PRELIMINARY STUDIES CONCERNING ELECTRICITY GENERATION IN MICROBIAL FUEL CELLS

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Abstract:
A microbial fuel cell (MFC) is a bioreactor that converts chemical energy of organic compounds into electrical energy through catalytic reactions of microorganisms under anaerobic conditions. The recent energy crisis has increased the interests in MFCs among researchers as a possible alternative way to generate electrical power or hydrogen from biomass without increasing the net carbon emission into the environment. MFCs can also be used in wastewater treatments to break down organic matters. Power output and efficiency are significantly affected by the types of microbes in the anodic chamber of the MFC, the fuel cell configuration and the operating conditions. Currently, the applications of MFCs are somehow limited because of their low power density in the range of several W/m^2. A miniaturised microbial fuel cell operated with mediator and different microorganisms was developed and the influence of the external load on the composition of the anodic biofilm microbial community was investigated in this MFC fed with glucose and Saccaromyces Cerevisae used as source of electrogens. The purpose of these preliminary studies was to determine the effect of external resistance on biofilm formation and power output. The internal resistance in MFCs can be affected by the anolyte and catholyte composition, pH, the electrode material and structure, the electrode polarisation and by the microbe's characteristics.

Keywords: microbial fuel cell, biofilm formation, microbial composition

1. INTRODUCTION

In recent years the intensive use of fossil fuels (oil, natural gas) has accelerated and this fact has triggered a global energy crisis. In this context, renewable bioenergy is considered as one of the ways to alleviate the energy crisis and avoid global warming.

Technologies based on microbial fuel cells represent the newest approach regarding the electricity generation from biomass or sewage using bacteria and microorganisms.

In addition, there is a trend of miniaturization and portability of computers, computing and communications devices. These applications require small and light energy power sources that are able to support operation over long periods of time, especially in remote places. Moreover, progress in medical sciences lead to the design and use of a growing number of implantable powered devices (pacemakers). These devices require energy sources that work for extremely long periods of time, as their replacement requires surgery. Ideally, the implanted devices could use natural body "fuels", and thus would continue to draw power as long as the patient lives.

Biofuel cells offer potential solutions to all these problems by taking from nature the power generation solutions and adapting to our own needs. They consume the available substrates from renewable sources and turn them into secondary products, with electricity generation.

Because MFC (microbial fuel cells) use concentrated chemical energy sources, they can be small and light, and fuel can be purchased even from a living organism (e.g. blood glucose).

The first observations and experiments on generating electricity using bacteria-based systems were performed by Potter et al. in 1911 [1, 2], but in the next half century only a few practical applications have taken place in this area. In the early '90s a lot of studies regarding microbial fuel cells have been carried out. However, the accomplished experiments have required the presence of chemical mediators or of electron carriers, capable of transporting electrons from the cell towards exogenous electrodes. The most important breakthrough occurred in 1999 when it was discovered that the presence of these mediators is not required for electron transport.

Biofuel cells use biocatalysts for the conversion of chemical energy into electrical energy. Because most organic substrates undergoes combustion with energy evolving biocatalytic oxidation of organic substances by oxygen or other oxidants, at the electrode interfaces, provides an effective way of converting chemical energy into electrical energy. Abundant organic raw materials such as methanol, organic acids or glucose, can be used as oxidation substrates and molecular oxygen or H_2O_2 can act as substrate for the reduction reaction.

2. OPERATING PRINCIPLE OF BIOCHEMICAL FUEL CELLS

The present world energy crisis has raised interest in researchers for microbial fuel cells as a possibility to generate electricity or hydrogen directly from biomass without carbon emissions into the environment. Also,

these fuel cells can be used successfully in industrial and sewage wastewater treatment to decompose organic matter. Another way to use these microbial fuel cells is the biosensors (sensors for monitoring the biological oxygen consumption).

As the power generated by microbial fuel cells is somehow low, it does not comply with the needs for a large scale wastewater treatment. In fact, the only microbial fuel cell with potential practical application is that of a sediment-based design which produces current by incorporating the anode in that sediment and it is connected through an electrical circuit to a cathode placed in the aerated seawater. This system is feasible to generate power for sensors and telemetry devices in remote ocean areas.

A microbial fuel cell is a bioreactor that converts chemical energy from chemical bonds of organic compounds into electricity, based on catalytic reactions of microorganisms under anaerobic conditions.

Terminal voltage and energy efficiency are significantly influenced by the type of microorganisms in the fuel cell anode compartment, its configuration and operating conditions. Currently, practical applications of microbial fuel cells are limited due to low specific power values obtained (a few hundred mW/m^2). Therefore efforts are being made to improve energy performance and reduce costs of construction and operation of these cells.

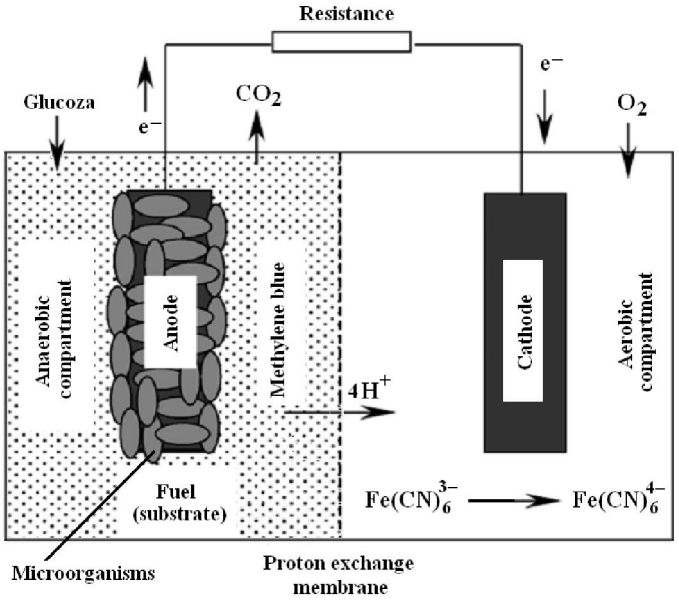

Fig. 1. Schematic representation of a microbial fuel cell [5]

Figure 1 presents a diagram of a typical microbial fuel cell for electricity generation. It consists of an anode and a cathode compartments, separated by a proton exchange membrane (PEM). The presence of oxygen in the anode chamber will inhibit the generation of electricity process. Therefore the system must be designed to maintain separate microorganisms and oxygen present in the catholyte. This separation can be achieved by placing a separating membrane, which provides charge transfer between the electrodes, forming two separate compartments: the anodic compartment, where microorganisms grow and the cathodic compartment, where the electrons react with catholyte [3, 4, 5].

The microorganisms from the anodic compartment of microbial fuel cell oxidize added substrate and generate electrons and protons in this process. Carbon dioxide results as a product of the oxidation reaction. However, there is no net carbon emission because carbon dioxide from renewable biomass comes from the atmosphere through the photosynthesis process. Electrons are transferred to the cathodic compartment through an external circuit and protons cross the exchange membrane shifting towards the cathodic compartment. Electrons and protons are consumed in the cathodic compartment, combining with oxygen to form water.

The electrolyte solution is an oxidizing agent, which takes electrons from the cathode. In the electron transfer circuit related to the microorganism cell one of the oxidizing agent is the oxygen. However, this is not very effective, as it would require large volumes of flowing gas; a more convenient solution is to use an oxidizing agent.

The connection of two electrodes in the external circuit is made of electrical wires, having inserted a resistive component (e.g. resistance, electrical bulb etc) and also the internal circuit contains a salt bridge or an ion exchange membrane. Selective membranes allow protons which are produced in the oxidation reaction to pass from anodic to the cathodic compartment [8, 9, 10].

Electricity generation is achieved by separating the microorganisms and the oxygen or any other final acceptor resulting in an anaerobic anodic compartment.

The electrochemical reactions taking place at the two electrodes are shown below using as an example the acetate ion:

Anodic reaction :

$$CH_3COO^- + 2H_2O \xrightarrow{\text{microorganisms}} 2CO_2 + 7H^+ + 8e^- \quad (1)$$

Cathodic reaction :

$$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O \quad (2)$$

The overall cell reaction is represented by the decomposition of the substrate to carbon dioxide and water, with concomitant generation of electricity as a by-product. Based on the two redox reactions at the electrodes, a microbial type bioreactor can generate electricity on electron flow from anode to cathode in the external circuit.

Generally, there are two types of microbial fuel cells: with mediators and without mediators. Microbial fuel cells without mediators are recently discovered and their operation is not very well explained, due to several factors affecting the optimal working conditions, such as: the nature of microorganisms used in the system, the type of ion exchange membrane, temperature. Usually, the used bacteria have electrochemically active redox enzymes such as cytochromes on the outer membrane that can transfer electrons outside.

Microbial fuel cells use inorganic mediators that enter in the electrons transport chain from cells and extract the transferred electrons. The mediator crosses the outer lipid membrane of the cells and the plasma wall, and then begins to release electrons from the electron transport chain, which normally would be taken over by oxygen or other intermediaries. The reduced form of mediator leaves the cell charged with electrons, which are carried to the electrode, where they are discharged; this electrode becomes the charge generator anode (negatively charged electrode). The release of electrons means that the mediator is oxidized to its original state, making it ready to repeat the process. It is important to note that this process may take place only under anaerobic conditions; if oxygen is present then it will capture all the electrons, because it has higher electronegative character than the mediator.

Under aerobic conditions, when microorganisms consume the substrate (sugar), they produce carbon dioxide and water. However, when oxygen is not present, the microorganisms produce carbon dioxide, protons and electrons, according to the following oxidation reaction:

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^- \quad (3)$$

In a microbial fuel cell, the anode is the final electron acceptor recognized by bacteria in the anodic compartment. Therefore, microbial activity is strongly dependent on anode redox potential. Recently, it was discovered that there is a Michaelis-Menten type dependence between anode potential and nominal generated power, so there is a critical value of anodic potential that can get the maximum value of nominal power for a microbial cell.

MFC's reach a maximum working voltage of 0.3 to 0.7 V. The value of generated voltage is a more difficult problem to predict than in case of a chemical fuel cell. In a microbial cell, it takes time for bacteria to colonize the electrode and to create enzymes and structures needed to transfer electrons outside the cell. Even the electric potential of a pure culture can not be predicted with precision.

In biological systems, the potentials are usually pre-adjusted to a neutral pH, because the cytoplasm of most cells has a pH = 7.

When the voltage is positive, the reaction that occurs is exothermic. In this case, the variation of Gibbs free energy is negative. Total voltage is represented by the difference between the anodic and cathodic potential:

$$E_{ref} = E_{cat} - E_{an} \quad (4)$$

The potential is corrected for standard conditions and for pH = 7.

If the system's thermodynamic limits the total produced power, it is expected that the measured anodic potential to approach the calculated maximum potential. Typically, the maximum cathodic potential is 0.4 V, with a working potential of 0.25 V, even using a platinum catalyst. With the exception of oxygen, the most widely used catholit is hexacyanoferatte Fe $(CN)_6^{3-}$:

$$K_3[Fe(CN)_6] + e^- \rightarrow K_4[Fe(CN)_6] \quad (5)$$

3. COMPONENTS OF MICROBIAL FUEL CELLS

The main challenge in achievement of a microbial fuel cell is to identify materials and design, which optimizes the energy generation process and the coulombic efficiency but also minimizes the costs of a reliable and reproducible system.

The main components of a microbial fuel cell are anode, cathode and selective membrane [11, 12, 13].

In Table 1 are presented the components of a microbial fuel cell and also the materials used for their construction.

Table 1. Basic components of microbial fuel cells

Components	Materials	Observations
Anode	graphite, graphite felt, carbon, Pt, Pt black, reticulated vitreous carbon	necessary
Cathode	graphite, graphite felt, carbon, Pt, Pt black, reticulated vitreous carbon	necessary
Anodic compartment	glass, polycarbonate, Plexiglas	necessary

Cathodic compartment	glass, polycarbonate, Plexiglas	optional
Proton exchange system	- proton exchange membrane: Nafion®, Ultrex, polyethylene, poly (styrene-co-divinylbenzene) - salt bridge, porous porcelain diaphragm	necessary
Electrocatalyst	Pt, Pt black, MnO_2, Fe^{3+}, polyaniline, electronic mediator immobilized on the anode	optional

1.1. The anode has reached the highest level of development through the use of graphite fibre brush-type electrodes. The selection of membranes or other material separating the anode – cathode compartments is an important step for the development of these devices, due to high cost and general effect of increasing internal resistance.

Anodic material must have the following characteristics: high conductivity, non-corrosive characteristics, high specific surface area, high porosity, cheap, easy to produce in larger dimensions.

Among the anodic materials it can be mentioned:

- carbon paper, carbon cloth, foams and RVC; These materials have high conductivity and are suitable for the growth of microorganism. Carbon paper is rigid and fragile, but easy to connect to a conductor. The stainless steel or titanium conductors have better behaviour than copper.
- graphite rods, felt, foam, granules, plates and sheets

Graphite rods were used in several studies especially due to their high conductivity and relatively well-defined surface, but present the disadvantage of a reduced surface area for growth of microorganisms. In terms of current density, this parameter was studied in comparison (Chaundhuri and Lovley, 2003) using Rhodoferax ferriducens in a two-compartment microbial fuel cell.

So it was found that the graphite felt produces 2.4 times more current than graphite rods, mainly due to the large surface area.

- fibres and graphite brushes
- conductive polymers
- metals and metal plating.

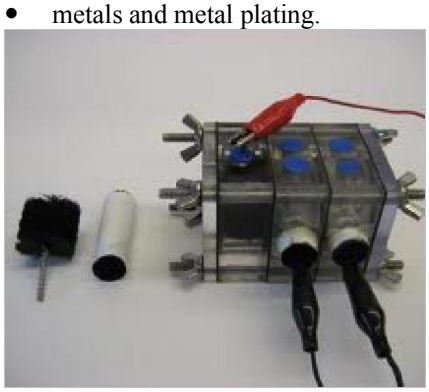

Fig. 2. Microbial fuel cell with carbon brush anode and tubular cathode [6]

3.2. In bicameral microbial fuel cells membranes are used to separate the anolit from the catholit. These membranes must be permeable to protons, but should be a barrier to the transfer of other chemical species between microbial fuel cell compartments. Membranes are used to reduce the organic substrate flow from the anode to the cathode and also the oxygen flow from the cathode to the anode (oxygen is toxic to exo-electrogene bacteria), improving coulombic efficiency.

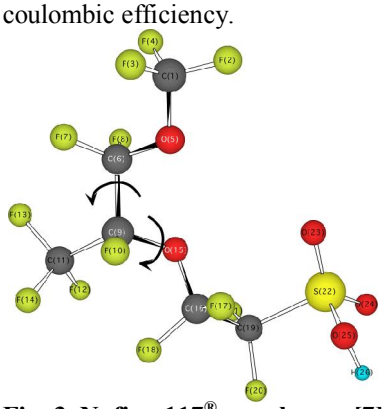

Fig. 3. Nafion 117® membrane [7]

The main disadvantages of separation membranes are the high cost and the fact that it slightly decreases the energy performance of the system as a result of increased internal resistance.

Membranes used in existing applications are of two types:

- cation exchange membranes: Nafion 117®, CEM
- anion exchange membranes

It was found that protons can be efficiently transported also by other chemicals used as pH buffer (phosphate anions).

- bipolar membranes;
- ultrafiltered membranes.

3.3. The presence of a catalyst is the necessary basic feature for the cathode. However, recent studies have shown promising results for the replacement of a noble metal catalyst by a transitional metal or other compounds that do not contain noble metals.

The mechanism of electrochemical reactions taking place at the cathode is difficult to determine because the electrons, protons and oxygen should converge on the catalyst surface in a three-phase reaction (solid catalyst, air and water). Catalyst placed on a conductive surface requires exposure to both water and air, so that protons and electrons from these phases to reach the same active reaction area of the catalyst.

At the cathode achievement are used the same materials as the anode, except that for the cathode is necessary, but not required, the presence of a catalyst (platinum for the reduction of O_2).

Among the cathode materials it can be mentioned:

- cathodes made of platinum or platinum-coated metal;
- metal cathodes;
- carbon cathodes with platinum catalyst;
- carbon cathodes with non-platinum catalyst.

in different geometrical shapes:

- plain carbon cathodes;
- tubular cathodes covered with carbon;
- bi-cathodes.

3.4. Microorganisms and exo-electrogens bacteria

Electrochemically active biofilms are of great importance in the natural environment, mainly in metals oxidation and reduction, in the minerals dissolution, in the carbon cycle and complexing phosphorus compounds and heavy metals.

Anaerobic bacteria have evolved over millions of years using various methods to reduce the compounds needed to maintain metabolism, all without the involvement of oxygen necessary for respiration [14, 15].

Most anaerobic bacteria can only transfer electrons to soluble compounds (nitrates, sulphates) that can diffuse through the cell membrane inside the cell.

Bacteria that have evolved have been able to use several different types of electronic acceptors, also transferring electrons outside the cell. Such bacteria are called exo-electrogens. Exo-electrogen bacteria differ in their capacity to carry electrons directly outside the cell, allowing them to function in a biochemical fuel cell.

Biochemical reactions inside the anaerobic bacteria may be carried out on different levels of temperature, depending on the tolerance of bacteria, from moderate temperature (15–35°C) to high temperatures (50–60°C) or low temperature (<15°C).

Exo-electrogen bacteria that produce electricity without mediators are:

- Gammaroteobacteria and Schewanella;
- Deltaproteobacteria and other members of Geobacteracae;
- Firmicutes and Clostridia;
- mediators generating bacteria;
- Pseudomonas Aeruginosa;
- Geothrix fermentans, from Geobacter genus type.

3.5. For any biological process substrate is important because it serves as a source of carbon and energy. Substrate affects not only the bacterial community full composition in anodic biofilm, but also microbial fuel cell performance, including the value of specific power and coulomb efficiency.

The most common types of substrates are:

- acetate – a simple substrate, extensively used as carbon source to induce electro-active bacteria; is preferred because of its inertia to other alternative types of microbial conversions at room temperature (fermentations and methane-genesis);
- glucose – another substrate commonly used in microbial fuel cells;
- lignocellulosic biomass.

Abundance and regenerative capacity of lignocellulosic materials from agricultural residues make of this type of substrate a potential source of cheap energy production. However, lignocellulosic biomass can not be used directly by microorganisms in MFC power generation. First this type of biomass must be converted to monosaccharide or other low molecular weight compounds. It was not found yet effective microorganisms to convert pentose (the main component of lignocellulosic hydrolysates) to bioethanol.

Other types of substrates involved in electricity generation are: synthetic waste water, waste water from the beer industry, starchy waste water, waste water from the paint industry, cellulose and chitin, inorganic substrates.

4. EXPERIMENTAL

4.1. Construction and operating conditions of the microbial fuel cell

Microbial fuel cell is achieved from polycarbonate and contains two circular compartments, anodic and cathodic, each having a volume of 27 mL, with an open side and two supply holes at the top, as in Figure 4. These compartments are assembled using screws, washers and nuts, being separated by a proton exchange membrane (Nafion®, DuPont USA), with an active area of 18 cm^2. Membrane was pre-treated by hydration in doubly distilled water for 24 hours before use. Each compartment contains one graphite electrode in the form of disc with a diameter of 4.8 cm (active surface area 18 cm^2).

The electrodes are provided with connecting wires that make the connection to external electrical circuit. Ohmic resistance of the electrodes and connecting wires is 3 kΩ, measured with an ohmmeter. The distance between electrodes is 3.4 cm.

Fig. 4. Components of the microbial fuel cell used in the experiments

The design shape of assembled microbial fuel cell is shown in Figure 5:

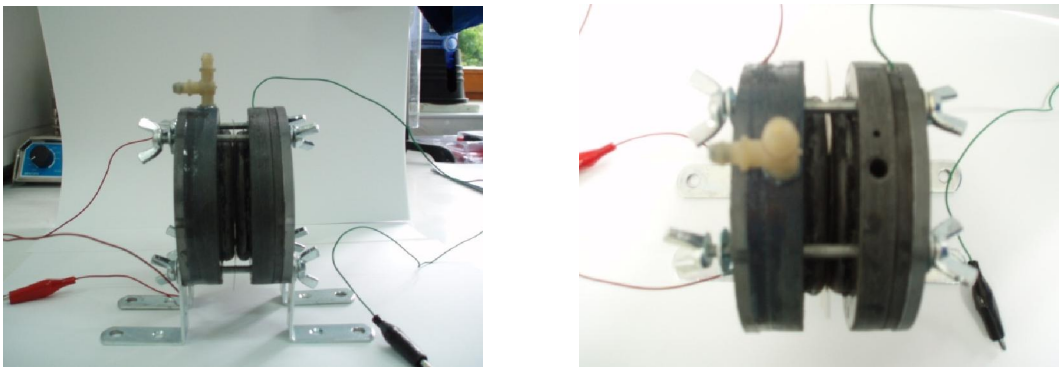

Fig. 5. The assembled microbial fuel cell

4.2. Electrolyte solutions and microorganisms

Anodic compartment was inoculated with a solution containing a suspension of microorganisms (Saccaromyces Cerevisae) in 0.1 M Na_2HPO_4 and NaH_2PO_4 buffer solution. The necessary nutrient for the feed of microorganism is based on 1M glucose solution and the mediator used is a 0.01 M methylene blue solution. After filling, the anodic compartment was closed to ensure an anaerobic environment. As a catholit in cathodic compartment was used a 0.02 M potassium hexacyanoferrate $K_3[Fe(CN)_6]$ solution.

Experimental measurements were performed at room temperature 25 ± 1°C. In order to form the biofilm on the anode surface, experimental measurements were initiated after a period of 30 minutes since inoculation.

Redox potential values of anodic and cathodic reactions are:

E_0 = +11 mV/Ag, AgCl (methylene blue) (6)

E_0 = +430 mV/Ag, AgCl (potassium hexacyanoferrate)(7)

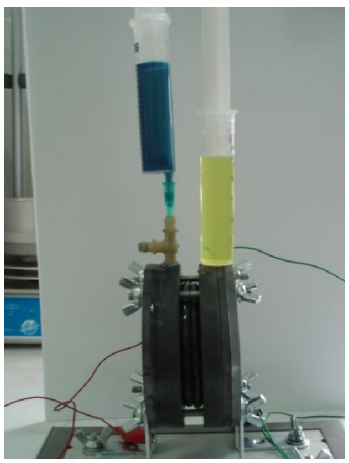

Fig. 6. Inoculation the two compartments of the fuel cell with the electrolytes

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4.3. Electrical and electrochemical measurements

In the external electric circuit one has placed a variable resistance (0 – 1000 Ω) connected to the two electrodes.

The nominal fuel cell voltage was monitored every 10 s by measuring the potential difference between anode and cathode, with VoltaLab 40 potentiostat, connected to a computer for data acquisition. Electrical power P was calculated with the formula:

$$P = I \cdot V \quad (8)$$

where V – electrodes potential measured versus Ag/AgCl saturated, V, I – current intensity, A.

Polarization tests were achieved by varying the external resistance, then reading current and voltage value at the stabilization point. Internal Ohmic resistance, which refers to the resistances amount of electrodes, electrolyte, membrane and interconnectors on charge transfer process was calculated according to the relation derived from Kirchoff's law for a circuit in that the value of electrical resistance connected to power source is known:

$$R_{int} = \frac{V_{OCP}}{I} - R \quad (9)$$

where V_{OCP} – open circuit voltage, I – current intensity at the resistance value R, R – electrical resistance.

5. RESULTS AND DISCUSSION

Figure 7 presents the nominal fuel cell voltage evolution under investigation. It can be noticed there is a sharp, gradual rise of the voltage in the first 30 minutes, followed by a further slower increase, then a period of stabilization around 500 mV values, approximately 2 hours after inoculation At the end of this investigation the anode and cathode surfaces were microscopically examined; on the anode was observed a thin layer, coloured in deep blue, which represents the biofilm formed by the microorganism's colony. This fact indicates that the tested microorganisms were oxidized the substrate and thus generating voltage.

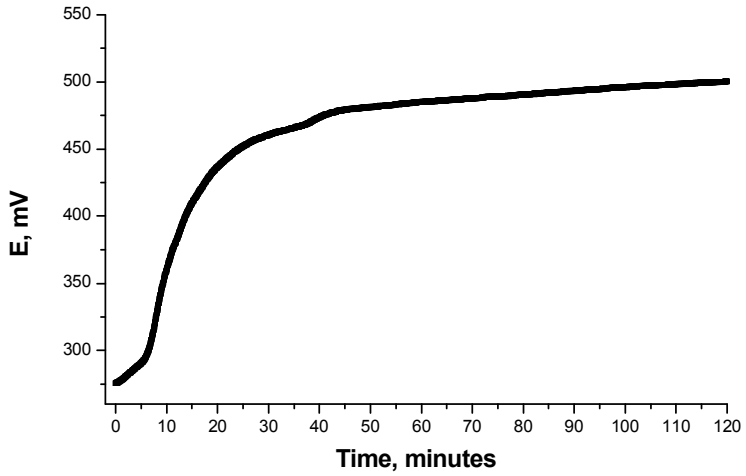

Fig. 7. Evolution in time of nominal fuel cell voltage

Figure 8 shows the experimentally determined nominal voltage and specific power, calculated according to relation (8), depending on current intensity values and implicit on variable resistance values of the circuit. From these curves can be observed that the maximum power value obtained is about 60 mW at a current intensity of 200 μA (external resistance R = 750 Ω). For this maximum value was calculated the internal resistance that is equal to 2 kΩ.

Since the cathodic reaction is potassium hexacyanoferrate reduction, the cathode potential remains constant, therefore changes in nominal fuel cell voltage is due to change in anode potential, thus biofilm formation and growth on anodic surface.

The low energy efficiency of fuel cell depends also on factors that can not be quantified, namely the accumulation of metabolites, biomass growth, diffusion through the substrate and electron-consuming competitive reactions (formation of gaseous hydrogen, methane gas, etc.) or reduction of gaseous oxygen that diffuses through the membrane.

5.CONCLUSIONS

Microbial fuel cells have gained much attention in recent years as a way to convert the organic waste, including wastewater or ligno-cellulosic biomass, into electricity. Electricity generation from microorganisms can become an important form of bioenergy in the future, since microbial fuel cells (MFC) allows the extraction of electricity from a wide variety of complex dissolved organic waste and also from renewable biomass sources. Variations in generated power values can be attributed to changes in the overpotential of activation, ohmic or concentration values within the investigated microbial fuel cell.

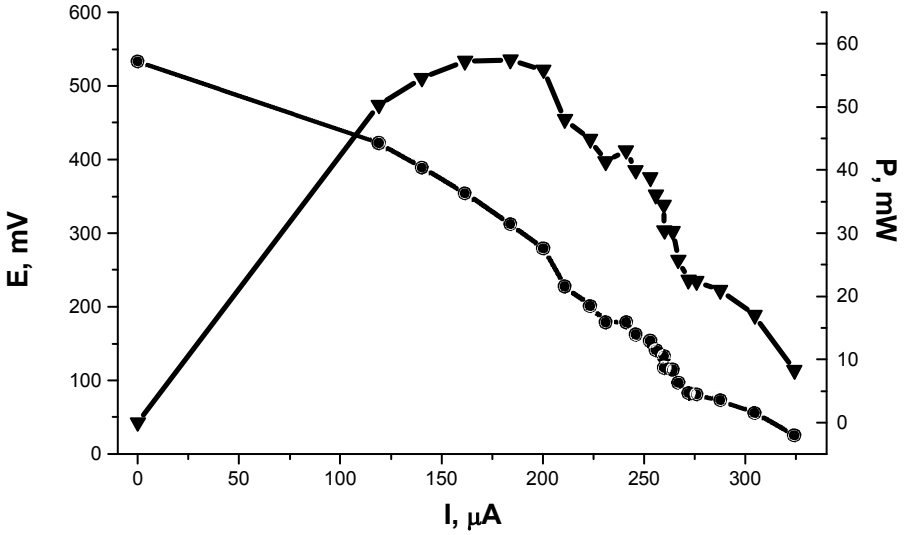

Fig. 8. The influence of external resistance values against nominal voltage and specific power

Variations in ohmic drop voltage and concentration overpotentials have relatively constant values as a result of similar operating conditions. Variations in generated power, as an indicator of energy performance, with external resistance value may be

associated with low levels of anodic activation overpotentials, which are influenced by the electrochemical activity of reducing microorganisms at the anode.

In conclusion, one may suggest that changes in anode potential depending on external resistance represent the main criterion for selecting the type of electrochemically active microorganism used.

It is expected that, over time, given the interest and resources invested in this area of research, generated power density to reach values that makes biochemical fuel cell used in other applications.

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