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# A Direct Electron Transfer-Based Glucose/Oxygen Biofuel Cell Operating in Human Serum

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## Abstract

We report on the fabrication and characterisation of the very first direct electron transfer-based glucose/oxygen biofuel cell (BFC) operating in neutral glucose-containing buffer and human serum. *Corynascus thermophilus* cellobiose dehydrogenase and *Myrothecium verrucaria* bilirubin oxidase were used as anodic and cathodic bioelements, respectively. The following characteristics of the mediator-, separator- and membrane-less, *a priori*, non-toxic and simple miniature BFC, was obtained: an open-circuit voltage of 0.62 and 0.58 V, a maximum power density of ca. 3 and 4 $\mu$W cm$^{-2}$ at 0.37 and 0.19 V of cell voltage, in phosphate buffer and human serum, respectively.

**Keywords:** Bilirubin Oxidase, Cellobiose Dehydrogenase, Direct Electron Transfer, Enzymatic Fuel Cell, Implantable Device

## 1 Introduction

It is inconceivable that already four decades ago, Beltzer and Batzold predicted a significant role of implantable biofuel cells (BFCs) operating on fuels and oxidants present in blood plasma as potential sources of electrical energy for artificial devices [1]. Much research and a large number of review articles on the design and characterisation of miniature BFCs based on mediated electron transfer (MET) have been published [2–8]. In spite of the obvious advantages of $\beta$-D-glucopyranose/oxygen BFCs (glucose/$O_2$ BFCs) based on direct electron transfer (DET) reactions as power sources for implantable devices (i.e. less-toxicity due to the absence of redox mediators, simple construction and ability for significant miniaturisation), there is no published work describing a mediator-, cofactor- and membrane-less BFC operating in human physiological liquids. Moreover, there are only few reports describing the performance of even well-studied mediator or/and cofactor-based glucose/$O_2$ BFCs in complex biological liquids, i.e. in human serum [9, 10]. Our previous intensive investigations of the DET properties of different oxidoreductases, including carbohydrate dehydrogenases [11, 12] and blue multicopper oxidases (BMCO) [13], allowed us to fabricate and characterise the very first DET-based glucose/$O_2$ BFC optimally working at pH 4.5 [14]. Importantly, the basic characteristics of the non-optimised DET-based BFC were significantly better compared to the MET-based lactose/$O_2$ BFC constructed using similar bioelements (cf. lactose/$O_2$ BFCs in Table 1). These DET- and MET-based devices, however, operated under acidic conditions because both bioelements used in the BFCs, viz. cellobiose dehydrogenase (CDH) from *Dichomera saubinetii* (*Ds*CDH) [11] or from *Trametes villosa* (*Tv*CDH) [12] and laccase (Lc) from *Trametes hirsuta* (*Th*Lc) [11] or from *Cerena unicolor* (*Cu*Lc) [12], have their bioelectrocatalytic activity optima at acidic pH values [14, 15].

CDH is an extracellular enzyme produced by a variety of different fungi. The enzyme consists of two domains connected by a linker region. The catalytic domain contains one flavin adenine

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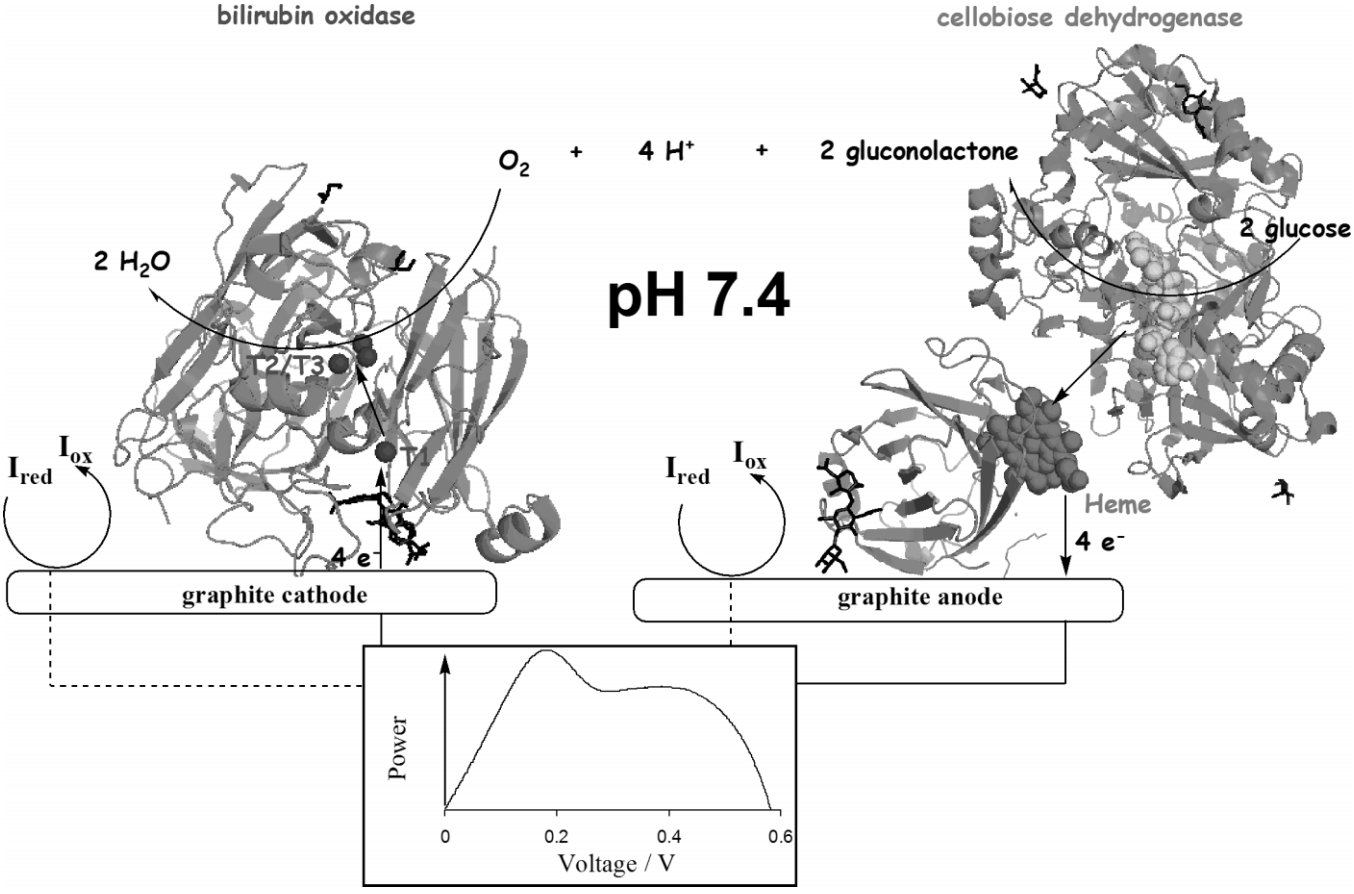


Fig. 1 A principal scheme of a mediator-, compartment- and soluble cofactor-less glucose-$O_2$ BFC operating in human serum and chloride-containing neutral buffers. The three-dimensional structure of BOx was visualised based on the structures of *B. subtilis* Lc (PDB 1UVW) using PyMOL v. 0.99. The flavin and heme domains of *Phanerochaete chrysosporium* CDH (PDB 1D7D and PDB 1NAA, respectively) represent the possible structure of *Ct*CDH. The protein globules are given as green ribbons and strands, carbohydrates are presented as black sticks, the four copper ions of BOx are shown as blue spheres, the FAD in CDH – yellow spheres, and the heme – red spheres.

dinucleotide (FAD) and the heme domain contains one heme *b* as cofactors (Figure 1, right part). CDH oxidises cellodextrins and lactose at the FAD domain and may, depending on its origin, also oxidise monosaccharides such as glucose [16]. The reduced FAD domain can be reoxidised directly by various electron acceptors or the electrons can be sequentially transferred to the heme domain, which in turn donates the electrons directly to the electrode acting like a 1 $e^-$ acceptor [17].

Our preliminary intensive studies allowed us to isolate and purify a new CDH from the ascomycete *Corynascus thermophilus* (*Ct*CDH), which has, in contrast to *Ds*CDH and *Tv*CDH used in the previously designed BFCs [14, 15] (Table 1), a high catalytic activity for glucose oxidation at neutral pH values.

BMCO is a group of copper-containing enzymes, such as ascorbate oxidase (AOx), bilirubin oxidase (BOx), sulochrin

Table 1 Comparison of CDH/BMCO-based BFCs.

| BFC | Anode/cathode | Conditions | Open circuit voltage (V) | Cell voltage (V) | Power output ($\mu Wcm^{-2}$) | Operation half-period (h) | Ref. |
|---|---|---|---|---|---|---|---|
| Glucose/$O_2$ 5 mM/0.25 mM | *Ds*CDH-DET/*Th*Lc-DET | $Cl^-$-free buffer, pH 4.5 | 0.73 | >0.5 | > 5 | > 38 | [14] |
| Cellobiose/$O_2$ 5 mM/0.25 mM | *Ds*CDH-DET/*Th*Lc-DET | $Cl^-$-free buffer, pH 4.5 | 0.76 | 0.55 | 11 | Not reported | [14] |
| Lactose/$O_2$ 5 mM/0.25 mM | *Ds*CDH-DET/*Th*Lc-DET | $Cl^-$-free buffer, pH 4.5 | 0.77 | 0.55 | 15 | Not reported | [14] |
| Lactose/$O_2$ 34 mM/0.25 mM | *Tv*CDH-MET/*Cu*Lc-MET | $Cl^-$-free buffer, pH ~ 4 | 0.6 | <0.3 | <2 | Not reported | [15] |
| Glucose/$O_2$ 5 mM/0.25 mM | *Ct*CDH-DET/*Mv*BOx-DET | PBS, pH 7.4 | 0.62 | 0.37 | 3 | >6 | Present study |
| Glucose/$O_2$ ~5 mM/~0.25 mM | *Ct*CDH-DET/*Mv*BOx-DET | Human serum | 0.58 | 0.19 | 4 | <2 | Present study |

  

oxidase (SOx), Lc and ceruloplasmin (Cp), catalysing the oxidation of different organic and inorganic compounds with the concomitant reduction of $O_2$ to $H_2O$ at different pH optima. The substrate specificity of these enzymes can be reflected in their trivial names, e.g. BOx is responsible for the oxidation of bilirubin to biliverdin [18, 19]. Any crystal structure of BOx has not yet been published; however, the biochemical, spectral, electrochemical and kinetic characterisations of *Myrothecium verrucaria* BOx (*Mv*BOx) have been done and some primary structures of BOx are also available (Table 2). Based on the similarity between BOx and other BMCO regarding their main biochemical, spectral and kinetic properties [18, 19], as well as in their primary structures (Table 2), it is widely held that the catalytic site of BOx consists of four copper ions per molecule, classified into three types denoted as T1, T2 and T3. The T2 and T3 sites form a trinuclear cluster, where one molecule of $O_2$ is bound and reduced to two $H_2O$ molecules [18]. The mononuclear T1 copper is able to accept electrons from reduced substrates (homogeneous reactions [18, 19]) or electrodes (heterogeneous reactions [13, 20]), which in turn are transmitted to the trinuclear centre *via* the highly conserved Cys-2His electron transfer (ET) pathway across a distance of ~13 Å. Such ligand arrangements are found in the structures of AOx [21], Lc [22] and Cp [23], and are also hypothesised to exist in BOx (Table 2; Figure 1, left part).

It should be emphasised that some characteristics of fungal BOx are very promising to design efficient and potentially implantable biocathodes. First, fungal BOx constitute high redox potential BMCO [24]. Second, these enzymes are not so strongly inhibited by chloride ions ($Cl^-$) and they remain very active at neutral pH, two properties which are shared with the low redox potential BMCO, e.g. plant and bacterial Lc. Indeed, in Figure 1 (left part) the three-dimensional structure of BOx is visualised using the available crystal structure from *Bacillus subtilis* Lc (*Bs*Lc), since the primary structures of both bacterial and fungal enzymes are also quite similar (Table 2).

Below, we describe the fabrication and characterisation of the very first mediator and cofactor free glucose/$O_2$ BFC based on adsorbed enzymes exhibiting direct anodic and cathodic bioelectrocatalysis, i.e. fungal *Ct*CDH and *Mv*BOx, operating in neutral chloride-containing buffers as well as in human serum (Figure 1).

Table 2 Comparison between the amino acid subsequences of *Mv*BOx and *B. subtilis* Cota Lc.

| Enzyme | Copper site subsequences |
|---|---|
| BOx (BAA02123, BAA03166) | $^{131}$V *H* L **H** G$^{135}$ |
| | $^{169}$L W Y **H** D **H** A M$^{176}$ |
| | $^{435}$T H P I *H* I **H** L$^{442}$ |
| | $^{494}$**H** C **H** N L I H E D H D M M$^{506}$ |
| Cota Lc (PDB 1UVW) | $^{104}$V *H* L **H** G$^{108}$ |
| | $^{150}$L W Y **H** D **H** A M$^{157}$ |
| | $^{418}$T H P I *H* I **H** L$^{425}$ |
| | $^{491}$**H** C **H** N L I H E D H D M M$^{503}$ |

The ligands of the T1, T2 and T3 coppers are underlined, italicised and bolded, respectively.

## 2 Experimental

### 2.1 Chemicals

Unless otherwise specified, all chemicals were purchased from Sigma–Aldrich GmbH (Schnelldorf, Germany). All solutions were prepared using water purified with PURELAB UHQ II system from ELGA Labwater (High Wycombe, UK) or with a Milli-Q system (Millipore, Milford, CT, USA).

### 2.2 Enzymes

*Mv*BOx was kindly provided by Amano Enzyme Ltd. (Nagoya, Japan), stored at –20 °C, and used without further purification. *Ct*CDH was purified from the culture supernatant of the ascomycete *C. thermophilus*, CBS 405.69 obtained from the *Centralbureau voor Schimmelcultures* (Baarn, The Netherlands). Cultivation and purification of the enzyme were similar to previously reported protocols for *Myriococcum thermophilum* CDH [17]. The homogeneous preparation of the enzyme was stored in a 50 mM acetate buffer, pH 5.5 at –80 °C.

### 2.3 BOx Catalytic Assay

The catalytic activity of *Mv*BOx in the absence and presence of urea, $HCO^{3-}$, $Mg^{2+}$ and $Ca^{2+}$ was determined spectrophotometrically (UV–Vis spectrophotometer Anthelie Advanced, Topac Inc., Cohasset, MA, USA) in a reaction medium containing 2 mM 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS, $\varepsilon$ = 36,000 $M^{-1}$ $s^{-1}$) or 5 mM ferrocyanide ($K_4[Fe(CN)_6]$, $\varepsilon$ = 1,040 $M^{-1}$ $s^{-1}$) in 10 mM phosphate buffer (PB) pH 7.4, at room temperature. The volume of the reaction mixture was 1 mL and the concentrations of different compounds in the reaction medium corresponded to their physiological concentration in human serum (*vide infra*).

### 2.4 Electrochemical Measurements

#### 2.4.1 Electrode Preparation

The enzyme-modified electrodes serving as working electrodes were made from rods of spectrographic graphite electrodes (SPGE, Ringsdorff Werke GmbH, Bonn, Germany, type RW001, 3.05 mm in diameter). The surface of the SPGE was prepared by first polishing with fine emery paper (Tufback Durite, P1200), then thoroughly rinsed with Millipore water and finally allowed to dry.

#### 2.4.2 Electrode Modification

The biocathodes and the bioanodes were prepared by placing an aliquot of 10 µL of enzyme solution (BOx, 10 mg $mL^{-1}$, CDH, 7.2 mg $mL^{-1}$) on the electrode surface and the solutions were air-dried at room temperature for 20 min. Before use, the electrodes were rinsed with Milli-Q water to remove weakly adsorbed enzyme.

  

*2.4.3 Electrolytes*

The main buffer was phosphate-buffered saline (PBS; 2 g of KCl, 80 g of NaCl, 17.8 g of $Na_2HPO_4 \cdot 2H_2O$, 2.4 g of $KH_2PO_4$ in 1 L of Milli-Q $H_2O$, sterilised by autoclaving), pH 7.4, with or without 5 mM glucose, which was dissolved in the buffer a few hours before use to allow mutarotational equilibrium. Human serum, originated from one apparently healthy male volunteer, was prepared as known in the arts and stored at +4 °C until use. For serum preparation, 9NC tubes from BD Vacutainer® (Plymouth, UK) were used. The serum was assumed to have a total protein, glucose, dissolved oxygen, urea, $HCO^{3-}$, $Mg^{2+}$ and $Ca^{2+}$ concentrations of about 70 mg $mL^{-1}$, 1 mg $mL^{-1}$, and 8 mg $L^{-1}$, 30 mg $L^{-1}$, 15 30 mg $L^{-1}$, 1 mg $L^{-1}$ and 1 mg $L^{-1}$, respectively [25–27]. The maximum physiological concentrations of two main possible interfering compounds (I, Figure 1), such as ascorbic and uric acids, were assumed to be 0.11 and 4.0 mM, respectively [27].

*2.4.4 Voltammetry*

Linear scan voltammograms and cyclic voltammograms (CVs) were recorded with a scan rate of 1 and 10 mV $s^{-1}$ in an electrochemical cell of 50 mL containing a Ag | AgCl | $KCl_{sat}$ (197 mV *vs.* NHE) reference electrode (Radiometer, Copenhagen, Denmark) and a platinum foil counter electrode operated by an electrochemical analyser (BAS CV 50W, Bioanalytical Systems, West Lafayette, IN, USA).

*2.4.5 Studies of pH-profiles of the Enzymes Absorbed on SPGE*

The CDH-modified SPGE was fitted into a Teflon holder and inserted into an electrochemical flow cell. The enzyme electrode was used as the working electrode, a Ag | AgCl (0.1 M KCl) electrode as the reference electrode and a platinum wire served as the auxiliary electrode. The electrodes were connected to a three-electrode potentiostat (Zäta Electronics, Lund, Sweden). A 0.1 M PB was used as the carrier and was propelled by a Gilson peristaltic pump (Minipulse 2, Villiers-le-Bel, France) at a flow rate of 0.5 mL $min^{-1}$. A working potential of +300 mV *versus* Ag | AgCl was applied to the working electrode [28]. One hundred microliters of a 20 mM glucose solution in buffer were injected into the carrier stream *via* a LabPRO six-port Rheodyne injection valve (PR700-100-01, Rheodyne, CA, USA) and the output signal was recorded on a strip chart recorder (Kipp and Zonen, type BD111, Delft, The Netherlands).

The dependence of the registered bioelectrocatalytic currents of $O_2$ reduction by BOx-modified SPGE on solution pH was studied previously [29].

*2.4.6 Open Circuit Potential Measurements*

For open circuit potential measurements, an Autolab PGSTAT 30 (EcoChemie, Utrecht, The Netherlands) equipped with GPES 4.9 software was used. The reference electrode was a Ag | AgCl | $KCl_{sat}$ electrode and the enzyme-modified electrodes were used as the indicator electrodes placed in a one-compartment 50 mL electrochemical cell. The equilibrium potential values were registered under air saturated conditions.

*2.4.7 Studies of the Biofuel Cell Performance*

In BFC studies, the Autolab PGSTAT 30 was used in potentiostatic mode. Polarisation curves were recorded at 1 mV $s^{-1}$ connecting the bioanode (CDH-modified SPGE) as the working electrode and the biocathode (BOx-modified SPGE) as a combined reference and counter electrode. For the time stability tests, an external load of 1 MΩ was connected between the two electrodes and the potential was measured with time without stirring. The power output of the cells in stability tests was calculated from the potential and resistance values according to Ohm's law. In this work all potentials are given *versus* NHE.

## 3 Results and Discussion

To fabricate a simple BFC producing electric power based on DET bioelectrocatalytic reactions occurring on both the anode and the cathode parts, two bioelements highly active at neutral pH (Figure 2), namely *Ct*CDH and *Mv*BOx, were exploited (Figure 1). One of the most attractive electrode elements for a potentially implantable BFC is a carbon material, which is cheap, abundant and biocompatible. To design this BFC, SPGE were chosen, as such electrodes are well-characterised [30] and widely used for bioelectrochemical studies of variety of enzymes including different CDH [17, 28, 31, 32] and BOx [20, 29], on which both bioelements showed excellent DET-based bioelectrocatalysis (Figures 2 and 3).

The proposed mechanisms of the bioelectrocatalytic reactions for both CDH and BOx directly adsorbed on SPGEs are

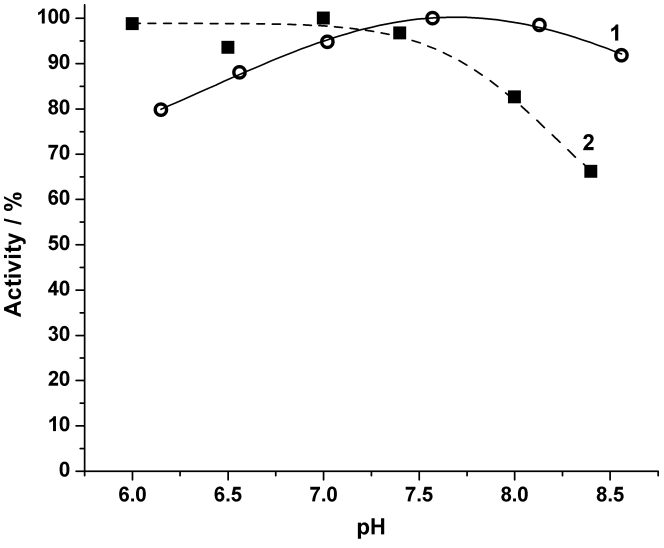


Fig. 2 Relative dependence of DET-based bioelectrocatalytic current on pH at *Ct*CDH- (circles, curve 1) and *Mv*BOx-modified SPGE (squares, curve 2).

  

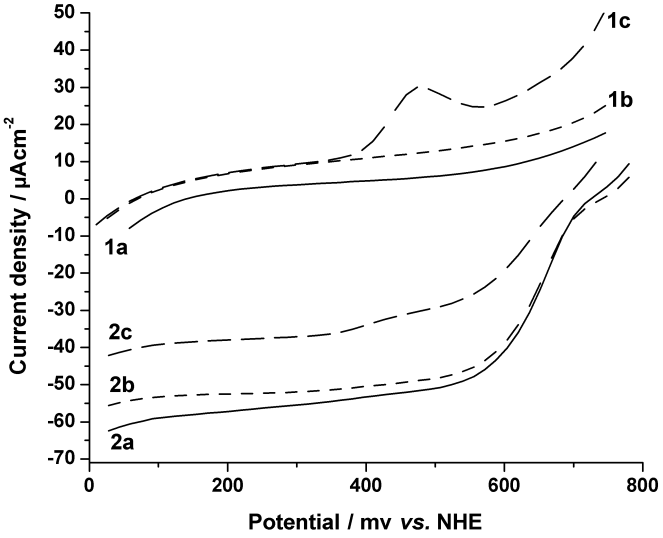


Fig. 3 Linear sweep voltammograms of CDH- (curves 1) and BOx-modified (curves 2) electrodes. The measurements were performed in batch mode in air-saturated serum (curves c) and PBS with (curves b) and without (curves a) 5 mM glucose.

described in our previous papers [11–13, 20]. A principal scheme of the designed BFC (Figure 1) is therefore similar to that of the already fabricated and characterised BFC operating in acidic solutions [14]. The major difference and novelty of the newly fabricated device is its ability to produce electric power in neutral chloride-containing solutions including complex biological fluids, such as human serum (*vide infra*).

As can be seen from the voltammograms (Figure 3), electrocatalytic currents at the electrodes modified with the enzymes start at 75 and 720 mV *versus* NHE for the *Ct*CDH- and *Mv*BOx-modified SPGEs, respectively. The starting potentials for $O_2$ bioelectroreduction (Figure 3) and the steady-state potential of *Mv*BOx-modified SPGE (Table 3) in the presence of $O_2$ coincide well at about 720 mV. This value is quite close to the half-wave potential of bioelectroreduction of $O_2$ (Figure 3), which is equal to the redox potential of the T1 site of *Mv*BOx, 670 mV *versus* NHE [24], the primary electron acceptor of the enzyme. The redox potential of the heme group, the electrochemically active domain of *Ct*CDH acting as the electron donor to the bioanode, is not yet known. However, based on the starting potentials of glucose bioelectrooxidation, on the steady-state potentials of *Ct*CDH-modified SPGEs in the absence and presence of glucose (Table 3), as well as on previous studies of other *Ct*CDHs from different fungi [11, 12, 14, 17, 28, 32, 33], one can assume that the redox potential of the heme group is close to 150 mV *versus* NHE. The differences between the steady-state potentials of the *Mv*BOx- and *Ct*CDH-SPGEs determine the maximal open circuit potential of the BFC operating in glucose-containing PBS and human serum, i.e. ca. 0.62 V and 0.58 V, respectively (Table 1).

The limiting biocatalytic current densities of the BOx-based biocathode significantly differed in buffer solution *versus* human serum, to some extent in agreement with previously published data concerning the reversible deactivation or even irreversible damage of BOx-catalysed homogeneous and heterogeneous reactions by serum components, e.g. the combination of urate/$O_2$ [34–36]. In contrast, the current output from the bioanode was almost the same in both serum and glucose-containing PBS solution (Figure 3).

The starting potentials for $O_2$ bioelectroreduction (Figure 3), the steady-state potential of *Mv*BOx-modified SPGE (Table 3) and the open circuit potential of the BFC were all decreased by ca. 40 mV in serum compared to simple neutral chloride-containing buffer solutions because of partial enzyme inhibition by several compounds presented in a complex natural buffer solution – human serum. Many organic compounds and inorganic ions presented in serum could affect the specific activity of *Mv*BOx, e.g. urea, $HCO_3^-$, $Mg^{2+}$, $Ca^{2+}$ (Table 4). It should be emphasised that the power output of the BFC increased insignificantly, when the glucose containing PBS was strongly mixed. This indicates a reaction limitation of the whole system by the catalytic activity of the immobilised enzymes and only to a limited extent by mass transfer limitation. Thus, partial inhibition of the enzyme resulted in detectable changes of the bioelectrocatalytic activity of adsorbed BOx, which influenced some basic parameters of CDH/BOx-based BFC (*vide infra*) including its open circuit potential.

Figure 4 shows the dependence of the cell voltage *versus* the power density of the designed BFC. In a quiet glucose-containing buffer solution, the maximum power density was 3.0 $\mu$W cm$^{-2}$ at 370 mV of the cell voltage. The shape of the curve was very similar to the previously reported voltage/power dependences of CDH/BMCO-based devices [14, 15]. The electrical power produced by *Ct*CDH-DET/*Mv*BOx-DET BFC was originated from two bioelectrocatalytic processes occurring simultaneously on the electrodes, viz. bioelectrooxidation of glucose on the anode and bioelectroreduction of $O_2$ on the cathode. In a quiet serum, one of the maxima on the voltage/power curve was also close to 3.0 $\mu$W cm$^{-2}$ (Figure 4). However, an additional maximum at 190 mV of the cell voltage appeared with the highest power density of BFC close to 4 $\mu$W cm$^{-2}$. Detailed electrochemical studies of

Table 3 Open circuit potentials (mV, *vs.* NHE) of SPGEs modified with CDH or BOx in air-saturated liquids.

| Conditions | Anode | Cathode |
|---|---|---|
| PBS, pH 7.4 | 155 | 720 |
| PBS, pH 7.4 + 5 mM glucose | 110 | 725 |
| Blood serum | 100 | 680 |

Table 4 Catalytic activity of *Mv*BOx ($k_{obs}$, s$^{-1}$) in the absence and presence of different organic and inorganic compounds.

| Compound | ABTS | $K_4[Fe(CN)_6]$ |
|---|---|---|
| PB, pH 7.4 | 31 | 63 |
| Urea | 25 | 56 |
| $HCO_3^-$ | 21 | 54 |
| $Mg^{2+}$ | 31 | 55 |
| $Ca^{2+}$ | 27 | 53 |

  

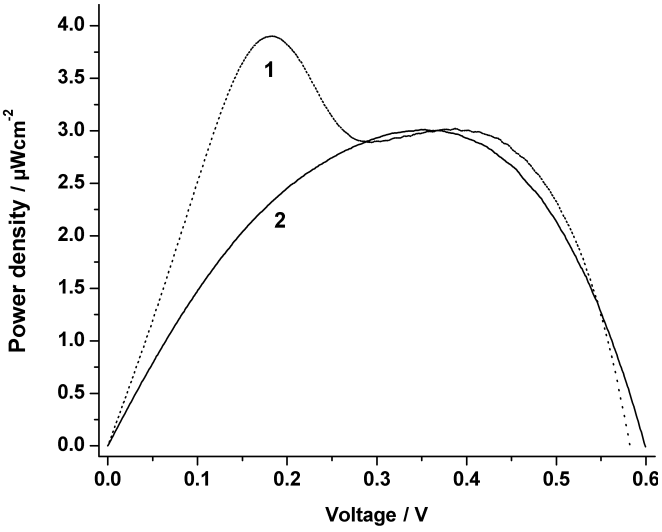


Fig. 4 Dependence of power density on operating voltage for the membrane-less BFC in quiet conditions. As fuels human serum (curve 1) and 5 mM glucose-containing PBS (curve 2) were used.

the anodic and the cathodic electrochemical reactions (Figures 3 and 5) explain significant differences in the BFC performance in complex human serum *versus* simple glucose-containing PBS solution (Figure 5).

In addition to the bioelectrocatalytic processes of glucose oxidation and $O_2$ reduction, non-enzymatic electrocatalytic reactions occur on both bioelectrodes submerged in serum. These non-enzymatic reactions correspond to electrochemical transformations of different electroactive compounds, e.g. electro-oxidation of uric and ascorbic acids (Figure 5), which are present in human serum at high physiological concentrations [27]. The peak height of the electro-oxidation wave of uric acid at a concentration of 0.4 mM (i.e. at normal concentration in serum of an adult male) coincides quite well with the peak height obtained by electro-oxidising serum from the apparently healthy male volunteer (cf. curves 3 and 4 in Figure 5). The electro-oxidation of ascorbic acid on SPGE starts at a potential of 140 mV *versus* NHE (Figure 5, curve 2). Both the starting potentials and current densities on the CVs of the SPGEs submerged in PBS with ascorbic acid and human serum coincide well in the potential window of 140–250 mV, i.e. at the initial part of the CVs recorded with the SPGE in PBS with ascorbic acid at a normal physiological concentration (cf. curves 2 and 4 in Figure 5). The shape of the CV of the SPGE in serum along with the significant current densities of the anodic and especially the cathodic waves (curve 4 in Figure 5) compared to the CVs of the SPGE recorded in PBS with ascorbic and uric acids point to the fact that some other electrochemically active compounds might be present in human serum. The nature of these compounds is unclear. However, the non-enzymatic electrochemical reactions of interfering substances (I; Figure 1) along with the bioelectrocatalytic processes occurring simultaneously on the electrode surfaces result in an additional maximum of 0.19 V on the power–voltage curve with the highest maximal power density of the device close to 4 $\mu$W cm$^{-2}$ (Figure 4).

An important factor of the designed BFC is its operational stability because a potentially implantable BFC should have a long lifetime. An estimated half-life of the BFC operating in simple PBS and human serum are ca. 6 and 1.5 h, respectively (Figure 6).

Both CDH and BOx are known to be stable biocatalysts. The substantial loss of the BFC power in the first few hours of operation is mostly related to the desorption of loosely bound enzymes from both bioelectrodes [14]. In serum, which has a very high concentration of human proteins and low molecular weight organic and inorganic compounds, two additional processes had a negative effect on the operating stability of BFC, viz. possible exchange of non-covalently bound

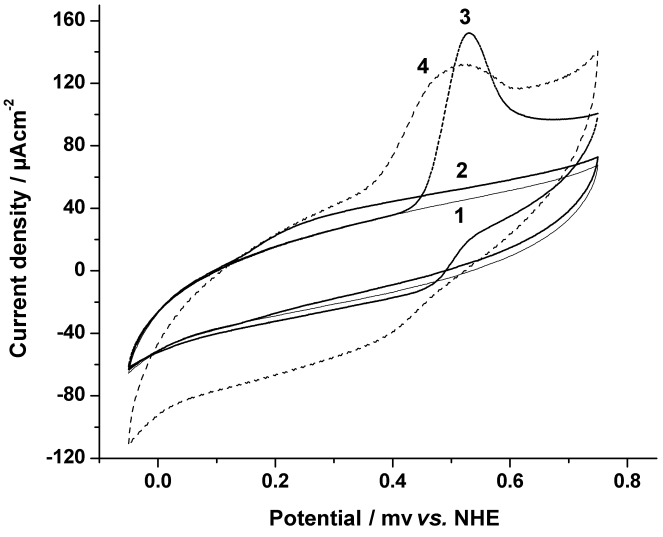


Fig. 5 CVs of an SPGE electrode in air-saturated PBS (curve 1), PBS with 0.11 mM ascorbate (curve 2), PBS with 0.4 mM urate (curve 3) and human serum (curve 4).

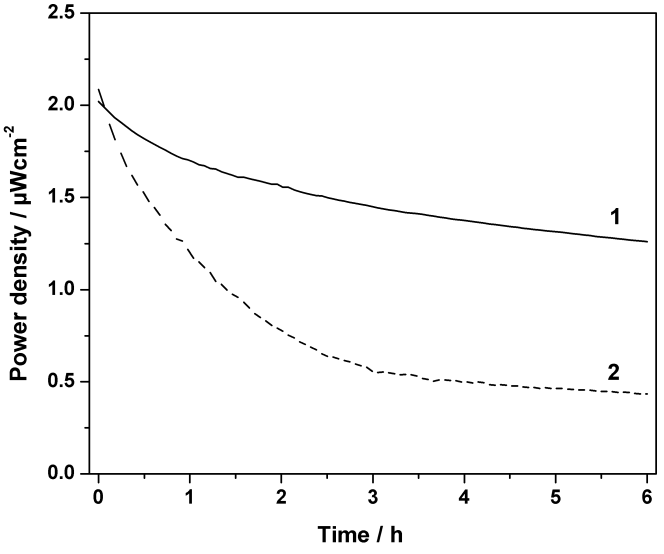


Fig. 6 Variation of power density of the BFC with time in air-saturated PBS, pH 7.4, with 5 mM glucose (curve 1) and human serum (curve 2).

  

enzymes (CDH and BOx) from both bioelectrodes and reversible deactivation/irreversible damage of the biocathode by the combination of urate/$O_2$ [34–36]. In addition, inhibition of adsorbed BOx by inorganic ions could also decrease the stability of the device (*vide supra*). After equilibration, i.e. enzyme desorption, inhibition, damage and exchange, the signal of the cell in both electrolytes, viz. buffer and even serum, is still substantial and it has reached a stabilised level (Figure 6).

In the future, covalent binding of CDH and BOx along with the usage of nanotechnological achievements might significantly improve both the operational stability and the power density of the BFC, e.g. as it was recently achieved for CDH-based bioanodes [37, 38] and Lc-based biocathodes [39].

## 4 Conclusion

Herein, we report the fabrication and characterisation of a mediator and soluble cofactor free, non-compartmentalised, glucose/$O_2$ BFC operating in human serum and PBS glucose-containing solutions. BFCs designed in this work and in our previous studies [14] can serve as simple not yet optimised models to construct non-toxic potentially implantable miniature DET-based glucose/$O_2$ BFCs working in physiological fluids of different compositions and pHs.

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