Inhibition of Electricity Generation by Micrococcus Luteus 1-I in a Biofuel Cell by Respiratory Poisons

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Keywords: Micrococcus luteus 1-I, biofuel cells, electricity inhibition, respiratory poisons.

Abstract: The article studied the effect of three respiratory poisons (sodium fluoride, sodium azide, 2,4-dinitrophenol) at concentrations of 5 and 20 mmol/L on the electrical characteristics of BFC operating on model wastewater with the bioagent *Micrococcus luteus* 1-I. It has been demonstrated that the inhibitors used in all tested concentrations had a negative effect on the power generation of BFC. It was shown that the degree of influence of respiratory poisons on voltage and current increased in the series sodium fluoride > sodium azide > 2,4-dinitrophenol.

1 INTRODUCTION

Biofuel cells (BFCs) are unique bioelectrochemical installations that convert the energy of chemical bonds in organic and inorganic molecules into electrical energy. Microorganisms are biocatalysts for this process (Idris et al., 2016). BFCs can use various wastes as a substrate, for example, sewage. This makes BFC technology extremely useful in terms of environmental conservation (Munoz-Cupa et al., 2021).

Today, very little is known about the mechanisms of electron transfer from microbial cells to the electrode and about the enzymes involved in these processes. It was expected that the study of the effect of enzymatic poisons would provide material for a possible interpretation and understanding of the sequence of events unfolding in the processes of electrogenesis in BFC. For these purposes, the use of poisons that can block individual elements of the respiratory chain, inhibit glycolysis, uncouple the processes of oxidative phosphorylation, lead to irreversible disturbances in ion exchange, and also induce oxidative stress is especially promising. The list of substances with the above properties primarily includes sodium fluoride, sodium azide, and 2,4-dinitrophenol (Parthasaradh and Kumari, 2018, Pradiptama et al., 2019, Johnston and Strobel, 2019, Zhang et al., 2022, Johnston and Strobel, 2020, Suresh et al., 2020).

The purpose of the study: to study the inhibitory effect of sodium fluoride, sodium azide and 2,4-dinitrophenol on the power generation of BFC with Micrococcus luteus 1-I.

2 MATERIALS AND METHODS

2.1 BFC and Electrodes

The effect of inhibitory substances on the generation of electricity was carried out in BFCs designed at the Research Institute of Biology of ISU (Kuznetsov et al., 2021). This is a two-chamber structure made of Plexiglas (Plexiglas XT 20070, 3 mm). The MF-4SK proton-exchange membrane (JSC Plastpolimer,

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Russian Federation) separates the chambers with a volume of 0.35 liters. The electrodes were URAL T-22P A carbon cloth (OAO Svetlogorskkhimvolokno, Republic of Belarus). The electrode dimensions were 16 × 4 cm (Fig. 1).

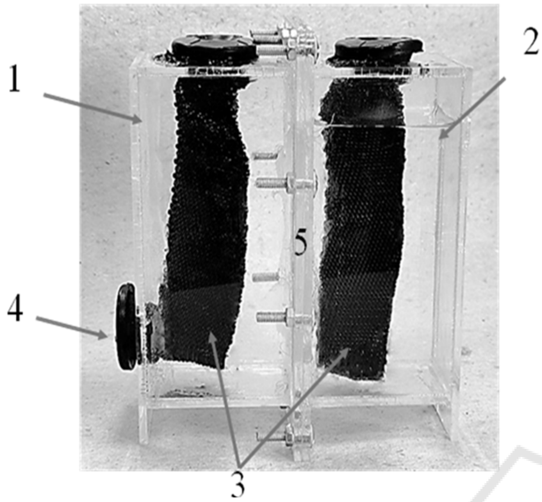

Figure 1: BFC design: 1 – anode chamber; 2 – cathode chamber; 3 – carbon cloth electrodes; 4 – rubber cap for anolyte sampling; 5 - proton exchange membrane.

2.2 Strains and Culture Conditions

Sterile model wastewater (MW) served as the anolyte (Stom et al., 2021). Tap water was used for catholyte (water intake in the Angara River upstream of the HPP dam). *Micrococcus luteus* 1-I was chosen as a bioagent. It was isolated from the activated sludge of the Angarsk petrochemical plant (Russia). The strain was deposited in the All-Russian Collection of Microorganisms (VKM) under the number VKM Ac-2637D. Bacteria were cultivated on fish peptone agar (FPA) (Stom et al., 1992).

In all experiments, 5 ml of washings from a one-day culture in saline (0.85% NaCl) were used. The washings were carefully suspended before inoculation. Sodium fluoride (NaF), sodium azide (NaN3) and 2,4-dinitrophenol (2,4-DNP) at concentrations of 5 and 20 mmol/L were chosen as biochemical inhibitors.

2.3 Generation of Electricity in BFC

The current in the MFC was recorded with a digital multimeter "DT-266". The voltage was measured using an automatic data recording system based on the microprocessor board "Arduino Mega 2560". BFC were thermostated at 30°C for 3 days.

2.4 Statistical Processing of Results

All experiments were performed in at least 5 independent experiments with 3 parallel measurements each. Statistical processing of experimental data was carried out using the Microsoft Excel software package. The results are the mean values for the sample. and their standard errors. Conclusions are made with the probability of an error-free forecast $P \geq 0.95$

3 RESULTS AND DISCUSSION

The used inhibitors at all concentrations had a negative effect on the power generation of BFCs with the bioagent *M. luteus* 1-I. However, the degree of this influence was different for each compound. So, NaF at a concentration of 20 mmol/L on day 2 reduced the voltage from 341 mV to 75 mV (about 5 times) and the short circuit current from 0.371 to 0.051 mA (more than 7 times), respectively. (Fig. 2 and Fig. 3).

Sodium azide had a stronger effect. Compared to fluoride, at the same NaN3 concentration on day 2, the voltage suppression was 3 times greater, and the current strength was 40 times greater (Fig. 4 and Fig. 5). 2,4-DNP at all concentrations completely suppressed the work of BFC (Fig. 6 and Fig. 7).

When discussing the data obtained in connection with the results of other authors, the works of J. K. Jan et al. They studied the effect of poisons on the operation of BFCs. It has been shown that the introduction of sodium azide at a concentration of 0.4 mmol/L into the biocathode leads to a decrease in the generated current from 3 mA to 2.8 mA. The authors suggested that the inhibitory effect is achieved due to disruption of extracellular electron transport (Jang et al., 2013). Based on this, one could expect an increase in the inhibitory effect on power generation in BFC at higher concentrations of sodium azide, which was confirmed in our experiments.

Influence of respiratory poisons on the power generation of BFC with *Chlorella pyrenoidosa*. studied Yi S. Hu et al. In the presence of the ATP synthase inhibitor resveratrol and the protonophore 2,4-DNP at a concentration of 1000 ppm (which is approximately equal to 5 mmol/L in our study), the current strength was not only not inhibited, but even increased. However, this change in the operation of the BFC was insignificant (Xu et al., 2015).

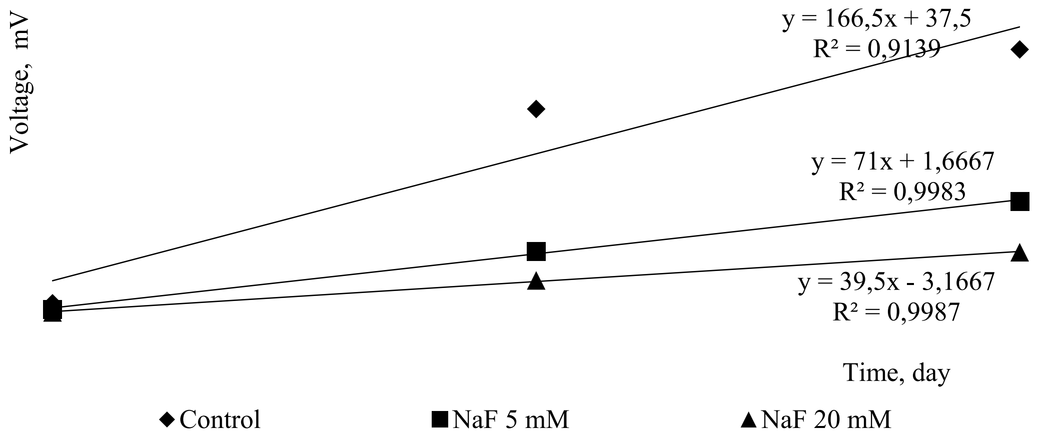

Figure 2: Dynamics of voltage generated in BFC by *M. luteus* 1-I strain in the presence of sodium fluoride (t = 30° C, electrode – carbon cloth, substrate – MW).

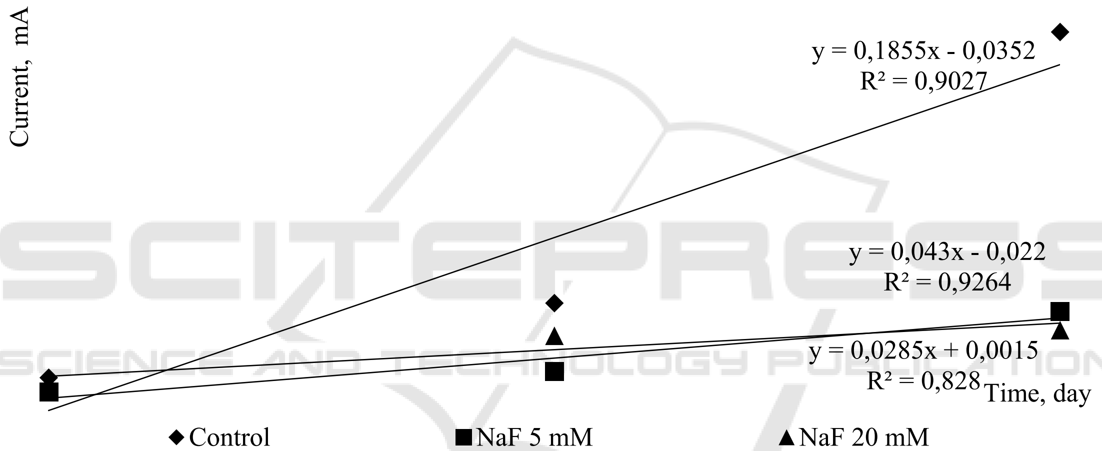

Figure 3: Dynamics of current generated in BFC by *M. luteus* 1-I strain in the presence of sodium fluoride (t = 30 °C, electrode – carbon cloth, substrate – MW).

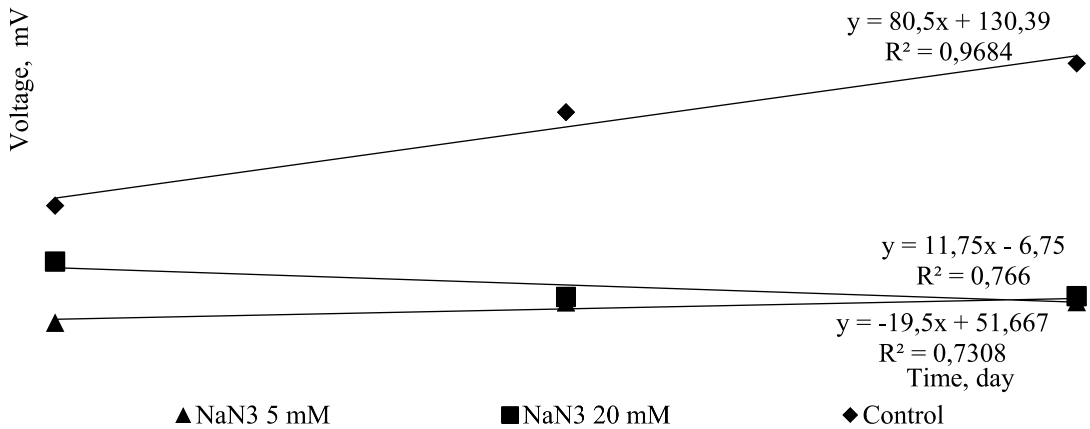

Figure 4: Dynamics of voltage generated in BFC by *M. luteus* 1-I strain in the presence of sodium azide (t = 30 °C, electrode – carbon cloth, substrate – MW).

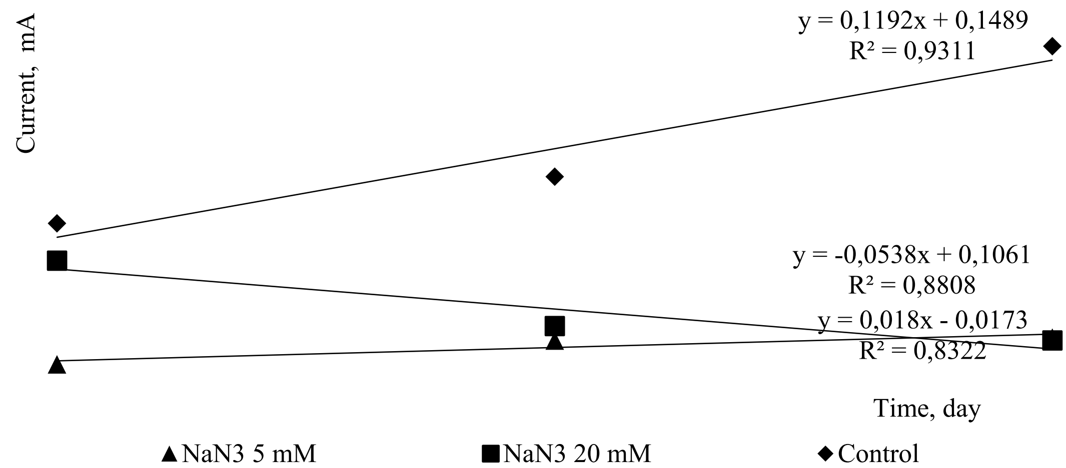

Figure 5: Dynamics of current generated in BFC by *M. luteus* 1-I strain in the presence of sodium azide (t = 30 °C, electrode – carbon cloth, substrate – MW).

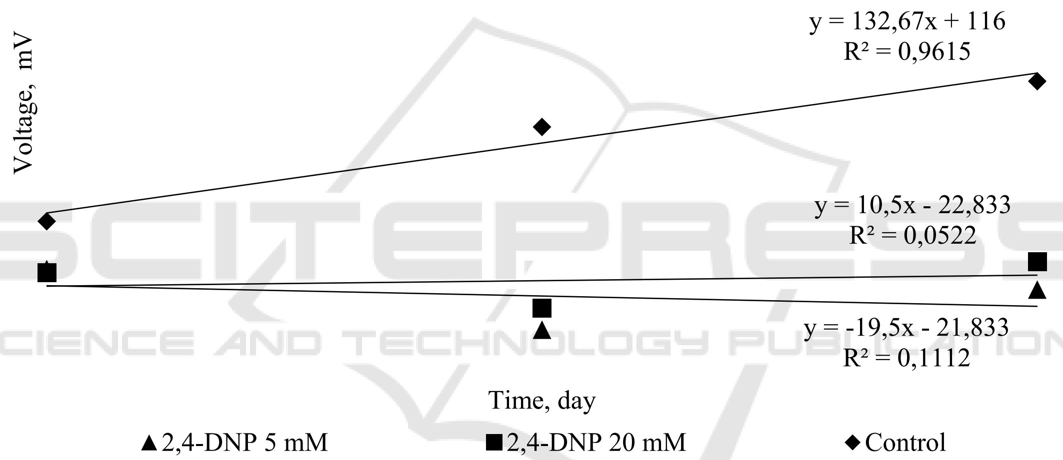

Figure 6: Dynamics of voltage generated in BFC by *M. luteus* 1-I strain in the presence of 2,4-DNP (t = 30 °C, electrode – carbon cloth, substrate – MW).

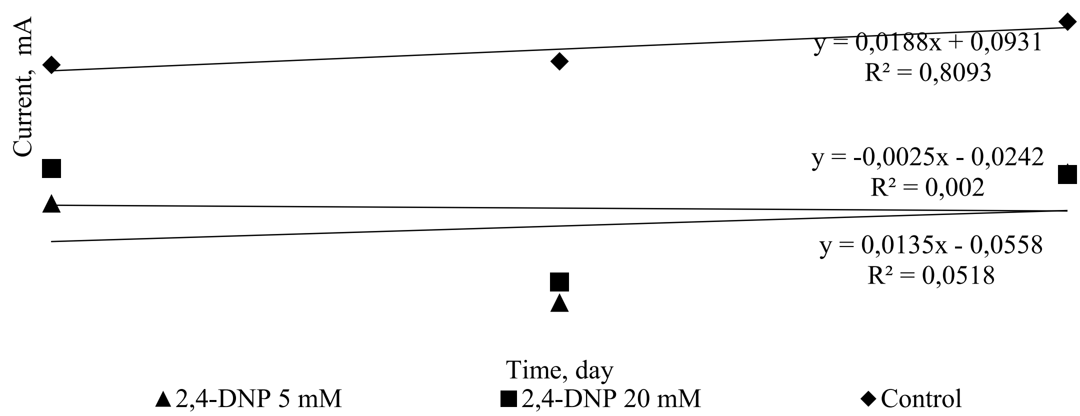

Figure 7: Dynamics of current generated in BFC by *M. luteus* 1-I strain in the presence of 2,4-DNP (t = 30 °C, electrode – carbon cloth, substrate – MW).

4 CONCLUSIONS

In this work, we studied the effect of three respiratory poisons and electron transfer inhibitors (NaF, NaN3, 2,4-DNP) on the power generation of BFC with *M. luteus* 1-I as an anode bioagent. It was found that all compounds in all tested concentrations (5 and 20 mmol/L) inhibit voltage and current. Moreover, the degree of inhibition of these indicators increases in the series sodium fluoride > sodium azide > 2,4-dinitrophenol.

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