### 2.1.3. Design of an Enzymatic Gas-Diffusion Electrode

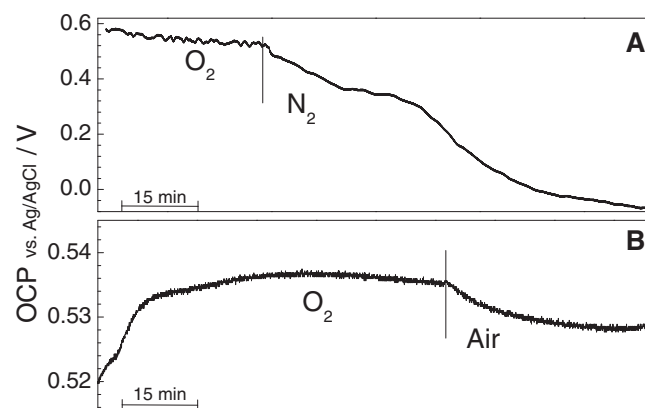
The limiting factor for catalytic as well as biocatalytic oxygen reductions is the transport of the gaseous reaction to the catalytic centers. Thus an efficient gas-diffusion electrode for enzymatic ORR becomes extremely important for designing biofuel cells. As discussed, an ideal gas-diffusion electrode is a layered design consisting of a gas-diffusion layer, a catalytic layer and if necessary a stabilizing (or binding) layer. The gas-diffusion biocathode described utilizes a carbon-PTFE composite material pressed onto a nickel mesh, which provides mechanical stability, and additionally serves as the current collector. A cross-section SEM image (Figure 5A) of the electrode shows the nickel wire imbedded within the gas-diffusion layer of about 500  $\mu\text{m}$

total thickness and in direct contact with the catalytic layer of  $\approx 100 \mu\text{m}$  thick. Figure 5B gives further insight into the architecture of the catalytic MWCNT-PTFE layer; specifically typical bundle-like structure of the carbon nanotubes within the pressed composites provides air-accessible pores. The final air-breathing cathode has a geometric surface area of about 1.3  $\text{cm}^2$ .

### 2.2. Characterization of Gas-Diffusion Electrode

To test the air-breathing properties of the gas-diffusion electrode, laccase was immobilized as an oxygen reducing biocatalyst directly onto the catalytic layer and the resulting open circuit potential monitored under oxygen, nitrogen and with ambient air. The air-breathing electrode is open to gases from the outside and to gas-saturated electrolyte from within. With oxygen present, the laccase-functionalized gas diffusion electrode shows a steady open-circuit potential (OCP) of 0.53 V (versus Ag/AgCl) which is close to the theoretical redox potential of the T1 copper center (Figure 6A). As expected, supplying nitrogen across cathode surface causes the OCP to drop to zero (i.e., the potential difference between carbon and the reference electrode). The slow decay over time ( $\approx 1 \text{ h}$ ) is attributed to small amounts of oxygen remaining in the electrolyte or carbon matrix. A comparable steady OCP of 0.536 V (versus Ag/AgCl) is observed under oxygen, with only a minimal 2% decrease to 0.528 V upon switching to static ambient air (Figure 6B).

In general, the OCP reflects the difference in redox potential between the laccase, or its specific copper centers, in direct contact with the carbon electrode (versus the reference electrode and without any external load connected, or electric current flowing). OCP, therefore can be used as first evidence for an established direct electron transfer (DET) contact but cannot be used for specific quantification of enzyme or substrate concentration. Therefore OCP measured under the influence of oxygen, nitrogen, and ambient air confirm effective air-breathing properties of our gas-diffusion electrode design with laccase as the oxygen reducing biocatalyst (Figure 6). The

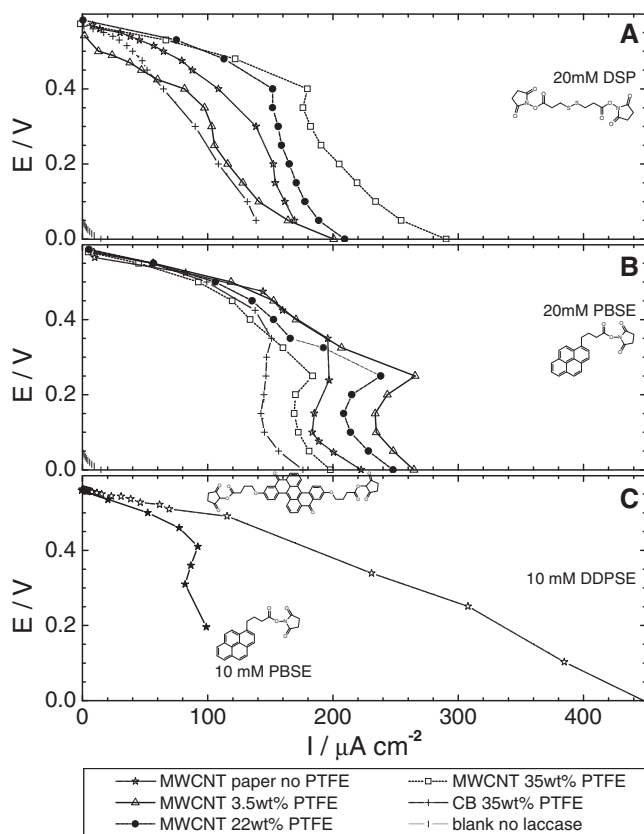


**Figure 6.** Monitoring the OCP of laccase adsorbed on a gas-diffusion electrode under the influence of (A) oxygen and nitrogen stream or (B) ambient air.

laccase-functionalized electrode is self-sustaining on oxygen supplied by ambient air alone and without any need of 'forced' gaseous convection.

### 2.3. Electrochemical Performance of Gas-Diffusion Electrodes

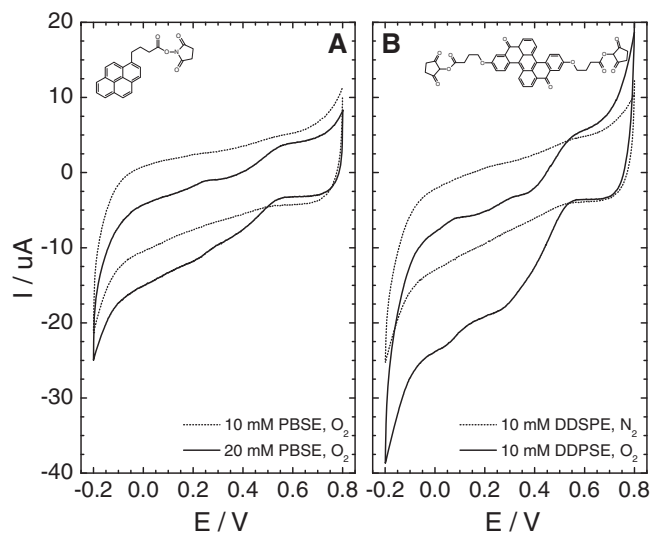
After characterizing the gas-diffusion layer for its air-breathing properties, we turned to then define the optimal hydrophilic-to-hydrophobic ratio of the carbon nanotube-based catalytic layer. The polarization behavior was determined for a range of gas-diffusion electrodes with MWCNT-PTFE layers, varying from 35–0 wt% of PTFE, and then compared to a pure carbon black/PTFE electrode (Figure 7). All polarization curves show almost no drop in potential close to the OCP (activation losses) after the initial polarization. The first segment (0.6 to 0.45 V) of the polarization curves reveals a nearly linear dependence of the applied potential over the current density. This area, defined as 'the ohmic region', covers a fairly wide current range, and represents the internal fuel cell resistivity loss. All three graphs in Figure 7 show an almost identical ohmic loss of about 0.1 V per 100  $\mu\text{A cm}^{-2}$ , regardless of the PTFE



**Figure 7.** Potentiostatic polarization curves of enzymatic oxygen reduction on gas-diffusion electrodes (air breathing) with a carbon black/35 wt% PTFE gas-diffusion layer and catalytic layers of different PTFE content (35–0 wt% PTFE). Laccase was crosslinked with (A) 20 mM DSP, (B) 20 mM PBSE, and (C) 10 mM DDPSE; 0.1 M phosphate buffer (PB), pH 6.3.

content and nature of crosslinking, indicating similar electrode and cell resistance. The higher current densities measured for the carbon nanotube-modified electrodes in comparison to the pure carbon black cathodes are attributed to the higher surface area. Finally, in lower potential ranges between 0.45 to 0 V, the mass-transport regime becomes dominating. The kinetics of the catalytic reaction is determined by the limiting oxygen supply probably caused by "flooded pores" inside the catalytic or gas-diffusion layer (as discussed above). In this area, the efficiency of ORR catalysis is influenced by orientation of the immobilized enzyme. Changes in immobilization chemistry are predicted to define protein orientation and will in turn affect catalysis. A comparison of three different crosslinkers (Figure 7A–C), demonstrates this effect. The aliphatic portion of DSP, for example, adsorbs nonspecifically to the carbon nanotube layer and covalently binds proteins via two amine groups on the protein surface. PBSE and DDPSE, in comparison, bind to carbon nanotubes through stronger  $\pi$ - $\pi$  stacking interactions, leaving the succinimide ester groups for covalent attachment of enzymes primary amine groups. As shown in Figure 7A, the measured current densities for DSP-crosslinked laccase decreases with increasing CNT content of the catalytic layer, indicating nonspecific adsorption of enzyme agglomerates that show preferential binding to the more hydrophobic PTFE composites. In contrast, the current densities for the PBSE and DDPSE tethered laccase are higher for less-teflonized composites with higher CNT ratios (Figure 7B,C). This observation further supports the idea of favored immobilization of biocatalysts by using a specific tethered crosslinker. As such, PBSE- and DDPSE-functionalized electrodes clearly show an extension of the mass-transport dominated regime compared to DSP (nonspecific adsorption) controls, as the multiwalled catalytic layer provides a higher surface area and favors the attachment of tethered crosslinker. The lower the PTFE content the more MWCNT surface area is accessible for enzyme, and as a result, the current densities achieved are almost double with MWCNTs (3.5 wt% PTFE) compared to carbon black. To further examine the concept of improved interaction of CNTs and tethered crosslinker we compared PBSE with DDPSE, which has a much larger area for interaction and for  $\pi$ - $\pi$ -stacking. For identical linker concentrations a significant increase in current density was observed on MWCNT paper modified with DDPSE, where current densities at 0.3 V were 3-fold higher for DDPSE over PBSE (Figure 7C). The advantages of DDPSE over PBSE are clear: i) it establishes stronger  $\pi$ - $\pi$ -interactions with the MWCNT surface, ii) it can covalently bind to two amine groups of the same enzyme and position the protein in a more defined orientation, and iii) the electron transfer distance is significantly reduced; about 1 Å shorter (Figure 1).

The presented design of a gas-diffusion electrode with tethered laccase as biocatalyst of oxygen reduction delivers approximately a current density of 0.5  $\text{mA cm}^{-2}$  (at 0 mV), which is quite comparable to other bio-cathodes. For example a "floating air" laccase cathode achieved current densities of 0.5  $\text{mA cm}^{-2}$  (at 500 mV)<sup>[17]</sup> and in previous work we reported 1  $\text{mA cm}^{-2}$  (at 0 mV)<sup>[20]</sup> for an air-breathing laccase cathode. In both cases much higher amounts of laccase were adsorbed to carbon black material without the use of crosslinker. Our presented approach is limited (due to the relatively high costs) to only 10 mM of



**Figure 8.** Cyclic voltammograms for laccase catalyzed oxygen reduction. Laccase was immobilized on MWCNT paper using (A) PBSE and (B) DDPSE, 0.1 M PB, pH 6.3, 10 mV s<sup>-1</sup>.

DDPSE and higher current densities should be possible by a higher surface coverage with enzymes.

To further support the preferential efficiency of DDPSE-functionalized carbon nanotubes for enzyme modification, cyclic voltammogram (CV) measurements were investigated for laccase tethered to MWCNT paper via alternative crosslinkers. Identical concentrations of crosslinker were selected and used for control experiments, the ORR activity was tested in aerobic and anaerobic conditions. The CVs in **Figure 8** show a dramatic increase in oxygen reduction current by using DDPSE as crosslinker. The onset potential for the enzymatic ORR is about 0.55 V (versus Ag/AgCl) which agrees with the reported OCPs. The CV in nitrogen purged solution shows no catalytic current, confirming the direct electrical communication between laccase and electrode.

### 3. Conclusion

Carbon black–PTFE based gas-diffusion electrodes provide a paradigm for efficient oxygen supply for multicopper oxidase-catalyzed ORR reactions. The introduction of MWCNT–PTFE composite materials, also provides a method to manufacture catalytic layers for gas-diffusion electrodes of various shapes and sizes. The modification with carbon nanotubes provides a higher surface area for enzyme immobilization and a better conductivity that results in reduced ohmic losses under polarization condition. Further, a MWCNT surface modification with tethered crosslinker, such as PBSE or DDPSE, clearly increases the DET efficiency between biocatalyst and carbon nanotube. The  $\pi$ - $\pi$ -stacking interaction provides a stronger binding between MWCNT and protein that through simple physisorption alone. Preliminary data also suggest that DDPSE's two covalent spacer arms covalently bind and position the enzyme closer the electrode surface and reduce the electron transfer distance.

### 4. Experimental Section

**Teflonization of Carbon Blacks and MWCNT:** A PTFE dispersion (4.5 g, 60 wt%, DuPont) was diluted in deionized (DI) water (150 mL) and carbon black (5 g, VulcanXC72R) was added to the 60 °C suspension under stirring. The hot mixture was stirred for 30 min and allowed to cool for about 2 h, during which time a precipitate forms. The filtrate was carefully washed with DI water, dried at 90 °C overnight and then heated to 150 °C for 30 min before slowly cooling to room temperature. A final grinding process results in a fine black powder without any visible PTFE residues with a PTFE content of 35 wt%. The MWCNT–PTFE (MWCNTs: 20–30 nm diameter, 10–30  $\mu$ m length, cheaptubes.com) composites were prepared at a range of concentrations, following the described protocol in a 1 vol% isopropyl alcohol (IPA)–water solution to achieve a better carbon nanotube distribution due to decreased surface tension than water alone: 4.5 g, 2.8 g and 0.45 g of 60 wt% PTFE dispersion were added to achieve composites with 35, 22, and 3.5 wt% PTFE, respectively.

**Preparation of Gas-Diffusion Electrode:** The carbon black PTFE carbon black composite material (80 mg, 35 wt% PTFE) was placed into a round-shaped die (0.75 inch diameter) and hand-pressed onto a nickel mesh to form the gas-diffusion layer. The carbon black or MWCNT–PTFE composite material (40 mg) was then placed on the gas diffusion layer to form the catalytic layer. Both layers were pressed for 5 min under pressure (1000 psi). For the binder-free MWCNT layer, a circle (0.5 inch diameter) of MWCNT paper (Buckeye Composites, OH, USA) was placed on top of the gas-diffusion layer and pressed together as before.

**Immobilization of Enzymes:** The crosslinkers DSP (dithiobis(succinimidyl) propionate), PBSE (1-pyrenebutyric acid *N*-hydroxysuccinimide ester), and DDPSE (4,4'-[(8,16-dihydro-8,16-dioxodibenzo[*a*,*j*]perylene-2,10-diyl) dioxo] dibutyric acid di(*N*-succinimidyl ester) were purchased from Sigma (St. Louis, MO, USA). The catalytic side of the gas-diffusion electrodes was exposed to the crosslinker solution (0.5 mL, 20 mM) in dimethyl sulfoxide (DMSO) for about 2 h. The activated electrodes were rinsed with DMSO and DI water and incubated at 4 °C overnight with laccase (0.5 mL, 8 mg mL<sup>-1</sup>) in phosphate buffer (0.1 M, pH 6.3).

**Electrochemical Measurements:** Electrochemical measurements were performed in a stackable 3-electrode cell as reported earlier<sup>[37]</sup> with a Pt-counter and a Ag/AgCl (3 M KCl) reference electrode. The stack cell has an inner diameter of 0.5 inch. The air-breathing gas-diffusion electrodes are placed on the bottom of the stack cell with the gas-diffusion layer exposed to the ambient air and the catalytic layer in contact with the internal electrolyte. All measurements were performed in phosphate buffer (0.1 M, pH 6.3).

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