Electrochimica Acta 126 (2014) 104–114

Contents lists available at ScienceDirect

Electrochimica Acta

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

New trends in enzyme immobilization at nanostructured interfaces for efficient electrocatalysis in biofuel cells

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ARTICLE INFO

Article history:
Received 24 May 2013
Received in revised form 15 July 2013
Accepted 16 July 2013
Available online 31 July 2013

Keywords:
Biofuel cell
Electrode modification
Nanostructuration
Electrochemistry
Enzyme

ABSTRACT

Biofuel cells, and among them enzymatic biofuel cells, are expected to take part in a sustainable economy in a next future. The development of such biodevices requires significant improvements in terms of efficiency of enzyme immobilization at the electrodes, so as to enable direct electron transfer, and to increase and stabilize the current densities. Many works during the last years aimed at reaching higher current densities, thus power densities, while increasing the long term stability of the enzymatic bioelectrodes. Search for new enzymes, wild type or mutants, new entrapment procedures, but also new electrode architectures, have been targeted. This review focuses on the materials developed and involved during the last few years to meet these demands *via* nanostructuration of electrode interfaces. Discussion is essentially focused on cases where direct electron transfer between enzymes and electrochemical interfaces are involved. After having introduced the main reasons for the need of nanostructuration, the materials and methods that are newly developed are described. The consequences on improved performances for enzymatic bioelectrodes are discussed, and finally major challenges for future research are addressed.

1. Introduction

In the current energetic context in which the depletion of usual fossil fuel resources is expectable, fuel cells have attracted great interest because they allow the use of renewable and inexpensive fuels with high energy density and safety. However, the need for noble metals such as platinum to catalyze the reactions that convert chemical energy into electricity is a limiting factor, as these metals are expensive, scarcely available and exhaustible. In the last century, technologies have emerged in which they are replaced by whole microorganisms or parts of these latter, leading to microbial, mitochondrial or enzymatic biofuel cells (BFCs). Some recent reviews by Walsh et al., Schuhmann et al., Minteer et al. and Cosnier et al. are particularly relevant in that latter domain [1–4].

In enzymatic BFCs (EBFCs), at least the catalyst at the anode is a redox enzyme. This kind of BFCs was first introduced in 1964 by Yahiro et al. [5] and although this field has undergone many developments since then, especially in the last decade, there is still scope for improvement. Enzymes are expected to be better candidates as biocatalysts than microbes, not only because of their excellent intrinsic properties such as specificity toward substrate, high catalytic activity with low overvoltage for substrate conversion, mild operating conditions like ambient T° and near-neutral pH, but also due to their low cost, renewability and biodegradability. All substances likely to be utilized by living microorganisms, like sugars, amines, alcohols, organic acids, hydrogen or large-molecular weight biomasses can be used as fuels, providing the specific enzyme for their transformation is identified and purified.

In the last three years, many different EBFCs relying on enzymatic reactions at both electrodes have been published, a high number that underlines the fast and continuous progresses made in this field. The most used enzymes in these very recent EBFCs are glucose oxidase (GOx) [6–15] which oxidizes glucose into gluconolactone at the anode, and laccase (Lac) [6,9,11,15–18] or bilirubin oxidase (BOD) [13,19,20], both multi-copper oxidases that perform the efficient reduction of oxygen into water at the cathode. Some various studies report also the use of hydrogenase (Hase) [21–24], alcohol dehydrogenase [25–27], cellobiose

Abbreviations: Au-NP, gold nanoparticle; AFM, atomic force microscopy; BFC, biofuel cell; BOD, bilirubin oxidase; CDH, cellobiose dehydrogenase; CNT, carbon nanotube; DET, direct electron transfer; EBFC, enzymatic biofuel cell; FAD, flavin adenine dinucleotide; FDH, fructose dehydrogenase; GC, glassy carbon; GDH, glucose dehydrogenase; GOx, glucose oxidase; Hase, hydrogenase; HOPG, highly-oriented pyrolitic graphite; ITO, indium tin oxide; Lac, laccase; MET, mediated electron transfer; MW-CNT, multi-wall carbon nanotube; PG, pyrolitic graphite; SAM, self-assembled monolayer; SEM, scanning electron microscope; SPR, surface Plasmon resonance; SW-CNT, single-wall carbon nanotube.

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http://dx.doi.org/10.1016/j.electacta.2013.07.133

Table 1
Some usual current densities reached at a planar electrode. Only currents due to DET are taken into consideration. ω is the rotation rate of the electrode.

Enzyme	Micro organism	Electrode material	Current density ($\mu A\,cm^{-2}$)	Reference
BOD	*Trachyderma tsunodae*	Glassy carbon (GC)	≈4 ($\omega = 0$)	[20]
CDH	*Phanerochaete chrysosporium*	Polycrystalline gold	≈0.5 ($\omega = 0$)	[69]
FDH	*Gluconobacter* sp.	GC	≈100 ($\omega = 0$)	[71]
Hase	*Aquifex aeolicus*	Pyrolytic graphite (PG)	20 ($\omega = 0$)	[72]
Hase	*Desulfovibrio gigas*	PG	125 ($\omega \to \infty$)	[73]
Lac	*Trametes* sp.	Highly Oriented PG (HOPG)	168 ($\omega = 0$)	[70]

dehydrogenase (CDH) [14,28], fructose dehydrogenase (FDH) [16,19,29,30], glucose dehydrogenase (GDH) [14,28,31–40], lactate dehydrogenase [41], malate dehydrogenase [27], urease [42] or trehalase [43,44], as catalysts respectively oxidizing dihydrogen, alcohols, cellobiose, fructose, glucose, lactate, malate, urea or trehalose at the anode, and polyphenol oxidase [45,46] or tyrosinase [10] reducing oxygen at the cathode. Other enzymes like pyruvate dehydrogenase at the anode [47] or peroxidases at the cathode had also been used formerly [48].

Some of the EBFCs mentioned above have been designed to work in physiological conditions [46,49–51] or in different physiological fluids such as human serum [52], plasma [14,53], human lacrimal liquid [28] or hemolymph [54], which is a challenge because of the low availability and slow diffusion of substrates as well as the presence of many possibly interfering compounds. They have sometimes even been successfully implanted in a lobster [55], in a rat [45,56], in insects [43,44,57], in a snail [54], in clams [58], thus demonstrating the possibility to extract energy directly from various living organisms, or from fruits [59]. For the sake of affordability, some others have been conceived to harvest substrates from commercially available beverages [25,38,39]. The use of cheap electrode materials like paper [39] has also been envisioned in the purpose of developing low-price disposable devices. More extreme environments like high temperatures [22,37] have been foreseen as well.

Power densities ranging from few tens of $\mu W\,cm^{-2}$ to almost $2\,mW\,cm^{-2}$ [16,25] and open circuit voltages as high as 1.1 V for a single cell [21,23] have been reported. Stability has been successfully evaluated on various scales: 24 h [16,21,50], one week [21,60,61], or even one month [51]. Miniaturization efforts have been done in a few cases [30,38,39,54,59,62] leading to systems as small as $5 \times 5 \times 1.1\,mm^3$ [30]. The ability to power tiny gadgets such as light emitting diodes [30,56,59,63], a watch [55], a digital thermometer [56], a small electrical motor [58] or pacemakers [55,64] has already been demonstrated for a couple of systems. An interesting study performed by Miyake et al. [30] is worth emphasizing, as it depicts the stacking of three similar mini fructose-O_2 cells in a single device, thus multiplying the low voltage of a single cell without altering the performances, and while remaining at the millimeter scale (OCV 2.09 V, P_{max} $2.55\,mW\,cm^{-2}$ at 1.21 V, $5 \times 5 \times 3.3\,mm^3$). These last developments open the route for effective use of EBFCs in low-power devices and in small portable applications. EBFCs might also be considered as suitable electrical power sources for implantable autonomous electronic devices, like drug-delivery or health-monitoring systems.

Despite these attracting properties and potential applications, the expansion of EBFCs still faces some limitations. Power densities remain in most cases quite low, and not sufficient to power the targeted applications. More critically, the long term stability of the devices is still a challenge. Except in rare exceptions where the enzymes remain in solution [42], this requires highly efficient and stable enzyme immobilization at electrodes. In many cases, electrode modifications with conductive nano- or micro-materials, leading to electrode nano- or micro-structuration, have proved to be valuable tools for enhancing the performances of enzyme bioelectrodes. The formation of enzyme-nanoparticle hybrids for electrochemistry has been envisioned since the end of the 1990s, and less recent results have already been reviewed in interesting papers by Willner et al. [65] or Xiao et al. [66]. This article covers the major contributions in the field over the last years. It does not focus on developed EBFCs but on enzyme electrodes that could be used in EBFCs. Since the topic could be rather extensive, only cases where DET was achieved are considered, essentially over the last five years. The main reasons for such modifications are first introduced, the materials and methods used are then described, and the performances obtained are finally discussed.

2. What is the purpose of electrode modifications by nano- or micro-materials?

2.1. Increase in current densities

One of the most cited motives for nano- or microstructuration of electrodes is to increase the current density and thus the delivered power density of the EBFC. At a planar electrode the current density is indeed limited by the intrinsic properties of the enzyme, according to the following equation:

$$J_{max} = k_{cat} \times n \times F \times \Gamma$$

where J_{max} is the maximal current density, n the number of electrons exchanged during the enzymatic reaction, k_{cat} the catalytic turnover of the enzyme, F the Faraday constant and Γ the coverage of enzyme. Let us consider the simplest case of a spheric enzyme with a molecular weight around 200 kDa that corresponds to a radius of 5 nm [67], a reasonable value of $k_{cat} = 1000\,s^{-1}$ and $n = 2$. A simple calculation gives a maximal current density $J_{max} \approx 1.2\,mA\,cm^{-2}$. With a voltage $V = 1.23$ V, which is the theoretical highest voltage reachable in aqueous electrolytes, this gives a maximal power density $P_{max} \approx 1.5\,mW\,cm^{-2}$, which is a quite limited value.

Moreover, it has to be taken into consideration that this equation describes the optimal case where both following conditions are fulfilled: (1) The electroactive surface area matches the geometric surface of the electrode and this electroactive surface area is totally covered by the enzyme; (2) the enzyme is oriented at the electrode in an optimal way so as to give 100% DET. It is therefore really unlikely that this optimal case might be observed. Besides, techniques that allow to assess correct orientations of electroactive enzyme are missing, which precludes the accurate knowledge of k_{cat}. As an example, only one article reports the quantification of Hases at self-assembled monolayers (SAMs) on gold thanks to electrochemistry coupled to scanning electron microscopy (SEM) [68]. Current densities reached at a planar electrode thus vary according to the kind of enzyme studied but are most often situated between $0.5\,\mu A\,cm^{-2}$ [69] and $168\,\mu A\,cm^{-2}$ [70] as described in Table 1.

Nano- or micro-structuration of the electrode would allow to increase the developed surface area of the electrode without modifying its geometric dimensions, whether by developing a volumic network where larger amounts of enzymes can be entrapped or by creating pores enabling the adsorption of more enzymes.