THE EVALUATION OF BACTERIAL CULTURES REDOX POTENTIAL IN MICROBIAL FUEL CELLS WITH DIFERENT CONFIGURATIONS

IACOB Matei-Tom, GHINEA Adrian, MOISESCU Cristina, BĂLAN Adriana, STAMATIN Şerban, ARDELEAN I. Ioan

Abstract. Microbial fuel cells are bio-electrochemical devices that generate electrical power by using the catalytic activity of microorganisms. The use of these fuel cells to measure the redox potential of microorganisms can become, in the future, a very important tool in monitoring the microbial cell activity. Finding a cheap and yet easy to use configuration can be a step closer for these systems to become common tools for analysing microbial behaviour. Normal procedures such as the sterilization of culture media and the maintenance of their sterility throughout the entire monitoring process can become an impediment when one wants to study closely the processes that take place in a pure microbial culture. Therefore, a simple set-up that gives good results has to be designed. The present study aims to evaluate the results obtained with two configurations of microbial fuel cells in order to see which configuration provides better results when measuring the redox potential of microbial cultures.

Keywords: bio-electrochemical systems, microbial fuel cell, redox potential.

Rezumat. Estimarea potențialului redox al unor culturi bacteriene prin utilizarea unor celule (pile) de biocombustie cu configurații diferite. Pilele de combustie microbiene sunt sisteme bioelectrochimice ce generează energie electrică cu ajutorul activității catalitice a microorganismelor. Folosirea pilelor de biocombustie pentru măsurarea potențialului redox al microorganismelor poate deveni o unealtă foarte importantă în monitorizarea activității celulelor microbiene. Găsirea unei configurații ieftine și totodată ușor de folosit poate constitui un pas înainte pentru ca aceste sisteme să devină unelte de uz comun pentru analiza comportamentului microbian. Proceduri normale precum sterilizarea mediilor de cultură și menținerea sterilității acestora pe tot parcursul procesului de monitorizare, pot deveni un impediment atunci când se dorește studierea îndeaproape a proceselor care au loc într-o cultură microbiană pură. Așadar, se impune necesitatea proiectării unui montaj simplu dar care să dea rezultate bune. Studiul de față își propune evaluarea rezultatelor obținute cu ajutorul a două configurații de celule de combustie microbiene, cu scopul de a vedea care configurație este mai adecvată pentru măsurarea potențialului redox al culturilor microbiene.

Cuvinte cheie: sisteme bioelectrochimice, pile de combustie microbiene, potențial redox.

INTRODUCTION

Microbial fuel cells are a type of bio-electrochemical systems, and they are used in various domains such as wastewater treatment, power generation, chemical compound synthesis etc. (ARDELEAN et al., 1983; KREYSA et al., 1990). This type of fuel cells can generate electric power by using microorganisms that liberate electrons while metabolising the organic matter (KATO, 2015).

A typical microbial fuel cell (MFC) usually comprises two compartments: (i) an anode compartment which contains an anode electrode, a bacterial culture medium, and one or more types of microorganisms, and (ii) a cathode compartment that contains a buffer solution and a cathode electrode. An ion exchange membrane is placed between the two compartments (LOGAN et al., 2006).

Fig. 1 shows the working principle of a typical MFC. The organic matter from the anode compartment is digested by the microorganisms and are liberating electrons. The electrons are collected by the anode and travel to the cathode through an external circuit. The electrons are used for the reduction of oxygen to water in the presence of the protons that passed through the ion exchange membrane.

This principle can work in one and two compartment configuration, the difference is that in the one compartment setup the cathode is placed with one side in direct contact with oxygen and not in the liquid as shown in Fig. 1 (LOGAN, 2009). Several microorganisms have been studied for power generation in microbial fuel cells (NEVIN et al., 2008; XING et al., 2008; ZUO et al., 2008; REZAEI et al., 2009). Mixed cultures of *Geobacter sulfurreducens* showed a smaller power density compared to the pure *G. sulfurreducens* (NEVIN et al., 2008). Other authors found that pure *G. sulfurreducens* generate less power density than the mixed culture and attributed this to the lower anode potential produced by the mixed culture (ISHII et al., 2008).

Shewanella is widely studied in the literature as it is perceived a standard in microbial fuel cells. Studies on *S. oneidensis* MR-1 showed that the anode potential changes with increasing current density (MANOHAR et al., 2008). Microbial fuel cells are a highly complex system in which the anode and cathode electrolyte as well as the electrochemical setup (1 or 2 compartment) plays an important role. The microorganisms are placed in an aqueous electrolyte medium which can influence the growth rate and metabolism. Direct comparison of power densities must be made in identical conditions (LOGAN, 2009) in order to relate the power densities to microorganisms alone.

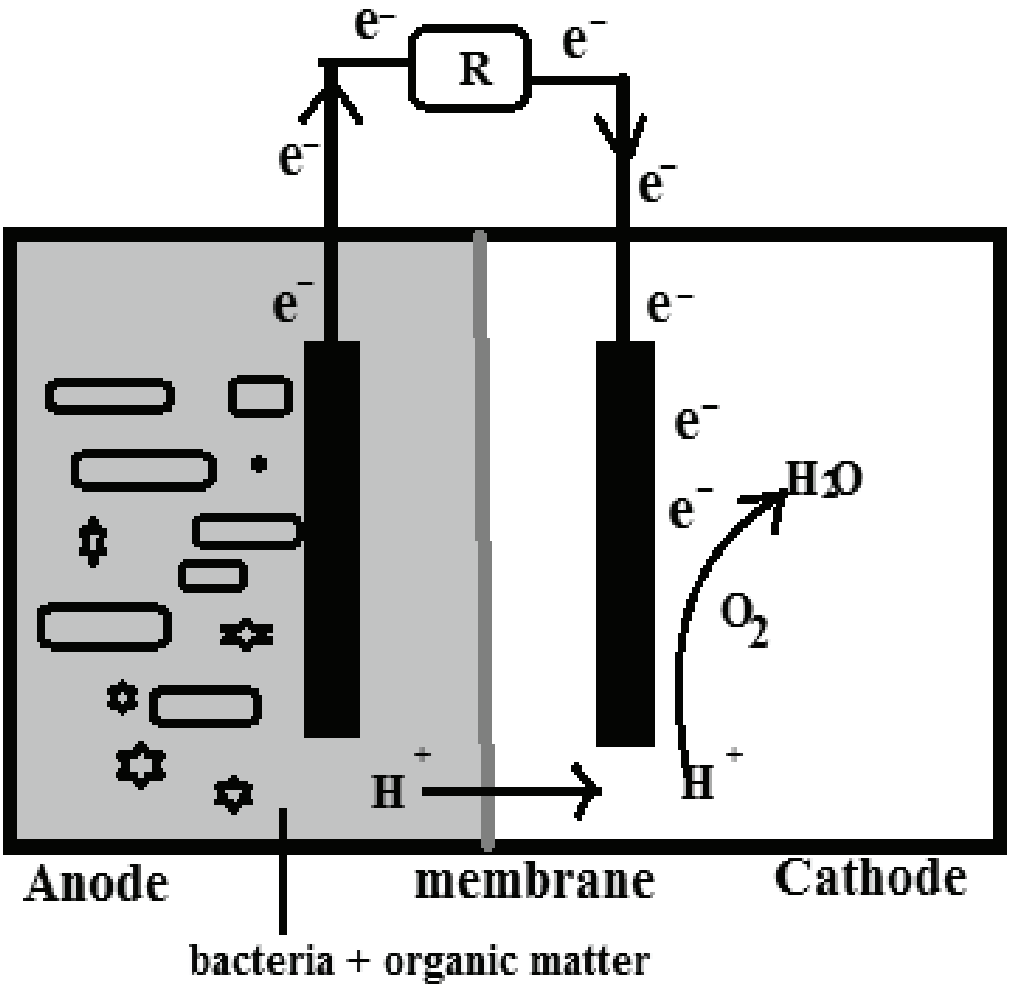

Figure 1. Representation of a typical microbial fuel cell (original).

The fact that electrons are liberated in the culture medium changes the electrochemical properties of the anode compartment. Redox potential (reported in Volts) is such a property which indicates the tendency of the medium to oxidize (positive values) or reduce (negative values) species present in the electrolyte. This redox potential can be measured with the help of a reference electrode (AIKENS, 2009). The redox potential of the electrolyte with microorganisms is related to growth (LEE & OLESZKIEWICZ, 2003; LEE & NIRMALAKHANDAN, 2011), metabolic pathways (TEMPEST & NEIJSSEL 1984), mutations (DU et al., 2007) etc. Moreover, the redox potential was shown to be related directly to the power production in a microbial fuel cell (WATSON & LOGAN, 2010).

This work aims at studying the differences in redox potential that arise from measurements in two different types of microbial fuel cell setups.

MATERIALS AND METHODS

Two different reactor configurations were used for this comparison. For a good comparison, conditions such as: temperature, amounts, medium type and culture batch were kept the same. Also, for measurements both the reference electrodes and carbon felt electrodes were the same type and dimensions (3x8 cm) in all configurations, as this excludes other types of errors.

One compartment fuel cell configuration. For the one compartment set-up we used a carbon felt electrode as measuring electrode and one calomel electrode as the reference electrode (Fig. 2a). The anode compartment was filled with 375 ml of a basal medium, modified after (ROH et al., 2006), inoculated with 1% bacterial culture (FAN & XUE, 2016).

Two compartments fuel cell configuration. For the two compartments set-up (Fig. 2b), in the anode compartment we used the same culture medium, the same amounts and the same type of electrodes as in the one compartment configuration. In the cathode compartment we used a Sorensen buffer solution (375 mL) and a carbon felt as cathode electrode (same as in anode). The two compartments were separated by an ion exchange membrane. In the external circuit between anode and cathode we connected a 100 ohms resistor as a consummator.

The reference electrode is an electrochemical device with the help of which you can make stable measurements of a potential of another electrode. Due to the changeable nature of the surfaces of different electrodes,

an electrode which will not change its potential is needed for accurate measurements, so that the potential can be measured in reference to it. In our experiments we used a saturated Hg/Hg_2Cl_2 reference electrode which was introduce in the medium before inoculation. Measurements were adjusted to the standard hydrogen electrode (SHE+244 mV). All the electrodes introduced in the microbial fuel cell should be biocompatible, chemically inert and good electricity conductors (MOROSAN et al., 2007). Using chemically active electrodes can perturb the redox reactions that are taking place and thus the measured results. Also, using non-biocompatible materials (e.g. copper) can be toxic and therefore inhibit the microbial metabolism (PHAM et al., 2009; ALINCY et al., 2018).

Bacterial strain and growth conditions. *Shewanella oneidensis* strain MR-1 (LMG 19005) was purchased from the BCCM/LMG Bacteria Collection and pre-grown aerobically aprox.24 h in Luria–Bertani broth containing yeast extract (5 g/L), sodium chloride (10 g/L), and tryptone (10 g/L), before inoculation. For monitoring the potential, we used an ADC 20-Pico Technology Ltd acquisition system connected to a laptop running PicoLog 6 software.

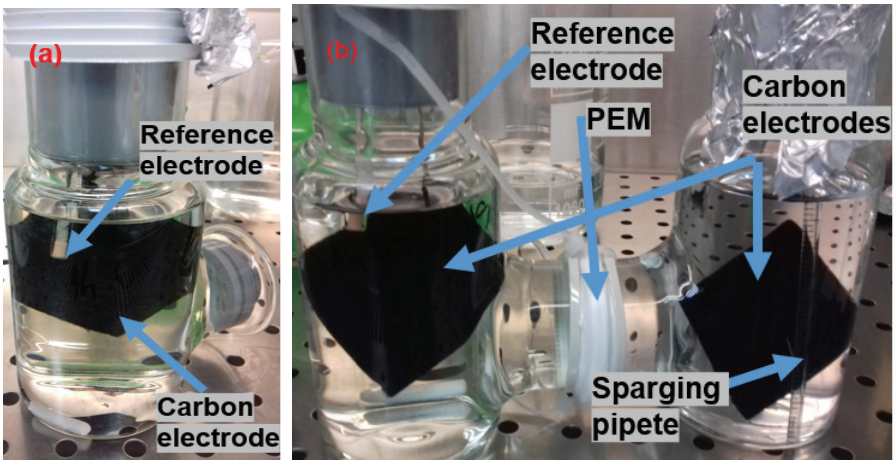

Figure 2. a) One compartment fuel cell configuration; b) Two compartment configuration (original).

RESULTS AND DISCUSIONS

Both configurations were inoculated and measured at the same time and kept next to each other in the incubator. That is why the recorded values are almost the same at the beginning, from the inoculation point; then they begin to change especially when the resistor is connected, but the growth start point, represented by the long drop in potential, remains very similar in both configurations. This and the fact that the same bacteria should have the same growth rate in the same conditions let us compare these systems better. A difference between the two configurations is that the two-compartment set-up is much harder to assemble than the one compartment setup. One of the causes for this is that we fit an ion exchange membrane which needs a lot of attention.

The line, shown in (Fig. 3a), represents the redox potential measured in the one compartment set and we see that it starts with the approx. 350 mV value which is the potential of the medium and it goes till around -300 mV which is the redox potential of the medium with the grown bacteria in it.

The line, shown in (Fig. 3b), follows the redox potential of the two-compartment set-up and starts around the same values as the other set-up, but the maximum value goes almost to -120 mV, smaller than in the one compartment set-up.

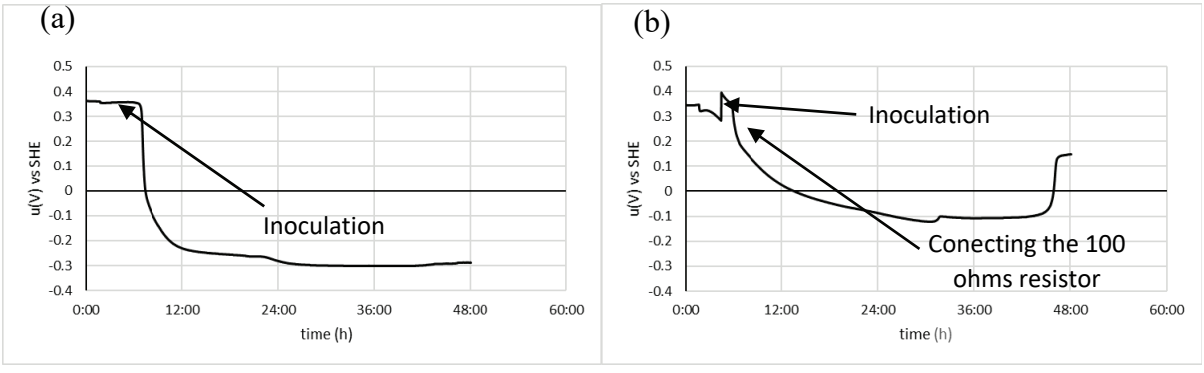

Figure 3. a) The evolution in time of the redox potential in one compartment configuration inoculated with *Shewanella oneidensis* MR-1 using a MBS medium; b) The evolution in time of redox potential in the two compartment configuration inoculated with *Shewanella oneidensis* MR-1 using a MBS medium and having a load of 100 ohms between the terminals of the anode and cathode.

Similar experiments (Watson & Logan 2010) show comparable results with the *S. oneidensis* MR-1 redox potential varying between -150 and -350 mV in different configurations indicating that the redox potential of this kind of bacteria is around these values. The fact that a current circulates through the resistor and that the two compartments configuration potential values are closer to zero clear indicates that a consummator affects the measured redox potential.

The negative values of redox potential mean that oxidations are taking place in the anode and electrons are liberated in the process. The fact that one configuration has smaller values doesn't mean necessarily that bacteria is consuming less resources but that the resistor is consuming electrons. The fact that we can measure a current in the two-compartment setup gives us the advantage that we can calculate the power generated by the microbial fuel cell. However, just for measuring the redox potential without wanting to find out more, the one compartment configuration is enough (PODDAR & KHURANA, 2011).

As a conclusion, the one configuration set-up gives better results when it comes to measuring the redox potential because it does not lose electrons through the resistor as the two-compartment setup does. The latter is harder to set and gives poorer results in terms of the redox potential, but we can harvest more information about the system by calculating the current that is passing through the resistor. Choosing a configuration is important depending on what you want to find out about the system that is why no configuration can be excluded permanently.

ACKNOWLEDGMENTS

This work was funded by the contract 76PCCDI/2018, Project "Eco-innovative technologies for recovering platinum group metals from used catalytic converters" (ECOTECH-GMP). The authors thank to Mrs. Cirnu Marinela for her technical skills and devotion.

REFERENCES

AIKENS D. 1983. Electrochemical methods, fundamentals and applications. *Journal of Chemical Education*. American Chemical Society Publisher. New York. **60**: 15-25.

ALINCY ANGELA, NAVANIETHA M., KRISHNARAJ R., SHAKAMBARI G., ASHOKKUMAR B., KATHIRESAN S., VARALAKSHMI P. 2018. Biofilm Engineering Approaches for Improving the Performance of Microbial Fuel Cells and Bioelectrochemical Systems. *Frontiers in Energy Research*. Springer. Berlin. **6**: 22-34.

ARDELEAN I., MĂRGINEANU D., VAIS H. 1983. Electrochemical conversion in biofuel cells using *Clostridium butyricum* or *Staphylococcus aureus* Oxford. *Bioelectrochemistry and Bioenergetics*. Elsevier. Paris. **11**: 273-277.

DU C., ZHANG Y., LI Y., CAO Z. 2007. Novel Redox Potential-Based Screening Strategy for Rapid Isolation of *Klebsiella pneumoniae* Mutants with Enhanced 1,3-Propanediol-Producing Capability. *Applied and Environmental Microbiology*. Elsevier. Paris. **73**: 4515-4521.

FAN L. & XUE S. 2016. Overview on Electricigens for Microbial Fuel Cell. *The Open Biotechnology Journal*. Scimago Press. Tokyo. **10**: 398-406.

ISHII S., WATANABE K., YABUKI S., LOGAN B., SEKIGUCHI Y. 2008. Comparison of Electrode Reduction Activities of *Geobacter sulfurreducens* and an Enriched Consortium in an Air-Cathode Microbial Fuel Cell. *Applied and Environmental Microbiology*. Elsevier. Paris. **74**: 7348-7355.

KATO S. 2015. Biotechnological Aspects of Microbial Extracellular Electron Transfer. *Microbes and Environments*. Scimago Press. Tokyo. **30**: 133-139.

KREYSA G., SELL D., KRÄMER P. 1990. Bioelectrochemical Fuel Cells. *Berichte der Bunsengesellschaft für physikalische Chemie*. Springer. Berlin. **94**: 1042-1045.

LEE Y. & OLESZKIEWICZ J. 2003. Effects of predation and ORP conditions on the performance of nitrifiers in activated sludge systems. *Water Research*. Elsevier. Paris. **37**: 4202-4210.

LEE Y. & NIRMALAKHANDAN N. 2011. Electricity production in membrane-less microbial fuel cell fed with livestock organic solid waste. *Bioresource Technology*. Elsevier. Paris. **102**: 5831-5835.

LOGAN B., HAMELERS B., ROZENDAL R., SCHRÖDER U., KELLER J., FREGUIA S., AELTERMAN P., VERSTRAETE W., RABAEY K. 2006. Microbial Fuel Cells: Methodology and Technology. *Environmental Science & Technology*. American Chemical Society. New York. 40: 5181-5192.

LOGAN B. 2009. Exoelectrogenic bacteria that power microbial fuel cells. *Nature Reviews Microbiology*. Nature Publishing Group. London. **7**: 375-381.

MANOHAR A., BRETSCHGER O., NEALSON K., MANSFELD F. 2008. The polarization behaviour of the anode in a microbial fuel cell. *Electrochimica Acta*. Elsevier. London. **53**: 3508-3513.

MOROSAN A., STAMATIN I., STAMATIN L., DUMITRU A., SCOTT K. 2007. Carbon electrodes for microbial fuel cells. *Journal of Optoelectronics and Advanced Materials*. INOE Publishing House. London. **9**: 221-224

NEVIN K., RICHTER H., COVALLA S., JOHNSON J., WOODARD T., ORLOFF A., JIA H., ZHANG M., LOVLEY D. 2008. Power output and columbic efficiencies from biofilms of *Geobacter sulfurreducens* comparable to mixed community microbial fuel cells. *Environmental Microbiology*. Elsevier. Paris. **10**: 2505-2514.

PHAM T., AELTERMAN P., VERSTRAETE W. 2009. Bioanode performance in bioelectrochemical systems: recent improvements and prospects. *Trends in Biotechnology*. Cell Press. London. **27**: 168-178.

PODDAR S. & KHURANA S. 2011. Geobacter: The Electric Microbe! Efficient Microbial Fuel Cells to Generate Clean, Cheap Electricity. *Indian Journal of Microbiology*. Springer. New Delhi. **51**: 240-241.

REZAEI F., XING D., WAGNER R., REGAN J., RICHARD T., LOGAN B. 2009. Simultaneous Cellulose Degradation and Electricity Production by Enterobacter cloacae in a Microbial Fuel Cell. *Applied and Environmental Microbiology*. Elsevier. Paris. **75**: 3673-3678.

ROH Y., GAO H., VALI H., KENNEDY D., YANG Z., GAO W., DOHNALKOVA A., STAPLETON R., MOON J., PHELPS T., FREDRICKSON J., ZHOU J. 2006. Metal Reduction and Iron Biomineralization by a Psychrotolerant Fe (III)-Reducing Bacterium, *Shewanella* sp. Strain PV-4. *Applied and Environmental Microbiology*. Elsevier. Paris. **72**: 3236-3244.

TEMPEST D. & NEIJSSEL O. 1984. The Status of YATP and Maintenance Energy as Biologically Interpretable Phenomena. *Annual Review of Microbiology*. Scimago Press. **38**: 459-513.

WATSON V. & LOGAN B. 2010. Power production in MFCs inoculated with *Shewanella oneidensis* MR-1 or mixed cultures. *Biotechnology and Bioengineering*. Nature Biotechnology Publishing. London. **105**: 489-498.

XING D., ZUO Y., CHENG S., REGAN J., LOGAN B. 2008. Electricity Generation by *Rhodopseudomonas palustris* DX-1. *Environmental Science & Technology*. American Chemical Society Publisher. New York. **42**: 4146-4151.

ZUO Y., XING D., REGAN J., LOGAN B. 2008. Isolation of the Exoelectrogenic Bacterium *Ochrobactrum anthropi* YZ-1 by Using a U-Tube Microbial Fuel Cell. *Applied and Environmental Microbiology*. Elsevier. Paris. **74**: 3130-31.

Iacob Matei-Tom, Ghinea Adrian, Moisescu Cristina, Ardelean I. Ioan
Institute of Biology Bucharest of the Romanian Academy, Spl. Independentei no. 296, sect. 6, 060031, Bucharest, Romania.
E-mails: tom.iacob@ibiol.ro, iacobtomita@yahoo.com; adrian.ghinea@ibiol.ro, adrianghinea95@gmail.com; cristina.moisescu@ibiol.ro, cristina.moisescu@yahoo.com; ioan.ardelean@ibiol.ro. ioan.ardelean57@yahoo.com

Bălan Adriana, Stamatin Şerban
Faculty of Physics, University of Bucharest, Atomiştilor 405, Măgurele – Bucharest, Ilfov County, Romania.
E-mails: andronie@3nanosae.org, adriana.balan@unibuc.ro; serban@3nanosae.org, serban.stamatin@unibuc.ro

Received: April 10, 2019
Accepted: September 02, 2019