# Hybrid electrocatalysts for oxygen reduction reaction: Integrating enzymatic and non-platinum group metal catalysis

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ABSTRACT

For the first time, oxygen reduction reaction has been demonstrated on a system which integrates enzymatic and non-platinum based catalysts simultaneously. This achievement is of a great importance as it offers the possibility of exploring concomitantly two very different types of catalysts, combining the advantages of both in enhancing oxygen reduction reaction rate. The engineered catalytic hybrid material not only possesses lower overpotentials compared to the purely non-PGM catalyst, but also is capable of achieving higher current densities in comparison to purely enzymatic catalyst. The hybrid catalyst undergoes oxygen reduction with the desired 4 electron transfer process, leading to the formation of water as a final product. The achieved current density of $1.2\,mA\,cm^{-2}$ is believed to be the highest reported for bilirubin oxidase -based gas-diffusion cathode reported so far.

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## 1. Introduction

The development of new generation of medical devices such as peacemakers, insulin pumps and biological sensors, designed for ensuring the survival of individuals with complex medical conditions, led to increased interest in the area of power sources, necessary for continual operation of such devices. The simplest solution, available to researchers at that time, was to introduce a primary battery as a power source. Unfortunately, batteries have finite operational lifetime and must be replaced regularly, which usually involves surgical intervention. With the high risk of introducing the constituents of the battery, that in case of an accidental leak could represent a serious toxic risk for the patient, the first peacemakers were powered by nickel-cadmium (NiCd) batteries [1]. Later, NiCd power sources were replaced with lithium-iodine (LiI) ones, which although having more advantages and being currently used, share the same drawbacks of the common batteries. The main disadvantage of any battery is the possibility of rapid discharge. When the capacity of the battery is running down, not giving enough time to be replaced, the life of the user is put at risk [2].

The development of enzymatic fuel cell (EFC) technology sparked immense interest in the area of alternative power sources, and not just for implantable medical devices [3,4]. Numerous applications were considered, especially for low power devices in off-grid locations [5,6]. EFCs were considered as potential power supplies for charging emergency response kits for camping enthusiasts, powering sensing devices for remote military surveillance or recharging cell phones in underdeveloped regions of the world. Case in point is Sub-Saharan Africa, where cell phones have become an essential part of communication and commerce in an area where more than 70% of the population does not have access to a stable source of electricity (the grid). This technology could use sugars from ubiquitous sources: fruits, tree sap and even sugar packages, to generate electricity and thus represents a good candidate to face the above-mentioned challenges.

Although EFCs theoretically have great application potential in numerous areas, so far they have been commercially explored only for the design of biosensors. The main reason for that is the low electrical output and short operational stability of these systems [7]. During the years of research in EFCs it has been shown that the performance of the bio-cathode is the limiting factor for the overall system operation [8]. As a result, great efforts have been dedicated to the optimization of the enzymatic cathodes, such as design of gas-diffusion electrodes with improved oxygen supply [9–12] and/or modification of the electrode surface for enhanced bio/nano interface interactions [9,13–16]. Currently, the highest performing

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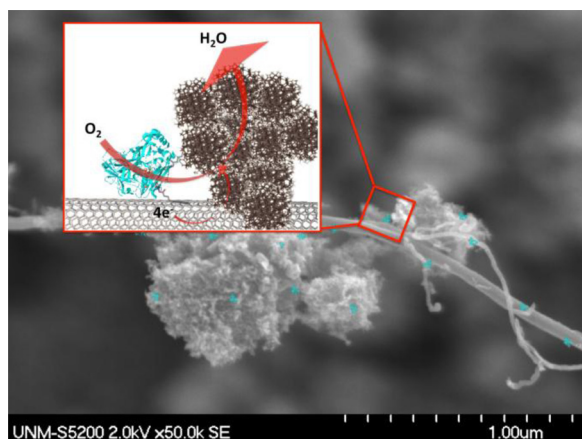[1] ISE member.

**Fig. 1.** Schematic representation of the incorporation of bilirubin oxidase with Fe-AAPyr catalyst, making a hybrid material capable of efficient oxygen reduction.

enzymatic cathodes, which rely on direct electron transfer mechanism, generate current densities in the range of 0.5–0.8 mA cm$^{-2}$ [10,11,16–18] under ambient air conditions and up to 2 mA cm$^{-2}$ with pure O$_2$ [18]. Attempts to utilize this technology in microbial fuel cells have conduced to successful outcomes by our group [19], opening the door for the usage in water purification systems that employ this advances.

The reported herein study is based on the utilization of exceptionally innovative approach for engineering a novel catalytic cathode material. This approach is based on the incorporation of non-platinum (non-PGM) catalyst materials with enzymes, both of which being capable of catalyzing oxygen reduction reaction. The transition metal-nitrogen-carbon material (M-N-C), 4-Amino-antipyrine and iron derived catalyst (Fe-AAPyr), belongs to the group of non-platinum based catalysts synthesized, characterized and optimized by our group [20–22]. This group of catalysts is considered to be most promising candidates for replacing Pt in proton-exchange fuel cells. M-N-C catalysts, synthesized from metal salt and nitrogen/carbon precursor molecules, are capable of caring out oxygen reduction reaction with efficiencies comparable to Pt, especially at high pHs [23]. The newly developed approach of making M-N-C type of catalysts based on the so called "Sacrificial Support Method" allows achieving high density of active sites, easily accessible to oxygen, as well as mitigating the problem with water management [24–26]. The low cost of and availability of the precursors used for the synthesis process makes M-N-C catalysts highly desirable materials for oxygen reduction reaction. This is of prominent importance when designing fuel cells that will be employed in commercial devices. Although M-N-C materials by themselves are state of the art cathodic catalysts, the oxygen reduction carried out by them is characterized with high overpotential at neutral pH, which limits their application in fuel cells operating in physiological conditions. To overcome the disadvantages of purely enzymatic and purely inorganic ORR catalysis, Bilirubin Oxidase and Fe-Aminoantipyrine(Fe-AAPyr) catalyst were incorporated into the design of enzymatic/inorganic "hybrid" electrode for ORR (Fig. 1), which demonstrates low overpotential and increased current densities in a broad range of potentials, under neutral conditions.

## 2. Methods

### 2.1. Catalyst synthesis

Fe-AAPyr catalyst was synthesized using the sacrificial support method (SSM) developed previously by our group [20,22]. This procedure consisted of wet impregnation of the carbonaceous support and iron precursors (4-Aminoantipyrine and Fe(NO$_3$) 3·9H$_2$O, both purchased from Sigma Aldrich) over the surface of fumed silica (Cab-O-Sil$^{TM}$ EH-5, surface area:400 m$^2$ g$^{-1}$). The impregnation occurred under sonication over an 8 hour period of time. Once the suspension was completely homogeneous, it was left to dry at 85 °C for 12 hours, resulting in a solid material that was manually ground using an agate mortar in to fine powder. The powder was then pyrolyzed under UHP nitrogen atmosphere (flow of 100 cc min$^{-1}$) at 800 °C, with a heating rate of 3 °C min$^{-1}$. After pyrolysis, the silica support was etched from the catalyst using a hydrogen fluoride solution (37%, Sigma Aldrich) over a 12 hours period. The excess of HF was washed by DI water until neutral pH and powder was dried overnight at $T$ = 85 °C.

### 2.2. Catalytic ink preparation

Suspensions of 9.5 mg ml$^{-1}$ Fe-AAPyr with various Fe-AAPyr: multi-walled carbon nanotubes (MWNT) ratios in water were prepared. Tetrabutylammonium bromide modified Nafion (TBAB-Nafion, kindly provided by Dr. Shelley Minteer from The University of Utah) was then added to the suspensions to provide adhesion of the ink to the electrode surface. The modified Nafion was dissolved in ethanol before the addition of the catalyst suspension and had a concentration of 79.3 mg ml$^{-1}$. The Nafion-containing suspension was sonicated for 30 minutes to ensure complete dispersion. The next step involved addition of 1-pyrenebutanoic acid, succinimidyl ester (PBSE), suspended in ethanol to a final concentration of 10 mmol dm$^{-3}$. The solution was left to react for 1 hour to allow for π-π stacking of the pyrene moiety of the PBSE and the carbonaceous material [27]. A stock solution (200 mg ml$^{-1}$) containing Bilirubin Oxidase (BOx, Amano, Japan) was prepared by dissolving the enzyme in 0.1 mol dm$^{-3}$ phosphate buffer solution (pH 7.5). A 2 μl aliquot of the BOx stock solution was mixed with 48 μl of the suspension from the previous step and left to react for 16–18 hours in order to allow appropriate time for enzyme immobilization.

### 2.3. Rotating ring disc electrode measurements

The rotating ring disc electrode measurements were performed in a 125 mL glass electrochemical cell using a WEB30-Pine bipotentiostat and a Pine Instruments Rotator (Pine Instruments, Raleigh, NC). The experiment was performed in a standard three-electrode setup with platinum wire and Ag/AgCl acting as counter and reference electrodes, respectively.

The working electrode was glassy carbon rotating ring disk electrode covered with 10 μl of the prepared ink. Electrode was allowed to dry completely under ambient laboratory conditions. The disc potential was swept from 0.8 V to −0.1 V vs. Ag/AgCl and the ring was polarized at 1.1 V vs. Ag/AgCl. The scan rate was 10 mV s$^{-1}$ and a rotating speed of 1600 rpm was chosen to minimize transport limitations. The electrochemical measurements were taken under saturated oxygen conditions. This was achieved by bubbling oxygen through the cell at room temperature and ambient pressure. The electrolyte used for the test was a 0.1 mol dm$^{-3}$ potassium phosphate buffer solution (pH 7.5). Potassium chloride (Sigma Aldrich) was added to this electrolyte to obtain a concentration of 0.1 mol dm$^{-3}$. This buffer solution was prepared in house by using Sigma Aldrich potassium mono-phosphate and potassium disphosphate and HPLC grade water (OMNISOLV EMD). The pH of the solution was verified by Omega PHB600R pH meter.