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A glucose/hydrogen peroxide biofuel cell that uses oxidase and peroxidase as catalysts by composite bulk-modified bioelectrodes based on a solid binding matrix

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Abstract

An improved composite bulk-modified bioelectrode setup based on a solid binding matrix (SBM) has been used to develop a glucose/hydrogen peroxide biofuel cell. Fuel is combined through a catalytically promoted reaction with oxygen into and oxidized species and electricity. The present work explores the feasibility of a sugar-feed biofuel cell based on SBM technology. The biofuel cell that utilizes mediators as electron transporters from the glucose oxidation pathway of the enzyme directly to electrodes is considered in this work. The anode was a glucose oxidase (GOx, EC 1.1.3.4)/ferrocene-modified SBM/graphite composite electrode. The cathode was a horseradish peroxidase (HRP, EC 1.11.1.7)/ferrocene-modified SBM/graphite composite electrode. The composite transducer material was layered on a wide polymeric surface to obtain the biomodified electrodic elements, anodes and cathodes and were assembled into a biofuel cell using glucose and H_2O_2 as the fuel substrate and the oxidizer. The electrochemical properties and the characteristics of single composite bioelectrodes are described. The open-circuit voltage of the cell was 0.22 V, and the power output of the cell was 0.15 $\mu W/cm^2$ at 0.021 V. The biofuel cell proved to be stable for an extended period of continuous work (30 days). The reproducibility of the biotransducers fabrication was also investigated. In addition, an application of presented biofuel cell, e.g. the use of hydrolyzed corn syrup as renewable biofuels, was discussed.

Keywords: Biofuel cell; Solid binding matrix; Spray-printing technology; Renewable fuels

1. Introduction

The bioelectrochemical generation of power is well known in the prior art [1,2]. It is also known that biological process of living organisms can be utilized to enhance the generation of electrical power [3–5]. This approach represents an interesting method to supply energy to low power electrically activated smart materials that can be directly used in a wide variety of applications such as microdevices, nanorobots, micropumps, pacemakers, neuromorphic circuits, etc. [6–8]. Several electrochemical cells utilizing the biological process of living organisms are shown in previous literature [9–11], but almost all of them utilize a free liquid cellular suspension in conjunction with the electrodes to generate electrical power. However, all of the prior art devices and process show serious drawbacks because of their complexity, size, limitation on power generation, poor reproducibility and reduced longevity due to the fact that the bacteria apparently were short lived and the cell could operate only few days before showing a marked decrease in ability to generate power. Another problem, although not stated but widely understood, is the health hazard posed by sludge battery. A sludge battery contains pathogens and this would pose a very real health concern if the battery leaks. Alternatively, a series of biocatalysts participate in the electron transfer chain between the fuel substrates and the electrode surfaces [12]. That is, instead of microorganisms, redox enzymes facilitate the electron transfer between substrates and electrode interfaces, thereby enhancing the cell current. Redox enzymes, electrocatalysts and electrobiocatalysts were used as dissolved forms into electrolytes [13] or as immobilized species on the electrode surfaces [14–16]. The recently reported setups have proved to be effective even though they may present important drawbacks such as a considerable variability of the bioactive electrodic layer, an uncertain storage and operational stability, tedious pre-

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treatments of special and expensive electrodic materials with a subsequent complex chemical and/or electrochemical functionalization, the absorption of many interfering species, etc.

In our laboratory, we are interested in developing new composite transducers for amperometric biosensors with improved environment within the transducer body based on solid binding matrices (SBMs) of defined molecular structure [17]. The SBMs are solid at room temperature and have a beneficial effect on the mechanical and electrochemical properties of the final transducer, thus securing structural and functional stabilization of the biocatalyst [18]. The replacement electrode materials (i.e. gold, platinum and glassy carbon) is important because that design offers a number of further advantages such as simplicity, lower cost, better mechanical flexibility and possibility of miniaturization.

The production of electrical power using low-cost biological matter has been previously demonstrated [19]. Basically, biofuels are renewable, sustainable, offer lower greenhouse gas emissions and reduce the demand for imported fuels. Cellulose material [20] and starch [21] derivatives represent just two examples of cheap, safe and flexible feedstocks capable of conversion into solid, liquid and gaseous fuels by physical, chemical and biological processes. Thus, we have employed hydrolyzed corn syrup, a cheap bulky material made from corn starch and industrially used for its sweetness, as a renewable biofuel.

The present work is related to a composite of flexible graphite material, which can be used in the making flow fields plates and electrodes, anodes and cathodes, for use in biofuel cells. The graphite plates as used herein represent the product of a spray-printing process [22–25] of synthetic graphite particles, suitably modified with a redox mediator and a biocatalyst, with a molecular binding agent that intercalates particles and leading thin conductive sheets supported on an inert polymeric foil. Our aim consists in the development and construction of immobilized glucose oxidase/ferrocene and peroxidase/ferrocene electrodes as an SBM-based conductive composite sheet. These bioactive plates provide the basic components to make an improved biochemical electrical power source that is reproducible, stable, economical, safe, extremely long lived, ''omnivorous'' with respect to different fuel class source, compact and mass-producible.

2. Experimental

2.1. Materials

Peroxidase from horseradish (HRP, EC 1.11.1.7 150 U/mg solid) and glucose oxidase type VII-S from *Aspergillus niger* (GOX, EC 1.1.3.4 180 U/mg solid) were obtained from Sigma (St. Louis, MO, USA). Hydrogen peroxide 30%, synthetic graphite powder and 2-hexadecanone were purchased from Fluka (Buch, Switzerland). Ferrocene (Fc) and D(+) glucose were purchased from Aldrich (Steinheim, Germany). Hydrolyzed corn syrup ADM 97/71 was obtained from ADM Corn Processing (Decatur, IL, USA). Proton exchange membrane (Nafion 117, perfluorinated membrane, 0.02 mm thick) was purchased from Aldrich and used as received. Silver ink Electrodag EL-010 was obtained from Acheson Colloids (Port Huron, MI, USA). Other reagents were commercially available as analytical grade. All solutions were prepared using bidistilled water that had been purified by Milli-Q plus system (Millipore, Molsheim, France). The inert polymeric foil of spray-printed electrodes was a flexible polyester sheet of 0.35 mm thickness.

2.2. Apparatus and electrochemical measurement

Cyclic voltammetry studies were carried out with a computerized electrochemical analyzer Amel 433/W (Milan, Italy) The output from the polarograph was transferred to a program emulating a chart recorder (Amel 6.x series, pro, Milan, Italy) via a data acquisition hardware unit (PC-compatible computer Olidata, Milan, Italy). Cyclic voltammograms were performed in 20 ml static 0.1 M potassium phosphate buffer at pH 7.0, containing 20 mM potassium chloride unless otherwise stated. Multimeter Electrometer Mod. 614 (Keitheyl, USA) was used for fuel cell electrical parameters (current, potential and resistance) measurements. A glass membrane pH electrode model PHI 2401 (Radiometer, Copenhagen, DK) was used to perform pH measurements. The electrode was connected with a pH meter model PHM 85 (Radiometer). Peekò three-way valves and Masterfex C/L peristaltic pump were obtained from Cole-Parmer Instrument (Nile, IL, USA). All experiments were carried out at room temperature (21 ± 2 °C).

Fig. 1 shows the process of the spray-printing thick-cover electrode fabrication. The spraying suspension was fed to the needle from a suitable glass flask with a screw-type Teflon stopcock. The top of the stopcock was given a conical shape, which allowed easy pressure mounting of standard injection needles. Before spraying, the clean polymeric support was inserted into the proper housing. Then, the support was exposed to the spray through a screen mesh yielding a 10-electrode template block. In the preliminary experiments, a modified gauge injection needle (0.3 mm o.d., 0.14 mm i.d.) was used as spraying tubing. Electrical contact was ensured by a silver track ($1.5 \text{ mm} \times 2$ cm) previously printed on the polymeric support [26].

2.3. Preparation of thick-cover spray-printed electrodes

For the anode, the graphite was first mixed with GOx. The enzyme (4.0% with regard to the final weight of the composite material) was dissolved in water and then the graphite powder was added. The suspension was mixed thoroughly until water was evaporated. Thick film anode

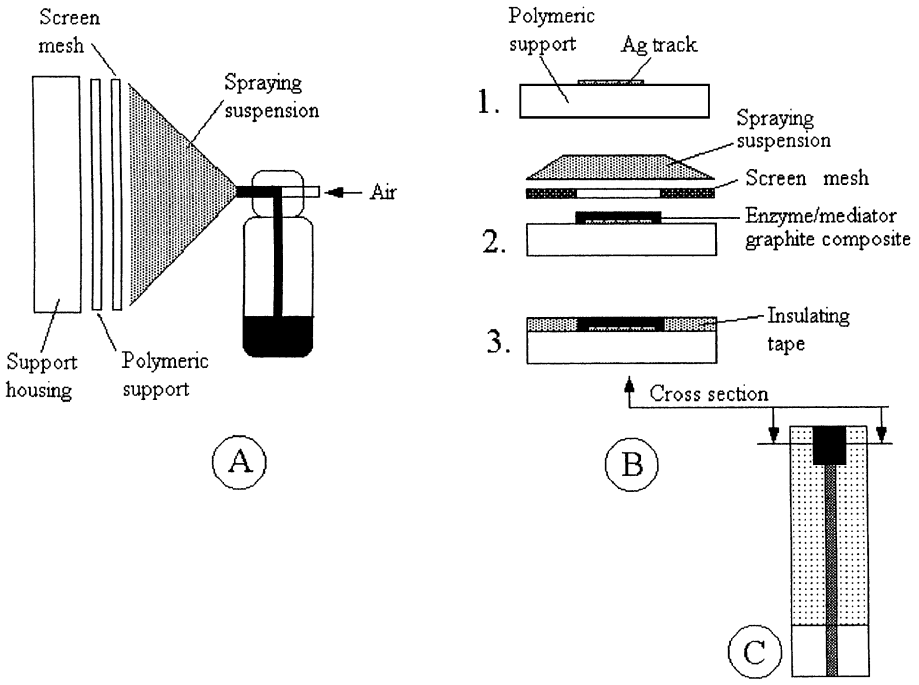


Fig. 1. (A) Diagram of spray-printing apparatus, (B) cross section of electrode fabrication procedure by using a suitable screen mesh and (C) schematic diagram of the spray-printing electrode strip. The electrode area was 0.032 cm^2. The drawing is not to scale.

was fabricated on a polyester sheet using a spray-printing process. The graphite suspension was obtained by adding 15 g of a 3.4% w/w 2-hexadecanone, 0.23% Fc, in chloroform to 0.5 g of GOx-modified graphite in a small glass vial. In the case of the cathode, the HRP was substituted to the GOX. The suspension components were mixed to give a suspension which could be sprayed through the mesh screen onto an inert polyester support. First, a suitable size piece of support sheet was cleaned carefully with ethanol and fixed to the spray-printing housing. The mixed graphite suspension was sprayed through the mesh screen producing 10-electrode template block. After this stage, the dry thin layer of modified graphite composite was smoothed carefully. The screen was then removed after yielding the printed electrode strip. The electrodes were cut off the substrate and covered with an insulating tape (RS components, Corby, Northants, UK) to define the working area. Precision of measurements was estimated using three electrodes taken from a single set of templates.

2.4. Prototype glucose/oxygen peroxide biofuel cell

A prototype fuel cell was fabricated from glass where the capacity of the anode and cathode were 20 ml, respectively. The anode and cathode compartments were separated by a Nafion 117, perfluorinated membrane 0.02 mm thick. The overall dimensions of the fuel cell were $6 \times 4 \times 3$ cm. The anode was a GOX/ferrocene-modified graphite composite plate with a geometric surface area of 0.032 cm^2. The cathode was a HRP/ferrocene-modified composite plate with a geometric surface area of 0.032 cm^2. The anolyte, (1 mM D(+) glucose, 0.5 g/l sodium benzoate, 0.1 M potassium phosphate buffer, pH 7.0) and catholyte (1 mM H_2O_2, 0.5 g/l sodium benzoate, 0.1 M potassium phosphate, pH 7.0) were purged with nitrogen for 20 min prior to operation of the fuel cell and blanketed with nitrogen during operation. The potential of the anode and cathode were referenced to a saturated calomel electrode (SCE) located within the anode and cathode compartments, respectively. The fuel cell was operated under external loads by placing a resistor (ranging between 1 Ω to 5 MΩ) in series between the anode and cathode.

3. Results and discussion

The present work reports the innovative production of a thin, laminar, plate-like enzyme graphite electrode made of modified graphite impregnated with a solution of a molecular binder and hardened under the action of the progressive networking subsequent to the organic solvent evaporation. Spray-printed electrodes have been reported to show different electrochemical behavior as compared with conventional electrodes. This was attributed to increased surface area of the electrode due to the specific structure of surface-printed electrodes [20]. Fig. 2(i) shows the cyclic voltammogram of the GOX-bulk-modified graphite composite electrode in the absence and in presence of added glucose. With glucose, an electrocatalytic anodic current was observed (curve b) implying the effective bioelectrocatalyzed oxidation of glucose by the biocatalytic enzyme electrode. Fig. 2(ii) shows the cyclic voltammograms of the HRP-bulk-modified

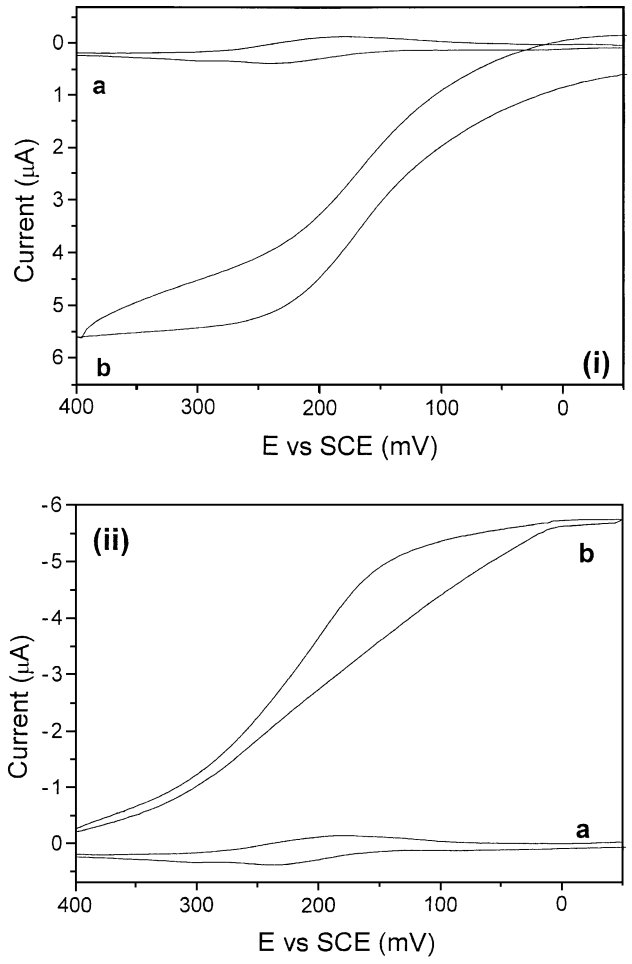
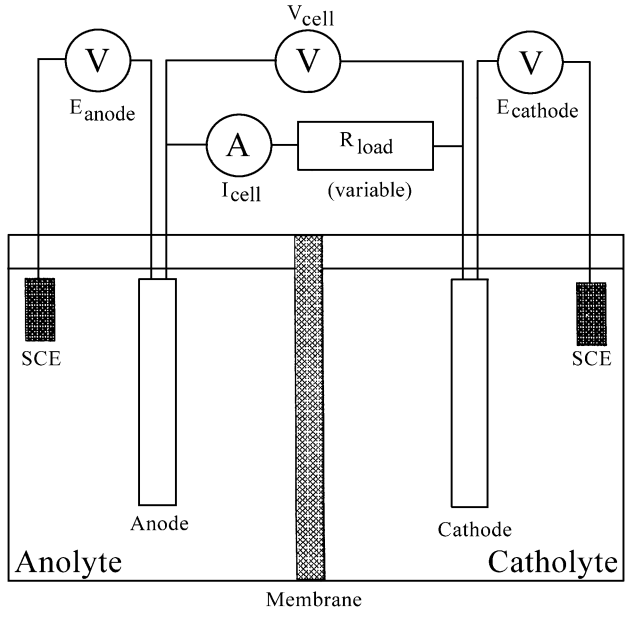


Fig. 2. (i) Cyclic voltammograms of (a) GOX/ferrocene-modified composite electrode and (b) in the presence of added glucose, 10 mM. (ii) Cyclic voltammograms of (a) HRP/ferrocene-modified composite electrode in background electrolyte and (b) in the presence of hydrogen peroxide, 8 mM. Background electrolyte: 0.1 M potassium phosphate buffer, pH 7.0, 20 mM potassium chloride.

graphite composite electrode in the absence of H_2O_2 (curve a) and in the presence of added H_2O_2 (curve b). The observed electrocatalytic cathodic current indicates the effective electrocatalytic cathodic current indicates the effective electrobiocatalyzed reduction of H_2O_2 by the biocatalytic enzyme electrode.

The electrocatalyzed reduction of H_2O_2 by the HRP electrode and the effective electrobiocatalyzed oxidation of glucose by the GOX electrode permit, in principle, the design of biofuel cells using H_2O_2 and glucose as the cathodic and anodic substrates, respectively (Scheme 1). Hence, the reaction on the anode electrode of the enzymatic fuel cell is the oxidation of the substrate by enzyme and the release of electrons at the anode itself. The enzyme glucose oxidase converts glucose to gluconic acid in the presence of ferricinium ions. Ferricinium, a redox mediator, readily captures electrons. Release of the electrons from the reduced form, ferrocene, becomes easy and fast. The mediator is not consumed in the reaction. On the cathode electrode, the



Scheme 1. Schematic configuration of the biofuel cell employing glucose and hydrogen peroxide as a fuel and oxidizer, respectively, and GOX- and HRP-modified graphite composite electrodes as biocatalytic anode and cathode, respectively.

enzyme peroxidase catalyze the reduction of hydrogen peroxide to water-oxidizing the heme group of the active site of the enzyme. The oxidized heme group needs to be reduced to keep activity and it may be done electrochemically or by an electron donor species [27]. The electron donor species, such as ferrocene, is oxidized. Ferricinium ions produced extract electrons from the electrode. For the optimization of the biofuel cell element, the potentials of the enzymes bulk-modified electrodes as a function of the concentration of the cathodic and anodic substrates were determined vs. the SCE reference electrode. Fig. 3 shows the potentials of the GOX electrode at different concen-

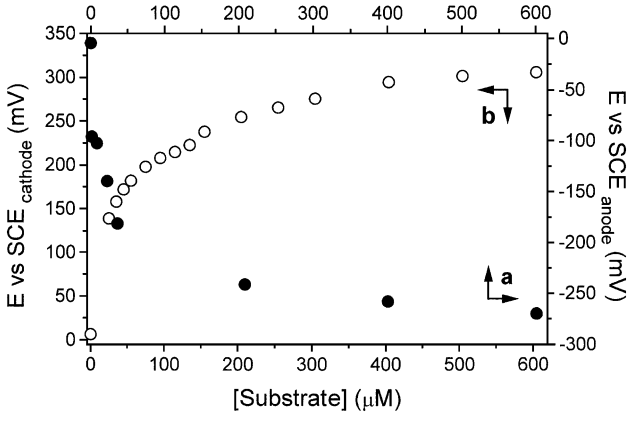


Fig. 3. (a) Potential of the GOX-modified graphite composite electrode as a function of glucose concentrations. (b) Potential of the HRP-modified graphite composite electrode as a function of hydrogen peroxide concentrations.

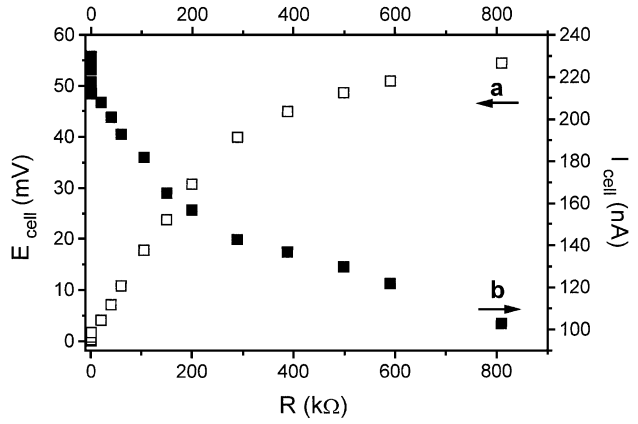


Fig. 4. (a) Biofuel cell voltage at different external loads. (b) Current development by the cell at different external loads. Data record using H_2O_2, 1 mM, as the substrate solution in the cathodic compartment, and glucose, 1 mM, as the substrate solution in the anodic compartment. The background electrolyte in both compartments was 0.1 M potassium phosphate buffer pH 7.0.

trations of glucose (curve a) and the potentials of the HRP electrode at different concentrations of H_2O_2 (curve b). The potentials of the GOX electrode and of the HRP electrode become more negative and more positive as the concentrations of the glucose and H_2O_2 are elevated, respectively. The potentials of the electrodes reach saturation at a high concentration of the substrates. For the specific system, the saturation potentials of the anode and the cathode are reached at ca. 0.6 mM of glucose and 0.6 mM of H_2O_2, respectively.

The evaluation of the reproducibility of the electrodes fabrication was based on statistical evaluation of the potential outputs values vs. SCE at a 0.6 mM concentration of the substrates. The RDS of the values of the potential outputs of the electrodes were 3.72% ($n=6$) and 4.03% ($n=6$) for the anode and the cathode, respectively. The enzyme/ferrocene bulk-modified graphite/SBM composite electrodes display excellent storage characteristics. Anode and cathode electrodes were stored under dry conditions at room temperature for 3 months. After that, they both exhibited a decrease in the potential output (0.6 mM substrates vs. SCE) of less than 4.0%. In this way, we have satisfactorily overcome the poor reproducibility and stability that are some of the main problems inherent in the fabrication biofuel cell electrodes. The obtained good reproducibility reflect the ability of the SBM-based electrodes to provide a homogeneous and functional distribution of all electrode components, which leads to mass producible electrode with a reproducible surface under controlled conditions.

Taking into account the geometrical electrode area (0.032 cm^2), the current generated by the cell at a loading resistance of 100 kΩ can be translated into the current density, 5.94 μA cm^{-2}.

The biofuel cell performance was examined at the concentration corresponding to 1 mM of each of the two substrate: fuel and oxidizer. Fig. 4a shows the cell voltage at variable external loads. The cell voltage increases, and at an external load of ca. 600 kΩ, it levels of to a constant value of ca. 310 mV. Fig. 4b shows the resulting current in the cell at the variable external loads. Upon increasing the

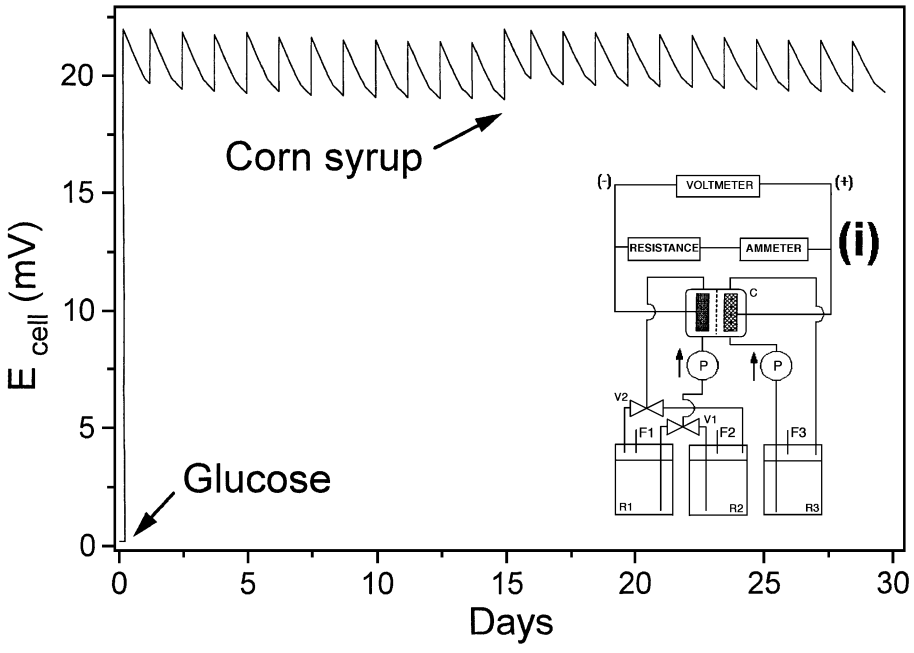


Fig. 5. Stability of the biofuel cell voltage operating at the load of 100 kΩ. Inset (i) represents a schematic diagram of the fuel cell of Scheme 1 to which a biofuel anolyte and an oxidizer catholyte vessels are attached and, thus, having a structure so that anolyte and catholyte are continuously circulated into the fuel cell. R1, glucose, 1 mM, anolyte vessel; R2, hydrolyzed corn syrup, 1.75% w/w, vessel; R3, hydrogen peroxide, 1 mM, catholyte vessel; V1, input three-way valve; V2, output three-way valve; P, peristaltic pumps; F1, F2, F3, external feeding holes. Background electrolyte: 0.1 M potassium phosphate buffer, pH 7.0.

external load, the current drops and is almost 100 nA at an external load of 800 kΩ. The power extracted from the biofuel element (P = Vcell × Icell) correspond to 0.15 μW cm^{-2} at an external load of 110 kΩ.

While the above biofuel cell is an effective and efficient fuel cell, its operation is dependent upon maintaining the anolyte and catholyte solutions in proper chemical balance or condition and disposing the byproducts of fuel cell reaction. Fig. 5, scheme (i) diagrammatically illustrates the system and apparatus for supplying and circulating fresh electrolytes to and through the cell element. The manner in which to attain practical, efficient operation of such a cell is to establish and maintain a continuous flow of fresh anolyte and catholyte through the cell and to recirculate and to dispose the used electrolytes.

The stability of the biofuel cell was examined as a function of time. Fig. 5 shows a long-term dynamic behavior of the cell element when the loading resistance is 100 kΩ. The same level of potential output lasted over 15 days. During the 15 days, pH was controlled and stated at 7.0. Every 30 h, the glucose and hydrogen peroxide were controlled by a glucose [28] and hydrogen peroxide [29] biosensors, and suitable amounts of fuel substance and oxidizer were fed to the reservoir R1 and R3, respectively, as one portion, which resulted in the oscillation in the performance of potential. The circulation rate in this case was 14 ml min^{-1}. The voltage decrease by 10.5% after 30 h of the cell operation. This decrease in the cell performance could originate from the depletion of the fuel substrate, interpenetration of the fuel and oxidizer into the respective counter compartment and the degradation of the biocatalysts embedded into the electrodes. The decrease in the voltage output could be compensated by recharging the reservoirs with the fuel substrate and oxidizer. The biofuel cell displays good working stability. After 360 h of continuous work, it exhibited a decrease in the maximum potential output of 2.32%. On the 15th day, the biofuel reservoir was changed by switching the V1 three-way valve from R1 to R2, where there was diluted corn syrup (1.75% in 0.1 M potassium phosphate buffer pH 7.0) instead of glucose solution, and the potential output was followed as described above. In the latter case, the consumed amounts of glucose were restored by the temporized addition of concentrated corn syrup. An increase in the potential output was detected (5.8%). A satisfactory working stability was confirmed. After 360 h of continuous work, it exhibited a decrease in the maximum potential output of 2.5%.

4. Conclusions

Fuel cells can produce electricity with a very high efficiency. They are versatile and can be used between a wide range of power values and, thus, be sited wherever electricity is needed. Fuel cell technologies are, therefore, also strong vectors for increasing the use of renewable energy sources. The present work explores the feasibility of a sugar-feed biofuel cell based on SBM technology.

The studied biofuel cell system is a mediator-bound fuel cell, where the biological part and the electrical part have been implemented into the same network represented by a new composite transducer with improved environment within the transducer body based on SBM of defined molecular structure. The SBMs are solid at room temperature and have a beneficial effect on the mechanical and electrochemical properties of the final transducer, thus securing structural and functional stabilization of the biocatalyst. The replacement of classical electrode materials is important because the presented design offers a number of further advantages such as simplicity, lower cost, long-term stability, better mechanical flexibility and possibility of miniaturization and mass production. In fact, the composite transducer material can be easily layered (printed) on a wide variety of surfaces (metals, glass, plastics, etc.), if necessary, cut with a suitable design and, e.g. layered on to obtain a battery of several biomodified electrodic elements. In the practical application and use of this kind of biofuel cell, a multiplicity or battery of cells (e.g. as a wafer-like setup) must be provided to generate useful and necessary amounts of electric power to several micro- or nanodevices such as artificial muscle transducers, sensomotor systems, neuroprostheses, etc.

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