

## ELECTRICAL CHARACTERISTICS OF A BIOBATTERY WITH STAPHYLOCOCCUS AUREUS

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**ABSTRACT.** A homemade biobattery was studied from electrical point of view. The open circuit voltage for a 0.2 mA short circuit current was of 0.6 V. The maximum power for an internal resistance of 1.1 kΩ and a concentration of 10<sup>9</sup> cells/cm<sup>3</sup> of *Staphylococcus aureus* was of 17.57 μW.

**Keywords:** biobattery, electrical power, bacteria, *Staphylococcus aureus*.

### INTRODUCTION

In the last years, microbial fuel cells (MFCs) have received a great deal of attention as a promising solution for renewable energy generation and waste disposal [1-6]. A MFC converts energy, available in a bio-convertible substrate, directly into electricity through the catalytic activities of microorganisms.

It can be said that the MFCs are a hybrid of biological and electro-chemical reactors. By this dualistic nature, MFCs offer the advantage of utilizing a wide range of organic compounds as fuel, and exploit the value of electro-chemical cells by direct generation of electricity [7].

In a MFC, electricity is being generated in a direct way from biowastes and organic matter. This implies that the overall conversion efficiencies that can be reached are potentially higher for MFCs compared to other biofuel processes.

Parameters influencing the overpotentials at the anode are the electrode surface, the electrochemical characteristics of the electrode, the electrode potential, and the kinetics together with the mechanism of the electron transfer and the current of the MFC.

Mediators are important in MFC cells that use microorganisms such as *Escherichia coli*, *Pseudomonas*, *Proteus*, and *Bacillus* species that are unable to effectively transfer electrons derived from central metabolism to the

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outside of the cell. Common electron shuttles include thionine, benzylviologen, 2,6-dichlorophenolindophenol, 2-hydroxy-1,4-naphtho-quinone and various phenazines, phenothiazines, phenoxazines, iron chelates and neutral red [8]. These electron shuttles are typically capable to cross the cell membranes, accepting electrons from one or more electron carriers within the cell, exiting the cell in the reduced form and then transferring electrons onto the electrode surface. The critical issue with mediated electron transfer is the diffusion of the shuttle out of the biofilm or the bacterial environment [9].

There is a report on the bacteria, *Rhodospirillum rubrum* that can be used in microbial fuel cells effectively without a mediator [10].

Oxygen is generally used as the electron acceptor for the cathodic reaction in MFCs. Graphite is a commonly used electrode material. To improve catalytic activity, graphite can be modified with platinum [11].

Various metals (copper, gold, palladium/cobalt, molybdenum, tungsten, and manganese) and their complexes have been investigated as replacements for expensive platinum in the cathode [11-13].

Because the power output of MFCs is low relative to other types of fuel cells, reducing their cost is essential, if power generation using this technology is to be an economical method of energy production. Further research is required to enhance the power production.

In this study, we investigate the performance of a biobattery with *Staphylococcus aureus* bacteria without mediator in the original arrangement of electrode materials at anode compartment because the biobatteries are less investigated at this moment.

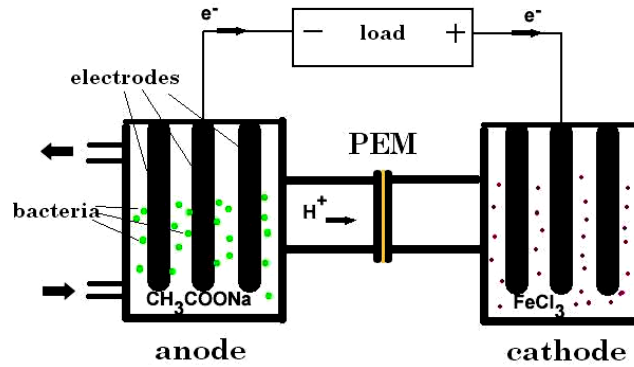
## RESULTS AND DISCUSSION

The purpose of the experiments was to measure the open circuit voltage, the short circuit current and to plot the power curve of the biobattery. The bacteria used in the experiment were *Staphylococcus aureus* and the substrate was the sodium acetate.

$\text{FeCl}_3$  was used as a cathode mediator in order to improve oxygen reduction kinetics [14-15] and acetate was used as substrate in the anodic compartment. The purpose of the experiment was to measure the voltage between the two compartments at 2 different concentrations of acetate in the absence and presence of the bacteria. The activation of the biobattery has been achieved by adding the bacterial solution. After a few seconds it was observed the bubbling process on the anode rods electrode material due to the carbon dioxide gas resulted from the bacterial activity.

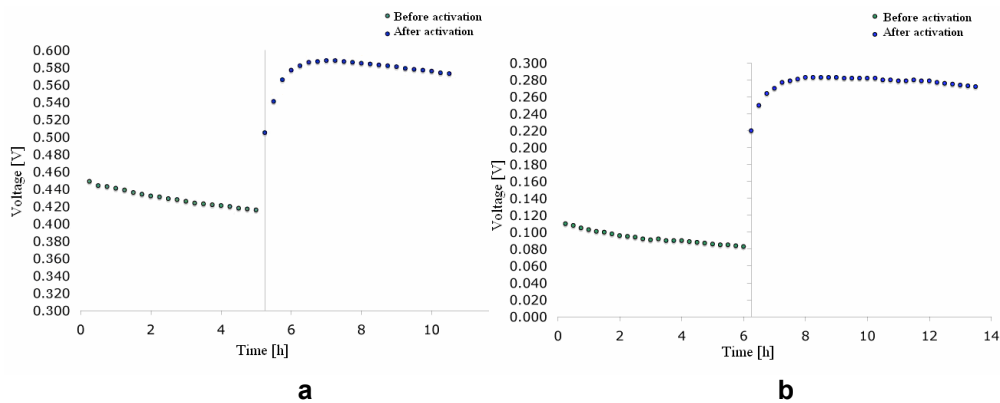
The pH of first solution was 8.6 and for the second solution 8.9.

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**Figure 1.** Biobattery representation

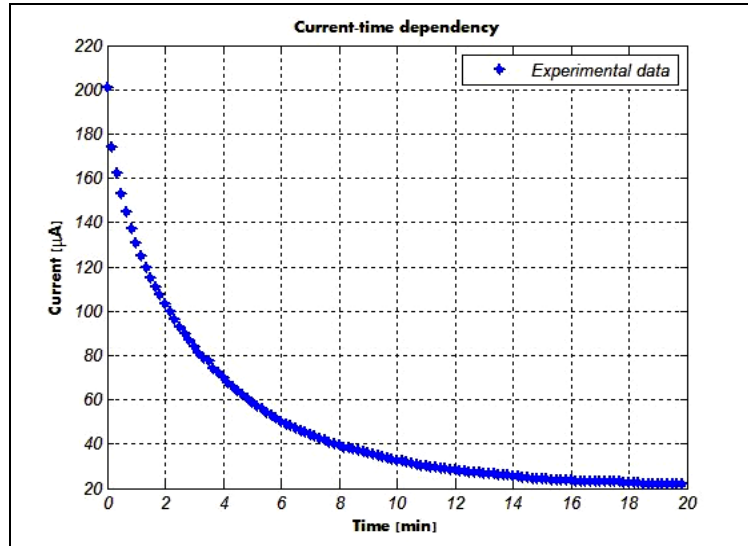
In the first experiment by monitoring the cell voltage for 5 hours, a drift of OCV (open circuit voltage) appears from 0.46V to 0.42 V before activation. A maximum peak of 0.6 V was measured after biobattery activation. The short circuit current was of 0.2 mA. (Figure.2a).



**Figure 2.** Voltage-time dependency.

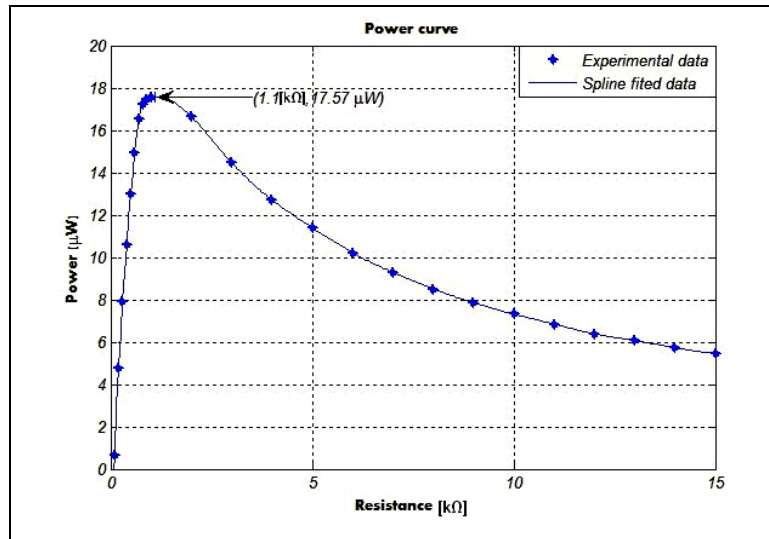
a) for 0.05M sodium acetate solution b) for 0.1M sodium acetate solution

In the second experiment before activation the same drift appear (about 40mV) and after activation we measured a maximum cell voltage of 0.3 V and the short circuit current of 0.2mA (Figure 2b). In both cases the open circuit voltage remains at the constant values of 0.56V respectively 0.26 V for one month. Current-time curve and power curve, was recorded for the biobattery with increased OCV.



**Figure 3.** Short circuit current-time dependency  
0.05M sodium acetate solution

Initially, after the biobattery activation, a peak current of about 200  $\mu\text{A}$  was registered. After 20 minutes, the limit current of about 20  $\mu\text{A}$  was reached in agreement with the last point of the power curve (Figure 4).



**Figure 4.** Power curve 0.05M sodium acetate solution

As we reduce the external resistance, a decrease of the voltage was recorded. In this way, we looked for having the smallest possible drop in voltage as the current is increased in order to attain the maximum power production over the investigated range of interest.

The internal resistance of the battery and the maximum power generated were calculated by using the Mathworks MATLAB® environment for processing the experimental data. Spline functions have been used to approximate the power curve. (Figure 4).

All of the equipment used was sterilized with hydrogen peroxide after the experiments.

## CONCLUSIONS

The maximum power of the biobattery was of 17.57  $\mu$ W, achieved at the internal resistance of 1.1 k $\Omega$  and for the external resistance of about 15 k $\Omega$  the minimum power reached was 5.45  $\mu$ W, in the case of 0.05M substrate concentration in anode compartment.

The value of the OCV remained at the constant value of about 0.56 V for a month. The difference which appears in OCV in both experiments can be explained by decreasing of the bacterial activity whit pH increase.

The biobatteries represent a viable method of generating electricity from chemical energy. Further research must be done with the purpose of reducing the dimensions of the biobattery for possible applications in medicine and other fields.

## EXPERIMENTAL SECTION

An electrochemical cell has been built with two chambers separated by a Nafion proton exchange membrane (Figure 1). Nine graphite rod electrodes of 6 mm in diameter and 10 cm in length have been used for each chamber. For the anode compartment a 300 ml sodium acetate solution 0.05 M and 0.1 M respectively, has been combined with 2 ml of *Staphylococcus aureus* ( $10^9$  cell/ml concentration). For the cathode compartment, 300 ml of 1 M FeCl<sub>3</sub> solution was used.

The circuits which contain a biobattery and multimeters were connected to a computer with continuous data acquisition software.

The used bacteria were *Staphylococcus aureus*. The bacteria posed no threat to the working environment because they were non sporogenous.

**Bacterial strains.** Both used bacterial strains, the UCLA 8076 (University of California, Los Angeles, USA) [16] and the 1190R (Simmelweis University, Budapest, Hungary) [17], were heterogeneous MRSA (methicillin-resistant *Staphylococcus aureus*) strains. These strains are preserved in glycerol (25% final concentration) at -80°C and prepared as follows: 5ml of overnight bacteria culture at 37°C in LB broth (Gibco BRL, Life Technologies, Paisley, Scotland)

were centrifuged in 15ml centrifuge tubes in a Sigma 1-18K centrifuge (Sigma Laborzentrifugen, Osterode am Harz, Germany –5,500 rpm, 10 min., room temperature) and the pellet was resuspended in 500µl sterile 50% glycerol.

The thawed bacteria were cultured overnight in 5ml of Mueller Hinton broth (Fluka, Buchs, Switzerland) into a Certomat BS-T incubation shaker (Sartorius Stedim Biotech, Aubagne, France) at 37°C, 150 rpm until the culture reached an OD<sub>600</sub> of 0.8-1.0 (Spekol UV VIS 3.02, Analytic Jena, Jena, Germany). A loop of bacterial suspension was passed on Mueller Hinton agar (Fluka, Buchs, Switzerland) to maintain the culture for further analysis. The bacteria were cultured also on blood agar plates (Fluka, Buchs, Switzerland) and confirmed using colony morphology method.

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