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Operation of a carbon nanotube-based glucose/oxygen biofuel cell in human body liquids—Performance factors and characteristics

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ABSTRACT

The performance of an enzymatic biofuel cell in physiological media depends not only on the electrode architecture but is also influenced by substances in these media which can be directly oxidized or reduced at the anode and/or cathode or have an impact on the biocatalytic processes.

For the anode construction carbon nanotubes are modified with a polyaniline film onto which pyrroloquinoline quinone dependent glucose dehydrogenase (PQQ-GDH) from *Acinetobacter calcoaceticus* is covalently coupled. The cathode is based on bilirubin oxidase (BOD) from *Myrothecium verrucaria* which is coupled to a PQQ-modified carbon nanotube electrode. The resulting biofuel cell achieves power maxima of more than 100 $\mu W/cm^2$.

Galvanodynamic performance measurements in urine and saliva show a significant loss of the maximum power density compared to an EBFC in 5 mM glucose containing buffer. The EBFC achieves 12% in urine and 18% in saliva. The initial open cell potential of about 710 mV for the EBFC is reduced to 400 mV in urine and to 665 mV in saliva.

To elucidate the effect of potential interfering substances in the real media the individual electrodes are investigated by cyclic voltammetry in human urine and saliva and also in the presence of urea, uric acid and ascorbic acid.

Reasons for the low power output in the both body liquids are the very low glucose concentrations. In urine the cell potential is significantly decreased because of the strong influence of an oxidation process at the cathode. The low anode performance in saliva can be attributed to the diminished biochemical catalysis.

1. Introduction

Over the last few years biofuel cells came into the focus of research due their ability to convert chemical energy from very different sources into electrical energy. One kind of biofuel cells based on microbes is used for the energy conversion from chemical compounds in sewage water [1]. Other biofuel cells are based on the conversion of chemical energy by means of enzymes [2]. Developments of this kind of fuel cells are characterized by rather small dimensions and hence their power output is limited. But they can be used as power supply for several in-situ-applications in medicine and environmental monitoring [3]. In-vivo biofuel cells for the operation of sensors or implantable devices can utilize energy-rich compounds that can be found in human body liquids such as serum, saliva, urine or tear fluid. In contrast to conventional fuel cells enzymatic fuel cells can convert their substrates efficiently in the range of the body temperature und at physiological pH values.

For the final electron transfer on the cathodic site most biofuel cells apply oxygen or peroxides as bio-oxidant. Hence the oxygen reducing multi-copper enzymes laccase and bilirubin oxidase [4,5] or peroxidases [6] are usually applied for the cathode construction. On the anodic site of an enzymatic fuel cell oxidoreductases oxidize a broad spectrum of energy-rich compounds e.g. glucose, cellobiose, fructose, pyruvate, ethanol, methanol or hydrogen [7] (Falk et al., 2012). While in the first biofuel from Yahiro et al. in 1964 [8] glucose oxidase catalyzes the anodic reaction in the last decade many biofuel cell designs apply glucose dehydrogenase, cellobiose dehydrogenase, fructose dehydrogenase, alcohol dehydrogenase and other dehydrogenases for the oxidation of

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the fuel mainly because these enzymes are not disturbed by the final electron acceptor oxygen, thus allowing the construction of membrane-free cells [9].

The performance of biofuel cells depends strongly on an efficient electron transfer. The electrical communication between the biocatalyst and the electrode can be realized by mediated (MET) or direct electron transfer (DET). In MET-based systems a certain potential difference between the enzyme and the mediator has to be ensured which results in a loss of cell voltage. Such a situation can be avoided using a direct electron transfer pathway. But also for DET the cell voltage can be reduced due to over potentials caused by a hindered charge transfer and/or mass transport.

Due to their good biocompatibility and high electrical conductivity different carbon materials are often applied for a direct enzyme electrode contact [10] and [11]. In order to create a high number of docking sites for the enzymes with good substrate accessibility favorably carbon nanotubes are used. Nanostructured carbon architectures have been reported in various versions as for example on gold [12], on glassy carbon [13], on silicon [14], as functionalized graphene [15] or as pressed CNTs in bucky paper [16]. Improved binding characteristics of the rather hydrophobic CNTs for enzymes can be achieved by chemical oxidation [17], by plasma treatment [18], by Π stacking [19], by electrochemical methods [20], by sonication [21] and [12] or a modification with conducting polymers [22]. Besides redox polymers with defined redox centers conducting polymers have been used for coupling the biocatalyst to the electrode and "wire" the enzymes to the electrode. Polythiophene [23], polypyrrole-derivatives [24] and polyaniline-derivatives [14] are characterized by a high biocompatibility which can result in a high activity of the bound biocatalyst.

Usually the development of biofuel cells is accompanied by the search for optimal conditions in the surrounding buffer. But in an in-vivo-application as power supply for sensors and medical devices the performance depends strongly on the composition of the physiological media. Early studies already showed the influence of uric acid – a normal constituent of sera – on BOD based cathodes [25]. But there are already several examples of biofuel cell applications in living organisms [26–29], which clearly demonstrate the general feasibility. But power output is often much smaller than in pure buffer under optimized conditions. The present study uses a glucose/oxygen biofuel cell with a PQQ-GDH/polyaniline/CNT-anode and a BOD/PQQ/CNT-cathode as reported previously [14] and investigates their performances in two body liquids – urine and saliva – in order to elucidate factors influencing the behavior of both electrodes. This shall help to understand the EBFC behavior and identify factors limiting the performance of the cell under such applied conditions.

2. Experimental

2.1. Materials

Samples from human urine and saliva have been obtained from in.vent DIAGNOSTICA GmbH, Germany. Bucky Paper (BP) has been provided from Buckeye Composites (NanoTechLabs, Inc.), USA. The apo-enzyme of soluble pyrroloquinoline quinone dependent glucose dehydrogenase (PQQ-GDH) is a kind gift from the Roche Diagnostic GmbH, Germany. It is recombinantly expressed in *E.coli*. Pyrroloquinoline quinone (PQQ) is provided from Wako Pure Chemical Industries. Poly(3-aminobenzoic acid-co-2methoxyaniline-5-sulfonic acid) – short PABMSA – has been received by a chemical oxidation process as described in Sarauli et al., 2013. Bilirubin oxidase (BOD), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS) are provided by Sigma Aldrich GmbH, Germany. Calcium chloride ($CaCl_2$), sodium hydroxide (NaOH), hydrochloric acid (HCl) and citric acid are from Carl Roth GmbH & Co. KG, Germany. 2-(N-morpholino)ethansulfonic acid (MES) is purchased from Applichem GmbH, Germany. Disodium hydrogen phosphate and sodium dihydrogen phosphate are provided from Merck KGaA, Germany. All aqueous solutions are prepared with purified water from the water system "ultra clear direct" (SG Water, Germany).

2.2. Anode preparation

For reconstitution of PQQ-GDH 2 mg of the apo-enzyme (20 μM) are dissolved in 1 ml 5 mM MES with 1 mM $CaCl_2$ containing PQQ in an equimolar ratio. This solution is incubated for 3 h at room temperature and stored in aliquots of 50 μl at −20 °C until use. After reconstitution the specific activity is determined to be 240 U/mg (solid). BP stripes with a width of 1 mm are incubated in a 5 mM PABMSA solution in 5 mM MES with 1 mM $CaCl_2$ at pH 6.5 for 1 h. For a covalent fixation the PABMSA modified BP-electrodes are transferred into a 100 mM EDC/25 mM NHS solution (in H_2O) for 15 min. After this they are incubated in the 20 μM PQQ-GDH solution for 1 h and washed 3× in 100 mM CiP, pH 7. For storage the PQQ-GDH/PABMSA-BP electrodes are kept at 4 °C.

2.3. Cathode preparation

3.75 mg of BOD (about 1.2 mg pure protein) with a specific activity of 22 U/mg(protein) are dissolved in 1 ml 100 mM citrate phosphate buffer (CiP), pH 7 with a final concentration of 20 μM and stored in aliquots of 30 μl at −20 °C. Before enzyme coupling 1 mm-BP stripes are transferred into a 5 mg/ml PQQ containing 100 mM CiP buffer (pH 7) and stored for 1 h at room temperature. Then the carboxylic groups of the PQQ are activated in an aqueous EDC/NHS solution (100 mM/25 mM) for 15 min. For the final covalent fixation the activated PQQ-BP-electrodes are incubated in the 20 μM BOD solution for 1 h and washed in 3× in 100 mM CiP, pH 7. The BOD-PQQ-BP electrode are stored at 4 °C.

2.4. Electrochemical characterization

The galvanodynamic measurements for the fuel cell characterization are performed with the potentiostat Reference 600 from Gamry Instruments, USA. A scan rate of 3 nA/s has been applied to the two enzyme electrodes because lower scan rates did not show any influence on the power density curves. For all fuel cell application measurements an 1 ml-home-made electrochemical cell has been used. All electrochemical measurements are performed at room temperature.

The voltammetric characterizations of the individual electrodes are performed in an 1 ml-measuring cell made by our workshop using the potentiostat CHI1230B, USA. A three-electrode configuration is used consisting of a working CNT electrode (8 mm^2), an Ag/AgCl, 1 M KCl reference electrode (Microelectrodes Inc., USA) and a platinum wire as counter electrode. For the measurements with and without glucose and in the presence of potential inferring substances 100 mM CiP pH 7 has been applied as electrolyte.

3. Results and discussion

3.1. EBFC behavior in urine and saliva

The biofuel which has been developed previously [14] is schematically shown in Fig. 1. The anode is based on carbon nanotubes modified with a sulfonated polyaniline to which PQQ-GDH is covalently coupled. This ensures electron flow from the substrate-reduced enzyme directly via the polymer to the