Journal of The
Electrochemical Society

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To cite this article: Sigita Bendinskaite *et al* 2024 *J. Electrochem. Soc.* **171** 105501

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Microbial Fuel Cell Based on *Ensifer meliloti*

Sigita Bendinskaite,[1] Ingrida Bruzaite,[2,3] Juste Rozene,[4] Tomas Mockaitis,[2] Antanas Zinovicius,[2,4] Inga Morkvenaite-Vilkonciene,[2,5,z] Almira Ramanaviciene,[6] and Arunas Ramanavicius[1,2,*,z]

[1]*Department of Physical Chemistry, Faculty of Chemistry and Geosciences, Vilnius University, Vilnius LT-03225, Lithuania*
[2]*Department of Nanotechnology, State Research Institute Centre for Physical Sciences and Technology, Vilnius LT-10257, Lithuania*
[3]*Department of Chemistry and Bioengineering, Faculty of Fundamental Sciences, Vilnius Gediminas Technical University, Vilnius, LT-10223, Lithuania*
[4]*Department of Mechatronics, Robotics, and Digital Manufacturing, Vilnius Gediminas Technical University, LT-10105 Vilnius, Lithuania*
[5]*Department of Electrical Engineering, Vilnius Gediminas Technical University, LT-10105 Vilnius, Lithuania*
[6]*NanoTechnas—Center of Nanotechnology and Materials Science, Institute of Chemistry, Faculty of Chemistry and Geosciences, Vilnius University, Vilnius LT-03225, Lithuania*

The world's growing energy crisis demands renewable energy sources. This issue can be solved using microbial fuel cells (MFCs). MFCs are biocatalytic systems which convert chemical energy into electrical energy, thereby reducing pollution from hazardous chemical compounds. However, during the development of MFCs, one of the most significant challenges is finding and assessment of microorganisms that generate sufficient redox potential through metabolic and catalytic processes. In this research, we have used *Ensifer meliloti (E. meliloti)* bacteria to design MFCs based on consecutive action of two redox mediators (9,10 - phenanthrenequinone (PQ) and potassium ferricyanide), which transferred charge between *E. meliloti* bacteria and graphite rod electrode. A viability study of *E. meliloti* culture showed that PQ significantly inhibits the growth of bacteria at 0.036 mM. Cyclic voltammograms were registered in the presence of 20 mM of potassium ferricyanide and different concentrations (0.036 and 0.071 mM, 0.11 mM, 0.14 mM, 0.172 mM, 0.32 mM) of PQ. Four days of lasting assessment of the microbial fuel cells in two-electrode systems showed that the maximal open circuit potential during the experiment raised from 174.9 to 234.6 mV. Power increased from 0.392 to 0.741 mW m^{-2}.
 [DOI: 10.1149/1945-7111/ad8037]

Manuscript submitted February 7, 2024; revised manuscript received September 5, 2024. Published October 14, 2024. *This paper is part of the JES Focus Issue on Trends and Biohybrid Approaches in Microbial Electrochemical Systems.*

Microbial fuel cells (MFC) have recently gained increasing attention for their advantageous performance and promising ecological aspects.[1–3] Recently, MFCs have been widely explored as a new source of green energy. The MFC is a bioelectrochemical device, which is capable of generating electricity by anaerobic oxidation of organic substrates.[4–6] Therefore, MFCs have attracted considerable attention as environmentally friendly energy conversion systems. Microorganisms applied in MFCs can generate electric energy by oxidizing the organic fraction of various environmental waste and/or renewable biomass.[7,8] Notably, some organic contaminants can be exploited as electron donors or acceptors for metabolic processes running in microorganisms. These contaminants can act as electron acceptors in microbe-catalyzed oxidation at the anode of MFC and, in such a way, they can be removed from the environment. Some organic pollutants can also act as electron donors in anode[9,10] or electron acceptors in cathode[11] compartments. Moreover, oxygen is widely exploited as an electron acceptor due to its high oxidation potential and accessibility. Several recent studies have evaluated some electron acceptors due to the requirement of more rapid anode/cathode reactions[12] and the development of MFC-based technologies in various applications.[13]

Combining MFCs with sensors capable of detecting important chemical and physical parameters is also relevant for various technological and practical purposes.[12,14] However, the power of electrical energy generated in an MFC highly depends on the charge transfer efficiency from the microorganisms to the electrode. Therefore, this issue is prioritized during the development of biofuel cells.[15] These include investigations on MFC electrode materials[16] and its' modifications,[17] applications of additional catalysts,[10] involvement of electron acceptors in MFC,[13] and applications,[18] metabolic and genetic cell engineering.[19,20] The emerging global energy cliff has prompted action to use organic waste as a biofuel.[21] MFC-based systems have also been used in a few new applications, such as hydrogen production, seawater desalination, biosensors, and microbial electrosynthesis.[22] With large amounts of research, more and more applications for MFC have been discovered over the years.[23] One of them is the application of biofuel cells in the design of self-powered biosensors, where biofuel cells are used simultaneously as an energy source and sensing element of biosensors.[1] This simplifies the design of the biosensing system by eliminating the need for a power supply.[24] The development of microbial self-powered biosensors relies on the capability of microorganisms to exchange electric charge with the electrode.[25] An important feature of microbial self-as-powered biosensors is their long operational stability. The continuous growth of new microorganisms replacing old/dead ones allows prolonged operation of the device.[26]

Biofuel cells have specific characteristics like conventional and anaerobic energy sources such as anaerobic bacteria reactors.[27] However, unlike anaerobic reactors, we know relatively little about the microbiology of MFCs.[28] Recently, biologically active substances that can stimulate bioelectricity production have been tested. These microorganisms are metabolites with good biocompatibility.[29] One of the microorganisms, *Saccharomyces cerevisiae*—yeast, has been used in MFC studies to determine their viability and ability to generate electricity.[10] High-precision sequencing showed that bacteria such as *Acinetobacter, Cyanobium*, *Pseudomonas,* and *Ottowia* were relatively abundant on the electroactive AB biofilm. In addition, bacteria grew well on the MFC anode. An economic evaluation showed that 0.0045 kWh m^{-3} of energy was used to treat one ton of wastewater.[30] These results confirm that after testing the MFC can improve aquaculture wastewater treatment at both technical and economic levels.

*Electrochemical Society Member.
[z]E-mail: inga.morkvenaite-vilkonciene@vilniustech.lt; arunas.ramanavicius@chf.vu.lt

Ensifer meliloti is a bacteria responsible for nitrogen fixation for the plants. The symbiosis between *Ensifer meliloti* (*E. meliloti*) and its legume hosts begins when the plant releases many betaines and flavonoids into the rhizosphere.[31] These compounds attract *E. meliloti* to the surface of the plant's root hairs, where the bacteria begin secreting nod factors. This initiates root hair curling. *Rhizobia* penetrates the root hairs and proliferates to form an infection thread. Through the infection thread, the bacteria move toward the main root. The bacteria develop into bacteroids within newly formed root nodules and perform nitrogen fixation for the plant. *E. meliloti* bacterium does not perform nitrogen fixation until it differentiates into an endosymbiotic bacteroid. A bacteroid depends on the plant for survival.[32] Leghemoglobin, produced by leguminous plants after colonizing *E. meliloti,* interacts with the free oxygen in the root nodule where the rhizobia reside. Rhizobia bacteria accumulate within symbiosomes in the root nodules of leguminous plants. The leghemoglobin reduces the amount of free oxygen present. Oxygen disrupts the function of the nitrogenase enzyme in the rhizobia, which is responsible for nitrogen fixation.[33] And these mechanisms could be adapted for the MFC design based on *E. meliloti* bacterium.

The cathode, one of the components of an MFC, also significantly influences the electrical characteristics of MFC. Dissolved oxygen, ferricyanide, potassium permanganate, or manganese dioxide are often exploited as cathode electron acceptors in many MFCs. In two-chamber MFC tests, it was found that replacing the basic aqueous solution in the cathode compartment with a solution saturated with oxygen and having a constant concentration of ferricyanide increases the power output of MFC by 1.5 to 1.8 times.[34]

The main aim of this research has been to evaluate the applicability of *E. meliloti* bacteria and better understand charge transfer aspects between bacteria and electrodes. Two redox mediators (9,10—phenanthrenequinone (PQ) and potassium ferricyanide) were applied to advance charge transfer efficiency.

Chemicals and Materials

Whatman Nuclepore (Sigma–Aldrich, Steinheim, Germany) track-etched membranes were purchased from Merck (Carrigtohill, Ireland), and graphite electrodes and chemicals were purchased from Sigma–Aldrich (Steinheim, Germany). The 0.05 M phosphate-acetate buffer solution (PBS), pH 6.8, was based on distilled water with dissolved 0.05 M of CH_3COONa, 0.05 M of NaH_2PO_4, 0.05 M of Na_2HPO_4 and 0.1 M of KCl. 9,10- phenanthrenequinone was dissolved in 97% ethanol purchased from "UAB Vilniaus Degtinė" (Vilnius, Lithuania). Potassium ferricyanide ($K_3[Fe(CN)_6]$; ⩾99.0%) was prepared in this PBS and was purchased from Riedel (Vilnius, Lithuania).

Bacteria strain and growth conditions.—The Bacteria *E. meliloti* strain was selected in this study. Gram-negative, nitrogen-fixing *E. meliloti* bacteria were obtained from the Lithuanian Research Centre for Agriculture and Forestry (Akademija, Lithuania) collection of microbial strains. Bacteria culture was cultivated in a 5 ml liquid medium consisting of 2 g l^{-1} of tryptone, 2 g l^{-1} of yeast extract, 1 g l^{-1} of NaCl, 0.3 g l^{-1} of magnesium sulfate and 0.28 g l^{-1} of calcium chloride (LBmc)[35] with continuous shaking at 180 rpm and 25 °C for ∼48 h. Then, 1 ml of bacteria culture was transferred to fresh LBmc medium when the optical density (OD) at 600 nm was ∼0.1 and cultivated for 120 h at 25 °C (OD at 600 nm is 1.0). After additional cultivation, the concentration of the bacteria culture was 1×10^8 CFU ml^{-1}.

Graphite electrode preparation.—The graphite electrode (rod with 150 mm length, 3 mm diameter, low density, 99.995% trace metals basis) was sanded with three sanding sheets of different grits and washed with distilled water and 97% ethanol. Then, one graphite electrode was placed in an Eppendorf tube with a suspension of bacteria *E. meliloti* culture. First, an Eppendorf tube was covered with an air-permeable filter, and the OD of the bacteria culture at 600 nm was 1.0. Next, the graphite electrode with bacteria *E. meliloti* culture suspension was incubated with shaking at 180 rpm and 25 °C for 72 h. Then, the bacteria *E. meliloti* culture-modified graphite electrode was used as the anode of MFC to study their performance.

Preparation of cells for viability assays.—*Agar well diffusion assay.*—The bacteria of the *E. meliloti* culture was inoculated in the flask ∼120 h). The culture's initial OD at 600 nm wavelength was 1.0 ± 0.02. Then, 0.1 ml of the bacteria culture suspension was transferred to a 9 cm Petri dish, and 10 ml of LBmc agar medium (2 g l^{-1} tryptone, 2 g l^{-1} yeast extract, 1 g l^{-1} NaCl, 0.3 g l^{-1} magnesium sulfate, 0.28 g l^{-1} calcium chloride, and 25 g l^{-1} agar) was added to the same plate and mixed smoothly. The holes (∼diameter 8 mm) were made on the cooled and frozen LBmc agar medium. Then 0.025 mL of 9,10-phenanthrene quinone (PQ) solution with 0.036 mM, 0.11 mM, and 0.32 mM concentrations was introduced in the well. Ethanol was used as a control solution. Petri dishes were incubated in a Laminar airflow box LaboGene SCANLAF Mars (Lillerød, Denmark) at 25 °C temperature for 24 h, and the clear zone was measured.

Dynamic growth curves.—For this experimental investigation, the bacteria of *E. meliloti* culture were inoculated in the presence of PQ solution with 0.036 mM, 0.11 mM, and 0.32 mM concentrations. Unaffected with PQ, bacteria culture cells served as the control. 1 mL of bacteria culture cells were transferred to a 9 ml fresh liquid LBmc medium (optical density at 600 nm was ∼0.1). Afterward, 100 μl of PQ solution was added to the prepared bacterial cell suspensions and incubated at 25 °C for 96 h. The OD cultures of the bacterial culture cells were determined within 96 h at 4 h intervals between measurements. Bacterial culture cells number was monitored on liquid LBmc media by measuring the light absorbance by the sample of the LBmc medium with the cells using Genesys 10S UV–vis spectrophotometer from Thermo Fisher Scientific (Mettler Toledo, Singapore).

Electrochemical measurements.—Potentiostat/Galvanostat Autolab PGSTAT 30 (Utrecht, Netherlands) and "NOVA" software were applied for electrochemical measurements. Measurements were performed at ambient temperature in phosphate/acetate buffer solution, pH 6.8, at aerobic conditions. Measurements were performed in a 50 ml volume three-electrode electrochemical cell, designed using components purchased from Metrohm AG (Herisau, Switzerland) with mechanical stirring. In the electrochemical cell, the graphite electrode was connected as a working electrode, the platinum electrode was used as a counter electrode, and the $Ag/AgCl/KCl_{(3\ M)}$ electrode was used as a reference electrode (Fig. 1). The electrochemical cell was placed in a Faraday cage with an Earth terminal (from Autolab, Utrecht, Netherlands). All cyclic voltammograms were recorded at the potential sweep rate of 0.1 V s^{-1} in the range from −0.6 V until +0.8 V. Three cyclic voltammogram cycles were performed, and the last one was plotted. The measurements were repeated after fifteen and thirty minutes. The concentration of the additional substance was then increased, and the exact measurements were taken.

A three-electrode setup was applied to conduct cyclic voltammetry. Cyclic voltammograms were recorded in PBS, pH 6.8, containing 20 mM of potassium ferricyanide (Fig. 4). A second redox mediator 9,10-phenanthrenequinone was added to the solution every ten minutes, increasing its concentration (0.036 mM, 0.07 mM, 0.11 mM, 0.14 mM, 0.17 mM, 0.32 mM). The scan rate of 0.1 V s^{-1} and potential step of 0.01 V was applied. The peak currents, determined from voltammograms vs PQ concentration,

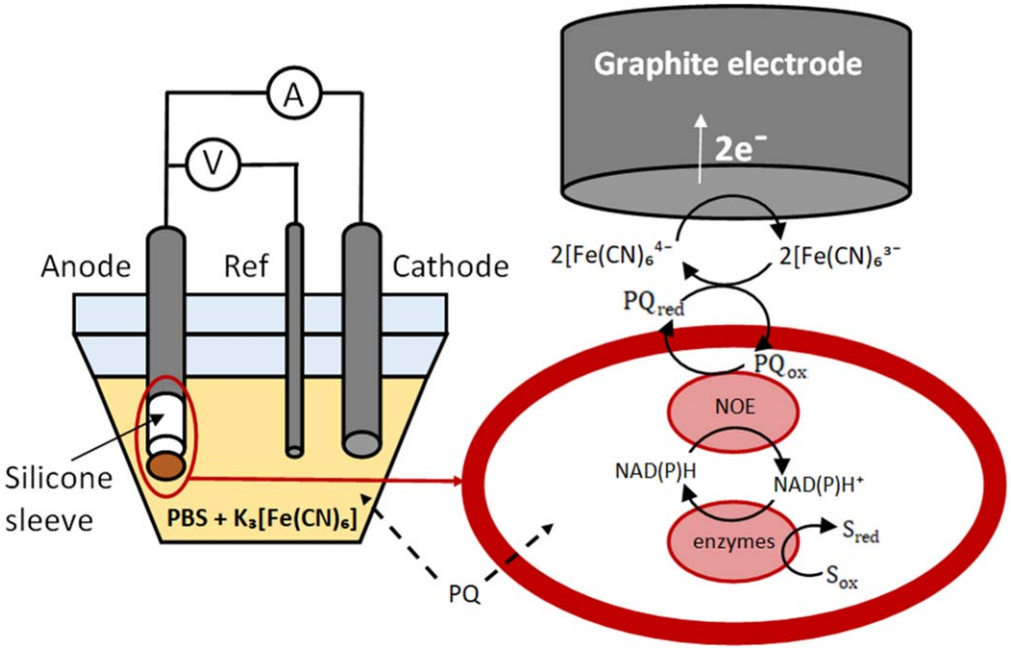

Figure 1. Schematic diagram of single-chamber microbial fuel cell based on *Ensifer meliloti* grown on graphite rod electrode structure. Cyclic voltammetry-based measurement of intracellular redox reactions using a dual redox mediator system was applied. The PQ diffuses to the outer part of the cell membrane, and it is reduced by NAD(P)H (a coenzyme involved in oxidation-reduction processes) at the inner side of the cell membrane. Then, the reduced form of PQ diffuses towards the outer side of the cell membrane and passes electrons to $[Fe(CN)_6]^{3-}$. The abbreviations "S_{red}" and "S_{ox}" indicate the reduced or oxidized form of the respective substrates. The acronym "*NOE*" stands for NAD(P)H oxidizing enzyme.

and then a change of potential vs time were fitted using Hill's function (Eq. 1).

To assess the performance of the designed microbial fuel cell, a two-electrode electrochemical cell based on a cathode consisting of a bare graphite rod and an anode consisting of a graphite rod modified by *E. meliloti* was applied. To simulate an external load and to evaluate the power density of the planned MFC, external resistances of 0.01 kΩ, 0.1 kΩ, 0.42 kΩ, 0.9 kΩ, 5 kΩ, 12 kΩ, 50 kΩ, 100 kΩ, 400 Ωk, 800 kΩ, 1100 kΩ, 1500 kΩ, 2100 kΩ, and 2600 kΩ were connected into an external electrical circuit during voltage measurements. All tests were conducted under aerobic conditions at +20 °C with stirring of phosphate acetate buffer, pH 6.8.

Microbial fuel cell design.—To track and analyze the electrochemical activity of the *E. meliloti* bacterium, the MFC based on two electron transfer mediator system was created (Fig. 1). Inside the anode compartment, protons, electrons, and carbon dioxide are emitted during the anaerobic oxidation of organic molecules such as acetate, glucose, lactate, and ethanol.[36] While the electrons go through the external circuit, the protons pass through the anode and into the cathode chamber. Electric current is produced by this charge exchange within the circuit.[13]

Calculations.—Electrochemical measurements were evaluated by using the Hill function:

$$J = \frac{C^n}{k^n + C^n} \quad [1]$$

Where J is current density, C is a concentration of selected substrate $K_3[Fe(CN)_6]$, or PQ, k is a constant, which is similar to PQ concentration at which half of the maximal current is observed, and ^{n} is a Hill coefficient.

Modified Hill function was used for the evaluation of time-dependent experiments:

$$J = \frac{C^t}{k^t + C^t} \quad [2]$$

Where t is a time of reaction.

Results and Discussion

The assessment of Ensifer meliloti cells viability.—The growth profile of PQ solution-treated and control bacteria *E. meliloti* culture grown for 96 h showed that PQ inhibits the growth of nitrogen-fixing bacteria (Fig. 2). Nitrogen-fixing bacteria *E. meliloti* prevalent in the surface of the root hairs and grow under environmental conditions.[36] The growth of bacteria was assessed by determining the optical density of the bacteria *E. meliloti* culture suspension OD at 600 nm. The results showed that *E. meliloti* is a slow-growing bacterium.[37]

As shown in Fig. 2A, the growth of *E. meliloti* bacteria was somewhat reduced at all concentrations of PQ in the solution. The results showed that optical density values for the bacteria growth curve at 0.11 mM and 0.32 mM PQ were insignificantly different. The optical density of bacteria *E. meliloti* culture suspension decreased 24 h after the inoculation. When the bacteria were treated with a solution containing 0.036 mM of PQ, the viability of the *E. meliloti* culture was less affected in comparison to the *E. meliloti* culture suspension treated with higher PQ concentrations. A solution containing 0.32 mM of PQ in LBmc liquid medium decreased the optical density of bacteria suspension compared to the control sample by 29.63 ÷ 39.7% (Fig. 2A).

Agar well diffusion[38] assay was used to determine if the PQ solution affected resistance to reactive oxygen species (ROS). Figure 2B represents the radius of the inhibition zone of the bacteria *E. meliloti* culture cultivated at 25 °C temperature.

PQ solutions with concentrations higher than 0.036 mM have a toxic effect on *E. meliloti* bacteria, as shown by the inhibition zones: 4.22 ± 0.02 mm for 0.036 mM PQ solution, 13.22 ± 0.02 mm for

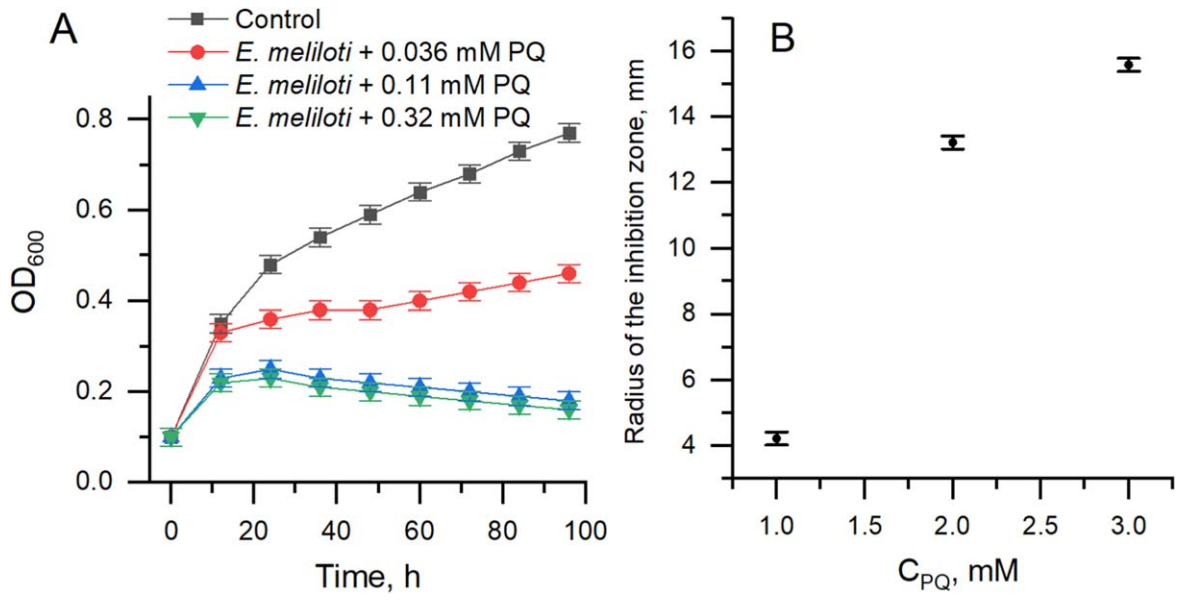

Figure 2. (A) Growth kinetic curves of *Ensifer meliloti* in LBmc liquid medium. Values are means of optical density determinations from triplicate cultures, and the bars represent standard errors. (B) Antibacterial activity of PQ solution evaluated by agar well diffusion method against bacteria *E. meliloti*.

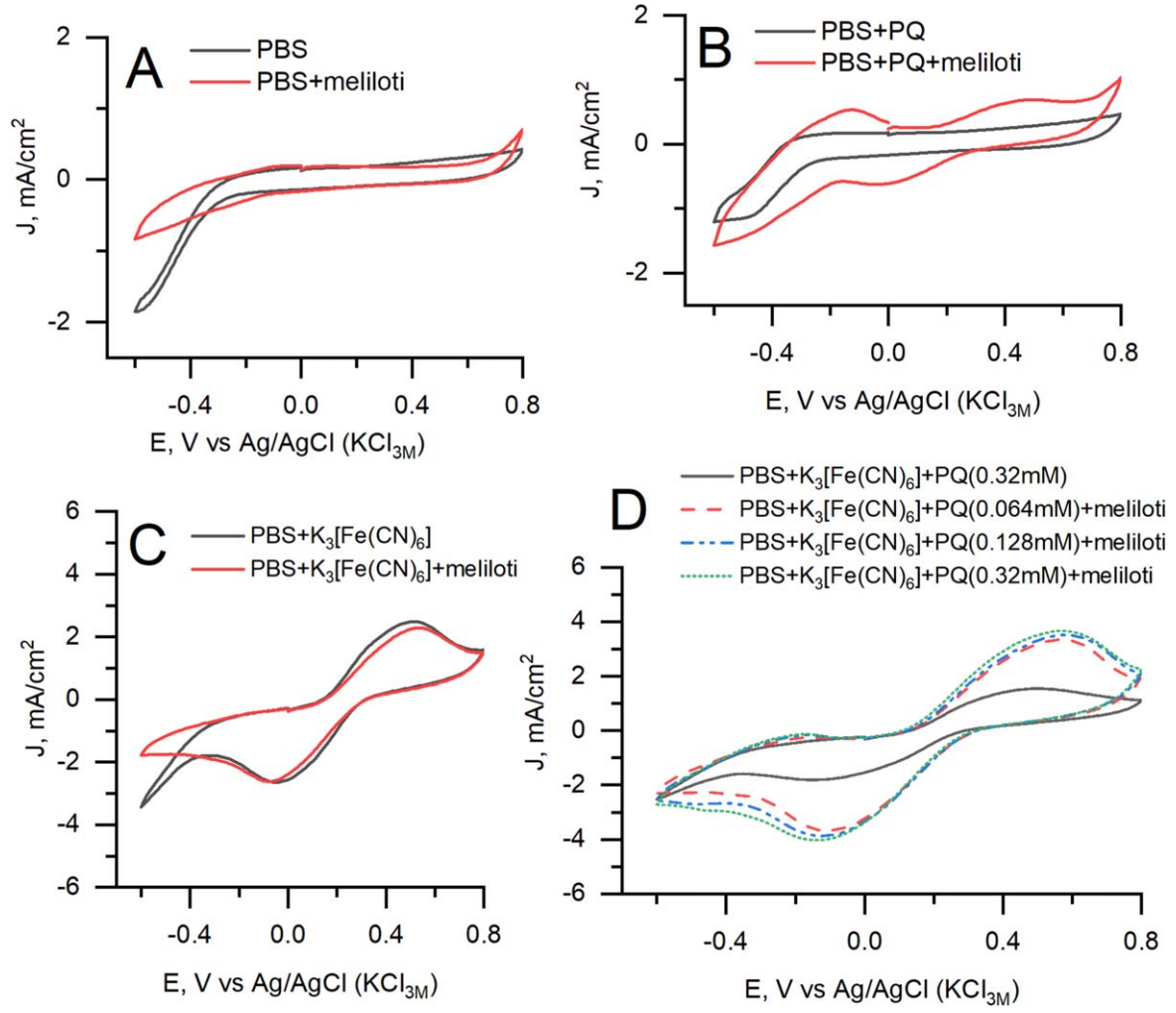

Figure 3. Cyclic voltammograms with and without *E. meliloti*. (A) in PBS. (B) in PBS with 0.32 mM PQ. (C) in PBS with $K_3[Fe(CN)_6]$. (D) in PBS, 19.23 mM $K_3[Fe(CN)_6]$, and different PQ concentrations. The scan rate of 0.1 V s^{-1} and potential step of 0.01 V was applied.

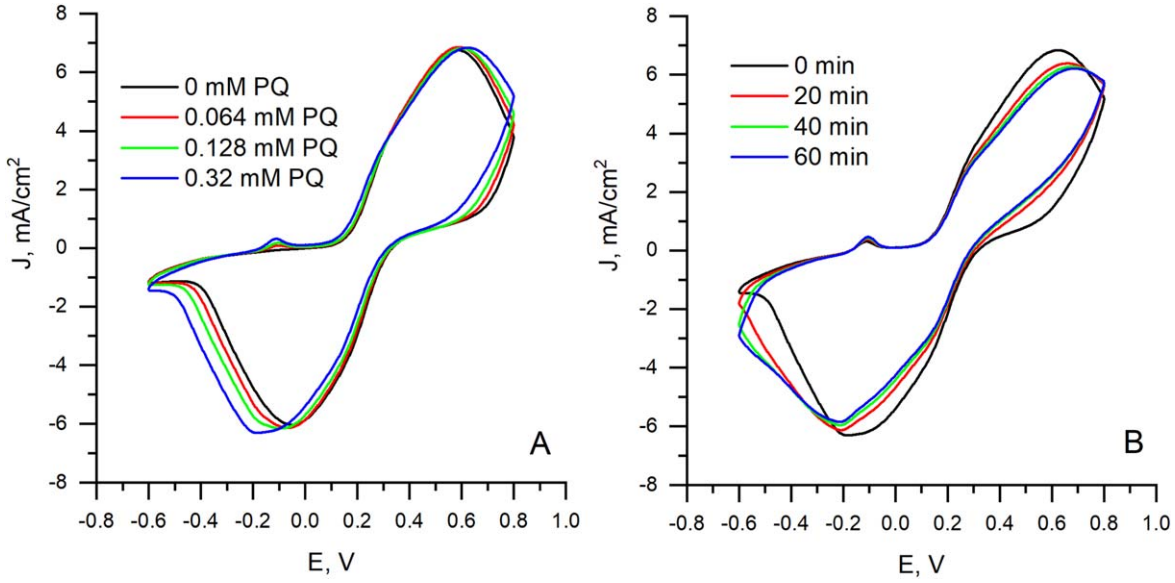

Figure 4. (A) Cyclic voltammograms at different concentrations of PQ. (B) Cyclic voltammograms at different time intervals with 0.32 mM PQ. The working solution was 19.23 mM potassium ferricyanide in PBS. The scan rate of 0.1 V s^{-1} and potential step of 0.01 V was applied.

0.11 mM PQ solution, 15.58 ± 0.02 mm for 0.32 mM PQ solution. Considering the radius of the zone of inhibition as a measure of antibacterial activity, bacteria *E. meliloti* was more resistant to solutions containing lower concentrations of PQ. The results of the bacteria viability test using the agar well diffusion method were very similar to that determined during "the dynamic growth" of cells determined by the determination of optical density curves. For this reason, PQ solutions of all concentrations were used in further MFC studies.

Electrochemical evaluation of microbial fuel cell.—Cyclic voltammograms recorded in phosphate/acetate buffer, pH 6.8 with the 19.23 mM potassium ferricyanide and different 9,10-phenanthrenequinone concentrations were registered (Fig. 3). In PBS, there is a small difference between the cyclic voltammograms recorded in PBS with and without *E.meliloti* (Fig. 3A). Two redox peaks were obtained by adding PQ to the PBS (Fig. 3B).; however, the current is as small as in the previous experiment. With only $K_3[Fe(CN)_6]$, the current density increased from 0.68 mA cm^{-2} to 2.46 mA cm^{-2}, but *E. meliloti* presence had no effect (Fig. 3C). Using two mediators and adding *E. meliloti* led to a current of 3.49 mA cm^{-2} (Fig. 3D). This shows that bacteria use PQ in their metabolic reactions, and $K_3[Fe(CN)_6]$ is needed for the performance of biofuel cells.

Cyclic voltammograms recorded in phosphate/acetate buffer, pH 6.8 with the 19.23 mM potassium ferricyanide and different 9,10-phenanthrenequinone concentrations were registered (Fig. 4A). The oxidation and reduction peaks of PQ increased together with concentration. When we measured cyclic voltammograms at different time intervals at the same 0.32 mM PQ concentration, we obtained a decrease in current at the oxidation peak (Fig. 4B).

The peak currents determined from voltammograms are shown in Fig. 5. Change of potential vs time was fitted using Hill's function (Eq. 1).[39] When PQ concentration increased, the current density values at both the cathodic (PQ reduction, at an interval from −0.168 V to −0.186 V) and the anodic (PQ oxidation, at an interval from −0.094 V to −0.108 V) peaks increased accordingly. When the concentration of PQ increased from 0 mM to 0.32 mM, the current density changed from 5.48 mA cm^{-2} to 6.31 mA cm^{-2} (Fig. 5A, reduction) and from 0.002 mA cm^{-2} to 0.31 mA cm^{-2} (Fig. 5B, oxidation). These results illustrate that as the concentration of PQ in the solution increased, the total oxidation potential decreased from 6.77 mA cm^{-2} to 6.21 mA cm^{-2} and shifted from +0.58 V to +0.69 V. The total reduction potential decreased from 5.97 mA cm^{-2} to 5.85 mA cm^{-2}, moving from −0.067 V to −0.21 V.

The assessment of microbial fuel cells was performed by time-lapse measurement after stabilizing PQ concentration at 0.32 mM. Measurements were performed for one hour every 10 min. Results were assessed by fitting experimentally obtained measurement results with those generated using Hill's function. The reduction peak (Fig. 4C) has decreased from −6.31 mA cm^{-2} to -5.85 mA cm^{-2} (range shifted from −0.18 V to −0.22 V), and the oxidation peak (Fig. 4D) has changed from 0.31 mA cm^{-2} to 0.46 mA cm^{-2} (range shifted from −0.11 V to −0.098 V).

The Hill's function coefficients "*k*," determined by reaction rate, were calculated. A higher coefficient "*k*" is related to a slower reaction rate. The peak currents, which were selected from voltammograms, vs PQ concentration as $k = 0.15$ (Fig. 4A), $k = 0.146$ (Fig. 4B), the "*k*" is very low, suggesting that the redox reaction rate is very high and the reaction itself is stable. The peak currents, which were observed over time at a steady-state concentration of PQ $k = 27.286$ (Fig. 4C); $k = 28.501$ (Fig. 4D), show that the "*k*" is large, suggesting that the redox reaction rate is very low, and the system is not stable.[39] Hill coefficient "*n*" in the peak currents from voltammograms vs PQ concentration ($n = 1.486$ (Fig. 4A); $n = 1.709$ (Fig. 4B), and the peak currents from overtime at a steady-state concentration of PQ ($n = 1.649$ (Fig. 4C); $n = 1.621$ (Fig. 4D) was determined higher than 1 which indicates that "positive cooperative binding" takes place in the system: when the first redox mediator moiety is bound to the enzyme, then enzyme's affinity for subsequent mediator molecules increases. Hence, Hill's coefficients indicate that if PQ concentration rises, the efficiency of charge transfer by PQ increases substantially.[39]

Performance of microbial biofuel cell.—To assess the performance of microbial biofuel cell measurements in the two-electrode-based electrochemical cell were performed, where an anode based on *E. meliloti* and a cathode based on a bare graphite electrode were used (Fig. 6). The measurements were carried out on four consecutive days. It was determined that the potential of biofuel cells raised from 169.8 mV to 233.4 mV at a load of 2.6 MΩ at 19.23 mM (Fig. 6A), and maximal open circuit potential increased from 174.9 mV to 234.6 mV (Fig. 6B) (both measurements were performed at 19.23 mM potassium ferricyanide and 0.32 mM PQ). The

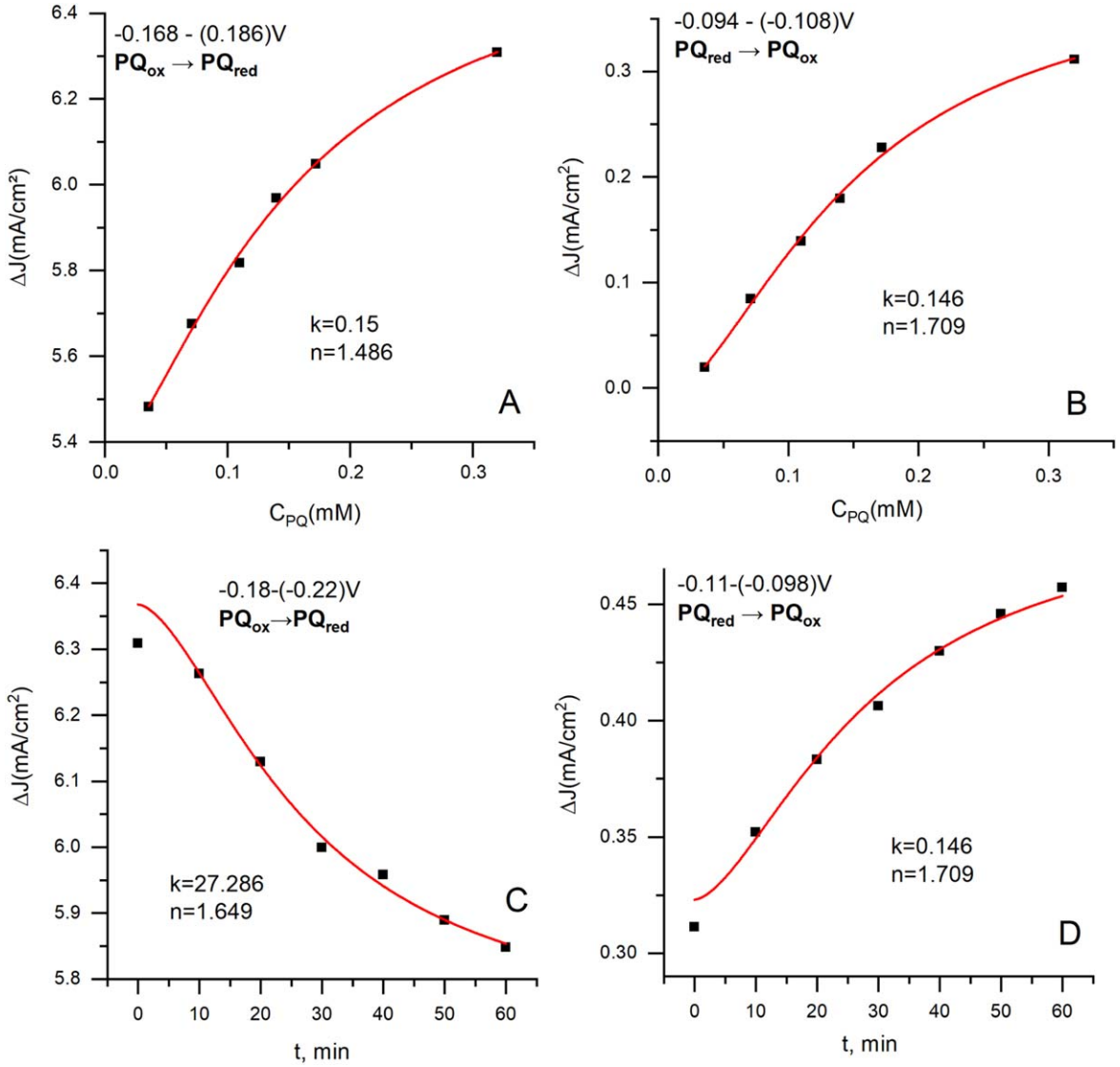

Figure 5. Results of the electrochemical measurements: (A) Cyclic voltammograms were registered using the *E. meliloti* electrode at different PQ concentrations in the buffer solution. Measurements were performed in a buffer solution with the potassium ferricyanide 19.23 mM and 9,10-phenanthrenequinone 0.32 mM. The peak currents, which were determined from voltammograms, vs PQ concentration were fitted using Hill's function (Eq. 1); (B) A reduction peak potential ranges from −0.168 V to −0.186 V; (C) Oxidation peak potentials range from −0.094 V to −0.108 V. Measurements were performed using a three-electrode-based electrochemical cell; (C) A reduction peak is observed over time at a steady-state concentration of PQ, where potentials range changed from −0.18 V to −0.22 V; (D) Oxidation peak observed over time at a steady-state concentration of PQ, where potential ranges changed from −0.11 V to −0.098 V.

maximal calculated power after four days raised from 0.55 mW m^{-2} at 152.8 mV to 15.372 mW m^{-2} at 72.2 mV (Fig. 6A,B). This effect can be related to PBS composition because it contains acetate, phosphate, and potassium ions, which can influence bacterial metabolism.[39]

Comparing the MFC efficiency data with other authors, we found that the highest power of 1624 mW m^{-2} was observed in a microbial fuel cell with rGO/SnO_2/Carbon cloth composite anode, platinum electrode, and *Escherichia coli* biomass (Table I). However, our research is the first attempt to use *E. meliloti* in biofuel cells; therefore, we achieved only 15.372 mW m^{-2}. This bacterium has huge potential for self-powered devices used for soil monitoring.

Conclusions

Research on microbial fuel cells based on electrocatalytic processes is becoming increasingly important in sustainable energy development. However, not all microorganisms involved in metabolic and catalytic processes can generate sufficient redox potential to transfer electrons to electrodes. Hence, in this study, we evaluated the adaptability of microorganisms such as *E. meliloti* for the design of MFC. To improve charge transfer, we have tested two redox mediator systems composed of potassium ferricyanide and 9,10-phenanthrenequinone. During the investigation of graphite rod electrode in a three-electrode system, the reduction current density changed from −5.48 mA cm^{-2} to −6.31 mA cm^{-2} and oxidation current density changed from 0.002 mA cm^{-2} to 0.31 mA cm^{-2} if the concentration of PQ added into electrochemical cell increased from 0 mM to 0.32 mM. When the concentration of PQ was constant at 0.32 mM, and measurements were performed in time, the overall reduction peak current density changed from 6.31 mA cm^{-2} to 5.85 mA cm^{-2}, and the peak potential increased from −0.18 V to −0.22 V. The oxidation peak current density increased from 0.31 mA cm^{-2} to 0.46 mA cm^{-2}. The peak potential has shifted from −0.11 V to −0.098 V from 0 min to 60 min. Four days of lasting assessment of microbial fuel cells in two-electrode systems showed that the maximal open circuit potential during the experiment raised from 174.9 mV to 234.6 mV. Power increased from

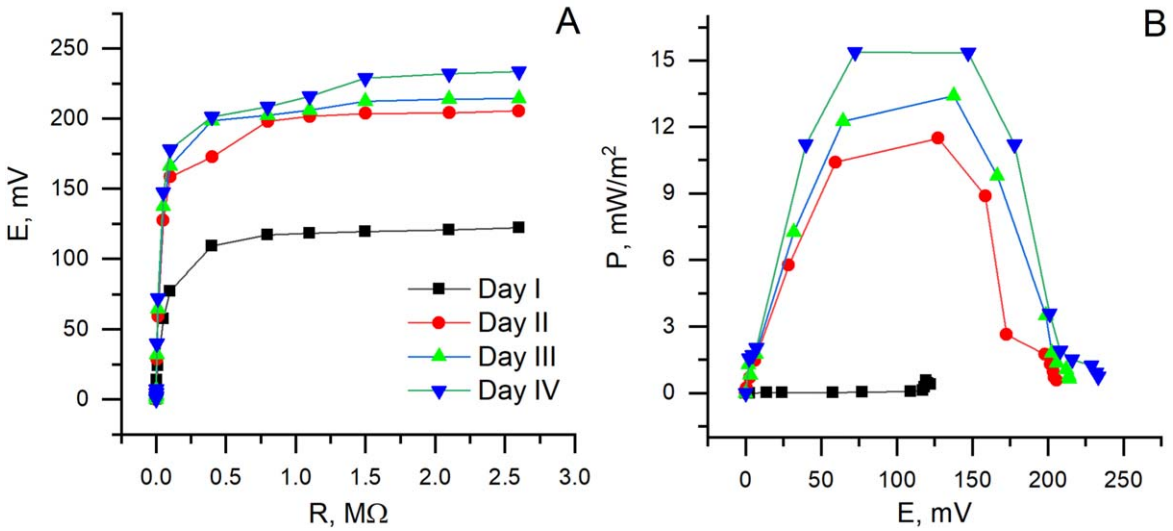

Figure 6. Result of microbial biofuel cells' performance: (A)—Potential dependence on applied external load; (B)—Calculated power density dependence on the generated potential in a single-compartment-based microbial biofuel cell, which consisted of the graphite anode with the immobilized *E. meliloti* and bare graphite cathode immersed in the phosphate-acetate buffer solution containing 19.23 mM potassium ferricyanide and 0.32 mM PQ. All measurements were performed in the two-electrode electrochemical cell.

Table I. Maximal power output data collected from references are reported in microbial biofuel cell investigations.

Anode	Cathode	Biomass	P_{max}	References
PQQ-dependent alcohol *dehydrogenase* (PQQ-GDH)	GOx/microperoxidase-8/GRE	—	3.5 μW cm^{-2} at 0.2 V	40
Carbon cloth	Carbon cloth with Pt coating	Oil sands tailings affected water	392 mW m^{-2}	41
PQQ-GDH	Alcohol oxidase/microperoxidase-8/GRE	—	1.5 μW cm^{-2} at 0.19 V	42
Graphite plate	Graphite felt	Anaerobic sludge	314 mW m^{-2}	43
Carbon cloth	Carbon cloth	*S. putrefaciens* CN32	679.7 mW m^{-2}	44
Carbon brush	Carbon cloth	Sludge mixture	4.25 W m2	45
Porous carbon cloth	Porous carbon cloth	*Proteus Hauser*	83.4 mW/m^2	46
Unpolished graphite	Rutile–coated graphite cathode	Anaerobic sludge	0.13 ± 0.03 mW m^{-2}	45
Carbon fiber felt	Carbon fiber felt	*Dysgonomonas* and *Klebsiella*	529 ± 12 mW m^{-2}	47
Carbon brush	Reduced graphene oxide	*Geobacter* and *Pseudomonas*	0.95 W m^{-2}	48
Porous carbon paper	Porous carbon paper	Aerobic sludges	213.93 mW m^{-2}	49
3D-Graphen	Carbon cloth	*E.coli*	1516 ± 87 mW m^{-2}	50
RGO/ Carbon cloth-PANI	Carbon felt	Anaerobic Sludge	1390 mW m^{-2}	51
Activated carbon cloth	Graphite foil	*D. desulfuricans* strain	0.51 mW cm^{-2}	52
S. oneidensis MR-1	Carbon cloth	*S. oneidensis* MR-1	145 mW m^{-2}	53
rGO/SnO2/Carbon cloth composite	Pt	*E.coli*	1624 mW m^{-2}	54
Carbon felt	Carbon felt	Mixed-culture sludge	8.67 mW m^{-2}	55
Activated charcoal	Activated charcoal	Algae biomass	207 mW m^{-2}	56
Carbon felt	Carbon felt	*E. coli*	181.1 mW m^{-2}	19
Carbon cloth	Carbon-based air-breathing cathode	*E. coli*	806 ± 7 mW m^{-2}	20
Graphite rod	Graphite rod	*Ensifer meliloti*	15.372 mW m^{-2}	This article

0.392 mW m^{-2} to 0.741 mW/m^2. These results indicate that electron transfer efficiency is still limited; therefore, the cell's internal resistance remains one of the most significant challenges in developing microbial fuel cells. Improving microorganism charge transfer would be one of the future objectives of increasing the productivity and efficiency of MFC.

Acknowledgments

This research was funded by the Research Council of Lithuania (LMTLT), agreement No S-MIP-22–87. Author Contributions: Conceptualization, I.M-V. and A.R (Arunas Ramanavicius); methodology, I.M.-V., I.B.; software, S.B.; validation, S.B., T.M. and I. B.; formal analysis, J.R., T.M.; investigation, S.B., I.B.; resources, I. M.-V. and A.R. (Almira Ramanaviciene); data curation, S.B., T.M.; visualization, S.B.; writing—original draft preparation, S.B.; writing —review and editing, S.B., I.B., J.R., T.M., A.R. (Arunas Ramanavicius), I.M.-V., A. R (Almira Ramanaviciene); supervision, I.M.-V. and A.R.; project administration, I.M.-V.; funding acquisition, I.M.-V. and A.R. (Almira Ramanaviciene). All authors have read and agreed to the published version of the manuscript.

ORCID

Almira Ramanaviciene https://orcid.org/0000-0001-5864-0359
Arunas Ramanavicius https://orcid.org/0000-0002-0885-3556

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