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THE PROGRESS IN BIO-ELECTRODE'S DESIGN: FROM BIOSENSORS TO BIOFUEL CELLS - AN OVERVIEW

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ABSTRACT

Bioelectrochemistry emerged as a multidisciplinary field, resulting from the symbiosis between biotechnologies and electrochemistry, which also benefits from the recent progress in material science and especially in nanotechnologies. The two mainstreams of this interdisciplinary research area are: i) the development of electroanalytical devices with embedded biological components for molecular recognition (biosensors); and ii) biofuel cells - small-scale energy producing devices that utilize at least one electrode reaction catalyzed by a biological catalyst.

The present work reports on some different approaches in engineering bioelectrodes' surface in order to meet the requirements of each particular application: for biosensors, in addition to the high sensitivity and selectivity, those are low noise level, low background current as well as a very small energy consumption, while the developments for fuel cell applications require long-term operational stability of the bio-catalyst as well as an ability to extract high power density from the bioelectrode. The design of a biofuel cell is illustrated by a three-step improvements made to a cellobiose dehydrogenase – laccase biofuel cell (fed on lactose as a fuel and air as oxidizing agent), in order to enhance its extractable power density through: i) improving the immobilization procedure of the enzyme used on the anode; ii) expanding the potential difference between anode and cathode by tuning the redox potential of the corresponding electrode processes; and iii) up to 200 times magnified current density as a result of engineered with nano-structures electrode surfaces.

Keywords: bio-electrodes, biosensor, biofuel cell; redox reactions.

Comparing bio-electrodes for applications in biosensing and in biofuell cells: The idea to derive electricity from biocatalysed redox reactions pioneered some four decades ago, when the first attempts to use bacterial metabolism in designing microbial biofuel cells (BFC) have been made [1]. Since now, however, the poor power characteristics of these devices limit their practical use to only few examples.

With the help of nanotechnology, this drawback has been partially overcome in the enzyme–based BFCs. Usually enzyme biocatalyst assemblies on electrode surfaces do not achieve significant electron transfer communication between the redox center and the conductive support, mostly because of the electrical insulation of the biocatalytic site by the surrounding protein shells. Despite the development of biofuel cell devices has not been extensive, research in biocatalytically modified electrodes, particularly for sensor applications, has provided substantial technological foundation for current biofuel cell development [2]. Technical requirements for biosensors and biofuel cells, concerning chemical and mechanical stability, selectivity, and cost of materials are practically overlapping. However, these two technologies diverge in the area of energy supply, in that sensors are generally energy-consuming cells and biofuel cells must be energy producers. This significant difference leads to differing technical requirements, primarily in the areas of current density and cell potential. First, similarly to an electrolysis cell, sensors operate at cell potentials greater than open circuit. Second, cell current must be minimized to minimize power consumption. Generally, sensors are designed with currents in the nanoampere to microampere range such that power consumption is very small even for cell potentials near 1 V. Often, cell potential in a sensor must also be minimized to avoid undesired side reactions.

In contrast, as an energy-producing cell, an enzymatic fuel cell has to generate maximum power, i.e. both current and potential must be high enough. Cell materials and structure must be designed such that overpotentials due to kinetics, ohmic resistance, and mass transfer are minimized and current density, particularly in terms of current per unit area, is maximized. A second issue that distinguishes biofuel cells from sensors is stability. Often, biocatalyzed electrochemical sensors are inexpensive enough to be single-use (disposable), and hence, long-term stability is not essential. Should stability be required, one approach is to encapsulate the biocatalytic species in a low-porosity hydrophilic material. Depending on the enzyme, entrapment of the molecule can result in reduced activity and often restrict the mobility of reactants and products, leading to mass-transfer limitations in the electrode. This might be a desired result in an amperometric sensor, where mass-transfer-limited signals are often linearly related to reactant concentration, but in an energy producing device is an unwanted effect since will result in lowered power output. Therefore, although sensor designs can act as starting point for biofuel cell development, the demands of high power and stability ultimately lead the biofuel cell design process down an independent path.

Some general principles in the design of a biofuel cell

Biofuel cell is a galvanic cell, which uses bio-catalysed electrochemical reaction(s) to produce electricity. A typical assembly of the conventional fuel cell consists of at least five elements: two electrodes- cathode at which the oxidizer is reduced electrochemically and anode where the electrooxidation of the fuel occurs; a semi-permeable membrane separating the electrode compartments so that the

crosstalk between the cathode and anode reactions is avoided; two nozzles for fuel and oxidiser supply, a case for placing all these elements, a seal, *etc.*. The construction of a biofuel cell, however, could be considerably simplified avoiding the membrane, case, seal, etc. and putting together only the two bio-electrodes in the working medium (Fig.1), provided that both biocatalytic electrodes share compatible working conditions with respect to pH optima, ionic strength, suitable electrolytes, etc. and the corresponding electrode reactions are highly selective towards their substrates i.e. any crosstalk between the electrodes is excluded. Either oxygen/air or hydrogen peroxide can act as an oxidizing agent in biofuel cells, while a vast variety of organic compounds, such as acids, sugars, alcohols, esters and many other renewable organic compounds are available as fuel.

As a rule the cathodic reaction occurs at a relatively high electrode potential and the anodic oxidation takes place at much lower potentials. The difference between these electrode potentials produces the cell voltage:

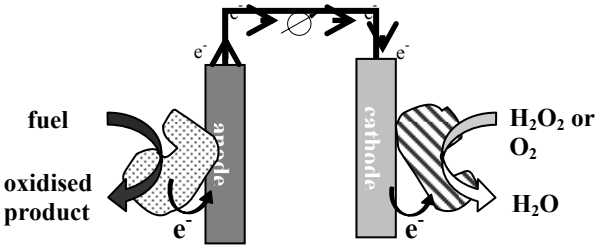

Figure 1. *Scheme of a membrane-less biofuel cell.*

$U_{cell} = E_c - E_a$. The bigger the difference, the higher the power output. The power extractable from a BFC is calculated from the expression: Power density = $U_{cell} \times i$, where $i = I/A_{electrode}$ is the resulting current I divided by the electrode area ($A_{electrode}$).

Optimisation of a cellobiose dehydrogenase/laccase biofuel cell

In this part is discussed how the architecture of a bio-electrode reflects its current density and how different electrode architectures affect the performance of an electrode reaction in particular and, in perspective, of the biofuel cell as a whole. The optimization process is illustrated with a membrane-free BFC assembled from an anode modified with cellobiose dehydrogenase (*Trametes villosa, Tv*CDH) and a laccase (*Cerena unicolor*)–based cathode. Cellobiose dehydrogenase (CDH) is a two-domain oxidoreductase consisting of a catalytic FAD-containing domain connected to a heme-domain via a flexible linker. Its catalytic action targets the oxidation of complex sugars – mainly di–saccharides, such as cellobiose and lactose. The extensive bio-availability of the later makes them promising renewable fuels for green energy production. On the cathode side of the biofuel cell, the electrode was modified with a high potential laccase as biocatalyst for the reduction of molecular oxygen to water *via* 4-electron transfer mechanism. Excellent direct electron transfer (DET) properties were previously proven for both cellobiose dehydrogenase [4,5] and laccase [6] enzymes, i.e. with both enzymes biocatalytic electrodes could be produced through a simple adsorption on the electrode surface. Carbonaceous surfaces such as graphite materials, are excellent for this purpose because they are very "friendly" with the immobilized proteins, allowing them to keep in adsorbed state their conformation close to the native one and hence to retain high heterogeneous catalytic activity. The very first attempt to construct a BFC was made

with one bio-anode working in direct electron transfer mode and a bio-cathode consisting of laccase entrapped into an Os-containing redox polymer with a high formal potential (Fig.2). The polymer matrix in which laccase was immobilized played a triple role: to extend the operational lifetime of the immobilized enzyme, to enhance the surface load of enzyme and to mediate the electron exchange between the graphite surface and laccase active site thus increasing the resulting cathodic current density several times as compared to just adsorbed enzyme. As it could be seen from Fig.2, however, such assembly results in very poor current density (in the nanoampere range) even upon addition of lactose concentrations exceeding 30 mM in intensely aerated buffer solutions.

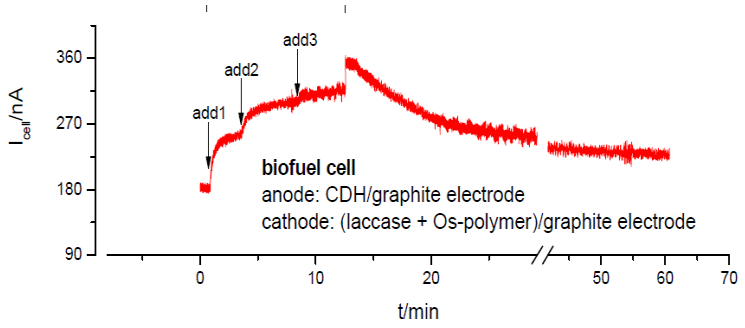

Figure 2. *Current output (original record) of a BFC assembled from adsorbed CDH- bioanode and laccase trapped in Os-redox polymer* **biocathode, pH 4.3.**

The entrapment of target enzymes within a three-dimensional network of a redox hydrogel, such as Os-containing redox polymers, is offering the advantage of a potentially extended operational lifetime of the enzymes by providing a stable micro-environment (constant pH, ionic strength, etc.) and fixation into a restricted conformation that will reduce the susceptibility of the individual enzyme molecule to unfolding and denaturation [7]. Moreover, these redox polymers are capable of connecting even remote enzyme molecules and, as a consequence, ensure an enhanced electron flow from the active sites of the polymer-entrapped enzyme molecules to the electrode, using Os-containing polymers as redox relays. Several Os-complex modified redox polymers with formal potentials ranging between -50 and +200 mV were tested for electrically wiring *Tv*CDH, and one Os-complex modified redox hydrogel with a formal potential of 80 mV lower than the formal potential of T1-Cu site of the laccase (+530 mV *vs.* Ag/AgCl at pH 3.0 [3]). The redox polymers employed for anode modification were tailored by varying the hydrophobic/hydrophilic properties of their polymer backbones as well as by introducing different flexible spacers between the Os-complexes and the polymer backbone aiming at optimal electron-transfer communication between the polymer entrapped enzyme and the electrode surface. In order to achieving an optimal performance of the BFC, several important issues were examined as related to the chosen polymers [8, 9]: the enzyme to polymer ratio, the pH working range, the lactose concentration and the type of oxidizing agent – air or pure oxygen. The obtained power characteristics of a biofuel cell with the improved bioanode performance as compared to the above example, are depicted at Fig.3.

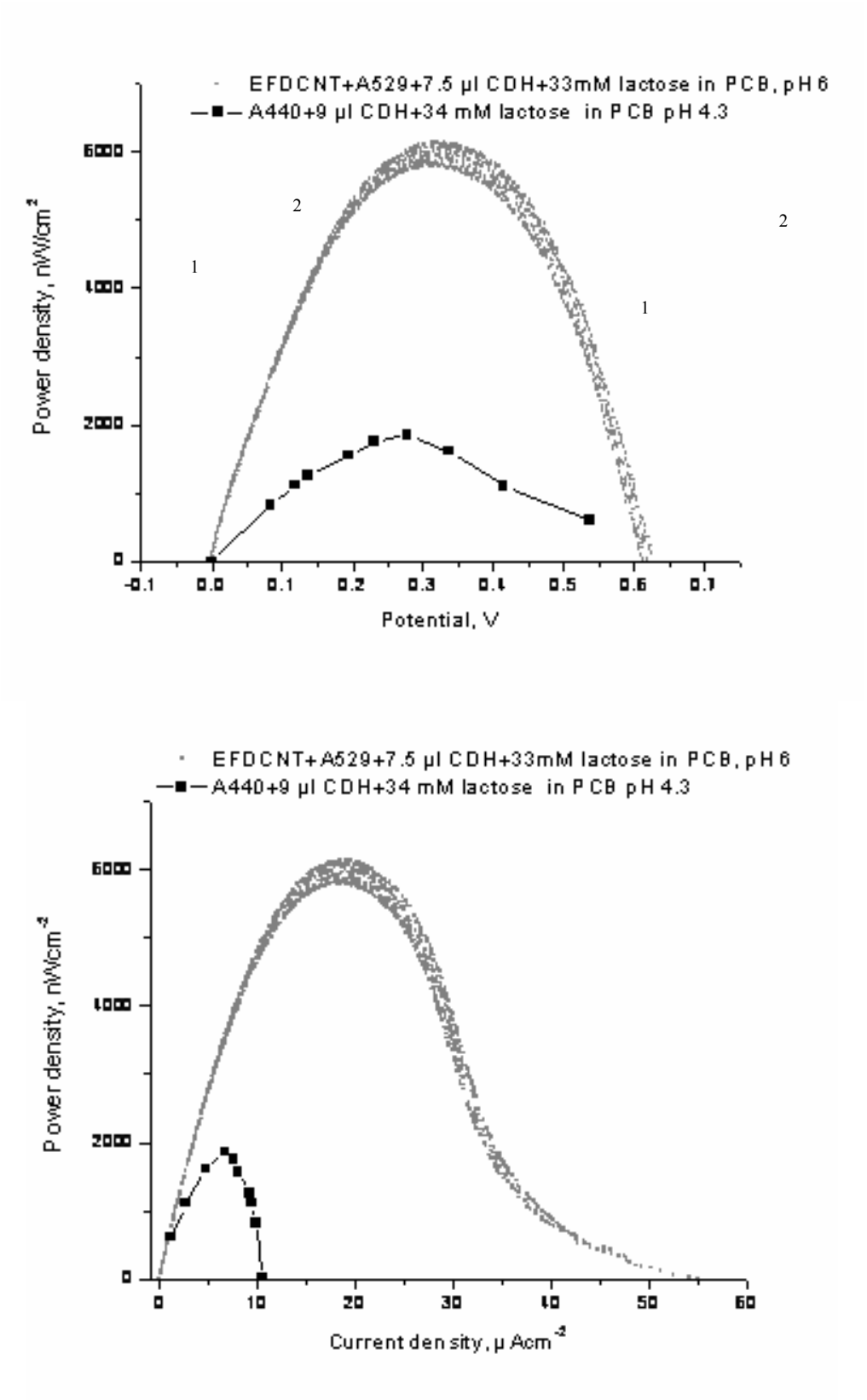

Figure 3. *Dependence of the power density on cell voltage (left) and on current density (right) for a BFC assembled from cellobiose dehydrogenase/Os redox polymers bioanode and laccase/Os redox polymer biocathode. The redox polymers used for entrapment of CDH were with formal potentials respectively ca. +200 mV (1) and -50 mV vs Ag/AgCl (2).*

The enhanced power output of so constructed biofuel cell is due mainly to expanded potential difference between both electrodes – from about 300 mV when using bioanode modified with positive-redox potential Os-containing polymer to ca. 600mV when using Os-containing redox polymer with negative potential for bioanode design.

Schematically the processes taking place at the polymer modified electrodes can be represented as follows:

– on the cathode side: oxygen is biocatalytically reduced to water via a 4-electron mechanism; simultaneously with the biocatalytic process, the active site of the enzyme switches from reduced to oxidized state at a high redox potential which is pH dependent; to regenerate the reduced active site of the enzyme, electrons are coming from the Os-redox polymer through a hopping mechanism with concomitant change of the oxidation state of the Os ions (OsIII / OsII). The redox potential at which the redox transformation of the polymer happens is only 50 mV less positive than the redox potential of the enzyme biocatalytic site and is pH independent. The electron flow initiating the cascade of reactions comes from the negatively charged electrode.

– on the anode: lactose, the substrate of CDH is oxidized to the corresponding lactone with a simultaneous change of the redox state of the cellobiose dehydrogenase enzyme active site. The redox potential of the later is pH dependent. Meanwhile, the electrons via a hopping mechanism are transferred through the Os-polymer to the electrode surface. Analogously to the above processes the redox potential of Os-polymer used for bioanode development is pH independent.

Coupling these two reactions into the suitable medium and supplying it with fuel (lactose) and oxidizer (air) ensures that the electrons will flow through the external circuit. However, despite the extended potential difference between the anode and cathode when tuning the redox potential of the anode to more negative values, the power extractable from this biofuel cell is not enough for practical use.

Further improvements of the bioanode design were made with the help of nanotechnologies. To create a highly developed electrode surface a bunch of carbon microfibers was grown on the top of a 5-mm graphite rod and then additionally these microfibers were branched through formation of carbon nanotubes on each particular fiber. As a result of such a modification, the electrode impedance sharply decreased and the electrode surface rose about 200 times. The enzyme layer was retained over the nanostructured surface using layer-by-layer electrode architecture, i.e. the negatively charged nanostructures were first covered with a layer of positively charged polymer, and then the negatively charged enzyme CDH was easily assembled onto polymer-covered surface due to the electrostatic interactions.

The resulting current of such a bioanode was found to exceed 2 mA in lactose rich solution – a value which is more than 1000 times higher than the ones obtained with previously considered electrode architectures. In terms to create a biocathode with comparable characteristics, so that the overall performance of the biofuel cell is

not limited by either electrode, further optimization of the cathode side should be considered.

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