# **ANODE BIOFILM FORMATION WITH APPLIED EXTERNAL VOLTAGE**

# **D.S. Koltysheva\* , K.O. Shchurska, Ye.V. Kuzminskyi**

Igor Sikorsky Kyiv Polytechnic Institute, Kyiv, Ukraine

\*Corresponding author: dinakoltisheva@gmail.com

#### Received 13 February 2023; Accepted 5 April 2023

**Background.** The formation of an exoelectrogenic biofilm in a microbial fuel cell (MFC) is an important stage, because it affects later on current generation by the system. The fermented residue after methanogenesis as an inoculum contains not only exoelectrogenic microorganisms, but also methanogens, which reduce the productivity of MFC. The use of current allows the formation of a biofilm enriched with exoelectrogenic microorganisms.

**Objective.** The purpose of our study was to establish the parameters of MFC under periodic application of external voltage.

**Methods.** A two-chamber H-type MFC with a salt bridge between the chambers was used for the study. The anolyte was stirred with a magnetic stirrer for 4 h a day and a 3V voltage was simultaneously applied to create selective conditions for exoelectrogenic biofilm growth.

**Results.** The application of external voltage stimulated the increase in the current and voltage of the MFC. With the periodic application of an external voltage, the MFC current increased to 788  $\pm$  40  $\mu$ A for the MFC with a resistor and without load. After disconnection and discharge, the MFC current dropped to  $189 \pm 10 \mu A$  for the MFC without load and to  $154 \pm 8 \mu A$  for the MFC with a resistor, respectively. Under the conditions of MFC operation without applying external voltage, the current was  $960 \pm 50 \mu A$  for MFC with an open circuit and  $672 \pm 35 \mu A$  for MFC with a closed circuit when a resistor is connected. For all MFC, the current gradually decreased over time. MFC demonstrated capacitive behaviour: after accumulating charge for 4 h, a discharge from  $622 \pm 30$  mV to  $462 \pm 23$  mV was observed. Microscopy showed fouling of the anode. Since the fermented residue after methanogenesis is mixed consortium, the anodic biofilm was also mixed consortium enriched with different species of exoelectrogens.

**Conclusions**. Periodic application of external voltage allowed to increase the current by 17% and double the voltage compared to MFC without external voltage supply. However, after disconnecting the external voltage source, the MFC gradually discharged, that is, the current and voltage decreased. The maximum value of the current of the MFC with an open circuit was 22% more than the MFC with a closed circuit.

**Keywords:** microbial fuel cell; external voltage; biofilm; bioanode. 

## **Introduction**

The use of fossil fuels causes environmental pollution, which creates a need for sustainable and environmentally friendly technologies for obtaining energy [1]. Microbial fuel cell (MFC) is a technology in which wastewater treatment and energy production are carried out simultaneously due to the bioelectrochemical conversion of substrates by exoelectrogenic microorganisms [2]. In a twochamber MFC exoelectrogenic microorganisms oxidize organic substances with the formation of electrons at the anode. Electrons from the anode through an external electrical circuit are transported to the cathode, and protons are transported through the membrane between the cathode and anode chambers. At the cathode electrodes, protons and the terminal electron acceptor, which in many cases is oxygen, combine to form water [3]. In the anode chamber, either exoelectrogenic anode bacteria represented by a pure culture or a microbial consortium can be used as bioagents that decompose organic compounds with the release of electrons. The microbial consortium is more resistant to changes in the qualitative and quantitative composition of the medium due to a wide range of compounds, which is able to transform [4]. The main role in the production of electricity is played by exoelectrogenic bacteria, which are directly attached to the anode [2]. Thus, for a more efficient conversion of the substrate into electrical energy, it is important to form a biofilm enriched with exoelectrogenic bacteria on the anode. The source of obtaining exoelectrogenic bacteria is a number of natural and artificial sources, in particular, such as river sludge, anaerobic sludge, sand, compost, soil, etc. [5]. The fermented residue after methanogenesis contains a significant number of exoelectrogenic bacteria, but on the other hand, it is also rich in other types of bacteria, in particular

<sup>©</sup> The Author(s) 2023. Published by Igor Sikorsky Kyiv Polytechnic Institute.

This is an Open Access article distributed under the terms of the license CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/), which permits re-use, distribution, and reproduction in any medium, provided the original work is properly cited.

methanogens [6]. Methanogenesis is known to inhibit bioelectrochemical reactions through competition for substrate [7]. It has been shown that electrical stimulation contributes to the biological oxidation of organic compounds. Guo *et al.* [8] obtained a specific power of 2,035.08 mW/m<sup>3</sup> in MFC with an applied voltage of 1.1 V, which is higher compared to the control without applied voltage. The applied voltage of 2V promotes the growth of anode biomass, and the consortium is enriched with bacteria of the genus *Shewanella* [9]. Similarly, Guo *et al.* [8] demonstrate that transiently applied voltage enriches Flavobacteriaceae and reduces Metganotrichaceae in the anodic consortium. The absence of a voltage source promotes direct interspecies electron transfer [10]. The applied voltage furthers to increase the driving force of substrate oxidation and bacterial adhesion to the anode surface [11]. Increasing the applied voltage by 1.2 V and above shows a negative effect on methanogenesis [12]. In contrast, an applied voltage of 0.39 V furthers more methane production [10]. Tice & Kim [13] used an applied voltage of 3.5 V to evolve oxygen and to inhibit methanogenesis. The application of the appropriate voltage facilitates the migration of a large number of protons and electrons through the proton exchange membrane and through the external circuit, respectively [14]. At a voltage of  $3 \text{ V}$  and above, free radicals are formed, so the anodic biofilm needs time to recover [15]. Pietrelli [9] demonstrated that an MFC under an applied voltage of 2 V accumulates charge and then discharges as a capacitor. The purpose of our study was to establish the parameters of MFC under periodic application of external voltage.

## **Materials and Methods**

A two-chamber H-type MFC with a salt bridge between the chambers was used for the study (Fig. 1). The volume of the cathode and anode chambers was  $1 \text{ dm}^3$  each. Each chamber is made of polypropylene. The salt bridge consisted of 2.4 g of agar, 8.947 g of KCl, 120 ml of distilled water [16]*.* The length of the salt bridge was  $105 \pm 5$  mm. The cameras were connected by a polyvinyl chloride tube  $\varnothing 8\times 3$ mm. The tightness of the entry was ensured by PG-11 cable entries. The electrode frame was made of a stainless-steel mesh that was wrapped with carbon fiber. The weight of the entire electrode was  $8.5 \pm 0.5$ g, the weight of the carbon thread on the electrode was  $4.5 \pm 0.5$  g. The dimensions of the electrodes were

 $100 \times 50$  mm ( $\pm 5$  mm), the visible area was 0.01 m<sup>2</sup>. Electrodes were washed with 1N HCl and 1N NaOH and distilled water and then left in distilled

water for 24 h before use [17]. The electrodes were connected by a stainless-steel wire with a 66  $\Omega$  resistor, as a small external resistor is useful for higher organic compound substrate utilization [2].

The anolyte consisted of 50 mM PBS buffer solution (pH 6.1) with sodium acetate  $(1 \text{ g}/\text{dm}^3)$  [18], FeCl<sub>3</sub> (200  $\mu$ M) [19] and 10 ml of vitamins and minerals. The concentrations of PBS components were (in grams per liter of distilled water): 4,58 Na<sub>2</sub>HPO<sub>4</sub>, 2,45 NaH<sub>2</sub>PO<sub>4</sub>, 0,31 NH<sub>4</sub>Cl, 0,13 KCl. The concentrations of components of the vitamins and minerals solution were (in milligrams per liter of distilled water): 1,5 retinol palmitate, 0,01 cholecalciferol, 60 ascorbic acid, 13 nicotinamide, 10 a-tocopherol acetate, 5 calcium pantothenate, 1,2 riboflavin, 1 thiamine nitrate, 2 pyridoxine hydrochloride, 0,003 cyanocobalamin, 0,4 folic acid, 20  $Mg^{2+}$  (magnesium lactate),  $15 \text{ Ca}^{2+}$  (calcium hydrogen phosphate), 12 P<sup>5+</sup> (calcium hydrogen phosphate), 10 Fe<sup>2+</sup> (ferrous fumarate),  $3 \text{ Zn}^{2+}$  (zinc sulphate),  $1 \text{ Cu}^{2+}$  (copper sulphate),  $1 \text{ Mn}^{2+}$  (manganese sulphate),  $0,1 \text{ Mo}^{6+}$  (sodium molybdate).

Fermented residue after methanogenesis (Department of Вioenergy, Bioinformatics and Environmental Biotechnology of the Igor Sikorsky Kyiv Polytechnic Institute) was used as a source of exoelectrogens (inoculum) (dry weight  $7.3 \pm 0.1$  g per anode chamber). Fermented residue after methanogenesis consists different microorganism in particular exoelectrogens and methanogens. This inoculum is enriched with microorganisms from



**Figure 1:** Scheme of a microbial fuel cell: *1* – anode chamber, *2* – cathode chamber, *3* – anode, *4* – cathode, *5* – polyvinyl chloride tube with a salt bridge in PG-11 cable entries, *6* – closed gas pipe, *7* – check valve, *8* – stainless steel wire, *9* – resistor, *10* – PG-9 cable entries

family Methanosarcinaceae, such a *Methanobacterium* sp., *Meathanosaeta* sp. and was described by Golub et al. [20]. For inhibition methanogens external voltage was applied to create selective conditions for exoelectrogenic biofilm growth. The anolyte was stirred with a magnetic stirrer for 4 h a day and a 3V voltage was simultaneously applied. Two 1.5 V AA batteries were used as a voltage source. During mixing, the resistor was replaced with an LED lamp with an amplifier (Fig. 2). To determine the influence of devices that consume electrical energy on electrical characteristics of the

MFC an LED (600  $\Omega$ ) or a resistor (66  $\Omega$ ) was connected. In addition, the LED was used as a voltage source indicator. The contents of the anode chamber were stirred to improve the mass transfer inside the anolyte.



**Figure 2:** Scheme of induction LED amplifier

The catholyte consisted of 50 mM PBS buffer solution (pH 6.1) with  $K_3[Fe(CN)_6]$  50 mM [18]. Voltage and current were measured with a multimeter (DT 830B, China). The dry mass of inoculum and electrodes was determined by drying them to a constant mass in a drying cabinet (LabExpert 3050 MC, China) at 105 °C. Weighing was carried out using an electronic scale (SPU 123 Scout Pro OHAUS, Switzerland). The experiment was carried out in three replicates. MFC without the addition of inoculum was used as MFC with an abiotic anode to determine the bioelectrochemical nature of the current in MFC. For comparison, an additional experiment was conducted without the application of external voltage, similar to the one described above with a closed and open circuit with the addition of inoculum in each. The difference was in the absence of external voltage application.

From 580 to 864 h (during 284 h), apply of external voltage was stopped to restore the biofilm [15]. MFC has capacitive behaviour. To check the capacitive behaviour, a voltage of 3V was applied for 4 h with simultaneous stirring, and the change in the open circuit voltage was measured. During all measurements, the resistor was replaced by a LED, and the open-circuit voltage was also measured. Microscopy was used to determine the presence of immobilization of microorganisms on the anode (XSP-139ТР Ulab ТM, China). Statistical data processing was carried out using the Microsoft Excel software package, determining the arithmetic mean and standard deviation.

## **Results**

With the periodic application of an external voltage, an increase in the MFC current was observed (Fig. 3). In the MFC with an abiotic anode that did not contain inoculum, the current did not exceed  $20 \pm 5 \mu A$  during the entire study. The MFC current gradually decreased from  $189 \pm 10$ to  $81 \pm 4 \mu A$  for the MFC without load and from  $154 \pm 8$  to  $91 \pm 5 \mu A$  for the MFC with a resistor when measured daily before applying an external voltage. During the cultivation, the anolyte was replaced twice (partially), but the addition of a new portion of the substrate did not cause a significant increase in the current when measured daily before applying an external voltage (Fig. 3, a,b). After 336 h from the start-up of the MFC, the decrease in current became slower. The current measured immediately after disconnecting the external voltage on the first day was  $178 \pm 9 \mu A$  for the MFC with LED, and  $660 \pm 33 \mu$ A for the MFC with a resistor and no load (Fig. 3c). On the second day, the current measured immediately after disconnecting the external voltage reached maximum values, namely 390  $\pm$  20  $\mu$ A for MFC with LED and  $788 \pm 40 \mu A$  for MFC with resistor and no load. The current gradually decreased to  $21 \pm 1 \mu A$  for MFC with LED, to  $123 \pm 6 \mu A$  for MFC with resistor and 131  $\pm$  7  $\mu$ A for MFC without load. After partial replacement of the anolyte, the current gradually increased to  $96 \pm 5 \mu A$  for MFC with LED, and to 295  $\pm$  15  $\mu$ A for MFC with a resistor and to 304  $\pm$  15  $\mu$ A for MFC without load.

For comparison, MFC without applying an external voltage with a closed and open circuit was studied. Already in the first day, the current increased to  $960 \pm 50 \mu A$  for MFC with an open circuit and to  $672 \pm 35$   $\mu$ A for MFC with a closed circuit when the resistor was connected (Fig. 4). The current gradually decreased to  $590 \pm 30 \mu A$  for MFC with an open circuit and  $460 \pm 23 \mu A$  for MFC with a closed circuit when the resistor is connected. After the light of the LED appeared on the 5th day, the measurement of the MFC current with the LED was started. The current when connecting the LED gradually increased.



**Figure 3:** Change in a microbial fuel cell (MFC) current over time before applying external voltage for MFC with a bioanode and for MFC with an abiotic anode: (a) with a resistor (66  $\Omega$ ) ( $\rightarrow$  - MCF with bioanode,  $\rightarrow$  - MCF with abiotic anode), (b) without load ( $\rightarrow$  – MCF with bioanode,  $\rightarrow$  – MCF with abiotic anode), (c) change in current after periodic application of an external voltage 3 V with a resistor ( $\leftarrow$  66  $\Omega$ ), with an LED ( $\leftarrow$  600  $\Omega$ ), and without load ( $\leftarrow$  open circuit). Arrow – replacement of 500 ml of anolyte



**Figure 4:** Change in a microbial fuel cell current over time without applying an external voltage with an open and closed circuit: (a) with a resistor (66  $\Omega$ ), (b) with an LED (600  $\Omega$ );  $\rightarrow -$  open circuit,  $\rightarrow -$  closed circuit

The current of the MFC immediately after disconnecting the external voltage of 3 V and the MFC with a closed circuit without applying an external voltage were compared (Fig. 5). The maximum value of the MFC current after disconnecting the external voltage was 17% more than the MFC operating without applying external voltage. A gradual decrease in current was observed for both MFC. When the LED was connected, there was a gradual decrease in the current after applying an external voltage, and a gradual increase for MFC with a closed circuit without applying an external voltage.

With the periodic application of external voltage for 48 h, an increase in voltage was observed up to  $406 \pm 20$  mV for MFC with LED and  $414 \pm 20$  mV for MFC without load (Fig. 6). Subsequently, the no-load voltage in the experiment increased to  $425 \pm 20$  mV for MFC with LED and



**Figure 5:** Change in a microbial fuel cell (MFC) current over time for MFC after periodic application of an external voltage 3 V and MFC with closed circuit without applying an external voltage: (a) with a resistor (66  $\Omega$ ), (b) with LED (600  $\Omega$ );  $\longrightarrow$  - MFC with closed circuit without 3 V,  $\leftarrow -$  MCF after 3 V



**Figure 6:** Change in a microbial fuel cell (MFC) voltage over time before applying external voltage for MFC with a bioanode and for MFC with an abiotic anode: (a) with an LED (600  $\Omega$ ), (b) without load, (c) change in voltage MFC without load after periodic application of an external voltage. Arrow – replacement of 500 ml of anolyte;  $\rightarrow$  – MCF with bioanode,  $\rightarrow$  – MCF with abiotic anode

 $444 \pm 20$  mV for MFC without load, while for MFC with an abiotic anode, it did not rise above  $80 \pm 5$  mV during the entire operating. The voltage remained relatively constant throughout the operation. The voltage measured immediately after disconnection of the external voltage source on the first day increased to  $1062 \pm 50$  mV for MFC without load, and decreased almost by half after 389 h. After partial replacement of the anolyte, the noload voltage gradually increased to  $952 \pm 45$  mV, and then began to gradually decrease again.

The voltage of MFC when LED connected without applying an external voltage was relatively stable during operation and averages  $450 \pm 22$  mV for MFC with a closed circuit and  $480 \pm 25$  mV for MFC with an open circuit (Fig. 7). Without load, the voltage gradually increased to  $740 \pm 37$  mV for MFC with open circuit and  $710 \pm 35$  mV MFC with closed circuit.

The voltage of the MFC immediately after disconnecting the external voltage of 3 V and the MFC with a closed circuit without applying an external voltage were compared (Fig. 8). The voltage was about 500 mV for the MFC with the LED after applying the voltage and without applying the voltage. The voltage without load was about 1000 mV for the MFC after applying the voltage, compared to 500 mV for the closed circuit MFC without the external voltage applied.

For 12 days (from 580 to 864 h), the applying of external voltage was stopped to restore the biofilm. During this time, the voltage and current of MFC did not change significantly. And after the external voltage was applied again, it did not significantly affect the MFC voltage and current. Hence, it can be assumed that the formed biofilm does not need to be restored.



**Figure 7:** Change in a microbial fuel cell voltage over time without applying an external voltage with an open and closed circuit: (a) with an LED (600  $\Omega$ ), (b) without load;  $\longrightarrow$  - open circuit,  $\longrightarrow$  - closed circuit



**Figure 8:** Change in a microbial fuel cell (MFC) voltage over time for MFC after periodic application of an external voltage 3 V and MFC with closed circuit without applying an external voltage: (a) with LED (600  $\Omega$ ), (b) without load;  $\rightarrow$  MFC with closed circuit without 3 V,  $\longrightarrow$  MCF after 3 V

When studying the capacitive behaviour of MFC, it was established that the open circuit voltage increased from  $413 \pm 20$  mV to  $622 \pm 30$  mV for MFC with bioanode and from  $65 \pm 3$  mV to  $260 \pm 13$  mV for MFC with abiotic anode (Fig. 9). After that, the external voltage source was disconnected, and during the first hour voltage decreased to  $462 \pm 23$  mV for the MFC with bioanode and to  $127 \pm 6$  mV for MFC with abiotic anode. Within 4 h after disconnecting the external voltage source, the open circuit voltage dropped to  $461 \pm 23$  mV for MFC with bioanode and to  $80 \pm 4$  mV for MFC with abiotic anode and remained constant, which indicated that the MFC had the ability to accumulate charge for a short time.



**Figure 9:** Effect a microbial fuel cell (MFC) open circuit voltage on capacitive behaviour of MFC. A voltage of 3 V was applied for 4 h from 912 to 916 h:  $\rightarrow$  – MCF with bioanode, MCF with abiotic anode

Microscopy of the anode carbon fiber from the MFC with bioanode and MFC with abiotic anode after operation of MFC allowed to establish the presence of flake-like structures on the carbon fiber from the MFC with bioanode (Fig. 10). Similar structures are observed during immobilization of aerobic or anaerobic sludge. Such structures were observed over the entire surface of the fibers and were visually tightly connected to the fibers.



**Figure 10:** Microphotograph anode in a microbial fuel cell: (a) carbon fiber without anode biofilm (magnification  $400\times$ ), (b) carbon fiber with anode biofilm (magnification  $200 \times$ )

## **Discussion**

When an external voltage was applied, the current increased in the MFC with a bioanode and with an abiotic anode, but the preservation of the current after disconnection of the external voltage in the MFC with a bioanode indicated that the biological component in the MFC is involved in the formation of the current. In the MFC with an abiotic anode, after disconnection of the external voltage, the current dropped to values of less than 20 μA, which was 5 times less than the current in the MFC with the inoculum. The gradual decrease in the current can be explained by the multi-layer fouling of the electrodes by microorganisms from the inoculum, which could lead to a decrease in the availability of the substrate for microorganisms. Also, the obtained data indicate that in the absence of stimulation and the application of external voltage, the fermented residue after methanogenesis reveals itself as an inoculum rich in exoelectrogenic microorganisms. Voltage, creating selective conditions, at the same time slightly suppresses the metabolism of microorganisms, forcing them to create multi-layered biofilms, and at the same time affects redox processes associated with electrolyte components. The dynamics of current values indicated the establishment of a relatively stable system of immobilized microorganisms, which were quite resistant to certain changes in the system, for example, to the addition of a substrate. On the other hand, this indicated the absence of further growth of the biofilm of exoelectrogenic microorganisms and the establishment of the limit of access of the substrate to exoelectrogens, since the current did not increase after the addition of a new portion of the substrate.

The flake-like structures that were observed during microscopy and the presence of current and voltage allowed to make assumptions about the immobilization of exoelectrogens on the anode. However, these flake-like structures were very dense, which can explain the decrease in current in MFC during cultivation due to the difficulty of mass exchange and a very dense network of microorganisms. Because of this, microorganisms that were located further from the surface of the carbon fiber and closer to the anolyte can be the first to consume the substrate, and due to the absence of mediators or morphological structures such as pili between these bacteria and those closer to the surface of the electrode, the availability of organic compounds for exoelectrogens on anode can be reduced.

The obtained results indicated that the external voltage has a positive effect on the MFC current generation. However, over time, the influence of external voltage significantly decreased due to the dense growth of the anode surface and redox processes on the electrodes. The fermented residue after methanogenesis contains a large number of organic compounds and microorganisms, which can lead to uneven biochemical transformation of organic substances by suspended microorganisms and immobilized on the anode, which in turn leads to fluctuations in the current and voltage values. Timmers *et al.* [21] note that voltage fluctuations can be caused by changes in anodic potential. Considering the fact that the fermented residue after methanogenesis consists of a large number of organic compounds by itself and the complete replacement of the anolyte was not carried out during the entire cultivation in MFC, a mixed potential, that is, an average potential of simultaneous biochemical redox reactions, could be observed. Saravanan *et al.* [22] note that external voltage affects enzyme activity, changes in zeta potential, and changes in electrode sediment viscosity. After a certain period of work, the substrates were depleted, and metabolites accumulated in the MFC, which reduced the activity of exoelectrogenic microorganisms [23]. However, the accumulation of the biofilm limited the arrival of new portions of the substrate to the exoelectrogenic bacteria directly on the electrode, as evidenced by the absence of an increase in current and voltage after the introduction of a new portion of the substrate. Conversely, during the cultivation, a stable biofilm was formed, which returned to constant values after the disconnecting of the external voltage. An external voltage of 3 V in combination with stirring allowed selectively activate exoelectrogens in the first days, as evidenced by the increase in current during the first 48 h. However, the influence of external voltage gradually decreased. The obtained results indicated that to stimulate the deposition of exoelectrogens on the anode in MFC, it is sufficient to apply an external voltage of 3 V with simultaneous stirring for 4 h per day for 2-3 days, since further applying of external voltage did not lead to a significant further increase in current and voltage. On the other hand, the fermented residue after methanogenesis contains a large number of exoelectrogenic microorganisms [5], and the effect of selective inhibition by the external voltage significantly reduces the total voltage and current generated by MFC. Thus, the effect of 3 V voltage for 4 h on the one hand allows creating selective

conditions for exoelectrogens and suppressing methanogenesis, but on the other hand reduces the overall current production by the system.

Power generation in MFC is related to the presence of exoelectrogenic genera in anodic biofilm. Eyiuche *et al.* [6] points that in anodic communities fed with acetate at a higher frequency detected *Geobacter* and *Desulfuromonas*, which have exoelectrogenic properties. Fermentative bacteria, such as *Clostridium* and *Escherichia coli*, which may be in fermented residue after methanogenesis also can produce electricity [24]. Since the in fermented residue after methanogenesis used as inoculum in this study consists many different species, the anodic biofilm was most likely enriched with different species of exoelectrogens.

The maximum values of the open circuit voltage in the experiment with an applied external voltage of 3 V and in the experiment without applying an external voltage were  $1062 \pm 50$  and  $710 \pm 35$  mV, respectively. However, after disconnecting the external voltage source the voltage decreased from  $1062 \pm 50$  mV to  $430 \pm 20$  mV, and during a long time the effect of the applied external voltage decreased. Such results indicate that the applying of external voltage has a short-term effect on the efficiency of generating electrical energy of MFC.

## **Conclusions**

Periodic application of external voltage allowed to increase the current by 17% and double the voltage compared to MFC without external voltage supply. However, after disconnecting the external voltage source, the MFC gradually discharged, that is, the current and voltage decreased. MFC demonstrated capacitive behaviour: after accumulating charge for 4 h, a discharge from 622 to 462 mV was observed. MFC demonstrated capacitive behaviour: after accumulating charge for 4 h, a discharge from  $622 \pm 30$  to  $462 \pm 23$  mV was observed. The maximum value of the current of the MFC with an open circuit was 22% more than the MFC with a closed circuit.

## **Interests disclosure**

The authors have no conflicts of interest to declare.

## **Acknowledgements**

We would like to extend our sincere thanks to PhD I.I. Levtun for his help in the manufacture of the LED amplifiers.

## **References**

- [1] Li M, Zhou M, Tian X, Tan C, McDaniel CT, Hassett DJ, Gu T. Microbial fuel cell (MFC) power performance improvement through enhanced microbial electrogenicity. Biotechnol Adv. 2018 Jul-Aug;36(4):1316-27. DOI: 10.1016/j.biotechadv.2018.04.010
- [2] Lin H, Wu S, Zhu J. Modeling power generation and energy efficiencies in air-cathode microbial fuel cells based on freter equations. Appl Sci. 2018;8(10):1983. DOI: 10.3390/app8101983
- [3] Kakarla R, Min B. Photoautotrophic microalgae scenedesmus obliquus attached on a cathode as oxygen producers for Microbial Fuel Cell (MFC) operation. Int J Hydrogen Energy. 2014;39(19):10275-83. DOI: 10.1016/j.ijhydene.2014.04.158
- [4] Shchurska K, Zubchenko L, Sobczuk H, Kuzminskyy Y. High exoelectrogenic biofilms formation in microbial fuel cells. Innov Biosyst Bioeng. 2019;3(4):246-52. DOI: 10.20535/ibb.2019.3.4.185159
- [5] Chabert N, Amin Ali O, Achouak W. All ecosystems potentially host electrogenic bacteria. Bioelectrochemistry. 2015;106(Pt A):88-96. DOI: 10.1016/j.bioelechem.2015.07.004
- [6] Eyiuche NJ, Asakawa S, Yamashita T, Ikeguchi A, Kitamura Y, Yokoyama H. Community analysis of biofilms on flame-oxidized stainless steel anodes in microbial fuel cells fed with different substrates. BMC Microbiol. 2017;17(1):145. DOI: 10.1186/s12866-017-1053-z
- [7] Nath D, Chakraborty I, Ghangrekar MM. Methanogenesis inhibitors used in bio-electrochemical systems: A review revealing reality to decide future direction and applications. Biores Technol. 2021;319:124141. DOI: 10.1016/j.biortech.2020.124141
- [8] Guo J, Cheng J, Wang J, Zhang Z, Xie X, Chu P. Effects of temporary external voltage on the performance and community of Microbial Fuel Cells. Water Sci Technol. 2020;81(9):1972-82. DOI: 10.2166/wst.2020.251
- [9] Pietrelli A. Electrical valorization of MFC: application to monitoring [dissertation on the Internet]. Université de Lyon, Università degli studi La Sapienza (Rome); 2019 [cited 2023 Feb 13]. Available from: https://www.researchgate.net/publication/334107134
- [10] Lee JY, Park JH, Park HD. Effects of an applied voltage on direct interspecies electron transfer via conductive materials for methane production. Waste Manag. 2017;68:165-72. DOI: 10.1016/j.wasman.2017.07.025
- [11] Li WW, Sheng GP. Microbial fuel cells in power generation and extended applications. In: Bai FW, Liu CG, Huang H, Tsao G, editors. Biotechnology in China III: Biofuels and bioenergy. Advances in biochemical engineering biotechnology, vol. 128. Berlin, Heidelberg: Springer; 2012. p. 165-97. DOI: 10.1007/10\_2011\_125
- [12] Joicy A, Seo H, Lee ME, Song YC, Jeong YW, Ahn Y. Influence of applied voltage and conductive material in diet promotion for methane generation. Int J Hydrogen Energy. 2022;47(18):10228-38. DOI: 10.1016/j.ijhydene.2022.01.075
- [13] Tice RC, Kim Y. Methanogenesis control by electrolytic oxygen production in microbial electrolysis cells. Int J Hydrogen Energy. 2014;39(7):3079-86. DOI: 10.1016/j.ijhydene.2013.12.103
- [14] Wang Y, Dong L, Zuo Y, Xu T, Ru Z. Improving hydrogen production from straw though simultaneous fermentation by applied voltage in microbial electrolysis cell. Trans Chinese Soc Agricult Eng. 2016;32(24):234-9.
- [15] Oh SE, Kim JR, Joo J-H, Logan BE. Effects of applied voltages and dissolved oxygen on sustained power generation by microbial fuel cells. Water Sci Technol. 2009;60(5):1311-7. DOI: 10.2166/wst.2009.444
- [16] Shchurska K, Kuzminskyi Y. Bioelectrochemical generation of hydrogen in a microbial fuel cell. 3. Experimental part. Vidnovluvana Energetika. 2012;(1):67-77.
- [17] Kokabian B, Ghimire U, Gude VG. Water deionization with renewable energy production in microalgae microbial desalination process. Renewable Energy. 2018;122:354-61. DOI: 10.1016/j.renene.2018.01.061
- [18] Lawson K, Rossi R, Regan JM, Logan BE. Impact of cathodic electron acceptor on microbial fuel cell internal resistance. Bioresour Technol. 2020;316:123919. DOI: 10.1016/j.biortech.2020.123919
- [19] Liu Q, Yang Y, Mei X, Liu B, Chen C, Xing D. Response of the microbial community structure of biofilms to ferric iron in microbial fuel cells. Sci Total Environ. 2018;631-632:695-701. DOI: 10.1016/j.scitotenv.2018.03.008
- [20] Golub NB, Shinkarchuk MV, Kozlovets OA, Morgun BV, Lakhneko OR, Stepanenko AI, et al. Determination of biogas producers in antibiotic-containing sewage. Water Air Soil Pollut. 2020;231(8):445. DOI: 10.1007/s11270-020-04805-6
- [21] Timmers RA, Strik DP, Hamelers HV, Buisman CJ. Long-term performance of a plant microbial fuel cell with Spartina Anglica. Appl Microbiol Biotechnol. 2010;86(3):973-81. DOI: 10.1007/s00253-010-2440-7
- [22] Saravanan A, Karishma S, Kumar PS, Yaashikaa PR, Jeevanantham S, Gayathri B. Microbial electrolysis cells and microbial fuel cells for biohydrogen production: Current advances and emerging challenges. Biomass Convers Biorefinery. 2020. DOI: 10.1007/s13399-020-00973-x
- [23] Wang Y, Wu J, Yang S, Li H, Li X. Electrode modification and optimization in air-cathode single-chamber microbial fuel cells. Int J Environ Res Public Health. 2018 Jun 27;15(7):1349. DOI: 10.3390/ijerph15071349
- [24] Guang L, Koomson DA, Jingyu H, Ewusi-Mensah D, Miwornunyuie N. Performance of exoelectrogenic bacteria used in microbial desalination cell technology. Int J Environ Res Public Health. 2020 Feb 10;17(3):1121. DOI: 10.3390/ijerph17031121

Д.С. Колтишева, К.О. Щурська, Є.В. Кузьмінський

КПІ ім. Ігоря Сікорського, Київ, Україна

## **ФОРМУВАННЯ АНОДНОЇ БІОПЛІВКИ ЗА ПРИКЛАДЕНЯ ЗОВНІШНЬОЇ НАПРУГИ**

**Проблематика**. Утворення екзоелектрогенної біоплівки в мікробному паливному елементі (МПЕ) є важливим етапом, оскільки він впливає на подальшу генерацію струму системою. Ферментований залишок після метаногенезу як інокулят містить не тільки екзоелектрогенні мікроорганізми, а й метаногени, які знижують продуктивність МПЕ. Використання струму дає змогу сформувати біоплівку, збагачену екзоелектрогенними мікроорганізмами.

**Мета.** Встановлення параметрів МПЕ за періодичного прикладення зовнішньої напруги.

**Методика реалізації.** Для дослідження використовували двокамерний МПЕ Н-типу із сольовим містком між камерами. Аноліт перемішували магнітною мішалкою протягом 4 год на день і одночасно прикладали напругу 3 В для створення селективних умов для росту екзоелектрогенної біоплівки.

**Результати.** Прикладення зовнішньої напруги спричиняє підвищення сили струму та напруги МПЕ. За періодичного прикладання зовнішньої напруги сила струму МПЕ зростає до 788 ± 40 мкА для МПЕ із резистором та без навантаження. Після відключення та розрядження сила струму МПЕ відповідно спадає до 189 ± 10 мкА для МПЕ без навантаження та до 154 ± 8 мкА МПЕ із резистором. За умов функціонування МПЕ без прикладення зовнішньої напруги сила струму становить 960 ± 50 мкА для МПЕ з відкритим конутром і 672 ± 35 мкА для МПЕ із замкнутим контуром при підключеному резисторі. Для всіх МПЕ сила струму поступово з часом знижується. МПЕ демонструє ємнісну поведінку: після накопичення заряду протягом 4 год спостерігається розряд від 622 ± 30 до 461 ± 23 мВ. Мікроскопія показала обростання анода. Оскільки ферментований залишок після метаногенезу є змішаним консорціумом, анодна біоплівка також була змішаним консорціумом, збагаченим різними видами екзоелектрогенів. **Висновки.** Періодичне прикладання зовнішньої напруги дає змогу збільшити струм на 17 % та напругу вдвічі порівняно з МПЕ

без подачі зовнішньої напруги. Однак після відключення зовнішнього джерела напруги МПЕ поступово розряджається, а саме зменшуються сила струму та напруга. Максимальне значення сили струму МПЕ з відкритим контуром на 22 % більше за МПЕ із замкнутим контуром.

**Ключові слова:** мікробний паливний елемент; зовнішня напруга; біоплівка; біоанод.