Journal of Power Sources 257 (2014) 272–279

Contents lists available at ScienceDirect

Journal of Power Sources

journal homepage: www.elsevier.com/locate/jpowsour

# Gold electrode modified with a self-assembled glucose oxidase and 2,6-pyridinedicarboxylic acid as novel glucose bioanode for biofuel cells

Malika Ammam [a,b,*], Jan Fransaer [b]

[a] Faculty of Mathematics and Natural Sciences, Products and Processes for Biotechnology – Institute for Technology, Engineering & Management, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands
[b] Department of Metallurgy and Materials Engineering (MTM), K.U. Leuven, Kasteelpark Arenberg 44, B-3001 Heverlee, Belgium

## HIGHLIGHTS

- 2,6-Pyridinedicarboxylic acid is a useful electron mediator for glucose oxidase.
- 2,6-Pyridinedicarboxylic acid and glucose oxidase were assembled on gold electrode.
- In presence of the mediator, glucose oxidation occur 461 mV earlier.
- The non-compartmentalized biofuel cell generated a power output of 25 μW $mm^{-2}$.

## ARTICLE INFO

*Article history:*
Received 18 November 2013
Received in revised form
30 January 2014
Accepted 3 February 2014
Available online 12 February 2014

*Keywords:*
Glucose oxidase
Self-assembled enzyme layers
Glucose bioanode
Biofuel cells

## ABSTRACT

In this study, we have constructed a gold electrode modified with (3-aminopropyl)trimethoxysilane/2,6-pyridinedicarboxylic acid/glucose oxidase (abbreviated as, Au/ATS/PDA/GOx) by sequential chemical adsorption. Au/ATS/PDA/GOx electrode was characterized by Fourier Transform Infrared Spectroscopy (FT-IR) and Electrochemical Impedance Spectroscopy (EIS). The data from FT-IR illustrated deposition of ATS, PDA and GOx on the surface of gold electrode. The latter has been confirmed by EIS which showed that the electron transfer resistance of the electrode increases after adsorption of each supplementary layer. Linear sweep voltammetry (LSV) in phosphate buffer solution containing 5 mM glucose displayed that compared to Au/ATS/GOx, oxidation of glucose at Au/ATS/PDA/GOx electrode starts 461 mV earlier. This gain in potential is attributed to presence of PDA in the constructed Au/ATS/PDA/GOx electrode, which plays some sort of electron mediator for glucose oxidation. The Au/ATS/PDA/GOx electrode was stabilized by an outer layer of polystyrene sulfonate (PSS) and was connected to a Pt electrode as cathode and the non-compartmentalized cell was studied under air in phosphate buffer solution pH 7.4 containing 10 mM glucose. Under these conditions, the maximum power density reaches 0.25 μW $mm^{-2}$ (25 μW $cm^{-2}$) for the deposited GOx layer that has an estimated surface coverage of ~70% of a monolayer.



## 1. Introduction

Enzyme-based biofuel cells (BFCs) utilize enzymes to catalyze chemical reactions, thus replacing traditional expensive metal electrocatalysts such as platinum employed in conventional fuel cells. Furthermore, by contrast to fuel cells where strong or harsh conditions maybe required for cell operation, enzyme biofuel cells generate electricity under mild conditions through the oxidation of renewable energy sources without greenhouse gas emissions or environmental pollution. In that respect, enzyme biofuel cells employ near-room temperature and neutral pH operation [1], which make them not only useful for biomass conversion but also as potential alternative power sources for *in vivo* applications for implantable biomedical devices like miniaturized sensors transmitters and artificial organs. To date, the vast majority of enzyme biofuel cells are based on the electroenzymatic oxidation of glucose

* Corresponding author. Faculty of Mathematics and Natural Sciences, Products and Processes for Biotechnology – Institute for Technology, Engineering & Management, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands. Tel.: +31 503639237; fax: +31 503632990.
*E-mail addresses:* m78ammam@yahoo.fr, m.ammam@rug.nl (M. Ammam).

http://dx.doi.org/10.1016/j.jpowsour.2014.02.007
0378-7753/

at the bioanode and oxygen reduction at the biocathode. However, assembly of enzymes on the electrode surfaces usually does not achieve significant electron transfer between the immobilized enzymes and the current collector or the electrode, mostly because of the electrical insulation of the active sites of the enzyme by the surrounding protein shells [2]. To overcome the problem, electron mediators are introduced to shuttle electrons between the active sites of the enzyme and the electrode surface [3].

Judicious choice of the electron mediator at enzyme bioanodes and biocathodes is essential to maximize BFC power output. Desirable characteristics for electron mediators include: rapid heterogeneous, enzyme-mediator and self-exchange electron transfer rates, for delivery of electrons; and chemical stability in oxidized and reduced forms, to ensure stability of catalytic turnover of enzyme reaction. To date, a large number of electron mediators have been investigated for enzyme biofuel cells with variable degrees of success [4–14]. In this paper, we introduce 2,6-pyridinedicarboxylic acid as a useful electron mediator for glucose oxidase modified gold electrode. The enzyme electrode exhibited reasonable current response and low starting potential of glucose oxidation, promising to be optimized and used as an efficient bioanode in glucose/$O_2$ biofuel cells.

## 2. Experimental

### 2.1. Materials

Ultrapure water milliQ grade with a resistivity of 18.2 MΩ cm was used for all the experiments. Glucose oxidase (GOx) crude from *Aspergillus Niger* (211 units $mg^{-1}$) was purchased from Sigma–Aldrich. Glucose (Glu) was purchased from Acros Organic and the glucose solution was prepared 24 h before use. $H_2O_2$ 60% and $H_2SO_4$ 90% from Acros Organic or Fisher. (3-Aminopropyl)trimethoxysilane 97% (ATS), 2,6-pyridinedicarboxylic acid 99% (PDA) and sodium polystyrene sulfonate (PSS) were from sigma. Phosphate salts ($NaH_2PO_4$ and $Na_2HPO_4$) and sodium chloride analytical grade were purchased from Acros Organic. The buffered saline pH 7.4, used for the testing of the GOx electrodes, was prepared from phosphate salts (0.1 M) and sodium chloride (0.15 M). Gold (Au) electrode rode 1 mm in diameter (surface area 0.78 $mm^2$) is used as a substrate for deposition and working electrode and was purchased from Goodfellow.

### 2.2. Equipment and procedures

Spectrum 100 FT-IR from Perkin Elmer was used for the Fourier Transform Infrared Spectroscopy (FT-IR) studies. A potentiostat from Solartron Instruments model SI 1287 was used for testing the enzymes electrodes using Linear Sweep Voltammetry (LSV). A three compartment electrochemical cell was used. The side arms contained an Ag/AgCl wire as reference electrode and a platinum counter electrode with surface area of 1 $cm^2$. The distance between the gold working electrode and the counter electrode is about 1.5 cm. Measurements were made at room temperature in 5 mL phosphate buffer solution pH 7.4. The gold electrode either before or after modification was polarized between −0.4 V and +0.5 for each LSV. For the Electrochemical Impedance Spectroscopy (EIS) measurements, the potentiostat was used in combination with an SI 1255 HF frequency response analyzer. Voltage and current measurements of the BFCs were carried out with two multimeters from Fluke 87 True RMS. The BFCs current and voltage were varied using a potentiometer (1 MΩ). The units of power density are expressed in μW $mm^{-2}$ because the final goal of these electrodes is to power miniaturized electronic devices for *in vivo* application of which small surface area electrodes are usually employed.

### 2.3. Preparation of the Au/ATS/PDA/GOx electrode

First, the clean and dried Au electrode under Nitrogen flow was treated with piranha solution (mixture of 90% $H_2SO_4$ + 60% $H_2O_2$ at proportion of 3:1 by volume) at a temperature above 90 °C for about 1 h. The electrode was then abundantly cleaned with ultrapure water and dried under nitrogen flow. This step allows a deep cleaning of the Au electrode and may also result in formation of thin layer of gold oxides on top of the gold electrode [15–17]. The clean oxidized and dried Au electrode was then treated with pure ATS by immersing the electrode in ATS solution for at least 15 min. The electrode was then removed, rinsed with ultrapure water and dried under nitrogen flow. Although at this stage it is not clear what happened during this step, our results indicate deposition of a layer of ATS on the gold electrode. On the other hand, presence of amine groups in ATS makes probably the electrode positively charged (Fig. 1) [18–22]. Other scenarios where for example the amino group of aminopropyltrimethoxysilane are adsorbed on hydroxylated surfaces prior to silylation are also possible. Afterward, the Au/ATS electrode was then immersed in 1 mL ultrapure water containing 5 mg PDA for at least 15 min. PDA is chemically adsorbed on ATS via probably electrostatic interactions (Fig. 1). This layer was found to be very important for mediation of glucose oxidation, perhaps due to the aromatic ring in PDA. Afterward, the Au/ATS/PDA was immersed in GOx solution containing 50 mg $mL^{-1}$ ultrapure water for at least 15 min. Though it is not yet clear how PDA is linked to GOx, our results demonstrate that both entities are close enough to communicate and yield better electron transfer. It is worth noting that similar behaviors have been observed elsewhere between pyridines or similar rings and proteins or enzymes [4,22–26]. Finally, the electrode was rinsed gently with ultrapure water and dried. The preparation of the other electrodes used for comparison such as Au/ATS/GOx followed similar process. In other words, the electrode was dipped in same solutions of (ATS, GOx) for 15 min then removed, gently cleaned with ultrapure water and dried.

## 3. Results and discussion

### 3.1. Characterization of Au/ATS/PDA/GOx electrode

Fig. 2 displays FT-IR spectra of Au electrode after modification with ATS (a), ATS/PDA (b) and ATS/PDA/GOx (c). All spectra have been taken with subtracted backgrounds. The chemical adsorption and polymerization of the silane ATS on the Au electrode is visible in the regions of 3521–2607 $cm^{-1}$, 1594–1275 $cm^{-1}$ and 1107–823 $cm^{-1}$ (Fig. 2a). The bands located at 1102 $cm^{-1}$, 1020 $cm^{-1}$ and 956 $cm^{-1}$ are assigned to the SiO–H and Si–O–Si groups [18]. The absorption bands at 907 $cm^{-1}$ and 837 $cm^{-1}$ revealed the presence of Si–O–H stretching and OH vibrations on the surface of Au. The two broad bands at 3352 $cm^{-1}$ and 1660 $cm^{-1}$ can be ascribed to the N–H stretching vibration and $NH_2$ bending mode of free $NH_2$ group, respectively [27,28]. In addition, hydrogen-bonded silanols also absorb at around 3200 $cm^{-1}$ and 3470 $cm^{-1}$ [27,29]. The presence of the anchored propyl group was confirmed by C–H stretching vibrations that appeared at 2935 $cm^{-1}$ and 2839 $cm^{-1}$. On the other hand, as shown in Fig. 2b, chemical adsorption of PDA on the Au/ATS electrode can mainly be distinguished in the region of 1777–1252 $cm^{-1}$, where supplementary enhanced bands appear. The latter will be assigned to presence of additional carboxylic and amine groups from the pyridine ring [30]. A zoom and comparison between the FT-IR spectra of ATS, PDA and ATS/PDA in the frequency range of 1800–1200 $cm^{-1}$ is depicted in Fig. 3 and for comparison, the main transmission bonds of each of ATS, PDA and ATS/PDA are mentioned in caption of Fig. 3. If covalent binding