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Development of a printable laccase-based biocathode for fuel cell applications

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Abstract

Laccases belong to the family of blue multicopper oxidases, which catalyze the four-electron reduction of dioxygen to water concomitantly through the oxidation of phenolic and other aromatic compounds. They are potential enzymes in many applications including biofuel cells to produce electricity through chemical reactions. We have tested here the incorporation of a high redox potential laccase from *Trametes hirsuta* in different types of conducting inks to produce dry printed enzyme electrode layers. ABTS was used as the redox mediator to shuttle the electrons between the surface of the cathodic electrode and the enzyme active sites. Our results demonstrate that the dry printed layers maintained their enzymatic activity even after several months. Furthermore, fuel cell prototypes could be constructed utilising an optimized printed laccase–ABTS layer as the cathode, and printed Zn layer as the anode. Under humidity controlled conditions, a cell voltage between 0.8 and 0.6 V could be maintained for several days under a 2.2 kΩ load. In addition, a corresponding stand-alone cell could be constructed where the cell voltage was maintained for 15 h under a load. These results offer a good starting point for further development of mass-producible, completely enzymatic printed biofuel cells.

Keywords: Biofuel cell; Printing; Enzyme; Oxidoreductase; Mediator; Ink; Carbon nanotube

1. Introduction

Biofuel cells are devices capable of directly transforming chemical to electric energy via electrochemical reactions involving enzymatic catalysis. Various oxidoreductases can be potentially applied as biocatalysts for the anodic or cathodic half cell reactions in biofuel cells (for recent reviews, see Refs. [1,2]). At the bioanode the fuel, such as sugar or alcohol, is oxidised with the help of a suitable oxidoreductase and the electrons are transferred to the anode. At the biocathode the electrons are then transferred to the electron acceptor, typically dioxygen or peroxide, through an enzymatic reaction. The electron transfer can happen either directly between the enzyme and the electrode, or more commonly through a mediated electron transfer where the redox mediators shuttle the electrons between the enzyme and the electrode.

Biofuel cells offer an inexpensive alternative to classical fuel cells, which rely on transition metals as catalysts. Due to the usage of biocatalysts, the cell can also be operated under mild conditions, i.e. near, or at room temperature and neutral pH. In addition, the wide variety of reactions catalysed by enzymes allows a wide range of possible fuel substances to be used. An ideal biofuel cell should generate both a high current and a high potential, and it should be stable enough for the application. Typically, glucose oxidases or glucose dehydrogenases have been used as the anodic enzyme, and peroxidases, laccases, or bilirubin oxidases as the cathodic enzyme in the published biofuel cell studies (for a review, see Ref. [3]).

Abbreviations: ABTS, 2,2′azino-bis(3-ethylbenzthiazoline-6-sulfonic acid); cnt, carbon nanotubes; CV, cyclic voltammetry; IJFC, Impinging Jet Flow Cell; *ThL*, *Trametes hirsuta* laccase; OCV, open circuit voltage; PVA, polyvinyl alcohol; COM, confocal optical microscope; PEM, proton exchange membrane; Ω/square, surface resistivity, independent of the size of a square or its dimensional units.

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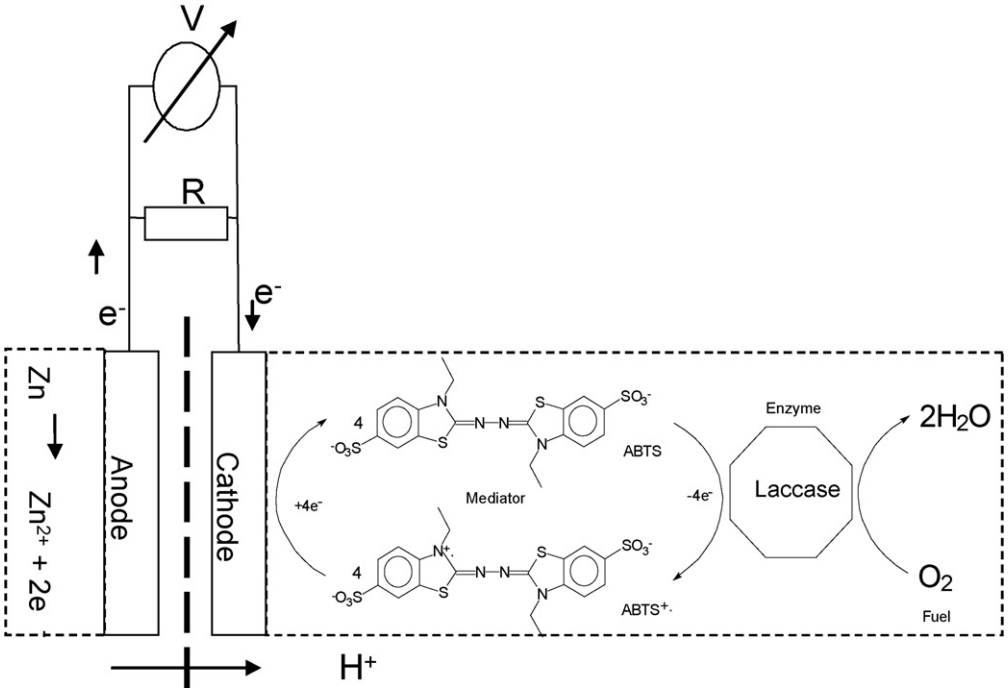

Fig. 1. Schematic structure of the laccase–Zn half fuel cell.

Fungal laccases (*p*-diphenol dioxygen oxidoreductases, EC 1.10.3.2) are oxidoreductases which contain integrated redox-centres in the form of four Cu-atoms, and have, depending on the enzyme, a relatively high redox potential for oxidation of phenolic and other aromatic compounds. Laccases catalyze the four-electron reduction of dioxygen to water concomitantly through the oxidation of substrate molecules (for a review, see Ref. [4]). Laccases are industrially interesting enzymes with application potentials in detergents, pulp bleaching, adhesives, fibre functionalization, detoxification, denim bleaching, textile dye decolourization and baking [5,6]. Furthermore, they have been shown to function in biosensors and in biofuel cells [7–9]. During recent years the three-dimensional structures of several different fungal laccases have been solved giving a more detailed insight in the function of these enzymes [10–12].

Printing enables cost efficient mass manufacturing of electronics and other functionalities on large and flexible substrates like plastic, paper and fabrics. Development of new printable functional materials can be harnessed in several application areas like displays, sensors, power sources and printed RFID [13]. Printed bio-electronic devices have been realised for example by Setti et al. [14,15], who have studied thermal inkjet printing as a tool to deposit enzymes in biosensor applications as a separate layer on top of the conductive surface. Some examples have also been presented describing the incorporation of the biocatalyst directly into the conducting layer as well as the improvement of the biocatalyst stability by ink additives [16–22].

In the work presented in this article we have studied the performance of printable biocathode based on a high redox potential fungal laccase from *Trametes hirsuta*. The properties of the printed biocathode were optimised by varying the ink composition and printing substrate. In the final phase the biocathode was tested and optimised in a printed fuel cell construction using a zinc-based anode (schematic structure in Fig. 1).

2. Materials and methods

2.1. Materials

ABTS (2,2′Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)) was obtained from Roche, ferrocene-carboxyaldehyde from Sigma, and methylsyringate from Aldrich. Silver-based, water-soluble ink and carbon-based, water-soluble ink were obtained from DuPont (product numbers BQ231 and BQ129, respectively). α-Sorbitol was from Sigma. Carbon nanotubes (multiwall, diameter 10–30 nm, purity >80%) were purchased from Hydrocell. The binder (Sicpa 1100) was kindly provided by SICPA. Polyvinyl alcohol (PVA) was obtained from Alfa Aesar. *T. hirsuta* laccase (*ThL*) was partially purified from concentrated culture supernatant using a DEAE anion exchange chromatography. The enzymatic activity of this purified protein preparation was 4400 nkat/ml (on ABTS at pH 4.5, 25 °C) and protein concentration 12.5 mg/ml (measured with Bio-Rad DC protein assay kit).

2.2. Preparation of inks and coatings

Three principal ink types were used as the basis for the enzyme-containing conducting layers (Table 1). Carbon-based ink (Ink 1) was prepared by mixing carbon nanotubes (0.17 mg carbon nanotubes/mg dry ink) and laccase diluted in 50 mM succinate buffer pH 4.5 (0.21 nkat laccase/mg dry ink) into the ink. The consistency of the ink was adjusted by the addition of 50 mM succinate buffer pH 4.5. Silver-based enzyme containing ink (Ink 2), used as a second ink type was prepared in a similar way using 0.24 mg carbon nanotubes and 0.26 nkat laccase/mg of dry ink. When specifically indicated, an electron transferring mediator (ABTS, methylsyringate or ferrocene-carboxyaldehyde), was added to Inks 1 and 2 as a solid powder (0.15 mg/mg carbon nanotubes). A non-commercial, carbon nanotube containing ink was used as the third alternative (Ink 3). This ink was prepared by carefully mixing carbon nanotubes with α-sorbitol (0.28 mg α-sorbitol/mg carbon nanotubes), polyvinyl alcohol (PVA) (0.05 mg/mg carbon nanotubes), binder (3.75 ml Sicpa 1100/mg carbon nanotubes) and laccase (0.86 nkat laccase/mg carbon nanotubes). If specifically