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# Enzymatic biofuel cell based on anode and cathode powered by ethanol

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ABSTRACT

Enzymatic biofuel cell based on enzyme modified anode and cathode electrodes are both powered by ethanol and operate at ambient temperature is described. The anode of the presented biofuel cell was based on immobilized quino-hemoprotein-alcohol dehydrogenase (QH-ADH), while the cathode on co-immobilized alcohol oxidase (AOx) and microperoxidase (MP-8). Two enzymes AOx and MP-8 acted in the consecutive mode and were applied in the design of the biofuel cell cathode. The ability of QH-ADH to transfer electrons directly towards the carbon-based electrode and the ability of MP-8 to accept electrons directly from the same type of electrodes was exploited in this biofuel cell design. Direct electron transfer (DET) to/from enzymes was the basis for generating an electric potential between the anode and cathode. Application of immobilized enzymes and the harvesting of the same type of fuel at both electrodes (cathode and anode) avoided the compartmentization of enzymatic biofuel cell. The maximal open circuit potential of the biofuel cell was 240 mV.



## 1. Introduction

The production of electrical power from low-cost biofuels is an important challenge in the energetics, since biofuels are renewable, sustainable, offer lower greenhouse-gas emissions and reduce the demand for common fuel sources, these are of special interest of modern-energetics (Pizzariello et al., 2002). As the majority of organic substrates undergo combustion with the evolution of energy, the biocatalyzed oxidation of organic substrates by oxygen at electrode interfaces provides a means for the conversion of the chemical energy to an electrical one (Willner et al., 1998a). Significant concentrations of ethanol and other alcohols are often present in the various biological substances (Lim and Wang, 2003). Basic fermentation processes might be applied to increase concentration of alcohols in these substances. The chemical energy of abundant biological substrates including polyhydroxylic (Arechederra et al., 2007) and monohydroxylic alcohols (Ikeda and Kano, 2001; Ramanavicius et al., 2005) may be converted into electrical current by biofuel cells. The advantage of biofuel cells when compared to standard fuel cells is that biofuel cells are able to operate at a low substrate concentration which can be even at the micromolar level (Pizzariello et al., 2002). In the future biofuel cells can be used as alternative energy supply sources for biosensors and for other bioelectronic devices (Willner et al., 2001). These cells may also be used as power sources of implantable devices (Barton et al., 2004). The combination of bioelectronics with nanotechnology allows integration of biofuel cells within the operating devices, while the nanotechnology offers novel perspectives for the miniaturization of bioelectronic devices and the increase of their efficiency (Willner et al., 2001; Wang et al., 2005). Microorganisms and/or enzymes are catalysts that are able to convert chemical energy to electrical energy (Katz et al., 1999), for this reason they are important subjects of biofuel cells (Bullen et al., 2006). The most efficient biofuel cell designs allow operation's without compartments dividing membranes (Chen et al., 2001; Ramanavicius et al., 2005). It allows applications of biofuel cells as portable power sources. Biofuel cells utilize biocatalysts for the conversion of chemical energy to electrical energy (Katz et al., 1999; Chen et al., 2001; Mano et al., 2002, 2003). The methodology based on the application of purified red-ox enzymes for the targeted oxidation and reduction of specific fuel and oxidizer substrates at the electrode supports and the generation of the electrical current output is used for the development of biofuel cells (Chen et al., 2001).

There are known several red-ox enzymes able gain electrons from various alcohols: methanol (Zhang et al., 2006a,b), ethanol (Ramanavicius et al., 2006a), glycerol (Lapenaite et al., 2006) and other alcohols (Ivnitski et al., 2006). Alcohol oxidases (AOxs) utilizes oxygen as natural electron acceptor thus producing hydrogen peroxide (Ramanavicius et al., 2006b; Malinauskas et al., 2005). Alcohol dehydrogenases are using NAD/NADH or artificial red-ox

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mediators based system for re-oxidation of its active site. It was demonstrated that both types of alcohol harvesting enzymes could be applied in the design of biofuel cells, however usually they are applied for designing of biofuel cell anode. The majority of oxidases and dehydrogenases require the application of red-ox mediators to establish direct electron transfer (DET) with electrode (Habermüller et al., 2000; Malinauskas et al., 2004).

Among the other types of enzymes, some pyrroloquinoline quinine (PQQ)-dependent red-ox enzymes were also employed for the construction of biofuel cells (Ikeda and Kano, 2003). However the majority of PQQ-dependent enzymes are unable to transfer electrons without additional red-ox mediators (Lapenaite et al., 2006; Habermüller et al., 2000). The detailed characterization of the interfacial electron transfer rates is essential in the construction of biofuel cells, since the enzyme and electronic conductors are foreign components in respect of one to the other, that leads to a lack of electric current between them. The modification of PQQ-dependent enzymes with covalently attached red-ox mediators can be applied to facilitate electron transfer from active site of enzyme towards electrode (Laurinavicius et al., 2004). An alternative to this is to apply DET-able enzymes (Gorton et al., 1999). The red-ox enzymes containing heme-c are very promising in this context (Freire et al., 2003; Ramanavicius et al., 2006a). Quino-hemoprotein-alcohol dehydrogenase from *Gluconobacter* sp. 33 (QH-ADH) demonstrates DET toward glassy carbon (Ikeda et al., 1993), other forms of carbon (Razumiene et al., 2002), gold (Ikeda et al., 1993) and conducting polymer polypyrrole (Ramanavicius et al., 1999). Another class of enzymes, heme-c containing peroxidases, are able for direct electron transfer and also are very promising for construction biofuel cell cathode (Willner et al., 1998a,b; Ferapontova and Gorton, 2001). Direct electrochemistry of microperoxidases with a gold electrode (Willner et al., 1998a), carbon electrodes (Ruzgas et al., 1996), along with platinum electrodes modified with carbon nanotubes (Wang et al., 2005), were investigated and exploited in the design of biofuel cell cathode. However, DET-based enzymatic biofuel cell utilizing the same substrate at both electrodes is still a challenge.

The aim of this study was to design basic, non-compartmentalized, mediator free biofuel cell based on enzymes exhibiting direct bioelectrocatalysis and able to convert the chemical energy of biological substrate – ethanol – at both cathode and anode.

## 2. Experimental

### 2.1. Chemicals

Alcohol dehydrogenase from *Gluconobacter* sp. 33 (E.C. 1.1.99.8) was isolated and purified at the Institute of Biochemistry (Vilnius, Lithuania). The enzyme had an activity of 171 U/ml and 7.6 mg/ml concentration of proteins. Microperoxidase-8 (MP-8) from horse heart 250 U/mg; AOx from *Pichia pastoris* (E.C.1.1.3.13), 50 U/ml, 25% glutaraldehyde and 96% ethanol were purchased from Sigma (Berlin, Germany). Carbon rod electrodes "Ultra F purity" 3 mm in diameter obtained from Ultra Carbon Division of Carbon USA, RAVEN-M were used. Carbon rod electrodes were sealed into epoxy to prevent the contact of electrode side surface with the solution.

### 2.2. Preparation of electrodes

Graphite electrodes modified with enzymes were prepared as follows: (i) rods of spectroscopic graphite were cut, and polished on fine emery paper, followed by rinsing the electrode surface with ethanol and water. Electrodes were dried at room temperature before coating with enzyme; (ii) enzyme solutions were deposited on the electrode surface and the coated electrodes were kept for 20 h over the 5% solution of glutaraldehyde at 4 °C in the closed vessel as it was previously described (Ramanavicius, 2007). In preparing the anode, 6 μl of QH-ADH solution was thoroughly distributed on the surface of the electrode and kept over glutaraldehyde solution. In preparing the cathode, 3 μl of MP-8 solution (10 mg/ml) was thoroughly distributed over the electrode and dried for 30 min. Afterwards, 3 μl of AOx solution (10 mg/ml) was deposited and the electrode was kept over glutaraldehyde. Control carbon electrode modified with MP-8 was prepared by deposition of 3 μl of MP-8 solution (10 mg/ml) and cross-linked by glutaraldehyde using the same cross-linking protocol.

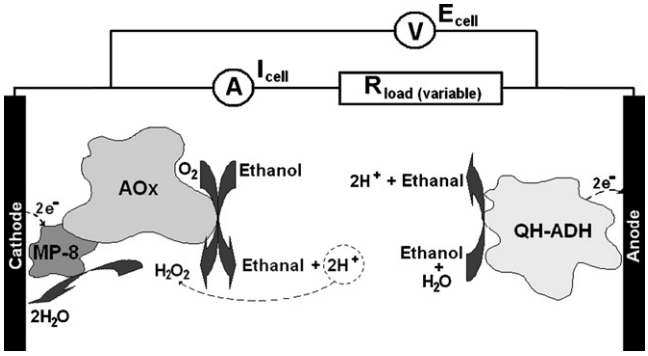


**Fig. 1.** Configuration of biofuel cell, using ethanol as a fuel for cathode and anode.

### 2.3. Electrochemical measurements

All electrochemical measurements were performed by potentiostat–galvanostat PGSTAT 30 using specialized software GPES3 v3,2 Echochemie/Autolab (Utrech, Netherlands). Two-electrode circuit was applied for registration of electrical potential between electrodes. Some potentiometric measurements were performed at open circuit; during some other experiments the resistors were switched into the external circuit (Fig. 1) to imitate circuit-load on biofuel cell.

## 3. Results and discussion

Current developments in nanotechnology enables serious thoughts about nano-devices and even nano-robots, but in many cases such systems require miniature power sources (Chen et al., 2001) otherwise such devices will be very limited in function and application. Major idea of the work presented here was to apply environmentally friendly and/or biodegradable materials. This concept does not allow us to apply any soluble red-ox mediators or hazardous red-ox polymers. Direct electron transfer exhibiting hemoproteins (Gorton et al., 1999) were selected for this study to avoid application of any additional environmentally and/or biologically hazardous red-ox materials. Enzymes MP-8 and QH-ADH were selected for the design of biofuel cell as the catalysts. These enzymes are able to transduce chemical energy into electrical one and to transfer electrical current directly to the carbon electrodes. Carbon is selected as a matrix-material, because various carbon forms are very often applied in various nanotechnological applications (Malinauskas et al., 2005). Moreover carbon is especially suitable for application in bioelectronic devices since it supports sufficient electron transfer due to proper enzyme orientation (Shleev et al., 2005). The QH-ADH is comprised of three subunits (Fre'bortova et al., 1998). The ability of QH-ADH to transfer electrons directly towards the electrodes was employed for generation of anodic current in amperometric biosensors (Ramanavicius et al., 1999) and later this process was applied in biofuel cell har-