SEDIMENT MICROBIAL FUEL CELL BASED BIOSENSOR FOR REAL TIME DETECTION OF Cu^{2+} IN INDUSTRIAL WASTEWATERS – OPERATION AND EFFECTIVENESS

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ABSTRACT. Sediment microbial fuel cells (SMFCs) are bio-electrochemical systems in which the anode is placed in the anaerobic sediment and the cathode is immersed in the surface layer of water. Natural exoelectrogenic bacteria decompose organic compounds in sediment, producing electrons and protons. The electrons reach the cathode through an external electrical circuit, while the protons pass through the soil layer, which acts as a kind of membrane. Oxygen is in many cases the preferred electron acceptor due to its presence in the cathode region and its high potential. Heavy metal ions and other compounds can also be reduced on the cathode, which will increase the energy generated. Based on the above characteristics, SMFCs would be suitable for application as biosensors for monitoring water pollution with heavy metals. In the present study, the possibility of application of SMFCs as biosensors for water pollution with copper has been studied. A high correlation was found between the concentration of copper ions in the range 0,1 – 100 mg/L and the voltage generated by SMFC. The constructed SMFC based biosensor showed wider detection limits for copper compared to other authors' studies as the coefficient of determination reached 0,9911. Native exoelectrogenic bacteria were represented mainly by *Geobacter, Clostridium, Anaeromixobacter* and *Bacillus.*

Key words: sediment microbial fuel cell, biosensors, heavy metals detection

Introduction

Anthropogenic water pollution with heavy metals (for example from mining, industry) has been receiving enormous attention worldwide due to their toxicity, persistence and bioavailability (Grattieri et al., 2017). The development of on-line warning sensors is of great importance for heavy metal discharge monitoring and emergency action (Kim et al., 2007). Conventional methods for monitoring water quality depend on periodic sampling and physicochemical laboratory analyses, through which detailed information on heavy metal contamination can be obtained. However, sampling may lag behind wastewater discharge, and sampling and analysis take a long time. All this makes it impossible to provide timely warning of heavy metal contamination (Sajana et al., 2014).

Microbial fuel cells (MFCs) are devices that convert chemical energy from organic substrates into electricity using electroactive microorganisms. Sensors based on microbial fuel cells are attracting huge interest because they achieve self-sustainability, low cost and online monitoring of water quality (Lorenzo et al., 2019). In most MFC-based sensors, the analysed water is fed into an anode chamber where microorganisms convert the organic matter into an electrical current. The microorganisms used can be very different, depending on the target process (e.g., denitrification, sulphate reduction, etc.). Because they usually occur in the anodic region, they are most often anaerobic (Deng et al., 2015). Toxic substances inhibit the metabolic activity of microorganisms, which leads to a reduced electric current. Since these sensors provide electrical signals based on the performance of anodic electroactive microorganisms, they have some major drawbacks. They need several weeks to acclimatise the electroactive microorganisms that are inoculated into the anode chamber of the MFC. This makes them impractical when monitoring an unexpected toxic shock in wastewater. Also, toxic contaminants would inhibit microorganisms and even cause irreparable damage, making it impossible to trace subsequent contamination. This makes it unrealistic to use this type of sensors for in situ monitoring of heavy metal pollution in aquatic environments (Schneider et al., 2016).

The application of MFC biosensors to heavy metals monitoring is not comprehensive enough. At present, the research on heavy metal sensors is mainly focused on single heavy metals. The research on MFC biosensors for combined heavy metals is still very limited (Zhang et al., 2022).

Sensors based on sediment microbial fuel cells avoid the inhibition of electroactive microorganisms (Alipanahi et al., 2019). The main advantages of the sensor include:

- Instead of using the inhibition of electroactive bacteria in the sediment, it captures heavy metal pollutants released into the water, reducing them on the cathode and raising the voltage (Wang and Jiang, 2019). The sediment adsorbs much of the heavy metals, protecting the electroactive microorganisms from being inhibited by heavy metal ions, (Alipanahi et al., 2018) thus allowing the sensor to function normally after shock contamination. Wu et al. (2018) reported Cu^{2+} shock only inhibited the exoelectrogenic activity in the surface soil (0–3 cm).
- The sensor uses natural electroactive bacteria living in the sediment, generating a base voltage, therefore the inoculation process can be avoided (Song and Jiang, 2018). The estimated number of bacterial species per gram of soil varies between 2000 and 8,3 million, and the abundance of organic matter, despite the variation between different soil types, is approximately 100 mg/g (Nandy et al., 2023). The organic matter degradation around the anode is normally enhanced by electroactive microbes like *Geobacter, Pseudomonas,* and *Desulfolobus* (Abbas et al., 2022).
- The sensor components are mounted in an integrated system; thus, monitoring is convenient and starts with placing the sensor in a wet area (Zhao et al., 2017).

The influence on the performance of SMFC systems of moisture content, soil texture and reactor design, has been investigated to maximise the power output and enhance the bioremediation capability. Nonetheless, to date the voltage

evolution during start-up in SMFCs has not been investigated in-depth yet, as it mainly depends on the time for acclimatisation and formation of a biofilm of electroactive microorganisms on the surface of the anode (Dziegielowski et al., 2023).

Based on the aforementioned advantages, the sensor would be suitable for monitoring repeated heavy metal pollution in waters. The application of the sensor involves long-term operation with various changes in temperature and weather conditions, and repeated contamination, therefore the performance of the sensor under such conditions is unknown, especially in terms of inhibition of electroactive microorganisms and interruption of the electrical signal of the sensor under repeated pollution (Song et al., 2019).

In the present study, we developed an SMFC-based sensor with an anode embedded in sediment and a cathode immersed in the surface layer of water for monitoring Cu^{2+} pollution, which is a typical heavy metal pollutant in aquatic environments. The aim of the study was to determine the effectiveness and operation of SMFC-based biosensor at different resistances and value of current.

Materials and methods

Soil Sampling

For the purpose of the study, river sediment was taken from Ivanyanska river, Bulgaria (42°42'27.1"N 23°11'46.4"E), from a depth of 20 - 30 cm. After taking the river sediment, the larger pieces were removed by sieving through 2 mm. In order to carry out physicochemical analyses, part of the sieved soil was air-dried. Soil pH was measured by pH-meter HANNA HI-9021 determined at 1 : 2.5 (soil : water). Soil electrical conductivity (EC) was measured by WTW LF 197-S determined at 1 : 5 (soil : water). The main soil physicochemical properties were as follows: pH 6,89, EC 142,31 µS/cm. The soil was classified as sandy clay loam, the Kjeldahl-N content was 0,214% and the dissolved organic carbon - 68,34 mg/kg. The amount of copper measured was 2,11 mg/kg.

Construction of SMFC biosensor

The sediment microbial fuel cell is a plastic container with a volume of 800 cm^3. At the bottom of the vessel is a stainless-steel electrode with an area of 32 cm^2. The vessel was filled with river sediment with a volume of 550 cm^3. The cell was then filled with pure water and a second 32 cm^2 stainless steel electrode was placed in the surface layer of the water. The physicochemical characteristics of water was pH 7,23, Eh 382 mV and EC 51 µS/cm measured by Vernier LabQuest Mini. A scheme of the laboratory model of the SMFC-biosensor is shown on Figure 1.

The test solutions pass through the cathode zone, where copper ions play the role of an additional electron acceptor and increase the voltage of the cell by changing the electrochemical parameters (Liu et al., 2020). Three identical SMFC-based biosensors were made – SMFC1, SMFC2, SMFC3. The anode and cathode of each biosensor were connected with an external 4000 Ω, 2000 Ω and 1000Ω resistor using copper wire. Then, they had been acclimated for 14 days, until the base voltage is reached and the electroactive microorganisms acclimatise.

The abundance of exoelectrogenic microorganisms was

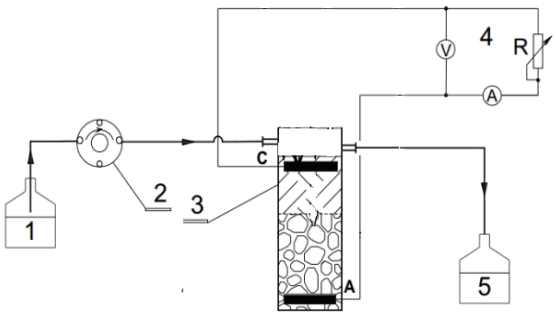


Fig. 1. The schematic diagram of the SMFC-based biosensor 1 – inflow, 2 – peristaltic pump, 3 – sediment microbial fuel cell, 4 – digital multi-metre, 5 – outflow, A – anode C – cathode

quantified by 16S rRNA gene. In the sediment *Geobacter* and *Clostridium,* were the most abundant. *Anaeromyxobacter*, *Bacillus, Pseudomonas* and *Desulfolobus* were also detected.

Cu^{2+} pollution

For the purposes of the study, synthetic solutions containing copper with different concentrations were prepared. $CuSO_4$ solutions were prepared in twelve concentrations. Six concentrations for the first part of experiment (including 10, 25, 50, 100, 250 and 500 mg/L Cu^{2+}) and ten concentrations for the second part of the experiment (0,1, 0,25, 0,5, 1, 2,5, 5, 10, 25, 50, 100 mg/L Cu^{2+}).

Electrochemical analysis

Polarisation and power curves of the individual SMFCs were obtained by varying external resistances sequentially from 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1,5, 1,3, 1, 0,7, 0,5, 0,3 to 0,1 KΩ using MCP lab electronics BXR-04 ResistorBox.

To determine the response of voltage signals to Cu^{2+} shock, the biosensors` voltage was measured and recorded with Vernier LabQuest Mini and Logger Lite software.

Results and discussion

After construction, the cells were allowed to acclimate and reach their baseline open circuit voltage. In 10 days, the voltage of SMFC-based biosensors reached a plateau of 540 ± 15 mV. On the eleventh day, the polarisation curves of the SMFC-based biosensor were measured. Data from the study are presented in Figures 2, 3 and 4.

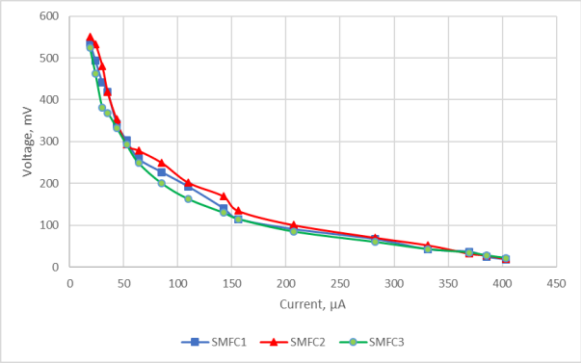


Fig. 2. Electrical characteristics of SMFCs

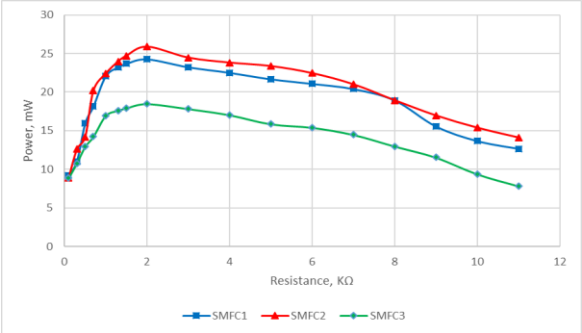


Fig. 3. Power dynamics (data from polarization curve) of SMFC-based biosensors

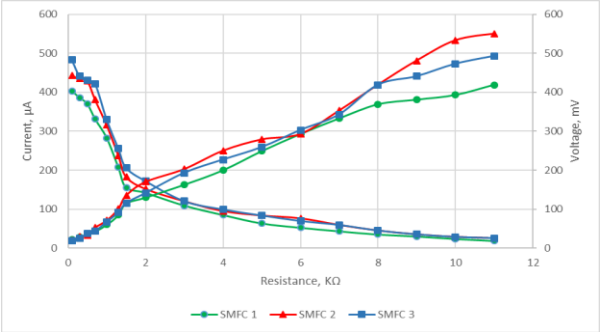


Fig. 4. Polarisation curves of SMFC-based biosensors

The graph in Figure 2 shows the relation between the voltage and current of the three SMFCs. The graphs show that the maximum power of 24,27, 25,86, and 18,47 mW were achieved at 2000 Ω external resistance for SMFC1, SMFC2, and SMFC3, respectively (Figure 3). At this resistance, the cells had a voltage of 141, 170, 130 mV and a current of 172,1, 152,1 and 142,1 μA, respectively (Figure 4).

Based on the obtained electrical characteristics, the three SMFC-based biosensors were switched to operate with an external resistor of 4000 Ω, 2000 Ω, and 1000 Ω, respectively (at these resistances the cells showed the highest power) for 14 days until the voltage reached a relatively stable plateau and acclimatiased the electroactive microorganisms. The results of the study are presented in Figure 5.

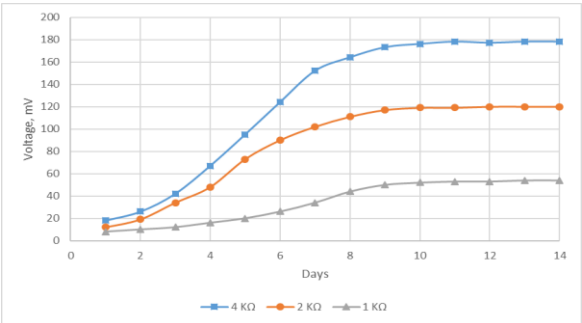


Fig. 5. Base voltage dynamic of SMFC-based biosensors

From the graph presented, it can be seen that after the ninth day, all three SMFC-based biosensors reach a base voltage of approximately 176 ± 2 mV, 117 ± 3 mV and 52 ± 2 mV for 4000 Ω, 2000 Ω and 1000 Ω, respectively. After stabilising the voltage, we moved on to the next part of the experiment. Synthetic copper solutions with increasing concentration – 10, 25, 50, 100, 250, 500 mg/L were successively fed to the three SMFC-based biosensors. The feed rate was 3 mL/s. Our preliminary experiments showed that the feed rate affect the speed of reaction of SMFC-based biosensor but not the value of the voltage change. This is due to the fact that the incoming copper ions, which are an additional electron acceptor in the cathode zone in addition to dissolved oxygen, change the electrochemical parameters of the cell until a constant concentration of Cu^{2+} is reached. (Liu et al., 2020). The faster the solution with copper ions occurs, the faster the electrochemical parameters change and the cell voltage increases. When feeding the individual solutions, the change in voltage after its stabilisation was taken into account. Based on the change in voltage at different copper concentrations, a standard curve is plotted. The graph is shown in Figure 6.

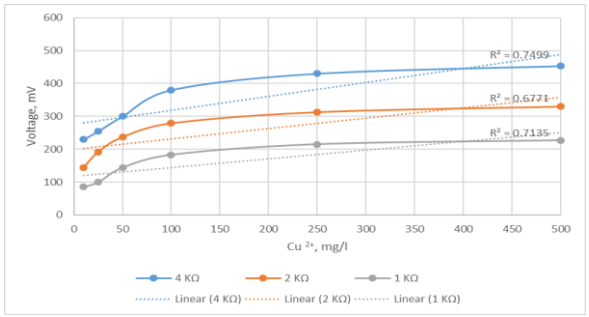


Fig. 6. SMFC-based biosensors standard curves

The graph shows a not very high coefficient of determination - R^2 = 0,7499, 0,6771 and 0,7135 for 4, 2 and 1 K Ω, respectively. Serious deviations were also observed of the graphs from the standard straight lines that could be used for analytical purposes in the studied interval.

When the concentration of copper increases above 100 mg/l, a weak sensitivity of SMFC-biosensors is observed, correspondingly a weak increase in the generated voltage compared to the concentration of copper ions.

In the interval 10 – 100 mg/LCu^{2+}, the sensitivity of SMFC-based biosensors is significantly higher, which is why additional research was done in interval 0,1 – 100 mg/L Cu^{2+}. The data from the study are presented in Figure 7.

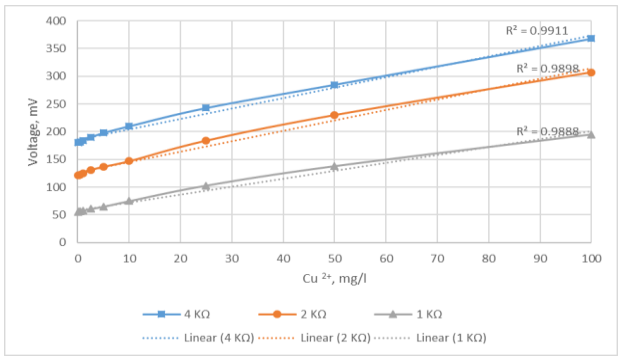


Fig. 7. SMFC-based biosensors standard curves

The graph shows a high coefficient of determination - R^2 = 0,9911, 0,9898 and 0,9888 for 4, 2 and 1 K Ω, respectively. A slight deviation is also observed of the graph from the standard straight lines in the studied interval. Solutions with copper concentrations of 0,1 to 0,5 mg/L cause minimal changes in voltage, which is why they are not clearly visible on the standard curve. This may be a limiting factor in the application of this type of biosensor in real conditions for the detection of very low concentrations of copper. However, the study has shown that the constructed SMFC-biosensor has wider

detection interval and larger voltage change ΔV compared to research by other authors (Liu et al., 2020, Wu et al., 2018).

Conclusion

In order to determine the possibilities for application of sediment microbial fuel cells as biosensors for real time monitoring of recurrent and/or toxic shock water pollution with copper, three SMFC-based biosensors were constructed. The devices were operated at three different resistances (4, 2 and 1 KΩ) to determine the effect on their performance. The influence of six concentrations of copper (10, 25, 50, 100, 250, 500 mg/l) on the change of the electrochemical parameters of the cells was studied. A high coefficient of determination was not achieved in the studied concentration range. Low sensitivity of the sensor was found in the interval 100 – 500 mg/L.

A high coefficient of determination of R=0.9911 was reached in the range 0,1 – 100 mg/L. The obtained results show that the SMFC-based biosensors can work effectively at all three tested resistances - 4, 2 and 1 KΩ, and the coefficient of determination were 0,9911, 0,9898 and 0,9888, respectively. The results have also shown that the constructed SMFC-biosensor has wider detection interval and larger voltage change ΔV at the studied concentrations.

Research has shown that sediment microbial fuel cells can be used as biosensors for recurrent copper contamination, but further research is needed on their design, inertia in changing electrochemical parameters and detection range. The behaviour of the SMFC-based biosensor in monitoring waters contaminated with more than one heavy metal should also be studied.

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