Glucose oxidase bioelectrodes in devices implanted in living plants for energy applications.

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Abstract. The growth of the world population and the lack of energy supply have led to the development of technologies to obtain alternative energy that has a minimal environmental impact, as is the case of fuel cells. In this work, the development of electrodes using glucose oxidase enzyme immobilized with functionalized carbon nanofibers on graphite rods is proposed for its application in a fuel cell implanted in living plants, specifically in cacti. The purpose is to convert solar energy to chemical and then, to electric energy, carrying out the glucose oxidation contained in these plants. The use of a living plant as a fuel cell comes from the idea of taking advantage of more efficiently the conversion of chemical energy, which comes from the plant’s photosynthesis until is converted into electrical energy.

1. Introduction

Fuel cells are defined as an oxidizing fuel anode and an oxidizing reducing cathode connected together by an external load and separated by an electrolyte [1-2, 3]. Unlike batteries that can supply power density only for a defined time, the fuel cell, thanks to absorbing new fuel supplies, can operate continuously, without combustion and polluting the environment. Being a continuous process by means of the oxidation-reduction reactions in the presence of a catalyst, it eliminates the products of the reaction. Ideally there is no change in the chemical composition of the electrolyte or both electrodes [4].

In the enzymatic fuel cells, biological catalysts as enzymes are used for the oxidation of the fuel at the anode and the reduction of the oxidant at the cathode. Enzymes are biological polymers that catalyze the chemical reactions that make life possible as we know it [5]. An important feature that they present is the high selectivity with the substrates to which they are bound, which follows the different models of enzyme-substrate binding that are determined by the form and the presence of specific functional groups in the substrate located in specific sites for the union [6]. The activity of these enzymes can be affected by different factors, starting with the temperature, being proteins, at a high temperature they become denatured or present a loss of activity, so that each enzyme has an optimal temperature [7].
The proper choice of the enzyme allows such reactions to occur under normal conditions (neutral pH, room temperature) compared to conventional fuel cells. In addition, the immobilization of enzymatic catalysts that are specific to a reaction (or class of substrate) on electrodes that are otherwise electro-catalytically inert, such as carbon, can eliminate the need for the required separator and housing components for conventional fuel cells [8-9].

Glucose oxidase catalyses the glucose oxidation reaction to gluconolactone which results in the in situ production of H$_2$O$_2$. The latter is used by peroxidase to oxidize hydrogen peroxide [10]. This enzyme is a glycoprotein dependent on FAD, which catalyzes the oxidation of β-D-glucose, via the D-glucan-δ-lactone, towards gluconic acid and hydrogen peroxide, using molecular oxygen as the final electron acceptor [11]. This reaction can be divided into a reductive and an oxidative step. In the middle of the reductive reaction, glucose oxidase catalyses the oxidation of β-D-glucose to D-glucono-δ-lactone, which is not hydrolyzed enzymatically to gluconic acid. Subsequently, the flavin adenine dinucleotide (FAD) ring of glucose oxidase is reduced to FADH$_2$. In the middle of the oxidative reaction, the reduced glucose oxidase is oxidized again with oxygen to produce H$_2$O$_2$. This enzyme is highly specific for β-D-glucose and only shows marginal activities with other sugars [12].

The methods of immobilization of enzymes are classified into two types: physical retention and chemical union. The creation of a device with the ability to be implanted in an organism as a plant is evaluated according to the adaptation and development capabilities it may have, the selection of a cactus is due to its known resistance and means of adaptation to the environment surrounds them, so that development of electrodes for the conversion of energy can be a promising alternative.

2. Experimental

2.1 Reagents

Glucose oxidase (EC 1.1.3.4 Type XS, lyophilized powder, 100,000-250,000 units / g solid _Aspergillus niger_), tetrabutylammonium bromide (TBAB), Nafion® (5% diluted in water), carbon nanofibers were purchased from Sigma-Aldrich.

2.2 Development of the electrodes.

Commercial carbon nanofibers (CNF) (Sigma-Aldrich) were functionalized by a treatment in a mixture of acids, HNO$_3$ / H$_2$SO$_4$ (1: 3) for 3 hours at 40 °C. The precipitate was washed with deionized water at pH 7.0 and dried at 90 °C for 8 hours to obtain functionalization of the carboxylic groups, which helped to immobilize the glucose oxidase enzyme. A ink conformed by the functionalized nanofibers, the enzyme, Nafion (5% diluted in water), tetrabutylammonium bromide (TBAB) and phosphate buffer solution 0.1M pH = 7, was deposited on graphite rods, creating the enzymatic anode for tests on fuel cell in plants and a graphite bar with commercial Pt / C as cathode.

2.3 Fuel cell evaluation.

The anode implanted in plants (cacti) was evaluated along with a platinum cathode to complete the fuel cell. The insertion of these electrodes may be possible in this kind of plants. The evaluation of the implanted fuel cell was performed exposing it with ultraviolet light during 2 hours so that the plant could increase the production of glucose (Figure 1).
3. Results and discussion

When the plant is exposed to ultraviolet light the power density obtained by evaluation of fuel cell increased in 37.26% (Figure 2), due to the fact that the photosynthetic process increases the production of glucose in the cactus, making it attractive for the generation of electrical energy from a plant resistant to high temperatures and low water requirements, being able in the future to be a source of energy to power energetically small electronic devices.

The stability of the fuel cell was tested by chronoamperometry technique (Figure 3) showing excellent behaviour as a function of the time and when the plant is exposed to ultraviolet light the current density generated by the fuel cell is increased.
4. Conclusions

An enzymatic bioanode based on glucose oxidase enzyme is developed to be implanted in a plant to carry out the conversion of light energy to chemistry and later to electricity, taking advantage of the ability of the enzyme to oxidize the glucose present in the plant, capable to generate 7.28 mW/cm² of power density after to exposed the plant 2 hours under ultraviolet light, this being important for future research for the development of fuel cells implanted in living plants.

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