Prototype of a SMFC-based biosensor for real-time monitoring of heavy metals polluted water

Rosen Ivanov
University of Mining and Geology, Bulgaria
r.ivanov@mgu.bg

Abstract: *Timely detection and treatment of water pollution is of utmost importance for preventing serious environmental pollution and toxicity to living organisms. Traditional monitoring methods require time for sampling and analysis, and those that provide real-time monitoring are expensive and have limited application. The development of a low-cost sensor for real-time water monitoring will enable wider application and quality management of more water bodies. Guided by this, we constructed and tested a prototype of a biosensor based on a sediment microbial fuel cell. The sensor showed high efficiency and sensitivity to the most common heavy metals pollutants from industrial, agricultural and domestic wastewater. The sensor is designed for both portable and desktop applications, as well as for integration into the technological schemes of enterprises for continuous monitoring.*
Keywords*: SMFC, BIOSENSORS, BIO-ELECTROCHEMICAL SYSTEMS, REAL TIME MONITORING*

1. Introduction

Water pollution poses a significant threat to aquatic ecosystems, the environment, and human health, necessitating continuous monitoring of their quality. Instrumental methods for monitoring water pollutants have high sensitivity; however, these techniques require expensive chemicals and equipment. They face challenges related to complex operational procedures. The intricate sample preparation processes, high equipment costs, and difficulties in data interpretation hinder the effective use of traditional physicochemical approaches for continuous water quality monitoring. [1] Additionally, the limitation associated with sampling frequency can lead to difficulty in capturing pollution events where wastewater with high concentrations of pollutants is discharged into the water, especially when this occurs periodically. Missed pollution events can cause serious threats to the ecosystem and human health due to the lack of timely notification and pollutant mitigation. In contrast to these traditional physicochemical methods that can only detect preselected chemicals, biosensor-based approaches provide a more comprehensive assessment of water pollution. By evaluating the overall physiological response of biological components to the water sample, they can detect both known and unknown toxic substances, complementing the shortcomings of traditional methods. [2] In this context, developing a sustainable, user-friendly, and cost-effective early warning indicator system for real-time detection of pollution is crucial for ecological consequences.

The microbial fuel cell (MFC)-based sensor is a highly promising self-powered and self-sustaining early warning system for water quality monitoring. These sensors are cost-effective, compact in design, and portable, making them suitable for real-time and on-site water quality monitoring. [3] Like traditional biosensors, MFC-based ones also require time for biofilm formation or immobilization of microorganisms on the electrode surface, but this sensor does not require sample purification before analysis. [4] Therefore, MFC-based biosensors take less time compared to traditional biosensors. The primary goal of the MFC sensor is to establish the relationship between the output signal and the impact of target analytical parameters or compounds (COD, BOD, VFA, and toxicity). Traditionally, MFC-based biosensors operate on the principle of reducing the electrical signal due to inhibition of electroactive microorganisms by contaminants passing through the anode zone of the cell. Because most MFC-based sensors rely on exoelectrogenic bacteria for detecting contaminants, they cannot monitor recurrent pollution unless the exoelectrogenic bacteria are restored or reinoculated. [2] To overcome this drawback, a new type of sensor based on sediment microbial fuel cells (SMFCs) has been developed for online and in-situ monitoring of recurrent water pollution. There are some significant advantages of SMFC-based biosensors, such as: eliminating the inoculation process because SMFC-based sensors use local soil exoelectrogens to produce baseline voltage; exoelectrogenic microorganisms overcome contaminant shock since the surface soil absorbs a larger portion of the contaminant concentration and the cathode detects the contaminants instead of the microorganisms; the sensor components are integrated systems. [5]

The SMFC sensor consists of an anode and a cathode, with the first placed in the sediment and the second submerged in the surface layer of water. Exoelectrogenic bacteria, which generate and deliver electrons to the anode, are widely distributed in the sediments of various wet zones, such as lake, coastal, and riverside wetlands. [6] Electrons flow from the anode to the cathode, which serves as the sensing element for detecting heavy metals and other contaminants in the water. The mechanism of the detection process is based on the reduction of contaminants at the cathode, leading to an increase in the electrical parameters of the sensor—primarily voltage and current. [7] As a result, the increase in voltage will be considered as a real-time signal during monitoring of a pollution event. Since SMFC sensors use the cathode instead of exoelectrogenic bacteria for detecting contaminants, they are capable of monitoring recurrent shocks. [8]

Compared to conventional biosensors, sediment microbial fuel cell (SMFC)-based biosensors exhibit advantages such as low cost, simple design, on-site usability, and long-term self-powered monitoring, making them attractive devices for long-term water quality monitoring. [9]

A number of environmental factors can influence the performance of SMFC-based biosensors, such as temperature, pH, and soil conductivity. [7] Most exoelectrogenic microorganisms are mesophilic, which is why these biosensors exhibit their highest performance at temperatures between 19 and 30 degrees Celsius and a pH around the neutral point. It is important to note that these factors do not significantly affect the sensitivity of the sensors, as the pollutant detection processes occur in the cathode zone, far from the electroactive microorganisms. [10]

In recent years, laboratory models of microbial fuel cell based biosensors have been developed to detect various environmental pollutants and factors, such as oil spills [11], dissolved oxygen [12, 13], heavy metals [14], chemical oxygen demand (COD) [15], sediment bulking [16], and others.

The research and design of new methods and strategies for constructing SMFCs that can be robust in various environments are also urgent tasks for the future development of SMFC-based biosensors. The development of inexpensive electrode materials that are resistant to fouling could be very important for future applications to meet the requirements for long-term (months/years) monitoring. [17] Improving analytical characteristics is always a key issue for SMFC-based biosensors. Additionally, field testing and scaling up are also important tasks for the practical application of SMFCs. Currently, most SMFC-based biosensors operate under laboratory conditions. However, in field applications, many parameters such as pH, temperature, oxygen concentrations, ionic strength, etc., affect their performance. Therefore, SMFC-based biosensors need to be further tested under real conditions, and field

evaluation is necessary before practical application. Moreover, scaling up is another major challenge for SMFCs. The development and operation of SMFCs with real-world application scale should also be considered in the future. [7]

Taking all this into account, we developed an SMFC-based biosensor entirely designed for practical application with the aim of subsequent commercialization. In the construction of the sensor, influencing parameters were taken into consideration and optimized so that the sensor can operate in the long term, work effectively in the field, have sufficiently high sensitivity to various heavy metals, and be made from inexpensive structural elements. Research has shown high efficiency both in laboratory conditions and during field tests.

2. Materials and methods

Construction of SMFC biosensor

The biosensor consists of a sediment microbial fuel cell composed of an anode and cathode part. The anode part represents a PVC pipe with a diameter of 50 mm and a length of 500 mm. The bottom of the pipe is closed with a PVC plug. The pipe is filled with riverside sediment. At the bottom of the pipe, there is an electrode made of stainless steel AISI 304 with an area of 240 cm², acting as the anode. In the upper part of the pipe, it is plugged with a perforated rubber cap with a clay layer, acting as a membrane between the anode and cathode zones. The cathode part represents a PVC power strip Ø50/50x87°, in which a bow-shaped electrode made of stainless steel AISI 304 with an area of 240 cm² is longitudinally arranged, acting as the cathode. The two structural elements are connected and form a SMFC-based biosensor.

The flow of the investigated water passes freely through the cathode zone. The two electrodes are connected via a copper wire and are connected to a digital voltmeter with individual power supply. In the electrical circuit, a potentiometer with a maximum resistance of 20 KΩ is also installed. (Fig. 2) The voltmeter measures the change in voltage generated by the biosensor when the investigated water passes through it.

Figure 1 shows a schematic diagram of the SMFC-based biosensor.

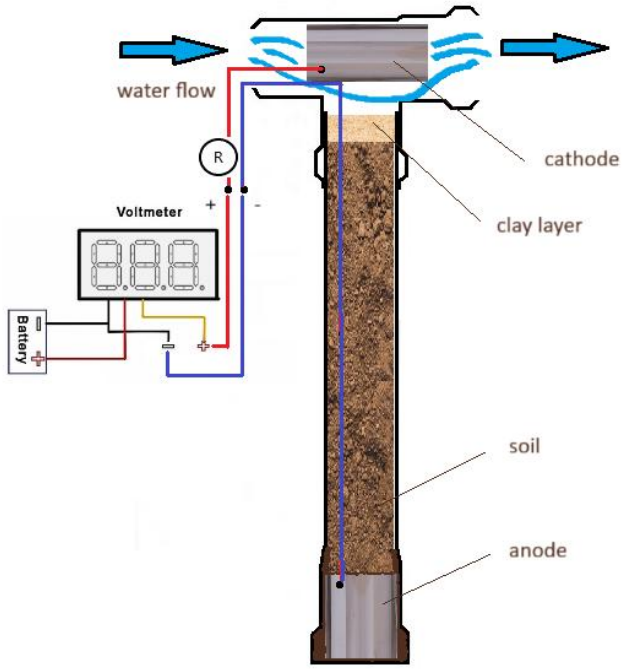

Fig. 1 *The schematic diagram of SMFC based biosensor*

All electronic components are assembled in a control unit with a user-friendly panel.

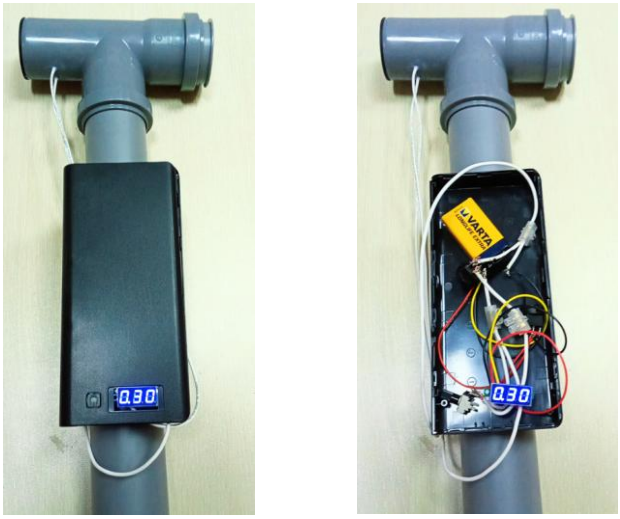

Fig. 2 *Photo of SMFC based biosensor*

Soil sampling

For the purpose of the study, riverside soil 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. The soil is characterized as alluvial-meadow with a high humus content of 6%. The soil has been classified as clay according to the Rutkowski method. 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 follow pH 6.42, EC 288.89 µS cm^{-1}. The Kjeldahl-N content were 0.59% and dissolved organic carbon 74.28 mg kg^{-1}. The amount of total nitrogen measured was 986.5 mg kg^{-1}, phosphorus – 20.2 mg kg^{-1}, potassium – 180.0 mg kg^{-1}. The abundance of exoelectrogenic microorganisms was quantified by 16S rRNA gene. In the sediment *Geobacter* and *Clostridium*, were the most abundant. *Anaeromyxobacter*, *Bacillus*, *Pseudomonas* and *Desulfolobus* were also detected. [18] All these indicators provide sufficient nutrients and substrate for degradation by exoelectrogenic microorganisms in the anode zone. This would ensure long-term and stable operation of the sensor.The soil thus prepared was then used to fill anode area of SMFC based biosensor. (Fig. 1).

Heavy metals pollution

For the purposes of the study, synthetic solutions containing Ni^{2+} ($NiSO_4.7H_2O$), Pb^{2+} ($PbCl_2$), Cu^{2+} ($CuSO_4.5H_2O$), Mn^{2+} $MnSO_4.H_2O$ and Zn^{2+} ($ZnSO_4.7H_2O$) with different concentrations were prepared. Heavy metals solutions were prepared in four concentrations – 5mg/l, 20 mg/l, 50 mg/l and 100 mg/l. Through the biosensor, solutions of individual heavy metals with increasing concentrations were sequentially passed. To determine the response of voltage signals to heavy metals ions shock, the biosensors` voltage was measured and recorded with Vernier LabQuest Mini and Logger Lite 1.9.4 software (Fig. 3)

Polarisation and power curves of the SMFC based biosensor were obtained by varying external resistances sequentially from 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 KΩ using MCP lab electronics BXR-04 ResistorBox. (Fig. 3)

Fig. 3 *Photo of investigation of the sensitivity of the biosensor to heavy metals*

3. Results and discussion

The conducted studies show that after filling anode area of the biosensor with riverside sediment, an increase in the open circuit voltage is observed. Figure 4 shows the increase in voltage over time until reaching a stable maximum open-circuit voltage of the biosensor.

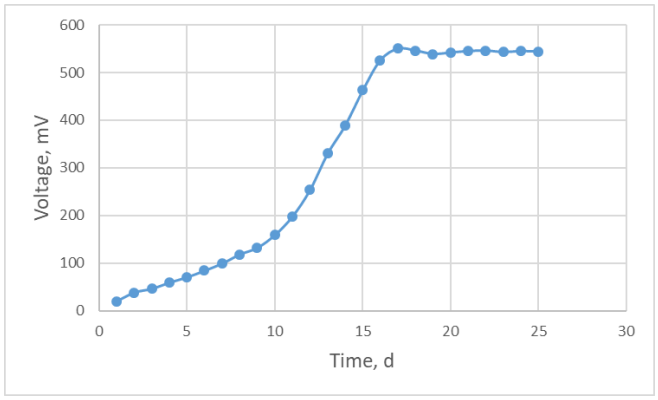

Fig. 4 *Reaching the optimal open-circuit voltage of the biosensor*

The graph shows that after filling the anode zone with river sediment, there begins a slow increase in the open-circuit voltage. The voltage gradually rises from 29 to 168 mV over a period of 10 days. This is followed by six days of significant voltage increase from 168 to 550 mV, after which the voltage remains stable within the range of 541-547 mV.

After stabilizing the parameters of the SMFC-based biosensor, its main electrical characteristics were measured. Figure 5 presents the polarization curve of the biosensor.

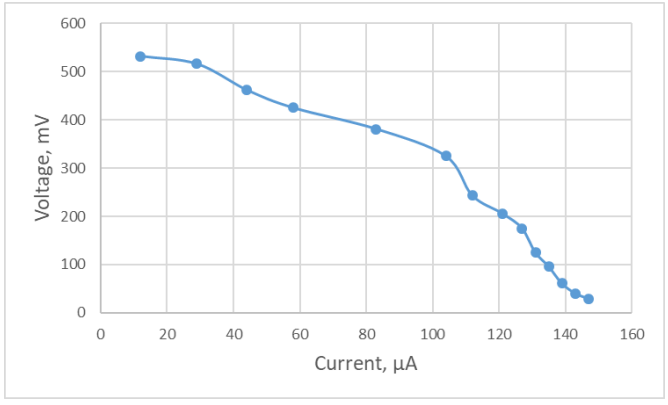

Fig. 5 *Polarization curve of biosensor*

The polarization curve was obtained through changing the resistance from 14 kΩ to 1 kΩ in steps of 1 kΩ. A gradual decrease in voltage from 535 mV to 29 mV was observed as the resistance decreased and the current increased, reaching up to 149 microamperes.

The polarization curve of the biosensor was used to construct the dynamics of the power density of the biosensor, which is presented in Figure 6.

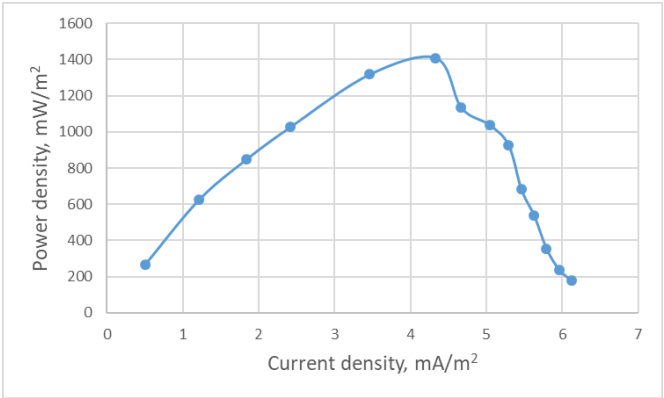

Fig. 6 *Power density dynamics of biosensor*

From the presented graph, it can be seen that the maximum power density of the constructed SMFC-based biosensor reaches 1408.33 mW. This power density is achieved at a current density of 4.33 mA/m² and a voltage of 325 mV. The applied resistance at the maximum power density is 9 KΩ.

The measurement of the main electrical parameters served for the optimal configuration of the biosensor with the aim of maximizing its sensitivity to the studied pollutants. In order to determine the sensitivity of the biosensor to contamination with various heavy metals, it was connected to computer software that recorded the real-time change in the sensor's voltage. Figure 7 presents graphs showing the change in the biosensor's voltage in response to different concentrations of heavy metals – Ni, Zn, Pb, Mn, and Cu.

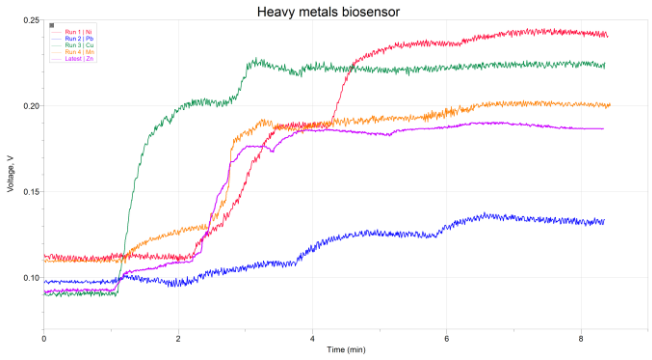

Fig. 7 *Dynamics of biosensor`s voltage to different concentrations of heavy metals*

The presented graph shows the change in voltage with respect to different concentrations of metal ions: nickel, lead, copper, manganese, and zinc. The data indicates that the biosensor exhibits the highest sensitivity towards copper ions. At just 5 mg/L of copper, the sensor's voltage increases by 120 mV within seconds, after which the voltage stabilizes at 206 ± 2 mV. Introducing a solution with a copper concentration of 20 mg/L leads to an increase in voltage by 25 mV, while introducing a solution with a copper concentration of 50 mg/L results in a voltage increase of 10 mV. Introducing a copper solution with a concentration of 100 mg/L does not cause any change in voltage, indicating that this concentration exceeds the sensitivity range of the biosensor for the examined concentrations.

The biosensor exhibits its lowest sensitivity towards lead ions. A concentration of lead ions of 5 mg/L results in a voltage increase of 5 mV, while a concentration of 20 mg/L leads to a voltage

increase of 10 mV. The sensor demonstrates higher sensitivity at concentrations of 50 mg/L and 100 mg/L, leading to voltage increases of 22 mV and 14 mV respectively.

For the remaining metals, similar voltage graphs are observed. For all three metals, introducing a solution with ion concentrations of 100 mg/L results in minor voltage increases, which can often be attributed to various environmental factors. Introducing a solution with a concentration of 5 mg/L does not result in a change in voltage for nickel, unlike zinc and manganese, where voltage increases of 12 mV and 21 mV are recorded, respectively. Introducing solutions with a concentration of 20 mg/L leads to the most significant voltage increases for all three metals: Ni – 77 mV, Zn – 66 mV, and Mn – 62 mV. Introducing solutions with a concentration of 50 mg/L does not result in a change in sensor voltage for manganese. For zinc, there is a voltage increase of 10 mV, while for nickel it is the most substantial – 45 mV.

4. Conclusion

Limiting environmental pollution with heavy metals inevitably involves developing and implementing cost-effective and sustainable monitoring methods and early warning systems. SMFC-based biosensors may be one of the most suitable alternatives, but numerous studies on their sensitivity, construction, and influencing factors are needed to ensure they can provide effective long-term solutions. The constructed and researched SMFC-based biosensor demonstrated good sensitivity to the studied metal pollutants. The highest sensitivity was found in detecting copper ions, with a significant increase in sensor voltage of 120 mV at copper concentrations of 5 mg/L. The lowest sensitivity of the biosensor was observed for lead ions. Based on the obtained results, we can conclude that the sensor would be most effective in monitoring recurring contamination with a known metal, provided that the electrical parameters (voltage, current, resistance) are optimally adjusted beforehand. This can be done quickly and easily through the control panel of the device, allowing its use both in laboratory conditions and on the field. The construction of the biosensor, consisting of a PVC tube, enables its integration into technological installations for continuous monitoring and early warning. For the widespread application of the sensor, additional research will be necessary regarding its long-term operation and the influence of environmental factors on its effectiveness.

Acknowledgements

This work was funded by Ecodiscover ltd. through the America for Bulgaria Foundation

5. References

1. O. C. Turker, A. Yakar, Sediment-based microbial fuel cell sensor for detecting boron mine effluent pollution in aquatic habitats, Microchemical Journal 218 (2025) 115355
2. T. Kumar, S. Naik, S. E. Jujjavarappu, A critical review on early-warning electrochemical system on microbial fuel cell-based biosensor for on-site water quality monitoring, Chemosphere 291 (2022) 133098
3. M. H. Do, H. H. Ngo, W. Guo, S. W. Chang, D. D. Nguyen, Y. Liu, S. Varjani, M. Kumar, Microbial fuel cell-based biosensor for online monitoring wastewater quality: A critical review, Science of the Total Environment 712 (2020) 135612
4. F. Ejeian, P. Etedali, H-A Mansouri-Tehrani, A. Soozanipour, Z-X. Low, M. Asadnia, A. Tahri-Kafrani, A. Razmjou, Biosensors for wastewater monitoring: A review, Biosensors and Bioelectronics 118 (2018) 66-79
5. L. Liu, Y. Lu, W. Zhong, L. Meng, H. Deng, On-line monitoring of repeated copper pollutions using sediment microbial fuel cell based sensors in the field environment, Science of the Total Environment 748 (2020) 141544
6. J. Dziegielowsky, M. Mascia, B. Metcalf, M. Di Lorenzo, Voltage evolution and electrochemical behaviour of Soil microbial fuel cells operated in different quality soils, Sustainable Energy Technologies and Assessments 56 (2023) 103071
7. S. Z. Abbas, J-Y. Wang, H. Wang, J-X. Wang, Y-T. Wang, Y. Yong, Recent advances in soil microbial fuel cells based self-powered biosensor, Chemosphere 303 (2022) 135036
8. S. Zhao, P. Liu, Y. Niu, Z. Chen, A. Khan, P. Zhang, X. Li, A Novel Early Warning System Based on a Sediment Microbial Fuel Cell for In Situ and Real Time Hexavalent Chromium Detection in Industrial Wastewater, Sensors 2018, *18*(2), 642
9. Ch. Li, X. Li, T. Li, Y. Su, W. Zhong, Ch. Han, Y. Jiang, H. Deng, Sediment microbial fuel cell monitoring repeated Cr(VI) shocks in different wetlands for a year: Performance and mechanism, Journal of Cleaner Production 452 (2024) 142147
10. Y. Hubenova, I. Bardarov, E. Hubenova, E. Slavcheva, Sediment microbial fuel cells capable of powering outdoor environmental monitoring sensors, Sensing and Bio-Sensing Research 46 (2024) 100695
11. Z. Dai, R. Yu, X, Zha, Z. Xu, G. Zhu, X. Lu, On-line monitoring of minor oil spills in natural waters using sediment microbial fuel cell sensors equipped with vertical floating cathodes, Science of the Total Environment 782 (2021) 146549
12. L. G. Olias, A. R. Otero, P. J. Cameron, M. Di Lorenzo, A soil microbial fuel cell-based biosensor for dissolved oxygen monitoring in water, Electrochimica Acta 362 (2020) 137108
13. N. Song, Z. Yan, H. Xu, Z. Yao, C. Wang, M. Chen, Z. Zhao, Z. Peng, C. Wang, H. Jiang, Development of a sediment microbial fuel cell-based biosensor for simultaneous online monitoring of dissolved oxygen concentrations along various depths in lake water, Science of the Total Environment 673 (2019) 272–280
14. R. Ivanov, P. Genova, P. Velichkova, Influence of environmental factors of long-term operation and effectiveness of SMFC-based biosensor for heavy metal polluted waters, International Scientific Journal "INDUSTRY 4.0", Vol.8 (2023) Issue 5, 182-185
15. R. Lu, Y. Chen, J. Wu, D. Chen, Z. Wu, E. Xiao, In situ COD monitoring with use of a hybrid of constructed wetland-microbial fuel cell, Water Research 210 (2022) 117957
16. C. Wang, H. Jiang, Real-time monitoring of sediment bulking through a multi-anode sediment microbial fuel cell as reliable biosensor, Science of the Total Environment 697 (2019) 134009
17. H. Wang, Y. Ye, J. Zhang, H. Ning, Y. Xiang, X. Song, W. Zhao, F. Guo, Power performance improvement in sediment microbial fuel cells: recent advances and future challenges, Int. J. Hydrog. Energy 48 (2023) 24426–24446
18. R. Ivanov, P. Genova, Sediment microbial fuel cell based biosensor for real time detection of Cu^{2+} in industrial wastewaters – operation and effectiveness, SEPRM 4 (2023) 43-45