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Is graphene worth using in biofuel cells?

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ABSTRACT

There is an enormous growth of interest in graphene, a two-dimensional carbon nanomaterial, exhibiting excellent conductivity, good mechanical and optical properties with an affordable cost. It was also found out that it can be integrated quite effectively with biocatalysts for fabrication of graphene-based biofuel cells (BFCs), where the biocatalysts are used for turning a chemical energy of substrates/biofuels into electricity. Like other nanomaterials, graphene can be applied for preparation of highly structured electrode interfaces, where high amount of biocatalysts can be loaded and thus the power output of a BFC can be increased. As a reflection of the fact that both graphene and BFCs are quite "hot topics" these days, the aim of this review is to cover and evaluate the current state of graphene applications in BFCs, either enzymatic or microbial, and also to answer the question whether it is indeed more favorable to use graphene instead of more common carbon nanotubes or metallic nanoparticles.

1. Introduction

One of the most significant tendencies in the industry and in the energy supply today is to minimize both economic inputs and environmental impacts of increasing good and energy production. One way how electric energy can be generated in a sustainable way is to use biocatalysts (isolated enzymes [1–3] or enzymes present within microbial cells [4–6]) to turn chemical energy into electricity by fabrication of biofuel cells (BFCs) [7–9]. Just like any conventional fuel cell, a BFC comprises two electrodes, an anode and a cathode with at least one of them prepared as a bioelectrode containing biocatalysts, labeled as a bioanode or a biocathode. On a surface of the former one, a supplied fuel is oxidized by a (bio)catalyst, i.e. it donates electrons to the (bio)anode surface. These electrons are subsequently employed in a (bio)catalyst-assisted reduction of a depolarizator (mainly oxygen) on a (bio)cathode surface (Fig. 1) [10,11].

A total catalytic activity of a biocatalyst immobilized on the electrode surface is the most important factor to achieve a high power output of the device. At the same time, immobilization protocol is equally significant, because only a biocatalyst docked in a proper orientation allowing a direct electron transfer (DET) with the conductive interface can effectively exchange electrons with the electrode. New promising concepts of addressing these two important issues were recently developed with a progress in a material science, namely with an introduction of a wide range of conductive nanomaterials.

Since more than three decades ago, it was known that carbon paste electrodes (CPE), i.e. graphite micro- and nanoparticles in a fixing matrix, provide a sufficient, electrically conductive, interface for immobilization of enzymes. Ikeda and coworkers [12] reported 50-fold larger amount of electrochemically active glucose oxidase (GOx) adsorbed on CPE compared to a plane graphite electrode surface [12,13]. In 1979, DET of laccase immobilized on a carbon black electrode has been reported [14]. Lately, more sophisticated nanomaterials have been synthesized and integrated with biocatalysts, namely 0D spherical nanoparticles such as fullerenes and especially 1D carbon nanotubes (CNTs) [15,16], possessing high electron conductivity across a bulk of the electrode interface because of their typical length in a micrometer range. Their application has allowed a fabrication of the most promising enzymatic BFCs described so far [17,18].

After a breakthrough study of Geim's lab describing first preparation of graphene [19], there has been a substantial effort to employ this nanomaterial consisted of one atom thick planar sheets of carbon, for electronic coupling of redox enzymes. It has been shown that the 2D carbon crystals of single graphene sheets possess very high in plane electron conductivity, which together with its simple and inexpensive fabrication and versatile processing protocols including surface patterning techniques [20,21], makes it currently the most promising material in electronics development and also for construction of bioelectrodes [22–24]. Graphene-based materials are mostly prepared by a physical exfoliation of graphite or by a chemical synthesis using e.g. a chemical vapor deposition

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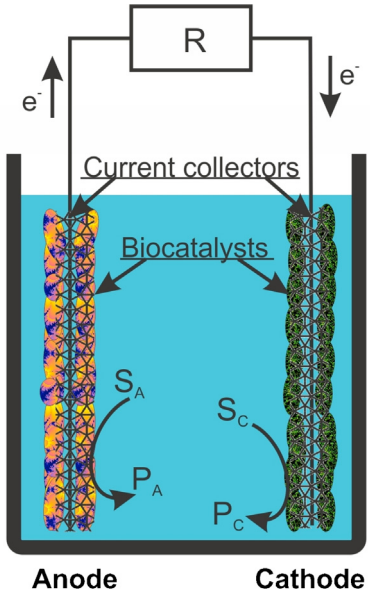

Fig. 1. A scheme of a membraneless, one compartment BFC with S and P denoting substrate and product, respectively, of anodic (index $_A$) and cathodic (index $_C$) biocatalytic reactions. An anodic substrate is a supplied biofuel, while ferricyanide or oxygen are the most common cathodic substrates, i.e. final electron acceptors.

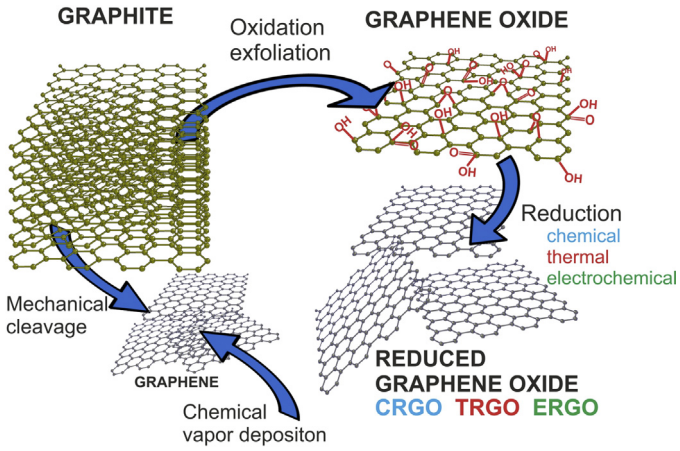

Fig. 2. A schematic illustration of possible ways for preparation of graphene and rGO.

(CVD) protocol. The latter method provides "pure" and high-quality graphene (G) with quite a high cost. Far less expensive fabrication method is reduction of a material labeled to as "graphene oxide" (GO), which is obtained by oxidation and subsequent exfoliation of graphite and contains numerous oxygen-rich moieties on the surface of particular sheets, what makes GO highly soluble. By a reduction process, conductivity of GO is substantially increased *via* restoring of disrupted conjugated sp^2 bonds responsible for graphene's excellent conductivity. Reduction is mostly performed thermally, chemically or electrochemically (Fig. 2) and reduced graphene oxide (rGO) obtained should be distinguished from "pure" synthesized graphene. Depending on a reduction process involved, different forms of graphene can be prepared i.e. chemically, electrochemically and thermally reduced GO, in further text abbreviated as CRGO, ERGO and TRGO, respectively. Properties of differently prepared graphene-based materials can vary dramatically with the applied method and it should be possible to tune graphene properties as required for a particular application. This important issue has been addressed in several extensive reviews [25,26].

A success of graphene based biosensors [27,28] points out to the fact that biocatalysts should be very effectively integrated with this nanomaterial in a way applicable for a BFCs fabrication. In spite of this assumption, one can be surprised with the current situation that there are only a few studies so far describing the construction of enzymatic BFCs. Integration of microbial cells with graphene is studied more intensively, since the number of published BFCs is almost three times higher compared to the enzymatic ones.

All enzymatic and microbial BFCs employing graphene-based materials published to date are covered in this review in order to evaluate their performance and benefits offered, when compared to other devices based on nanomaterials, especially CNTs. In such comparison, not only power/current densities are taken into account, but also other factors including flexibility and cost-effectiveness of a fabrication process. This should help to suggest perspective ways of their fabrication and processing that will allow full exploitation of unique properties of graphene-based materials in fabrication of BFCs.

2. Enzymatic biofuel cells

To introduce graphene-based enzymatic BFCs (EBFCs) described so far, a summary is provided in Table 1, with main operational and construction characteristics of the reported devices. Further details, comparison and evaluation of particular EBFCs are provided in the following sections.

2.1. Direct and mediated electron transfer of biocatalysts

While new amazing features and applications of graphene were sought very intensively, its employment in the fabrication of electrode interfaces suitable for immobilization of bioelectrocatalysts was studied, as well. There are 100+ papers describing enzymatic electrodes based on various forms of graphene with a vast majority of studies being focused on GOx, as a model enzyme. In 2009, first papers focused on assembling of GOx and graphene in a biosensing device were published [29–32], while the first reports on the same components applied in a BFC were described one year later [33,34]. Since this period, five more graphene-based EBFCs have been reported [35–39], all of them with GOx for anodic oxidation of glucose and laccase (Lac) for cathodic reduction of oxygen (Table 1).

GOx contains a flavin adenine dinucleotide (FAD) cofactor surrounded by the protein and a glycan structure [40–42], restricting an efficient electron exchange between the enzyme's active site and the electrode surface, which is absolutely a necessary prerequisite for a good performance of such a bioelectrode. It should be noted that even if GOx-based mediatorless biosensors with a turnover catalytic reaction have been described, the catalytic current could not necessarily originate from a DET between the cofactor and the electrode. Rather it is a result of a non-enzymatic reaction of hydrogen peroxide or oxygen involved in a catalytic cycle of GOx on the electrode surface [43]. On the other side, there are studies describing a cathodic DET of GOx, when a current response is generated *via* reduction of FAD cofactor, which is continuously reoxidized by oxygen. This reduction occurred on electrode surface in an absence of a substrate and in such case, a decrease of the current response (a cathodic current) is observed upon glucose addition [44–46]. Obviously, this is not a system applicable in construction of biofuel cells.

Such an approach of course will allow to construct GOx-based biosensors, but it is not applicable for BFC' anodes because 1) GOx passes electrons more effectively to oxygen than to the electrode as can be judged from heterogeneous electron transfer (ET) rate constants k_S of GOx listed in Table 2 *vs.* value of $k_{CAT} = 1095\ s^{-1}$ representing a homogeneous ET rate between GOx and oxygen [47]