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INFLUELNCE OF THE CONSTRUCTION OF A BIOSENSOR BASED ON SEDIMENT MICROBIAL FUEL CELLS ON ITS SENSITIVITY TO WATERS CONTAMINATED WITH HEAVY METALS

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ABSTRACT. Sediment microbial fuel cells (SMFC) based biosensors can be a simpler and cheaper alternative to traditional methods for monitoring and early warning of water pollution with heavy metals. However, to make the transition from the laboratory to application in the environment, more research is needed on the influence, durability, and cost-effectiveness of their constructive elements. In the present study, three types of SMFC-based biosensors were constructed - with stainless steel electrodes without a separator, with graphite electrodes without a separator, and with graphite electrodes and a bentonite separator placed in the sediment. The last variant showed the highest sensitivity to the investigated pollutants - copper, chromium, nickel and zinc, as well as the highest correlation coefficient R^2 - over 0.99.

Key words: sediment microbial fuel cells, biosensor, real time monitoring, heavy metals

Introduction

With the rapid development of the industry, wastewater monitoring has become an extremely important issue worldwide. Traditional detection methods, such as spectrophotometry and atomic absorption spectrometry (AAS), have high selectivity and sensitivity, but cannot be used for in situ monitoring due to their high cost and complex sample pretreatment requirements (Turker and Yakar, 2025).

Among various emerging solutions, in recent years, microbial fuel cell (MFC)-based sensors have been envisioned as a promising low-cost and energy-efficient tool for online monitoring of water and soil contaminants. For most MFC-based sensors, exoelectric bacteria are inoculated and acclimated in the anode chamber to determine pollutant toxicity. If a pollutant inhibits the metabolic activity of exoelectric bacteria, the electrical signals (voltage or current) of these sensors weaken. This is considered the basic principle of sensors based on microbial fuel cells (Chauhan et al., 2025). However, the use of exoelectric bacteria for pollutant detection has serious drawbacks, as the sensors cannot monitor repeated fouling unless exoelectric bacteria are reconstituted or re-inoculated and acclimatized after each fouling event. Unfortunately, recovery from toxic shock or acclimatization usually takes several days to several weeks. To overcome these drawbacks, a new type of sediment microbial fuel cell (SMFC)-based sensors is being developed for monitoring water pollution events (Wang et al., 2023a). The main advantages of the sensor include a cathode instead of exoelectric bacteria in the sediment detecting pollution in the waters; the sediment adsorbs a large part of the contaminants, thus the exoelectric bacteria remain protected from inhibition and the sensor can work normally after a shock; the sensor uses naturally occurring exoelectric bacteria in the sediment to generate a base voltage; therefore, the inoculation process can be omitted; the sensor components are assembled into an integrated system, thus monitoring is conveniently started by placing the sensor in the monitored water (Li et al., 2024).

SMFCs are characterised by simplicity, low cost and maintenance, which facilitate the transfer of the technology from the laboratory to the environment (Sun et al., 2024). Sediment microbial fuel cells have great potential as cost-effective and sustainable sensors. The technology relies on endogenous electroactive microorganisms that convert chemical energy stored in biodegradable matter in the soil directly into electricity (Bose et al., 2026). Organic compounds in the sediment serve as a source of renewable fuel. Electrons are generated from the oxidation of organic matter near the anode by natural microorganisms present in the sediment. These electrons are transferred to the cathode where they reduce oxygen (Danhassan et al., 2023).

Recently, SMFCs have attracted the attention of many researchers due to the possibility of their use as biosensors for heavy metals (Abbas et al., 2026), soil toxicity, BOD (Abbas et al., 2022), oil spills (Dai et al., 2021), sediment bulking (Wang and Jiang, 2019), dissolved oxygen - DO (Azri et al., 2026), hexavalent chromium (Zhao et al., 2018) and others. However, a number of factors must be addressed before SMFC technologies can be applied on a larger scale. Previous research has focused on environmental factors, long-term stability, and other factors affecting peak voltage generation and scaling of SMFCs. Long start-up times are also one of the limitations that slow down their industrialisation. SMFC start-up refers to reaching a stable base voltage and generating electricity (Song and Jiang, 2018). Previous studies have made significant efforts to reduce the internal resistance of the cell and improve the performance of SMFCs at the laboratory scale by modifying anode surfaces, changing the anode configuration, using multiple anodes, optimising the depth and orientation of the anode in the sediment and increasing the oxygen supply to the cathode. Earlier investigations have shown that anode configuration and distribution could significantly affect SMFC performance. For example, increasing the area of a single anode will lead to higher electricity production, but it is challenging to accommodate large electrodes in cells (Wang et al., 2023b). Over the years, researchers have worked on the development of a variety of SMFCs. Many researchers have focused on increasing the current by modifying electrode materials (Wang et al., 2019) and investigating the effect of the distance between the electrodes (Simeon and Freitag, 2022).

Despite the development of platinum electrodes and other expensive materials, ultimately a major goal is to keep SMFC-based biosensors cheap and simple to operate devices so that they can gain widespread application in pollution monitoring and early warning (Nandy et al., 2023).

In the present study, the influence of two relatively low-cost stainless steel and graphite electrodes on the sensitivity and performance of SMFC-based biosensors for monitoring and early warning of water pollution with heavy metals was investigated. Also, for the first time, the influence of a separating layer of bentonite located in the sediment on the performance of a SMFC-based biosensor for monitoring water contaminated with heavy metals was investigated.

Materials and methods

Soil sampling

The river sediment used in the study was taken from Ivanyanska river, Bulgaria (23°11'46.4"E 42°42'27.1"N), from a depth of 20-30 cm. After taking the sediment, pieces larger than 2 mm were removed by sieving. For the purpose of physicochemical analyses, part of the sieved soil was air-dried. Soil pH was determined by pH-meter HANNA HI-9021 measured at 1:2.5 (soil : water). Soil electrical conductivity (EC) was determined by WTW LF 197-S measured at 1:5 (soil : water). The main physicochemical parameters of the soil were as follows: pH 6.68, EC 154.45 μS/cm. The soil was classified as sandy clay loam, the Kjeldahl-N content was 0.21% and the dissolved organic carbon 66.14 mg/ kg. The amount of copper measured was 59.5 mg/ kg, chromium – 20.2 mg/ kg, nickel – 13.0 mg/ kg, and zinc – 105 mg/ kg. The sediment microbial fuel cells-based biosensors were filled in with the soil prepared in the above-mentioned way.

Description of the laboratory installation

A scheme of the laboratory installation of a sediment microbial fuel cell-based biosensors is shown in Figure 1.

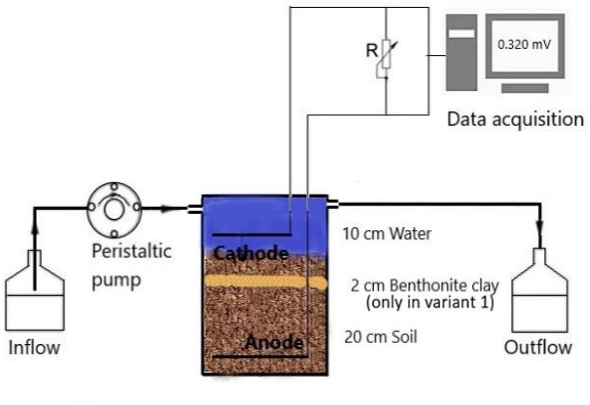


Fig. 1. Scheme of the laboratory installation of SMFC-based biosensors with different electrodes

For the purpose of the study 12 sediment microbial fuel cell-based biosensors were prepared in three variants. The sediment microbial fuel cell is a plastic container with a volume of 800 cm^3. In the first variant at the bottom of the vessel is a stainless-steel electrode AISI 304 (18% Cr, 10% Ni) 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 AISI 304 was placed in the surface layer of the water. In the second variant, graphite electrodes, also with an area of 32 cm^2, are placed in the sediment and in the surface layer of the water. The third variant is a plastic container with a volume of 800 cm^3. There is a graphite electrode with an area of 32 cm^2 at the bottom of the vessel. The cathode was made of graphite with the same area. The vessel was filled with river sediment with a volume of 510 cm^3. There is a layer of bentonite with a thickness of 2 cm in the middle of the sediment layer. The bentonite plays the role of a kind of membrane between the anode and cathode areas, which is aimed at achieving a better separation of the processes in the two areas.

The physicochemical characteristics of water were pH 7.06, Eh 213 mV and EC 58 μS/ cm measured by Vernier LabQuest Mini.

The heavy metal solutions pass through the cathode zone, where heavy metal ions play the role of an additional electron acceptor and increase the voltage of the cell by changing the electrochemical parameters. Initially, clean water was supplied to the cells until a constant base voltage was reached. The anode and cathode of each of the biosensors were connected with an external 1000Ω resistor using copper wire. Then they had been acclimated for 14 days, until the base voltage is reached and the electroactive microorganisms acclimatize. *Geobacter*, *Clostridium, Anaeromyxobacter*, and *Bacillus* were the most abundant in the sediment. *Pseudomonas*, *Desulfolobus* and *Desulfovibrio* were also isolated. The abundance of electroactive microorganisms was quantified by 16S rRNA gene.

After that, the heavy metal solutions were passed to the individual cells and the change in cell voltage was continuously recorded. Based on the correlation between voltage change and heavy metal concentration, standard curves were drawn.

Heavy metals pollution

For the purposes of the study, synthetic solutions containing copper ($CuSO_4.5H_2O$), chromium ($K_2Cr_2O_7$), zinc ($ZnSO_4.7H_2O$) and nickel ($NiSO_4.7H_2O$) with different concentrations were prepared. Solutions were prepared in eight concentrations – 0.5, 1, 2.5, 5, 10, 25, 50 and 100 mg/L.

Electrochemical analysis

To determine the response of voltage signals to heavy metals shock, the biosensors` voltage was measured and recorded with Vernier LabQuest Mini and Logger Lite software.

Results and discussion

In order to determine the influence of various design features on the performance of the SMFC-based biosensors, after stabilization of the base voltage, the passage of a synthetic copper solution through the three sensor variants began. The change in the voltage of the sensors during the passage of the solutions with different concentration was recorded, after which standard curves were drawn based on the obtained results. The graphs are presented in Figure 2. In the case of the cell with graphite electrodes and a separator placed in the sediment, the greatest change in voltages ΔU was observed as a function of Cu^{2+} ions concentration when passing the solutions of different concentration, which shows that this variant is the most sensitive to copper contamination.

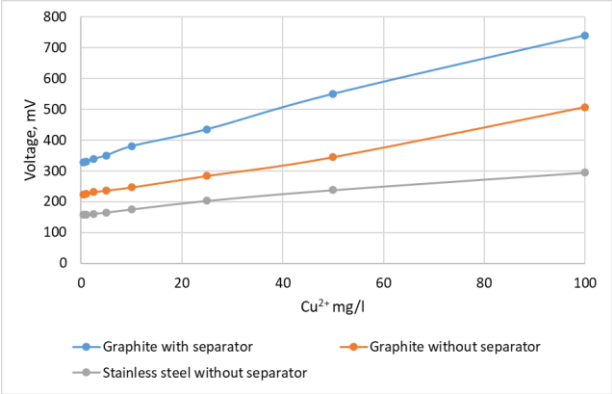


Fig. 2. Standard curves of three variants SMFC-based biosensor for copper contamination

From the presented graph, it can be seen that the biosensor with a stainless-steel electrode without a separator has the lowest baseline voltage, as well as the smallest voltage change ΔU relative to the concentration of copper ions. Nevertheless, both parameters are significantly higher compared to studies of other authors (Liu et al., 2020) on the sensitivity of SMFC-based biosensors to copper ions.

The next part of the experiment aimed to establish the influence of the design features of the SMFC-based biosensor in monitoring chromium-contaminated waters. Figure 3 shows the standard curves obtained when the chromium solutions were passed through the different variants of the sensor.

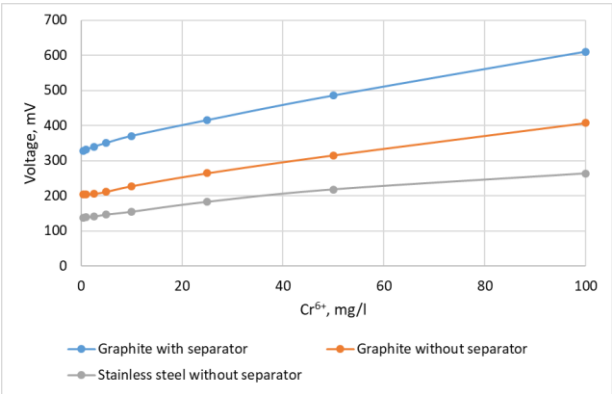


Fig. 3. Standard curves of three variants SMFC-based biosensor for chromium contamination

Again, the sensor with graphite electrodes and separator exhibits the largest change in voltages relative to the concentration of the investigated metal, corresponding to a high sensitivity to chromium ions. In this case, ΔU for a concentration of 100 mg/L Cr^{6+} ions reaches 412 mV. By comparison, when studying the sensitivity of SMFC-based biosensors to Cr^{6+} ions, this parameter varied between 25 and 150 mV (Zhao et al., 2018; Li et al., 2024).

The results from studies carried out to establish the sensitivity of SMFC-based biosensors to nickel-contaminated waters are presented in Figure 4.

The variant with graphite electrodes and separator has the highest base voltage and voltage change to different nickel ions concentrations.

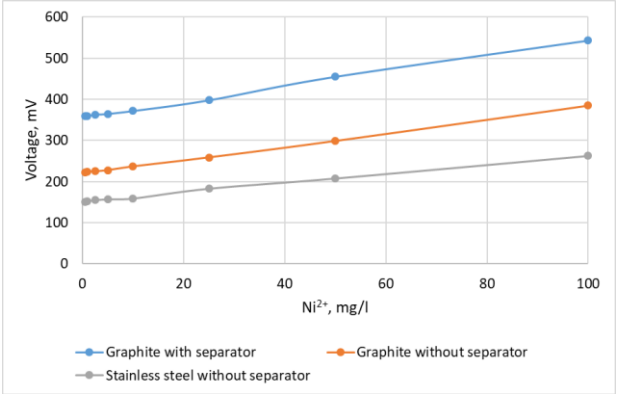


Fig. 4. Standard curves of three variants SMFC-based biosensor for nickel contamination

The last part of the experiment was aimed at establishing the influence of the structural features of the SMFC-based biosensors on their sensitivity to zinc-contaminated waters. The results obtained from the study are presented in Figure 5.

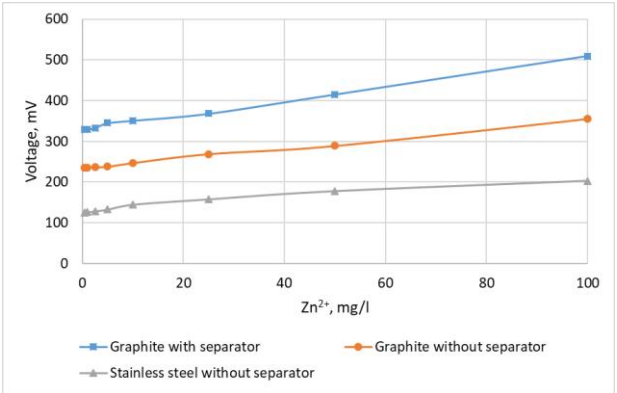


Fig. 5. Standard curves of three variants SMFC-based biosensor for zinc contamination

Smaller changes in the voltage of the sensor are observed during the passage of solutions with different concentrations of zinc ions, compared to the ions of other metals. The SMFC-based biosensor with graphite electrodes and separator again showed the highest sensitivity to zinc-contaminated waters.

From the presented graphs, it can be seen that heavy metal solutions with concentrations of 0.5 and 1 mg/L do not lead to a significant change in the biosensor`s voltage. ΔU for these concentrations varies between 1 and 4 mV, which may be attributed to side factors and cannot be indicative of heavy metal pollution.

From the obtained graphs, it can also be seen that the placement of a separator significantly increases both the baseline voltage and the sensitivity of the biosensor, expressed through ΔU. Placing a separator between the anodic and cathodic zone implies several key benefits, such as optimising ion transport, reducing oxygen penetration into the anodic zone, controlling chemical processes in the anodic and cathodic zones, and others (Bose et al., 2026). This resulted in more stable electrochemical parameters of the cell, and thus to improved sensitivity of the SMFC-based biosensor.

In order to provide better comparability of the obtained results, Table 1 presents the parameters of the linear regression and the correlation coefficient of the investigated SMFC-based biosensors.

Table 1. *Linear regression parameters and correlation coefficient of the SMFC-based biosensors*

Variant	Slope	Intercept	R^2
Cu^{2+}			
Graphite with separator	4.15	330.88	0.9981
Graphite without separator	2.79	219.7	0.9949
Stainless steel without separator	1.41	159.83	0.989
Cr^{6+}			
Graphite with separator	2.82	335.06	0.9943
Graphite without separator	2.08	203.98	0.9952
Stainless steel without separator	1.29	141.45	0.9768
Ni^{2+}			
Graphite with separator	1.87	356.43	0.9976
Graphite without separator	1.62	220.61	0.999
Stainless steel without separator	1.13	150.99	0.9972
Zn^{2+}			
Graphite with separator	1.77	329.55	0.9959
Graphite without separator	1.19	234.3	0.9954
Stainless steel without separator	0.79	130.49	0.9489

From the data presented in Table 1, high correlation coefficients are determined for all investigated variants. Nevertheless, for all four studied metals, the lowest R^2 value is obtained for the SMFC-based biosensor variant with stainless steel electrodes without a separator. Similar correlation coefficients have been reported by other authors when studying the sensitivity of SMFC-based biosensors towards copper and chromium (Liu et al., 2020; Li et al., 2024). The same trend is observed for the other two presented parameters – slope and intercept. These are highest for the SMFC-based biosensor variant with graphite electrodes and a separator, and lowest for the variant with stainless steel electrodes without a separator. The presented data further indicate that the constructed SMFC-based biosensors exhibit greater sensitivity to copper and chromium ions. This is evident from the obtained parameters for slope and intercept, which are significantly lower when investigating the sensitivity towards nickel and zinc ions. This is largely due to the nature of the studied metals and their standard reduction potentials.

Conclusion

Real-time monitoring and early warning of water pollution with heavy metals is extremely important for environmental protection. The construction of cheap and simple sensors will allow their widespread use and the timely detection and treatment of contaminated water. The present study aimed to determine the influence of construction of SMFC-based biosensors on their performance and sensitivity to waters contaminated with heavy metals. For this purpose, three variants of SMFC-based biosensors were constructed and investigated - with stainless steel electrodes without a separator, with graphite electrodes without a separator, and with graphite electrodes and a bentonite separator placed in the sediment. The sensitivity of the biosensors to four types of pollutants - copper, chromium, nickel and zinc - was investigated. The obtained results showed a high efficiency of the constructed biosensors to the investigated pollutants. A high correlation coefficient was achieved with all three biosensors variants. The most sensitive to the investigated pollutants was the SMFC-based biosensor with graphite electrodes and a separator placed in the sediment. For this variant, the highest values of the linear regression parameters – slope and intercept – were also established. The effect of a separator on the performance of a SMFC-based biosensor was investigated for the first time, and the results showed an increase in the base voltage of the sensor and an increase in the sensitivity of the biosensor to heavy metal water contamination. The studied SMFC-based biosensors display greater sensitivity to copper and chromium ions compared to nickel and zinc ions. The obtained results will contribute to a better understanding of SMFC-based biosensors and the construction of cheap and simple sensors for environmental application.

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