



**MINING AND GEOLOGY UNIVERSITY “ST. IVAN RILSKI”,
SOFIA**

**Faculty of Geology and Exploration
Department of Engineering Geoecology**

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**TREATMENT OF MINE WASTEWATER USING MICROBIAL ELECTROLYSIS
CELLS**

A B S T R A C T

of a dissertation submitted for the award of the
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**Supervisor:
Assoc. Prof. Dr. Anatoliy Angelov**

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The dissertation materials are available for review in the office of the Student and Academic Mobility Sector, MU "St. Ivan Rilski", Rectorate, 3rd floor, room No. 79, phone: +359 2 8060 209.

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I. GENERAL CHARACTERISTICS OF THE DISSERTATION

Relevance of the Research Problem

The mining industry is among the major industrial sources of wastewater characterized by high concentrations of sulfates, dissolved heavy metals, and low pH values. Their discharge into the environment leads to contamination of water bodies and soils, disruption of ecological balance, and poses risks to human health. Therefore, the development of effective and sustainable technologies for their treatment is of significant importance for the modern mining industry.

Conventional wastewater treatment methods are effective; however, they are associated with high operating costs and limited opportunities for resource recovery. Consequently, there is growing interest in innovative technologies that combine wastewater treatment with the recovery of valuable products.

In recent years, bioelectrochemical systems, and particularly microbial electrolysis cells (MECs), have been considered a promising technology for the treatment of mining wastewater. These systems provide conditions for the simultaneous removal of sulfates and heavy metals while improving treatment efficiency through the process of dissimilatory microbial sulfate reduction occurring in the anode compartment.

Aim of the Dissertation, Main Objectives, and Research Methods

The main objective of the dissertation is to investigate and evaluate the potential of various bioelectrochemical system configurations - microbial fuel cells (MFCs), microbial electrolysis cells (MECs), and microbial desalination cells (MDCs) - based on the process of dissimilatory microbial sulfate reduction in the anode compartment, for the effective treatment of model solutions and wastewater containing high concentrations of sulfate and copper ions. The study aims to achieve an optimal sulfate reduction rate and high sulfate and copper removal efficiencies, as well as to utilize waste organic substrates from the distillery industry as a sustainable source of electrons.

To achieve this objective, the following tasks were defined:

1. To design and configure laboratory-scale installations incorporating bioelectrochemical systems operating in both batch and continuous modes, including microbial fuel cells (MFCs), microbial electrolysis cells (MECs), and microbial desalination cells (MDCs).
2. To investigate the influence of design parameters, including the number of chambers, electrode surface area, and separator area, on the functional characteristics of bioelectrochemical systems.
3. To perform a comparative assessment of a two-chamber microbial fuel cell and a microbial electrolysis cell based on microbial sulfate reduction with respect to the treatment efficiency of model acid mine drainage solutions.
4. To evaluate the performance of a three-chamber microbial desalination fuel cell (MDFC) in terms of sulfate and heavy metal removal efficiency.
5. To determine the effect of the initial sulfate concentration in treated acid mine drainage on sulfate removal efficiency in the cathode and anode compartments of an MFC.

6. To assess the applicability of waste organic substrates - distillery stillage and vinasse - as electron donors for the process of microbial sulfate reduction in the anode compartment of microbial fuel cells (MFCs).

7. To analyze the performance of bioelectrochemical systems (BESs) in the utilization of waste organic substrates generated by the distillery industry.

To accomplish these objectives, various electrochemical, physicochemical, and analytical methods were employed. Laboratory experiments were conducted using microbial fuel cells and microbial electrolysis cells under different operating conditions. Changes in sulfate, sulfide, COD, and metal ion concentrations, as well as the main electrochemical parameters of the systems, were monitored. System performance was evaluated using indicators such as pollutant removal efficiency, sulfate reduction rate, current density, power density, Coulombic efficiency, and energy efficiency.

Scientific Novelty

The scientific novelty of the dissertation lies in expanding the knowledge regarding the application of bioelectrochemical systems based on the process of dissimilatory microbial sulfate reduction for the treatment of mining wastewater. The effects of various design and operational parameters on sulfate reduction, heavy metal removal, and electrochemical activity generation were investigated.

New experimental data were obtained concerning the influence of sulfate concentration, membrane type, operating mode, and organic substrate on the technological and electrochemical performance of bioelectrochemical systems. The relationships between dissimilatory microbial sulfate reduction, electron transfer processes, and metal ion removal mechanisms were elucidated.

Regularities governing the operation of microbial fuel cells and microbial electrolysis cells fed with different carbon sources and treating sulfate- and metal-containing media were identified. The obtained results contribute to a better understanding of the mechanisms determining the efficiency of bioelectrochemical systems in wastewater treatment and provide a basis for their further optimization and practical application.

Practical Significance

The results obtained in this dissertation broaden the possibilities for applying bioelectrochemical systems to the treatment of wastewater containing sulfates and heavy metals. The investigated technological solutions can be used in the development of energy-efficient methods for mining wastewater treatment, while simultaneously reducing the concentrations of major pollutants and enabling the recovery of valuable components.

The experimental data obtained may serve as a basis for the design, optimization, and scale-up of bioelectrochemical systems operating on the principle of microbial sulfate reduction. The established relationships between system design parameters, the organic substrates used, and process efficiency provide a foundation for selecting appropriate operating conditions for practical implementation of the technology.

The results of this research can be applied both in scientific investigations aimed at advancing bioelectrochemical technologies and in engineering practice for the development of innovative solutions for the treatment of mining wastewater and other industrial effluents containing sulfates and metal ions.

Dissemination of Results

The main results of the dissertation have been disseminated through publications in scientific journals and presentations at national scientific forums. They have also been presented and discussed at scientific conferences in the fields of mining engineering, environmental engineering, biotechnology, and wastewater treatment technologies.

Publications

The principal achievements and results of the dissertation have been published in five scientific papers, one of which is a single-author publication. Detailed information on the publications is provided in the section “LIST OF PUBLICATIONS RELATED TO THE DISSERTATION”.

Structure and Scope of the Dissertation

The dissertation comprises 145 pages and includes an introduction, eight chapters addressing the defined research objectives, a list of the main contributions, a list of publications related to the dissertation, and a bibliography. A total of 162 literature sources are cited, all of which are published in Latin script. The dissertation contains 45 figures and 22 tables. The numbering of figures and tables in the abstract corresponds to that used in the dissertation.

II. CONTENT OF THE DISSERTATION

CHAPTER 1. INTRODUCTION

Worldwide, enormous financial resources are spent each year on wastewater treatment, while these processes consume significant amounts of energy. In addition, global energy demand continues to increase substantially, leading to growing energy shortages and adverse impacts on the environment. The diversification of energy sources has been a major global concern over the past two decades. At present, the planet is facing a critical stage in terms of environmental degradation, making it essential to implement adequate corrective measures. Increasing public awareness and commitment to energy security are further stimulating the search for alternative solutions.

Bioelectrochemical systems, such as microbial fuel cells (MFCs) and microbial electrolysis cells (MECs), enable the utilization of organic pollutants present in wastewater for the production of electricity, hydrogen, and other value-added products, while simultaneously achieving wastewater treatment. In these systems, microorganisms catalyze oxidation and reduction processes through electron transfer between the anode and the cathode.

MFCs and MECs have demonstrated high efficiency in the degradation of organic matter in wastewater and offer significant opportunities for resource recovery, making them promising technologies for sustainable wastewater management.

CHAPTER 2. AIM OF THE DISSERTATION AND MAIN OBJECTIVES

The main objective of the dissertation is to investigate and evaluate the potential of various bioelectrochemical system configurations - microbial fuel cells (MFCs), microbial electrolysis cells (MECs), and microbial desalination cells (MDCs) - based on the process of dissimilatory microbial sulfate reduction in the anode compartment, for the effective treatment of model solutions and wastewater containing high concentrations of sulfate and copper ions. The study aims to achieve an optimal sulfate reduction rate and high sulfate and copper removal efficiencies, as well as to utilize waste organic substrates from the distillery industry as a sustainable source of electrons.

To achieve this objective, the following tasks were defined:

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7. To analyze the performance of bioelectrochemical systems (BESs) in the utilization of waste organic substrates generated by the distillery industry.

CHAPTER 3. MATERIALS AND METHODS

3.1 Design of Laboratory Installations

3.1.1 Design of Laboratory-Scale Bioelectrochemical Systems for Investigating the Influence of Structural and Operational Parameters

For the implementation of the experimental study, three different laboratory-scale installations were designed, constructed, and configured, as presented in Figures 23, 25, and 27 and designated as Laboratory Installation - Variant 1, Variant 2, and Variant 3, respectively. The purpose of employing several experimental configurations was to evaluate the influence of structural and functional parameters on the performance and efficiency of the bioelectrochemical system.

The main structural difference between Variants 1 and 2 lies in the geometry and configuration of the bioelectrochemical system (BES). In both cases, the system was designed in a “sandwich” configuration, where the anode and cathode compartments were separated by an ion-selective membrane. Variant 1 employed a cylindrical bioelectrochemical cell, providing uniform electric field distribution and symmetrical conditions for the electrochemical processes. In contrast, Variant 2 was designed with a rectangular geometry, allowing easier component assembly, better control of the active electrode surface area, and greater flexibility for system scale-up.

Despite the differences in design, identical materials were used for the main functional components of both experimental configurations. Graphite electrodes were selected as the electrode material due to their high electrical conductivity, chemical stability, and biocompatibility. Separation of the anode and cathode compartments was achieved using an AMI 7001 anion-exchange membrane, which ensured selective anion transport and minimized electrolyte mixing, thereby contributing to the stability and efficiency of the bioelectrochemical system.

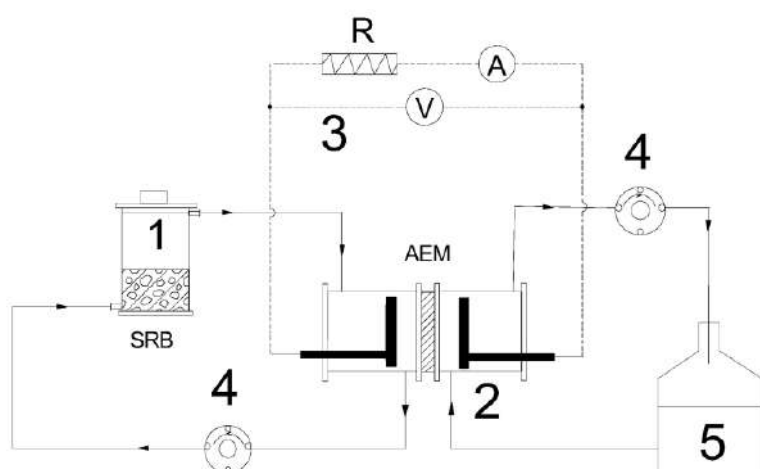


Figure 23. Schematic diagram of the laboratory installation - Variant 1: (1) SRB bioreactor; (2) BES operating as a microbial fuel cell (MFC); (3) external electrical circuit (load circuit) of the BES; (4) recirculation pump for the anode and cathode compartments of the BES; (5) buffer tank in the cathode compartment of the BES.

In Variant 2, the electrode surface area was optimized and was approximately one order of magnitude greater than that of Variant 1 (0.02 m^2 and 0.002 m^2 , respectively). Another significant difference was observed in the surface area of the anion-exchange membranes: 0.007 m^2 in Variant 1 and 0.01 m^2 in Variant 2.

Both laboratory installations incorporated identical sulfidogenic bioreactors, in which approximately half of the total reactor volume (0.7 dm^3) was filled with 0.3 kg of modified zeolite. The bioreactors were connected in series with the anode compartments of the cells. The modified zeolite served as a support material for the biofilm of sulfate-reducing bacteria (SRB) and other metabolically associated microbial communities.



Figure 24. Photograph of the microbial fuel cell used in Variant 1.

Figure 24 shows a photograph of the BES operating as a microbial fuel cell (MFC) in Variant 1. All vessels used in the laboratory installation, including the bioelectrochemical system itself, were manufactured from plexiglass and polypropylene. A buffer tank with a volume of 750 dm^3 was installed in the cathode recirculation loop to facilitate sampling and online process monitoring. The cathode compartment of the BES was filled with a solution containing high concentrations of sulfate and copper ions.

In the second configuration, the sandwich-type BES (Figures 25 and 26) consisted of a microbial fuel cell arrangement in which the anode, ion-selective separator, and cathode were positioned in close succession. The primary objective of this design was to significantly reduce internal electrical resistance and improve ion and mass transfer, resulting in higher current densities and more stable electrochemical performance compared to conventional two-chamber systems.

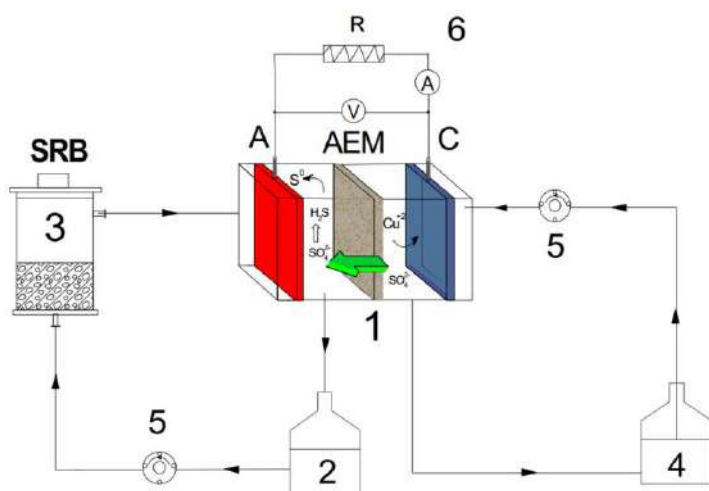


Figure 25. Schematic diagram of the laboratory installation - Variant 2: (1) BES; (2) buffer reservoir containing the nutrient medium for sulfate-reducing bacteria (SRB); (3) sulfate-reducing bacteria bioreactor; (4) buffer reservoir containing CuSO_4 solution; (5) recirculation pumps; (6) external electrical circuit (load circuit) of the BES.



Figure 26. Photograph of the sandwich-type bioelectrochemical system used in Variant 2.

In the third laboratory-scale configuration (Figures 27 and 28), a three-chamber bioelectrochemical system was constructed to operate as a microbial desalination cell (MDC).

Three-chamber microbial desalination cells represent a specialized class of bioelectrochemical systems designed for the simultaneous treatment of organically loaded wastewater and the reduction of salinity in aqueous solutions. A typical MDC configuration consists of an anode chamber and a cathode chamber separated by an intermediate desalination chamber. The three compartments are divided by a cation-exchange membrane and an anion-exchange membrane, which ensure selective ion transport and prevent the direct mixing of electrolytes.

The electric field generated between the anode and cathode serves as the driving force for the directed migration of dissolved ions from the desalination chamber. Under its influence, cations migrate through the cation-exchange membrane toward the cathode compartment, while anions migrate through the anion-exchange membrane toward the anode compartment. As a result, the concentration of dissolved salts in the intermediate chamber gradually decreases, leading to effective desalination of the treated water without the need for an external energy supply.

Microbial desalination cells integrate three major processes within a single system: biological wastewater treatment, electricity generation, and desalination.

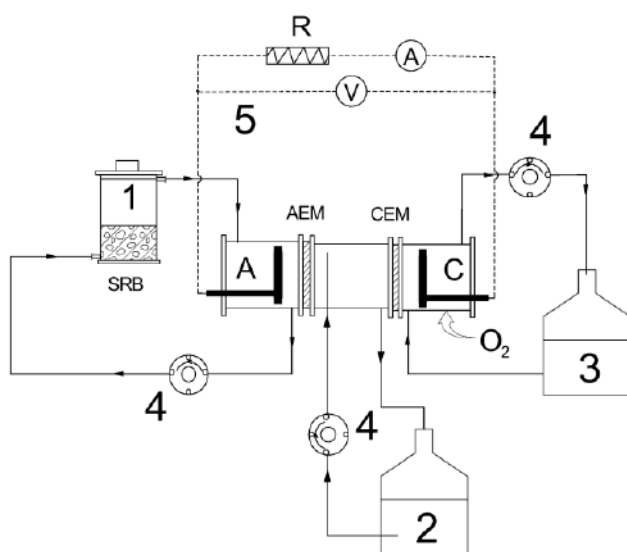


Figure 27. Schematic diagram of the laboratory installation of a three-chamber bioelectrochemical system operating as a microbial desalination cell (MDC): (1) bioreactor for microbial sulfate reduction; (2) buffer tank for the desalinated solution; (3) catholyte buffer tank; (4) recirculation peristaltic pumps; (5) external electrical circuit (load circuit) of the BES.



Figure 28. Photograph of the laboratory-scale three-chamber bioelectrochemical system operating as a microbial desalination cell (MDC).

The constructed bioelectrochemical system (Figures 27 and 28) was similar to the two-chamber system used in Variant 1; however, unlike that configuration, it included an additional intermediate chamber positioned between the anode and cathode compartments. The volume of all three chambers was identical (300 mL each), ensuring comparable hydraulic conditions and facilitating the analysis of the influence of design parameters on system performance. The chambers were separated by two membranes: a cation-exchange membrane (CEM, CMI 7001) and an anion-exchange membrane (AEM, AMI 7001S). These membranes provided selective ion transport while preventing the direct mixing of electrolytes between adjacent compartments.

One of the key design parameters of a bioelectrochemical system is the distance between the electrodes, as it directly affects internal ohmic resistance, voltage losses, and the overall energy efficiency of the process. In the two-chamber configurations, this distance was 30 mm, whereas in Variant 3 it was increased to 100 mm due to the inclusion of the additional intermediate (desalination) chamber. The distance between the membranes and the corresponding electrodes was maintained at 10 mm in all three configurations to minimize the influence of this parameter and ensure better comparability of the experimental results. This spacing was selected to prevent direct contact between the membranes, which may occur during prolonged operation as a result of membrane deformation in the direction of charge-carrying ion transport (Ramirez-Nava et al., 2021).

3.1.2 Design of a Laboratory-Scale Microbial Fuel Cell with a Gas-Diffusion Air Cathode for Investigating Waste Organic Substrates (Distillery Stillage and Vinasse) as Electron Donors for Microbial Sulfate Reduction

A laboratory-scale installation incorporating a fundamentally different bioelectrochemical system design from the previous three configurations was also investigated. In this configuration, an air cathode was employed.

Bioelectrochemical systems (BESs), including microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) equipped with air cathodes, are devices in which the cathodic reaction occurs through the direct reduction of oxygen from atmospheric air. This approach eliminates the need for liquid catholytes and energy-intensive aeration, thereby simplifying system design, reducing operational costs, and improving overall energy efficiency.

An air cathode typically consists of a multilayer electrode comprising a conductive carbon support, a catalytic layer, and a hydrophobic gas-diffusion layer. The kinetics of oxygen reduction under neutral pH conditions remains a limiting factor; therefore, both noble-metal and platinum-free catalysts are commonly employed in air-cathode designs. Their use aims not only to improve cathode potential and

current density but also to enhance the long-term stability, durability, and economic feasibility of the cathode.

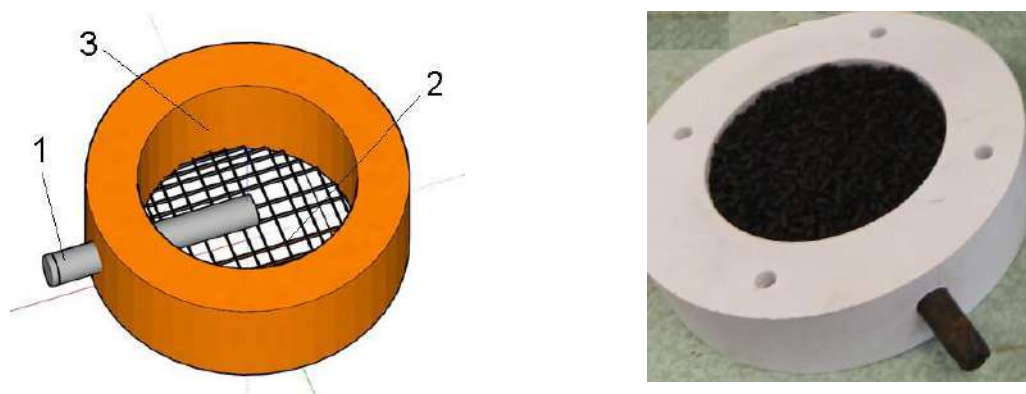


Figure 29. Design of the gas-diffusion air cathode: (1) graphite electrode with a diameter of 8 mm; (2) polypropylene mesh retaining the granular activated carbon within the cathode chamber; (3) cylindrical compartment (60 × 24 mm) containing activated carbon in contact with atmospheric oxygen, the graphite electrode, and the cation-exchange membrane.

In microbial fuel cells equipped with an air cathode, the system operates in a self-sustaining mode, in which the oxidation of the organic substrate in the anode compartment is directly coupled with electricity generation through an external electrical circuit. In this way, the chemical energy stored in organic waste compounds is directly converted into electrical energy without the need for an external energy input.

In microbial electrolysis cells, the air cathode is used less frequently; however, it may contribute to reducing the overall energy demand when an external voltage is applied.

For the present experimental study, air-cathode-based bioelectrochemical systems were constructed as cylindrical reactors fabricated by 3D printing from ABS material, with a geometric volume of 500 cm³ (Figures 29 and 30).

In the microbial fuel cell, a graphite rod with a diameter of 8 mm and a length of 100 mm was used as the anode, providing an active surface area of 0.030 m². Graphite was selected as the anode material because of its high electrical conductivity, chemical stability, and excellent biocompatibility, which favor the stable development of an electroactive microbial biofilm.

A cation-exchange membrane (CEM) with a diameter of 56 mm and an effective surface area of 0.0028 m² was used as a separator between the anode and cathode compartments. The membrane ensured selective cation transport while minimizing undesirable electrolyte mixing.

The cathode compartment was designed as a porous air cathode composed of granular activated carbon with a particle size of 2-4 mm and a layer thickness of 24 mm, providing a large active surface area and favorable conditions for the oxygen reduction reaction. A graphite rod with a diameter of 8 mm was positioned within the cathode chamber, with 40 mm of its length in direct contact with the activated carbon, ensuring efficient electron transfer from the catalytic layer to the external electrical circuit.

The gas-diffusion air cathode was constructed according to the design presented in Figures 29 and 30. The cathode housing was fabricated from 3D-printed ABS material, with dimensions of 60 × 24 mm and a volume of 0.068 dm³. One side of the cylindrical compartment was covered with a polyethylene mesh, while the internal volume was filled with granular activated carbon forming a porous catalytic layer.

A graphite rod with a diameter of 8 mm was installed in the side section of the cathode compartment, providing electrical contact with the activated carbon and facilitating efficient electron transport to the external electrical circuit. On the opposite side of the cylindrical compartment, a cation-exchange membrane (CMI 7001) was attached to both the anode and cathode housings and maintained in direct contact with the activated carbon filling the cathode chamber.

To ensure the supply of molecular oxygen to the reaction surface, side openings with a diameter of 2 mm were incorporated into the polyethylene mesh section, allowing atmospheric air to diffuse toward the activated carbon.

According to the laboratory installation scheme (Figure 31), the substrate (1) is supplied to the system by means of a peristaltic pump (2), which provides a precise and stable flow rate. The incoming solution first passes through a pre-treatment reactor (3), where homogenization of the composition and equalization of the concentrations of the main components are achieved. This minimizes fluctuations in the influent characteristics and provides stable and controlled operating conditions for the microbial fuel cell.

Subsequently, the flow enters a pH control reactor (4) equipped with a dosing system (5) for the automated addition of reagents for pH adjustment.

The preconditioned solution is then fed into the anode chamber of the microbial fuel cell (6), where microbial sulfate reduction takes place under strictly anaerobic conditions, accompanied by the oxidation of the organic substrate. As a result of these processes, electrons are generated, transferred to the anode, and subsequently transported through the external electrical circuit (7) to the cathode.

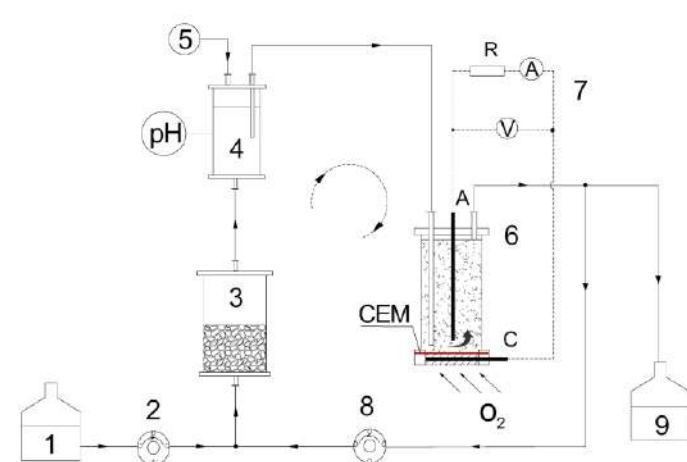


Figure 31. Schematic diagram of the laboratory installation based on a bioelectrochemical system with an air cathode for investigating different waste organic substrates (distillery stillage and vinasse) as electron donors in the microbial sulfate reduction (MSR) process: (1) feed solution; (2) dosing pump; (3) bioreactor for microbial sulfate reduction; (4) buffer tank for pH adjustment; (5) 1 N NaOH solution; (6) microbial fuel cell with an air cathode and a cation-exchange membrane (CEM); (7)

external electrical circuit (load circuit) of the BES; (8) recirculation pump; (9) effluent solution.

For the purposes of the experiment, three identical laboratory installations (Figure 31), each equipped with a separate microbial fuel cell, were constructed. The design of the installations included an anaerobic fixed-bed bioreactor for microbial sulfate reduction (MSR) (3), a microbial fuel cell (MFC) with an air cathode (6), and a buffer tank (4) for pH adjustment.

The geometric volume of the fixed-bed anaerobic bioreactor (3) was 0.5 dm³, of which 0.3 dm³ constituted the liquid phase. Natural zeolite (clinoptilolite) with a particle size ranging from 2.5 to 5.0 mm was used as the biomass carrier, according to the specifications described in previous studies (Angelov et al., 2013; Bratkova et al., 2019). For the pretreatment of 350 g of zeolite, a solution containing NH₄Cl (10 g/L), K₂HPO₄ (5 g/L), and MgSO₄·7H₂O (4 g/L) was used. Recirculation of the liquid phase within the laboratory installation was achieved using a recirculation pump (8).

The volume of the buffer tank (4) was 0.4 dm³ and was selected to accommodate the periodic sampling required for chemical analyses. Consequently, the total liquid volume in each laboratory installation was approximately 1.2 dm³, including the volume of the anode chamber of the MFC.

Prior to operation, the anode compartment and the sulfidogenic bioreactor were filled with a modified Postgate B medium, with a total medium volume of 1.1 dm³. The microbial inoculum for all three experimental variants consisted of 50 mL of a mixed culture of sulfate-reducing bacteria (SRB). Mixing and homogenization within the anode compartment of the microbial fuel cell were achieved using a recirculation pump operating at a flow rate of 5 dm³/h.

3.1.3 Laboratory Installation for Comparing the Performance of MEC and MFC Systems in the Treatment of Synthetic Acid Mine Drainage

The experiments were conducted using a laboratory-scale bioelectrochemical system designed to operate in two functional modes - as a microbial fuel cell (MFC) and as a microbial electrolysis cell (MEC) (Figure 32). This flexibility enabled a comparative evaluation of the processes under self-sustaining conditions and under externally assisted operation.

For this purpose, a two-chamber BES configuration was employed, consisting of two cylindrical compartments, each with a geometric volume of 0.2 dm³, separated by a Fumasep FAM anion-exchange membrane (AEM). Prior to the experiments, the membrane was conditioned in a 0.5 M NaCl solution at 25 °C for 24 h. This treatment was intended to stabilize its ion-exchange properties and ensure reproducible operating performance.

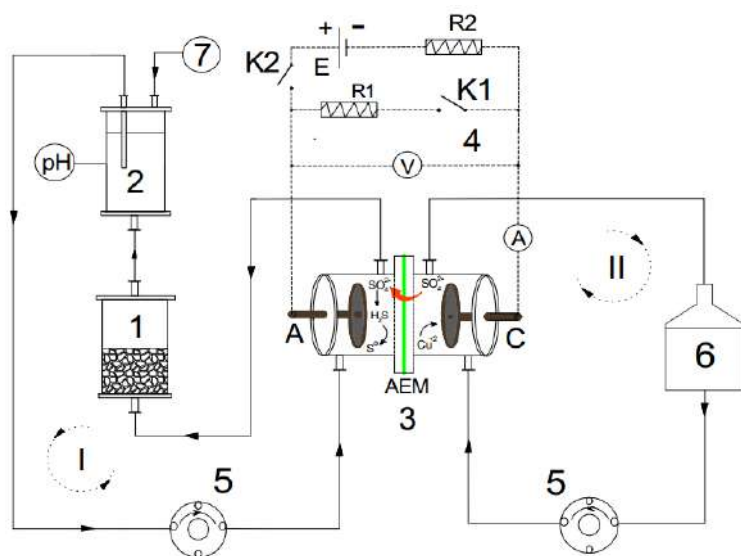


Figure 32. Schematic diagram of the laboratory setup for studying model SR systems: 1 - bioreactor for sulfate-reducing bacteria (SRB); 2 - buffer tank for pH adjustment; 3 - BES operating in two modes - microbial fuel cell (MFC) and microbial electrolysis cell (MEC), equipped with an anion-exchange membrane; 4 - external load circuit of the BES; 5 - recirculation pump for the anode and cathode compartments of the BES; 6 - buffer tank in the cathode compartment of the BES; 7 - 1 N NaOH solution.

As electrodes in the BES, graphite cylindrical bodies with a diameter of 45 mm and a thickness of 6 mm were used, connected via graphite rods with a diameter of 6 mm and a length of 48 mm. The total geometric surface area of each electrode was approximately 0.0038 m², and the distance between them was 15 mm, while the area of the anion-exchange membrane was 0.0028 m².

In the investigated BES (Fig. 32), a flexible electrical configuration was implemented, which through switching of the external circuit (switches K1 and K2) allows operation both in microbial fuel cell (MFC) mode and microbial electrolysis cell (MEC) mode. In this way, the same apparatus configuration can be used both for self-sustained electricity generation and for processes requiring external energy input.

In MEC operation mode, a classical external load resistor is not used, since the system operates under applied external voltage. In this case, the equivalent electrical resistance of the circuit is defined by the ratio between the applied voltage and the resulting current.

In the present study, the load resistances were selected as follows: $R_1 = 200\ \Omega$ for MFC mode and $R_2 = 10\ \Omega$ for MEC mode.

3.2 Operational modes of the laboratory installations

3.2.1 Operation of laboratory installations for studying the influence of BES design parameters

The three BES configurations were designed and operated in batch (cyclic) mode. In this mode, the process proceeds after a single filling of each system, without continuous inflow or outflow during the experiment.

Identical bioreactors were used in all three variants for the implementation of microbial sulfate reduction (MSR). The bioreactors were filled with modified Postgate B medium, with a volume of 400 mL in the sulfidogenic bioreactor and in the anode chambers of the BES - 300 mL for variants 1 and 3, and 100 mL for variant 2. Standardization of the biological and chemical media minimized the influence of external factors and ensured a reliable assessment of the impact of design and electrochemical parameters on system performance.

Inoculation in all three experimental variants was performed by adding 50 mL of a mixed culture of sulfate-reducing bacteria (SRB). The period required for the formation of an active SRB biofilm on the anode surface ranged between 30 and 40 days. After biofilm development, the experimental runs were initiated.

In all variants, the anolyte consisted of modified Postgate B medium for sulfate-reducing bacteria, containing 1 g/L MgSO_4 as a sulfate source and 3 g/L lactate as an organic electron donor and carbon source. The pH was adjusted to 7.5 using 1N NaOH to ensure optimal microbial activity.

The catholyte composition in variants 1 and 2 was identical and included: 1 g/L MgSO_4 , pH = 2.5-3.0, and CuSO_4 solution (Cu^{2+} - 1018 mg/L, SO_4^{2-} - 2.03 g/L, H_2SO_4 - 0.5 g/L). In variant 3, some differences in catholyte composition were applied depending on the specific experimental setup described in the Results and Discussion section. In variant 3, the middle desalination zone was pre-filled before the experiments with a CuSO_4 solution containing Cu^{2+} - 1018 mg/L, SO_4^{2-} - 2.03 g/L, and H_2SO_4 - 0.5 g/L.

3.2.2 Operation of MFC with a gas-diffusion air cathode for studying the utilization of waste organic substrates from the distillery industry

According to the laboratory setup configuration (Fig. 8), anaerobic fixed-bed reactors were pre-inoculated with sulfate-reducing bacteria (SRB), previously cultivated on a medium containing lactate as the sole carbon and energy source.

In the initial stage, microorganisms were cultivated in batch mode, where after reaching low residual sulfate concentrations ($< 0.3\ \text{g/L}$), approximately 70% of the liquid phase volume was replaced with fresh medium. The biofilm formation process on the saturated zeolites lasted 40 days until stable microbial activity was achieved.

After this period, the three bioreactors were operated independently and fed with three different organic substrates used as electron donors in the microbial sulfate reduction process, as follows:

- **First feeding solution:** lactate-based modified Postgate B medium containing 3.0 g/L Na-lactate, 0.25 g/L K_2HPO_4 , 0.5 g/L NH_4Cl , 2.0 g/L Na_2SO_4 , 0.1 g/L $CaCl_2$, 4.0 g/L $MgSO_4 \cdot 7H_2O$, 0.25 g/L yeast extract, pH 7.5. This variant served as a control.

- **Second feeding solution:** distillery stillage (pre-diluted with distilled water at a 1:1 ratio) with the same salt composition as the modified Postgate B medium described above, adjusted to pH 7.5.

- **Third feeding solution:** vinasse (pre-diluted with distilled water at a 1:1 ratio) with the same salt composition as the modified Postgate B medium, adjusted to pH 7.5.

The influence of sulfate volumetric loading rate on microbial process kinetics was investigated using seven hydraulic retention times: 21, 31, 60, 72, 86, 108, and 144 hours over a 3-month experimental period. After reaching steady-state conditions for each retention time, 2 - 4 samples were collected from the treated wastewater and analyzed for sulfate concentration, COD, and H_2S .

This approach enabled monitoring of microbial sulfate reduction dynamics and evaluation of process efficiency under different volumetric conditions, providing information on the influence of contact time and loading rate on biochemical transformation rates.

3.2.3 Operational modes of the installation for evaluating treatment efficiency of model wastewaters comparing MEC and MFC

In the laboratory setup shown in Fig. 32, cultivation of sulfate-reducing bacteria (SRB) in the MSR bioreactor was performed as described previously for the other laboratory systems.

The BES was operated in batch mode for 96-hour periods, investigating two modes - microbial fuel cell (MFC) and microbial electrolysis cell (MEC). In both cases, continuous recirculation of the liquid phase (5 L/h) was applied in both anode and cathode loops to ensure medium homogeneity.

In the anode compartment, heterotrophic microbial sulfate reduction was carried out, where distillery stillage served as an electron donor, and pH was maintained close to 7.5 by periodic adjustment. The cathode compartment was filled with model acid mine drainage.

The BES was operated in two modes - MFC and MEC. In MFC mode, the system operated with an external load resistor determined from polarization curves, while in MEC mode an external voltage of 0.7 V was applied from an external power supply to enhance electrochemical reactions and ion transport through the anion-exchange membrane.

3.3 Analytical and electrochemical methods

Electrical parameters of the microbial fuel cells were measured using a digital multimeter combined with a precision potentiometer (0-11 k Ω range) as the external load resistance.

pH, temperature (T), redox potential (Eh), and electrical conductivity (EC) were measured using a Regio multifunctional device with appropriate sensors and electrodes and integrated software for online monitoring.

Sulfide and sulfate concentrations were analyzed using Nanocolor test kits and a spectrophotometric $BaCl_2$ method.

Organic loading was evaluated by COD measurements according to APHA standard methods.

The composition of organic acids, alcohols, and sugars was determined by high-performance liquid chromatography (HPLC).

Energy characteristics were evaluated by constructing polarization curves and determining maximum power (Pmax), current (I), and open circuit voltage (OCV). In MEC mode, a stabilized adjustable DC power supply was used to apply an external voltage in the range of 0.6 - 0.8 V.

The rate of microbial sulfate reduction (MSR) was determined differently for batch and continuous modes due to differences in mass transfer mechanisms.

In batch mode with an anion-exchange membrane (AEM), sulfate migration from the cathode to the anode was taken into account when calculating MSR rates. Therefore, the initial sulfate concentration was defined as the sum of the initially measured concentration and the amount of sulfate ions migrated through the membrane over the corresponding period. The MSR rate was calculated as the ratio between the reduced sulfate amount and process time.

In continuous mode, the MSR rate was calculated as the difference between inlet and outlet sulfate concentrations divided by the process time.

The time required to reach steady-state current and power values varied depending on the applied external resistance and organic substrate. For the substrates used, it ranged from 10 s to 20 s at 11 kΩ and from 90 s to 120 s at 10 Ω. Power and current density calculations were based on the anode surface area of the fuel cell. For MEC operation, an external voltage from a stabilized power supply (HY30050) was applied.

3.4 Determination of BES energy efficiency

The evaluation of the energy efficiency of bioelectrochemical systems (BES) is essential for assessing their applicability in wastewater treatment and simultaneous energy production. It allows analysis of how effectively the system converts the chemical energy of organic compounds into electrical energy. Key indicators such as coulombic efficiency (CE), voltage efficiency (VE), and overall energy efficiency (EE) are used to provide a comprehensive understanding of the electrochemical behavior and energy performance of the system.

For CE determination, a modification of Equation 3 was used, according to Equation 5-

$$CE = \frac{M \cdot I \cdot t}{F \cdot b \cdot V_{an} \cdot \Delta COD} \times 100\% \quad (1),$$

Where: M = 32 is the molar mass of O₂; t (s) is the hydraulic retention time (HRT); I is the average current during the experiment (A); F (Faraday's constant) is 96,845 C/mol; b = 4 is the number of electrons required for the oxidation of 1 mol of O₂; ΔCOD is the difference between the initial and final COD values (gO₂/L); and V_{an} is the volume of the anode chamber.

Accordingly, the voltage efficiency (VE) was determined using Equation 6:

$$VE = \frac{U_{cell}}{E_{teor}} \cdot 100, \% \quad (2),$$

Where: U_{cell} is the average measured voltage of the MFC during the experiment (V), and E_{teor} is the theoretical electromotive force (EMF) of the cell (V).

Voltage efficiency indicates what fraction of the theoretical electromotive force of the cell is actually realized in the system.

The overall energy efficiency (EE) compares the actual electrical energy produced with the chemical energy of the removed organic matter (COD) and can be determined from:

$$EE = \frac{E_{elect}}{E_{chem}} \cdot 100 \quad (3),$$

Where: E_{elect} is the electrical energy, determined as:

$$E_{elect} = U_{avg} \cdot I_{avg} \cdot t \quad (4)$$

Where: U_{avg} is the average voltage (V), I_{avg} is the average current (A), and t is the operating time (s). E_{chem} represents the chemical energy of the removed COD. The relationship between COD and the energy content of organic matter is often expressed as:

$$E_{chem} = \Delta COD \cdot V_{an} \cdot 14 \text{ , (kJ/gCOD)} \quad (5),$$

Where: ΔCOD is the removed COD (g O_2/L), V_{an} is the volume of the anode chamber (L), and 14 kJ/g COD is the approximate energy content of the organic matter.

Based on the applied indicators - coulombic efficiency, voltage efficiency, and overall energy efficiency - a comprehensive evaluation of the energy performance of the bioelectrochemical system can be performed. These parameters allow monitoring of the efficiency of conversion of the chemical energy of organic matter into electrical energy, as well as assessment of the potential of the BES for simultaneous wastewater treatment and energy recovery.

CHAPTER 4. RESULTS AND DISCUSSION

4.1. Investigation of the influence of design and operational parameters on BES performance

Within this group of studies, different design configurations of two-chamber and three-chamber microbial fuel and microbial electrolysis cells were developed and experimentally tested, based on the process of direct microbial sulfate reduction (DMSR) occurring in the anode zone of the BES. The main objective was the treatment of model aqueous solutions with high concentrations of sulfate and copper ions, as well as the evaluation of the influence of design parameters on the rate of sulfate reduction and the removal efficiency of SO_4^{2-} and Cu^{2+} .

Microbial fuel and electrolysis cells based on DMSR represent an integrated technological approach in which simultaneous treatment of sulfate- and metal-rich wastewater streams and recovery of part of the chemical energy of the organic substrate in the form of electrical energy are achieved. A major advantage of these systems is that no external mediators are required, as microbially produced hydrogen sulfide acts as an electron mediator. It is oxidized at the anode to different sulfur species, including elemental sulfur (S^0), thereby directly participating in the electrochemical cycle of the system.

The accumulation of elemental sulfur on the anode surface during long-term operation of such BES can lead to deterioration of the electrochemical performance of the system. Additionally, the design of MFCs often does not provide optimal conditions for microbial biomass development. In the cathode zone, different electron acceptors may be used, with oxygen from air most commonly serving as the terminal electron acceptor, which upon reduction forms water.

Such systems maintain high potential for sustainable treatment and valorization of sulfate- and metal-rich aqueous solutions.

4.1.1. Results from investigations of a two-chamber microbial fuel cell based on the MSR process for the treatment of model solutions for sulfate and copper ion removal (Variant 1).

The obtained experimental results (Tables 5, 6 and Fig. 34) were focused on the treatment of model solutions with high concentrations of copper and sulfate ions in a two-chamber microbial fuel cell (MFC), based on the MSR process and separated by an anion-exchange membrane.

The experiments were conducted under static operating conditions, and to maintain a constant system volume, losses due to evaporation caused by significant temperature fluctuations in the working environment (24-34 °C) were compensated. This ensured relatively stable hydrodynamic conditions and enabled proper comparison between experimental series.

The volumes of the recirculating solutions in the cathode and anode chambers, including the associated buffer tanks, were identical - 600 mL each. The system efficiency was evaluated over a 10-day operating period, and changes in sulfate and copper ion concentrations, as well as the electrochemical parameters of the fuel cell, are summarized in Tables 5 and 6.

Table 5. Main operational parameters in the anode zone of the MFC for Variant 1.

Day	SO ₄ , mg/l	MSR rate, mg/l. h	pH	EC, mS/cm	Eh, mV	H ₂ S, mg/l	COD, gO ₂ /l
0	552	-	7.25	3.61	-375	220	8.77
5	350	4.31	7.6	3.49	-335	206	3.07
10	195	4.17	7.85	3.37	-235	44	2.15
Reduction degree, %	64.5%	-	-	-	-	-	75.4%

The obtained data allows a quantitative assessment of the processes of microbial sulfate reduction, cathodic reduction of metal ions, as well as the overall treatment and energy efficiency of the developed bioelectrochemical system.

Table 6. Main operational parameters in the cathode zone of the MFC for Variant 1.

Day	SO ₄ , mg/l	pH	EC, mS/cm	Eh, mV	Cu, mg/l
0	1995	2.38	3.19	355	1045
5	1680	2.83	2.64	315	1023
10	1351	3.35	2.01	277	997
Reduction degree, %	32.2%	-	36.9%	-	4.6%

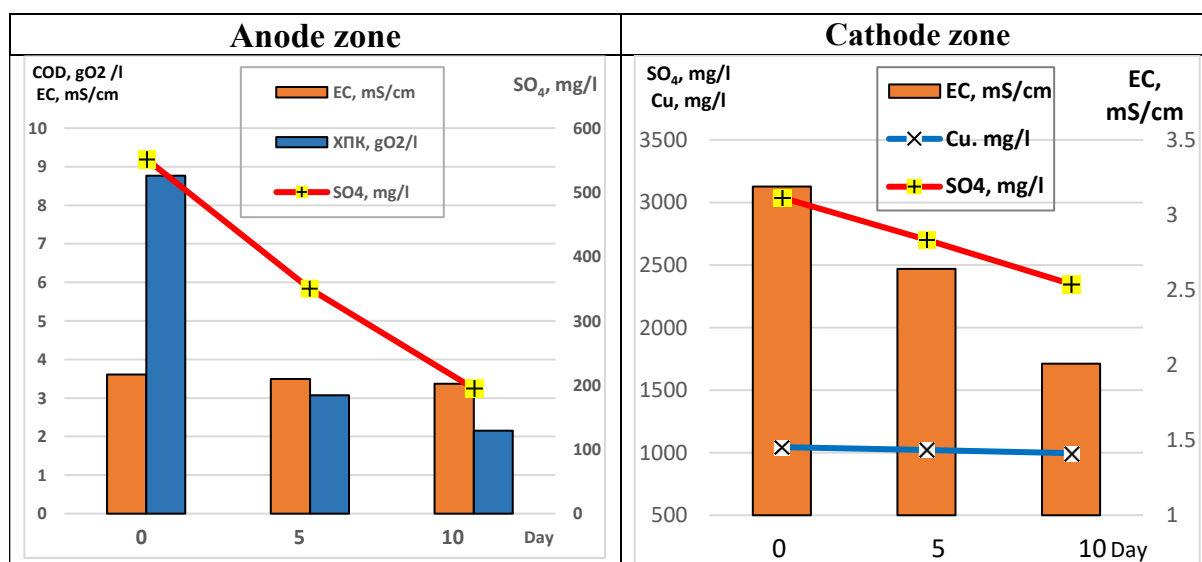


Figure 34. Variation of the main operational parameters in the anode and cathode zones for Variant 1.

The obtained results show a decrease in sulfate concentration in both the anode and cathode zones of the MFC over a period of 10 days, which is attributed to the ongoing microbial sulfate reduction process in the anode zone and the transport of sulfate ions through the anion-exchange membrane (AEM) of the MFC. The same trend is observed with respect to the electrical conductivity of the medium in both the anode and cathode zones (Tables 5, 6 and Fig. 34), confirming the conclusion that this type of microbial fuel cell with an anion-exchange membrane can be effectively used for the treatment of wastewater with high sulfate concentrations.

Regarding the presented values of Eh, copper ion concentration in the cathode zone, and H₂S and COD in the anode zone, they are consistent and support the obtained results.

In the conducted experiments, the sulfate concentration in the anode zone decreased by approximately 64.5% (due to the microbial sulfate reduction process), while in the cathode zone the decrease was 32.2% (due to sulfate mass transfer through the membrane) over a period of 10 days. It should be noted that the rate of microbial sulfate reduction in the anode zone is significantly higher than the rate of sulfate mass transfer through the AEM membrane from the cathode zone. The reduction in copper ion concentration in the cathode zone was only about 4.6%.

4.1.2. Results from the investigation of the influence of electrode and separator area

In the conducted studies using the laboratory setup - Variant 1 and Variant 2 (Figs. 23 and 25) - the focus was directed towards comparing the performance of a two-chamber microbial fuel cell with different geometric electrode surface areas, namely 0.002 m² and 0.02 m² for Variants 1 and 2, respectively. The comparison was performed both in terms of sulfate and copper ion removal efficiency and in terms of the electrochemical performance of the microbial fuel cell (MFC).

The construction of the fuel cell (Variant 2) was optimized with respect to the membrane and electrode surface area, where identical graphite plates with dimensions 100 × 100 × 6 mm were used as both cathode and anode, and an anion-exchange membrane with dimensions 100 × 100 mm was applied. The volumes of the anode and cathode chambers were identical-100 mL each, and the volumes of the buffer tanks were also equal-500 mL each.

The obtained results (Tables 7 and 8) were compared with the data from Variant 1 (Tables 5 and 6 and Fig. 34).

Table 7. Variation of sulfate (SO_4), electrical conductivity, and COD in the anode zone of the MFC for Variant 2.

Day	SO_4 , mg/l	MSR rate, mg/l . h	EC, mS/cm	COD, gO_2/l
0	498	-	3.60	8.44
5	212	8.73	3.42	2.67
10	60	7.28	3.25	2.05
%	87.9%	-	-	75.7%

Table 8. Main operational parameters (sulfates, electrical conductivity, and copper ions) in the cathode zone of the MFC for Variant 2.

Day	SO_4 , mg/l	EC, mS/cm	Cu, mg/l
0	2030	3.22	1045
5	1316	2.53	863
10	1088	1.91	760
%	46.4%	40.9 %	27.3 %

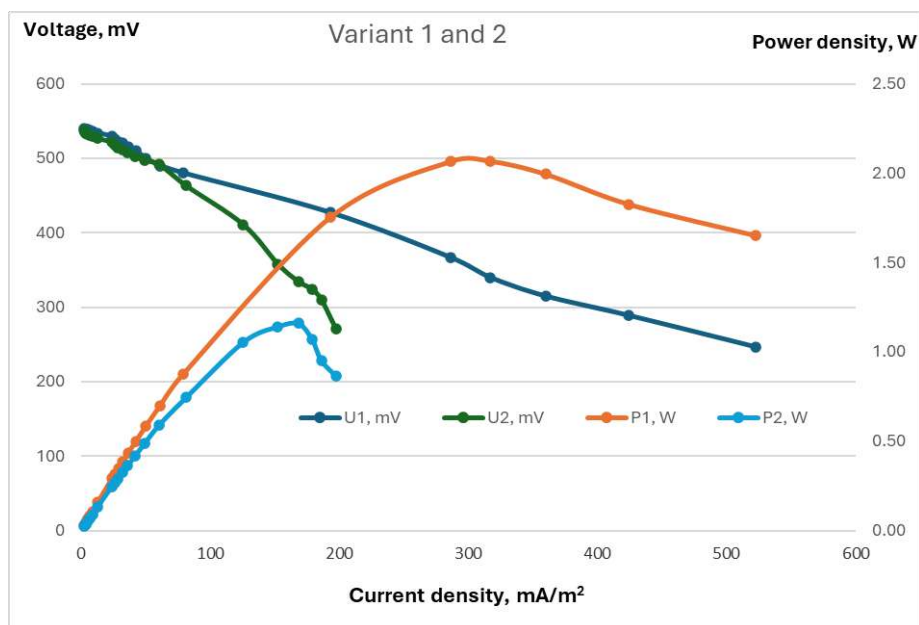


Figure 35. Polarization and power density curves at different electrode surface areas - Variants 1 and 2, where: U1 (mV) - voltage for Variant 1, U2 (mV) - voltage for Variant 2, P1 (W) - power for Variant 1, P2 (W) - power for Variant 2.

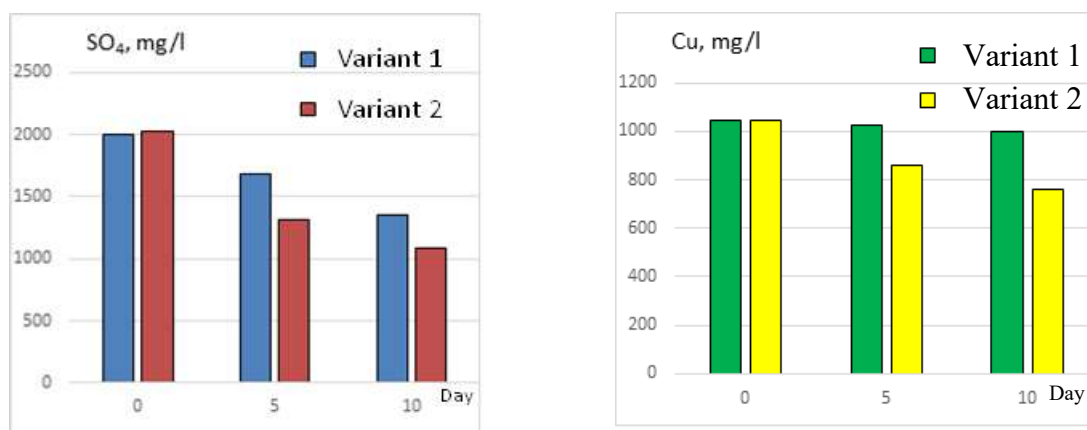


Figure 36. Comparison of the variation in sulfate and copper ion concentrations in the cathode zone for two different cathode surface areas (Variants 1 and 2).

The obtained results (Fig. 36) demonstrate a significant influence of the electrode and separator surface area on the efficiency of the bioelectrochemical system. For instance, when comparing Variant 1 with Variant 2, the sulfate removal efficiency increased from 64.5% to 87.9% in the anode zone, and from 32.2% to 46.4% in the cathode zone, respectively. Similar trends were observed for the removal of copper ions in the cathode zone, where the efficiency increased from 4.6% to 27.3%. Comparable results were also observed regarding the decrease in electrical conductivity in the cathode zone, where this parameter varied from 36.9% (Variant 1) to 40.9% (Variant 2).

Another significant effect of the electrode surface area was observed on the power output of the fuel cell in both investigated variants, where it is clearly evident that Variant 2 exhibited considerably higher power and current density values compared to Variant 1 (Fig. 11). Accordingly, $P_2 = 1.99$ W and $I_2 = 317$ mA/m² were obtained for Variant 2, while $P_1 = 1.07$ W and $I_1 = 168$ mA/m² were recorded for Variant 1.

4.1.3. Results of the Study on the Efficiency of Sulfate and Copper Ion Removal: Comparison between a Microbial Fuel Cell (MFC) and a Microbial Electrolysis Cell (MEC)

In this study, a microbial fuel cell (MFC) and a microbial electrolysis cell (MEC) were compared. Both systems were implemented within the same volume of the bioelectrochemical system and operated with identical electrode and separator dimensions. The bioelectrochemical system was tested under two operating modes: microbial fuel cell (MFC) mode with an external resistance of 200 Ω , and microbial electrolysis cell (MEC) mode by applying a constant external voltage of 0.7 V between the anode and cathode of the electrolysis cell and using an external resistance of 10 Ω (Fig. 32).

Table 9. Values of the technological parameters used for the comparison between the microbial electrolysis cell (MEC) and the microbial fuel cell (MFC).

Anode zone								
	SO ₄ , mg/l		MSR rate, mg/l. h		EC, mS/cm		COD, gO ₂ /l	
Day	MEC	MFC	MEC	MFC	MEC	MFC	MEC	MFC
0	520	498	-	-	3,55	3,60	8.48	8.44
5	201	215	13.9	8.5	3,27	3.42	1.55	2.67
10	40	60	9.5	5.8	3,01	3,25	1.08	2.05
%	92,3%	87.90%	-	-	-	-	87.60%	75.70%

Cathode zone						
	SO ₄ , mg/l		EC, mS/cm		Cu, mg/l	
Day	MEC	MFC	MEC	MFC	MEC	MFC
0	2055	2030	3,30	3,22	1035	1045
5	690	1316	1.23	2.53	450	863
10	250	1088	0.85	1.91	50	760
%	87.80%	46.40%	74.20%	40.90%	95.10%	27.30%

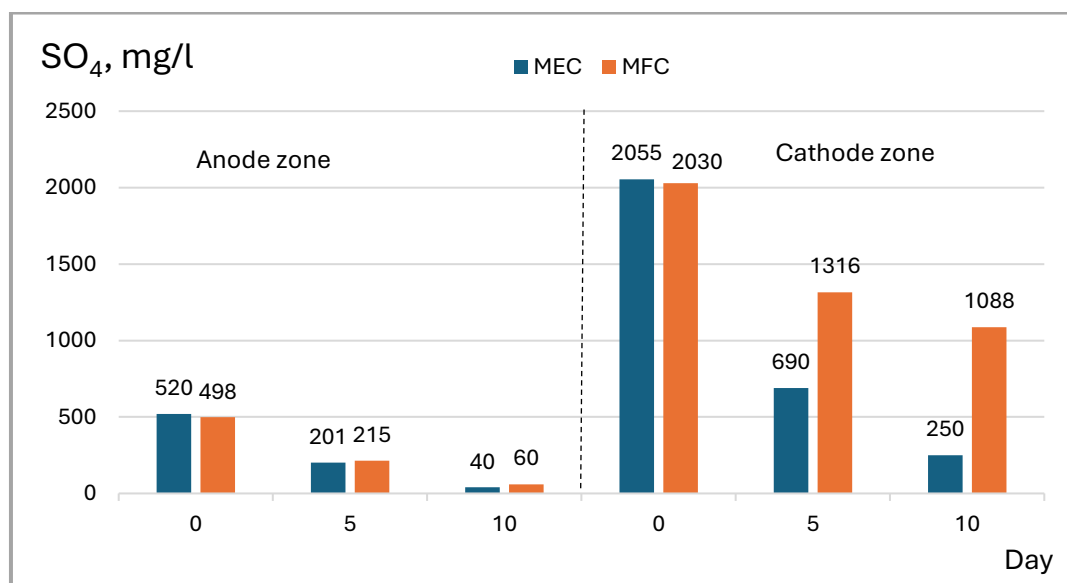


Figure 37. Comparison of sulfate concentration changes during the operation of the bioelectrochemical system (BES) as a microbial fuel cell (MFC) and a microbial electrolysis cell (MEC).

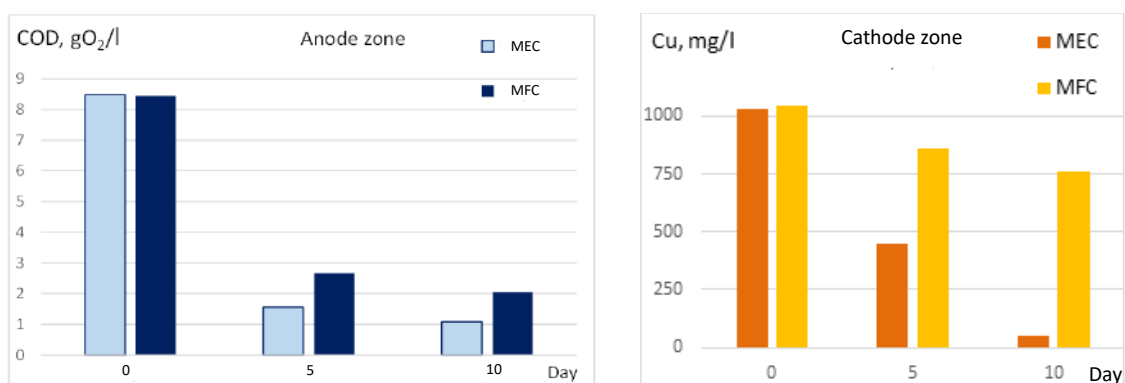


Figure 38. Comparison of changes in copper ion concentrations and COD during the operation of the bioelectrochemical system (BES) as a microbial fuel cell (MFC) and a microbial electrolysis cell (MEC).

The experiment was conducted under static conditions. The technological solutions and growth medium used for sulfate-reducing bacteria (SRB) were as follows: in the cathode chamber - 2 g/L SO_4^{2-} and 1 g/L Cu^{2+} , and in the anode chamber - 1 g/L sulfate in the form of MgSO_4 (modified SRB growth medium). The obtained results are presented in Table 9 and Figures 37 and 38.

Analysis of the obtained results (Table 9 and Figures 37 and 38) demonstrated a significant improvement in the performance of the bioelectrochemical system (BES) when operated in microbial electrolysis cell (MEC) mode compared to microbial fuel cell (MFC) mode. This is evidenced by the higher sulfate removal efficiency achieved in the cathode chamber, reaching 87.8% in the MEC configuration compared to 46.4% in the MFC configuration over a period of 10 days. Even more pronounced differences were observed regarding the reduction of copper ion concentration in the cathode chamber, where the MEC achieved a removal efficiency of 95.1%, compared to 27.3% for the MFC over the same 10-day period.

4.1.4. Results of the Study on the Performance of a Three-Chamber Microbial Desalination Fuel Cell (MDFC) (Laboratory Setup - Variant 3)

Desalination studies using synthetic solutions were carried out in a three-chamber microbial desalination fuel cell (MDFC) based on the sulfate reduction process, employing an AMI-7001S anion-exchange membrane and a CMI-7000S cation-exchange membrane. The MFC configuration, electrodes, and membranes were identical to those used in the previous experimental variants.

The experiments were conducted under static conditions at room temperature (22-24°C), with evaporation losses compensated throughout the study. The volumes of the recirculating solutions in the cathode, anode, and desalination (intermediate) chambers, including the buffer vessels, were equal, 600 mL each. The performance results of the fuel cell are presented in Table 10.

Synthetic solutions were prepared according to Figure 25 with the following compositions:

1. Anode chamber - modified SRB growth medium containing 1 g/L MgSO_4 , pH 7.5;
2. Intermediate (desalination) chamber - CuSO_4 solution containing $\text{Cu}^{2+} = 1018 \text{ mg/L}$, $\text{SO}_4^{2-} = 2.03 \text{ g/L}$, and $\text{H}_2\text{SO}_4 = 0.5 \text{ g/L}$;
3. Cathode chamber - H_2SO_4 solution containing 0.5 g/L H_2SO_4 .

An external resistance of 200 Ω was applied throughout the experiment.

Table 10. Values of the main operational parameters of the three-chamber microbial desalination fuel cell (MDFC).

Anode zone (MFDC - SRB)						
Day	SO ₄ , mg/l	Cu, mg/l	pH	EC, mS/cm	Eh, mV	COD, gO ₂ /l
1	450	-	7.59	3.93	-355	8.51
4	216	-	7.66	3.66	-330	4.89
7	110	-	7.70	3.54	-95	3.05
10	95	-	7.81	3.47	-77	2.44
Desalination zone (MFDC - CuSO₄)						
Day	SO ₄ , mg/l	Cu, mg/l	pH	EC, mS/cm	Eh, mV	-
1	1995	1045	2.38	3.93	426	-
4	1350	734	2.62	3.21	385	-
7	1100	469	2.84	2.85	354	-
10	984	325	2.98	2.77	325	-
Cathode zone (MFDC - H₂SO₄)						
Day	SO ₄ , mg/l	Cu, mg/l	pH	EC, mS/cm	Eh, mV	-
1	440	189	2.47	2.02	422	-
4	431	173	2.53	1.72	415	-
7	398	112	2.75	1.34	355	-
10	377	98	2.89	1.21	345	-

In this configuration of a three-chamber bioelectrochemical system operating as a microbial desalination fuel cell (MDFC), promising results were also obtained. The results indicate that the rate of microbial sulfate reduction in the anode chamber was significantly higher than the mass transfer rate of sulfate ions through the anion-exchange membrane, which appears to be the limiting factor for the microbial sulfate reduction process.

At the same time, a substantial fraction of the dissolved copper ions migrated through the cation-exchange membrane (CEM) into the cathode chamber, with the residual copper concentration in the desalination chamber decreasing to 98 mg/L after the 10-day experimental period.

The reduction in copper ion concentration in the intermediate (desalination) chamber reached approximately 69.2%, while the sulfate concentration decreased by 51.1% over the same 10-day period. To further improve the desalination efficiency, additional experiments should be conducted by operating the system in microbial electrolysis cell (MEC) mode through the application of an external voltage and the use of lower external resistance values.

4.1.5. Results of the Investigation of the Effect of Initial Sulfate Concentration in Treated Acid Mine Drainage on Sulfate Removal Efficiency in the Cathode and Anode Chambers of the MFC

The main objective of this study was to evaluate the effect of the initial sulfate ion concentration in the catholyte on their migration through the AEM, the electrochemical performance of the MFC (voltage, current, and power output), the reduction of copper ions in the cathode chamber, and the overall treatment efficiency of acid mine drainage (AMD).

To achieve these objectives, an identical experiment was conducted using the laboratory setup presented in Figure 25, with three different initial sulfate concentrations in the catholyte (4.5, 9.0, and 12.0 g/L) representing synthetic AMD. The experiments were carried out over a period of 96 hours (4 days) under identical hydraulic, temperature, and electrochemical conditions, allowing for a reliable comparison of the obtained results.

Prior to each experiment, the membrane was replaced with a new one, and the electrodes were mechanically cleaned to remove accumulated deposits of elemental sulfur and copper that could affect the electrochemical activity of the system. Winery distillery stillage was used as the electron donor for the microbial sulfate reduction process. The stillage was diluted 1:1 with distilled water and neutralized to pH 7.5 before use. Its application served the dual purpose of providing a carbon source for sulfate-reducing bacteria and enabling the valorization of an organic waste stream.

The experiments were performed in batch mode with a duration of 96 h. The anolyte was recirculated at a flow rate of 5 L/h, while the pH was maintained within the optimal range for sulfate-reducing bacterial activity. A synthetic acid mine drainage solution was used as the catholyte, containing three different initial sulfate concentrations (4.5, 9.0, and 12.0 g/L) and approximately 150 mg/L of Cu^{2+} ions. The catholyte pH was adjusted to 2.0 to simulate the typical conditions of real AMD.

The obtained results demonstrated effective migration of sulfate ions through the membrane from the cathode to the anode chamber, where dissimilatory microbial sulfate reduction and oxidation of the produced biogenic H_2S occurred. It was found that the initial sulfate concentration had a significant effect on the electrochemical characteristics of the system and on the efficiency of ion transport and pollutant removal processes.

Table 11. Dynamics of sulfate ions, electrical conductivity, pH, and copper ion concentration in the cathode chamber of the MFC at three different initial SO_4^{2-} concentrations.

	Parameter	0 h	24 h	48 h	72 h	96 h	Overall removal efficiency, %
Variant 1	SO_4^{2+} , mg/l	4574	4049	3685	3484	2870	37.25
	Cu^{2+} , mg/l	150.7	110.2	70.1	37.2	16.3	89.18
	EC, mS/cm	12.98	10.91	10.05	9.39	8.58	33.90
	pH	2.0	1.8	1.9	2.3	2.6	-
Variant 2	SO_4^{2+} , mg/l	9050	7880	6830	5900	5250	41.99
	Cu^{2+} , mg/l	150.7	70.4	45.7	16.1	9.4	93.76
	EC, mS/cm	18.12	15.20	13.20	11.70	10.22	43.60
	pH	2.0	2.2	2.4	2.6	2.8	-
Variant 3	SO_4^{2+} , mg/l	12100	10200	8100	7500	6400	47.11
	Cu^{2+} , mg/l	150.7	44.3	16.7	8.2	2.1	98.61
	EC, mS/cm	24.15	19.90	16.60	13.40	11.56	52.13
	pH	2.0	2.4	2.7	2.9	3.1	-

As the sulfate concentration increased, a corresponding increase in sulfate migration through the AEM was observed, accompanied by a decrease in catholyte conductivity. According to the obtained results, sulfate removal efficiency increased from 37.25% to 47.11%, while the catholyte conductivity decreased from 33.90% to 52.13%. At the same time, the removal of copper ions (Cu^{2+}) also increased, from 89.18% to 98.61%, most likely as a result of their reduction on the cathode surface.

In the anode chamber (Table 12), an intensive microbial sulfate reduction process was observed, catalyzed by the sulfate-reducing bacteria (SRB) present in the anolyte. This process was sustained by the migration of sulfate ions through the AEM, which served as the terminal electron acceptor during sulfate reduction. Evidence of dissimilatory microbial sulfate reduction (DMSR) was provided by the increase in H_2S concentration in the anolyte from 180 mg/L to 550 mg/L for the first and third synthetic AMD variants, respectively. Elevated H_2S concentrations may become toxic to the SRB community itself, potentially inhibiting the sulfate reduction process. Such an effect has been reported by Feng et al. (2016).

Simultaneously, a decrease in COD was observed, from an initial value of 41.20 g O_2 /L to 32.10 g O_2 /L and 29.70 g O_2 /L for Variants 1 and 3, respectively. These results indicate a more complete utilization of the organic substrate (winery distillery stillage) with increasing sulfate concentrations in the catholyte.

Table 12. Dynamics of sulfate ions, COD, electrical conductivity, and hydrogen sulfide concentration in the anode chamber of the MFC at three different initial SO_4^{2-} concentrations.

	Parameter	0 h	24 h	48 h	72 h	96 h
Variant 1	SO_4 , mg/l	933.4	540.4	397.9	193.6	144.2
	MSR rate, mg/l. h		38.3	29.7	25.4	25.5
	COD, g O_2 /l	41.20	39.60	37.62	33.20	32.10
	EC, mS/cm	12.05	13.30	14.52	16.35	17.95
	H_2S , mg/l	0	90	120	160	180
Variant 2	SO_4 , mg/l	945	705	640	506	430
	MSR rate, mg/l. h		58.8	52.6	46.5	44.9
	COD, g O_2 /l	41.20	37.50	35.52	32.10	30.10
	EC, mS/cm	12.11	13.90	15.40	17.61	19.23
	H_2S , mg/l	0	140	205	280	330
Variant 3	SO_4 , mg/l	950	910	830	790	750
	MSR rate, mg/l. h		80.8	85.8	66.1	61.5
	COD, g O_2 /l	41200	36.30	34.30	31.10	29.70
	EC, mS/cm	12.15	14.65	16.60	19.55	21.51
	H_2S , mg/l	0	240	360	480	550

With respect to the electrochemical performance of the system (Figure 40), an increase in sulfate concentration resulted, as expected, in higher electrical conductivity in both the cathode and anode chambers of the MFC. This, in turn, led to an increase in both anodic and cathodic current generation, as well as enhanced power output. These expected changes in the electrochemical parameters were confirmed by the measured polarization and power density curves obtained for the three investigated variants (Figure 40).

Accordingly, the maximum current density increased from 286 to 339 mA/m², while the maximum power density increased from 89 to 125 W/m² for Variants 1 and 3, respectively, corresponding to an increase of approximately 40.4%.

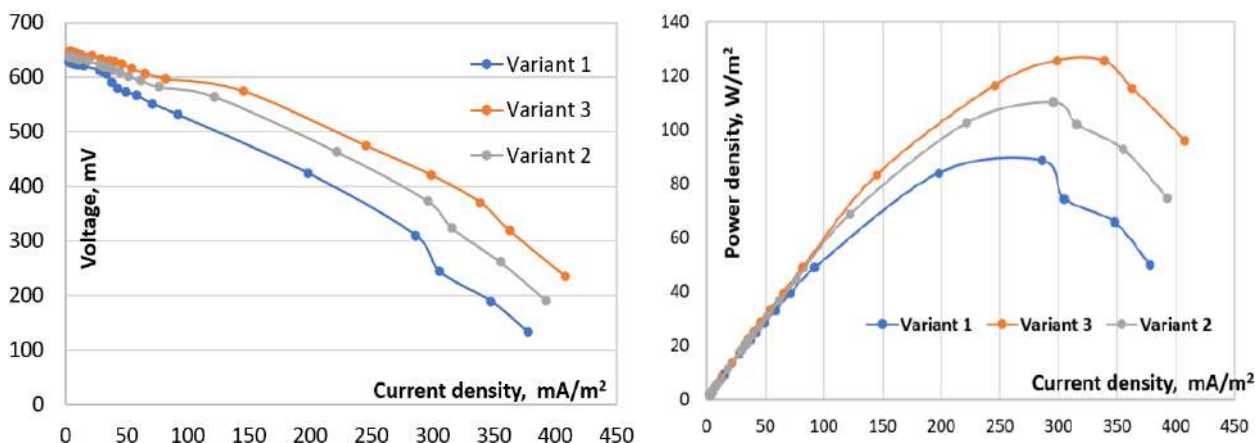


Figure 40. Polarization curves and power density curves for the three investigated variants after 96 hours of operation.

4.1.6. Results of the Comparative Evaluation of MFC and MEC Systems Based on Microbial Sulfate Reduction for the Treatment of Synthetic Acid Mine Drainage

This series of experiments was conducted using the laboratory setup presented in Figure 33. Synthetic acid mine drainage (AMD) was used in the study, with a chemical composition closely resembling that of real AMD generated in regions with intensive mining activities involving polymetallic ore deposits.

Winery distillery stillage was used as both the electron donor and carbon source for the microbial sulfate reduction (MSR) process. Distillery stillage is a complex substrate remaining after alcohol distillation from fermentation media and is characterized by high COD values (35,000-75,000 mg O₂/L). Previous studies have demonstrated its suitability for utilization in microbial sulfate reduction processes (Gonçalves et al., 2007).

The sulfate content of the distillery stillage ranged from 0.5 to 0.9 g/L, making it a suitable substrate for MSR. Nevertheless, in order to achieve an optimal COD/SO₄ ratio during the initial inoculation of the bioreactor, additional sulfate was supplied in the form of MgSO₄·7H₂O to reach a sulfate concentration of 1.5 g/L in the medium.

To ensure effective adaptation of the sulfate-reducing bacteria (SRB) to the distillery stillage, a preliminary cultivation stage was carried out in continuous-flow mode with a hydraulic retention time of three days over a period of one month in the bioreactor volume. Following this adaptation period, the

anodic circuit of the BES (bioreactor, buffer tank, and anode chamber) was filled with fresh distillery stillage without the addition of supplemental sulfate.

Simultaneously, the cathodic volumes (cathode chamber - 0.2 dm³ and buffer tank - 0.6 dm³) were filled with synthetic acid mine drainage having the chemical composition presented in Table 13.

The BES experiments were performed in batch mode over periods of five days. During operation, the liquid phase was continuously recirculated through both the anodic and cathodic loops using peristaltic pumps (5) at a flow rate of 5 L/h (Figure 33). The pH of the anolyte was adjusted daily to 7.5 by dosing a 1 N NaOH solution into the buffer vessel (position 7 in Figure 33).

Table 14. Dynamics of the technological parameters in the anolyte of the BES operated in MFC and MEC modes over a period of 96 hours.

	Parameters	0 h	24 h	48 h	72 h	96 h
MFC mode	pH	7.77	6.43	7.86	6.88	8.33
	EC, mS/cm	17179	18446	19980	20020	15380
	ORP, mV	-308	-375	-382	-409	-366
	COD, gO ₂ /l	44.5	41.3	41.1	40.6	40.1
	SO ₄ , mg/l	974	543	218	398	140
	HS ⁻ , mg/l	10.4	87.2	132.7	215.3	178.4
MEC mode	pH	6.89	7.21	7.43	7.56	7.57
	EC, mS/cm	15380	17055	18698	19290	19236
	ORP, mV	-366	-312	-368	-325	-403
	COD, gO ₂ /l	43.8	40.07	39.3	38.2	37.4
	SO ₄ , mg/l	331	426	188	93	65
	HS ⁻ , mg/l	12.5	4.2	4.1	3.8	2.8

The obtained results reflect the occurrence of two interconnected and parallel processes within the BES: heterotrophic microbial sulfate reduction in the anode chamber, using winery distillery stillage as both an electron donor and a source of organic carbon, and electrochemical reduction of the metals present in the acid mine drainage (AMD), occurring in the cathode chamber.

The occurrence of these processes is enabled by the electrical potential generated between the anode and cathode in the MFC configuration and by the externally applied voltage in the MEC configuration. As a result, a forced transport of anions through the separator (anion-exchange membrane, AEM) takes place, with sulfate ions being the predominant migrating species in the case of AMD. After passing through the AEM from the cathode to the anode chamber, sulfate ions enter a zone of active microbial sulfate reduction, where they are rapidly reduced to H₂S and, at pH values around 7.5, are predominantly present in the dissolved form as HS⁻. The produced H₂S subsequently acts as an electron-transfer mediator to the anode surface, where it is oxidized, depending on the operating conditions, to sulfur species with higher oxidation states, including elemental sulfur (Rodrigues et al., 2020).

In this series of experiments, the BES performance was investigated under two operating modes—microbial fuel cell (MFC) and microbial electrolysis cell (MEC)—operated in batch mode for a period of 96 hours, using winery distillery stillage as the substrate for microbial sulfate reduction.

The obtained results (Tables 14 and 15) allow the analysis and interpretation of the processes occurring in the anode and cathode chambers of the BES under both MFC and MEC operating modes.

Under MFC operation, the results from the anode chamber (Table 14) indicate the transfer of sulfate ions from the cathode chamber over the 96-hour period, as evidenced by the reduction in sulfate concentration within the anodic compartment. The oxidation-reduction potential (ORP) remained within the range of -384 to -409 mV throughout the experiment, indicating strongly reducing environmental conditions. The sulfate concentration gradually decreased from 974 mg/L on Day 1 to 140 mg/L on Day 5, demonstrating that the rate of microbial sulfate reduction exceeded the rate of sulfate migration through the AEM. At the same time, the HS^- concentration increased from 10.4 to 215 mg/L, indicating that the anodic current was insufficient to oxidize the electron-transfer mediator (HS^-) to a significant extent on the anode surface.

Table 15. Dynamics of the technological parameters in the catholyte of the BES operated in MFC and MEC modes over a period of 96 hours.

	Parameter	0 h	24 h	48 h	72 h	96 h
MFC mode	pH	2.82	2.77	3.2	3.63	3.68
	EC, mS/cm	4284	3658	3136	2928	2605
	ORP, mV	250	210	225	204	229
	SO_4 , mg/l	2752	2596	2363	2129	1989
	Cu, mg/l	77.7	66.3	50.6	35.5	24.1
	Zn, mg/l	10.2	-	9.19	-	9.03
	Fe, mg/l	10.8	-	10.5	-	10.4
	Mn, mg/l	4.6	-	4.5	-	4.5
	Al, mg/l	4.5	-	4.4	-	4.4
MEC mode	pH	2.81	4.14	4.74	4.97	5.69
	EC, mS/cm	4272	3590	3055	2650	2118
	ORP, mV	229	240	206	203	178
	SO_4 , mg/l	2797	2370	2205	1910	1459
	Cu, mg/l	77.3	56.3	40.2	22.1	3.3
	Zn, mg/l	10.4	-	7.4	-	4.3
	Fe, mg/l	10.8	-	10.5	-	0.5
	Mn, mg/l	4.5	-	4.4	-	4.4
	Al, mg/l	4.5	-	3	-	0.6

Particular interest is associated with the performance of the BES operating in MEC mode in the anode chamber (Table 14). Under these conditions, a substantial decrease was observed in the concentrations of both hydrogen sulfide (HS^- , from 12.5 to 2.8 mg/L) and sulfate ions (from 331 to 65 mg/L). In addition, the COD value exhibited a greater decline under MEC operation (to 37.4 g/L) compared to the MFC configuration (to 40.1 g/L). These results indicate an increased rate of microbial sulfate reduction (MSR) when the system operates in MEC mode compared with MFC mode.

The dynamics of the technological parameters in the cathode chamber of the BES under both operating modes are consistent with the results discussed above and clearly demonstrate the influence of the operating mode on process efficiency. During the 96-hour experimental period, sulfate removal from AMD reached 27.7% under MFC operation, whereas a significantly higher removal efficiency of 47.8% was achieved in MEC mode.

Regarding the heavy metals present in the AMD, operation in MFC mode resulted in relatively low removal efficiencies, with the concentrations of all metals, except Cu^{2+} , remaining nearly unchanged. A significant reduction was observed only for copper ions, for which the removal efficiency reached 69.0%. In contrast, operation in MEC mode resulted in substantially higher removal efficiencies, namely: Cu^{2+} - 95.7%, Zn^{2+} - 58.7%, Al^{3+} - 86.7%, and $\text{Fe}^{2+}/\text{Fe}^{3+}$ - 95.4%.

No significant change in the concentration of manganese ions (Mn^{2+}) was observed under either MFC or MEC operating conditions. This finding indicates a limited tendency of manganese to undergo reduction or precipitation under the investigated experimental conditions.

The observed results can be explained by two principal mechanisms affecting the speciation and behavior of metal ions in the treated mine water. The first mechanism is related to the electrochemical potential of the metals, which determines their ability to undergo electrochemical reduction and deposition on the cathode surface. The second mechanism is associated with changes in pH, which influence the solubility and precipitation of metal compounds.

For heavy metals commonly present in AMD (Fe, Cu, Ni, Zn, Cr, Cd, etc.), it has been demonstrated that, under suitable conditions, they can be reduced and deposited on the cathode surface in microbial fuel cells and microbial electrolysis cells (MFCs/MECs) (Kumbhar et al., 2021). Metals with positive reduction potentials, such as Cu^{2+} , Cr^{4+} , and Co^{2+} , can be effectively deposited on the cathode during MFC operation. In contrast, metals with lower reduction potentials, such as Cd^{2+} , Ni^{2+} , and Zn^{2+} , require the application of an additional electrical potential through an external voltage source, as demonstrated under MEC operation (Tao et al., 2014).

The results obtained in the present study (Table 15) experimentally confirm this behavior. During MFC operation, effective removal was observed only for Cu^{2+} (69.0%). In MEC mode, substantially higher removal efficiencies were achieved, including Cu^{2+} (95.7%), Al^{3+} (86.7%), and $\text{Fe}^{2+}/\text{Fe}^{3+}$ (95.4%), together with partial removal of Zn^{2+} (58.7%).

The removal of Cu^{2+} and Zn^{2+} is most likely associated with their electrochemical reduction followed by deposition on the cathode surface. In MEC mode, this process is enhanced by the externally applied voltage, whereas in MFC mode it proceeds without additional energy input. In contrast, the reduction in Al^{3+} and $\text{Fe}^{2+}/\text{Fe}^{3+}$ concentrations is attributed primarily to their precipitation as sparingly soluble hydroxides resulting from the increase in pH within the cathode chamber, an effect that is more pronounced under MEC operation.

With regard to manganese ions (Mn^{2+}), no significant concentration changes were observed under either operating mode. During the experiments, an increase in catholyte pH was recorded in both configurations, reaching 3.68 under MFC operation and 5.69 under MEC operation. Similar increases in pH during AMD treatment in BES systems have also been reported by other authors (Ostermeyer et

al., 2023) and may be regarded as an additional beneficial effect contributing to the improvement of treated wastewater quality.

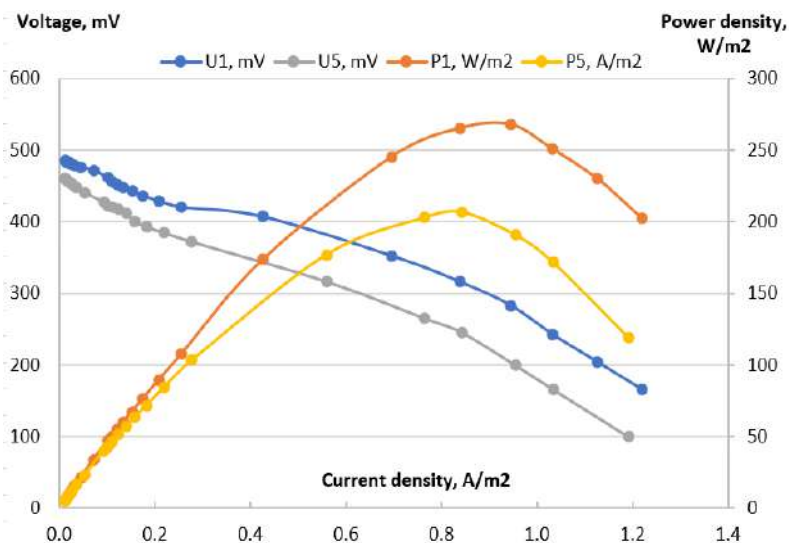


Figure 41. Polarization curves and power density curves in MFC mode, measured on Days 1 and 5 of the experiment.

When analyzing the electrochemical performance of the BES, it is important to consider the changes in the maximum power density and current density values observed between the beginning and the end of the experiment. These changes are illustrated by the polarization and power density curves (Figure 41), measured on the first and fifth days of operation in MFC mode.

The results show that the maximum power density decreased from $P_1 = 268 \text{ W/m}^2$ to $P_5 = 207 \text{ W/m}^2$, while the maximum current density simultaneously decreased from $I_1 = 0.9 \text{ A/m}^2$ to $I_5 = 0.8 \text{ A/m}^2$. These changes can be attributed to several factors, including variations in pH and electrical conductivity in both the anolyte and catholyte, changes in H_2S concentration in the anolyte, and the influence of the type and concentration of electron acceptors present in the cathode chamber (e.g., oxygen, Cu^{2+} , etc.).

4.1.7. Results of Studies Using Waste Organic Substrates (Distillery Stillage and Vinasse) as Electron Donors for the Microbial Sulfate Reduction Process in the Anode Chamber of the MFC

Prior to the use of waste organic substrates from the alcohol production industry—namely distillery stillage and vinasse—as electron donors for the microbial sulfate reduction process in the anode chamber of the bioelectrochemical system (BES), their initial characterization was performed. The vinasse was supplied by a private winery located in the vicinity of Pazardzhik, while the distillery stillage was obtained from “Kehlibar AD”, Svetovrachene.

Vinasse is characterized by a high organic load expressed as chemical oxygen demand (COD), typically ranging between 15 and 65 $\text{g O}_2/\text{L}$, low pH values (3.5-5.0), and a well-balanced content of macro- and micronutrients. The high concentration of dissolved solids originates from the raw materials used during production, including sugarcane, sugar beet, sweet sorghum, grapes, and agave. In addition, the organic matter present in vinasse is readily biodegradable, making it suitable for anaerobic treatment processes such as microbial sulfate reduction and biomethanation (Liamleam et al., 2017).

Distillery stillage is also characterized by high COD values, ranging from 10 to 100 $\text{g O}_2/\text{L}$, and even lower pH values (1.8-3.5). Consequently, its disposal without prior treatment poses a significant environmental risk due to its high pollution potential. Therefore, both vinasse and distillery stillage

represent waste streams with substantial pollutant loads and require appropriate treatment before discharge into the environment.

Table 16. Chemical composition of the distillery stillage.

Distillery stillage	Parameter	Value
	pH	3.46
	Dry matter, %	3.42
	COD, gO ₂ /l	57.3
	SO ₄ , mg/l	465.23
	D(+) cellobiose, g/l	0.52
	D(+) glucose, g/l	0.28
	D(+) xylose, g/l	0.13
	D(+) mannose, g/l	0.53
	D(+) galactose, g/l	0.03
	L(+) arabinose, g/l	0.07

Table 16 presents the parameters of the supplied distillery stillage. The wastewater is characterized by a high organic load (57 g O₂/L) and a diverse sugar composition. Based on the interpretation of the obtained results, and in order to ensure data comparability, as well as due to the significantly lower COD values in the standard growth medium for sulfate-reducing bacteria (Postgate B), it was decided to operate with at least a two-fold diluted waste organic substrate.

Table 17. Chemical composition of vinasse.

Vinasse	Параметър	Стойност
	pH	3.33
	Dry matter, %	3.08
	COD, gO ₂ /l	70.5
	SO ₄ , mg/l	0.169
	D(+) cellobiose, g/l	0.51
	D(+) glucose, g/l	0.6
	D(+) xylose, g/l	2.23
	D(+) mannose, g/l	0.4

From the results presented in Table 17, it can be seen that vinasse exhibits an even higher organic load (70 g O₂/L) and a rich sugar composition. The concentration of xylose is significantly higher compared to distillery stillage, due to the plant-based origin of the raw material. For this reason, it was decided to conduct the experiments using a three-fold diluted substrate.

The main results of the investigation of waste organic substrates (distillery stillage and vinasse) as electron donors in the anode chamber of the BES are presented below. In the laboratory setup (Figure 31), the bioreactor for microbial sulfate reduction (MSR) was pre-inoculated with sulfate-reducing bacteria (SRB) and cultivated according to the “Materials and Methods” section using Postgate B medium. After the formation of active biomass, the reactor was loaded with one of the three electron donor variants - lactate (control), vinasse, or distillery stillage - according to Section 3.2.2 of the “Materials and Methods”.

The data on the main operational parameters of the wastewater for each of the three investigated variants at seven different contact times are presented in Tables 18, 19, and 20.

Table 18. Technological parameters at different contact times during operation of the laboratory setup for DMSR with control sample (Variant 1).

Lactate		Feed solution	21h	31h	60h	72h	86h	108h	144h
	pH	7.51	6.9	7.67	7.52	7.66	7.69	7.71	7.99
	Eh (mV)	32.1	-380	-397	-414	-409	-405	-377	-367
	EC (mS/ cm)	11.12	11.30	10.75	10.796	10.77	10.92	10.80	9.21
	SO ₄ ²⁻ (g/1)	3.050	0.766	0.735	0.657	0.583	0.537	0.505	0.503
	S ²⁻ (mg/1)	-	198.7	235.4	577.1	448.1	346.4	240.2	35.5
	COD (g/1)	6.45	5.6	4.9	4.6	4.2	3.5	2.1	1.9

Table 19. Technological parameters at different contact times during operation of the laboratory setup for microbial sulfate reduction using distillery stillage (Variant 2).

Distillery stillage		Feed solution	21h	31h	60h	72h	86h	108h	144h
	pH	7.51	6.54	6.96	7.03	7.22	7.26	7.43	7.62
	Eh (mV)	21.1	-395	-398	-412	-401	-357	-383	-375
	EC (mS/cm)	17.520	16.133	15.93	16.03	16.17	15.79	15.36	15.13
	SO ₄ ²⁻ (g/1)	3.542	0.246	0.105	0.088	0.087	0.082	0.061	0.053
	S ²⁻ (mg/1)	-	141.1	269.6	640.7	565.6	480.1	267.4	243.5
	COD (g/1)	27.2	19.5	19.1	18.5	18.1	17.6	15.3	15.3

Table 20. Technological parameters at different contact times during operation of the laboratory setup for microbial sulfate reduction using vinasse (Variant 3).

		Feed solution	21h	31h	60h	72h	86h	108h	144h
Vinasse	pH	7.51	6.63	6.71	6.86	6.87	6.98	7.15	7.51
	Eh (mV)	22.5	-366	-416	-420	-378	-355	-354	-312
	EC (mS/cm)	16.155	15.16	15.59	15.49	15.44	14.263	14.268	13.44
	SO ₄ ²⁻ (g/l)	3.482	0.323	0.312	0.010	0.183	0.097	0.061	0.025
	S ²⁻ (mg/l)	-	146.8	173.6	402.9	368.7	171.8	187.6	151,62
	COD (g/l)	30.1	19.5	18	17.8	16.6	18	15.6	14

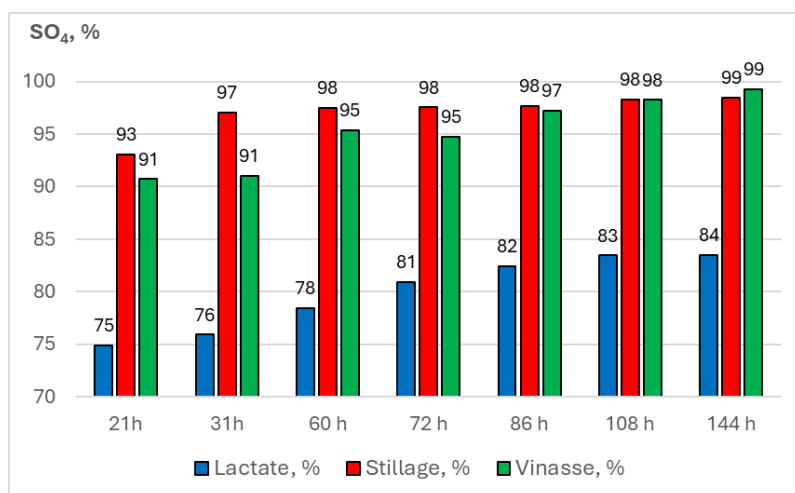


Figure 42. Sulfate removal efficiency at seven different contact times for three electron donor variants used in the microbial sulfate reduction (MSR) process in the anode chamber of the MFC.

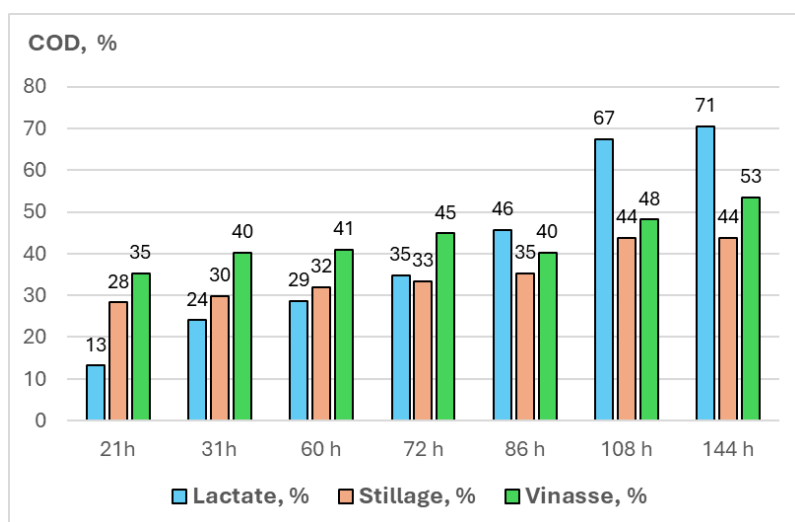


Figure 43. COD removal efficiency at seven different contact times for three electron donor variants used in the microbial sulfate reduction (MSR) process in the anode chamber of the MFC.

In the experiments conducted with the three different electron donors - lactate (control), distillery stillage, and vinasse - the pH values were monitored at seven different contact times. A decrease in pH below 7.0 was observed at the shortest contact times (21 and 31 hours) for the vinasse and distillery

stillage variants. This is likely due to the more complex organic composition of these substrates compared to lactate, as well as increased production of organic acids and reduced alkalinity resulting from lower concentrations of HCO_3^- ions.

Confirmation of these results is provided by the lower concentrations of S^{2-} at pH values below 7.0, which can be explained by the fact that a larger fraction of the microbiologically generated hydrogen sulfide is present in the fully protonated form (H_2S) and is released into the gas phase.

Table 21. Volumetric loading rates of sulfate and COD, and sulfate and COD removal in the laboratory systems.

Electron donor	HRT (h)	Volumetric sulfate loading (g SO_4^{2-} /l). h	Sulfate reduction rate SO_4^{2-} (mg/l . h)	Sulfate removal efficiency (%)	Volumetric organic loading (g/l. h)	COD removal efficiency, (%)
Lactate	21	0.124	93.3	75.43	0.256	13.18
	31	0.084	66.1	76.42	0.173	24.03
	60	0.043	34.2	78.92	0.090	28.68
	72	0.036	36.1	81.30	0.075	34.88
	86	0.030	25.0	82.77	0.063	45.74
	108	0.024	20.2	83.80	0.050	67.44
	144	0.018	18.0	83.86	0.037	70.54
Distillery stillage	21	0.141	130.8	93.05	1.079	28.31
	31	0.095	92.4	97.04	0.567	29.78
	60	0.049	48.0	97.52	0.293	31.99
	72	0.041	41.0	97.54	0.244	33.46
	86	0.034	33.5	97.68	0.204	35.29
	108	0.027	26.9	98.28	0.163	43.75
	144	0.020	20.2	98.50	0.122	44.12
Vinasse	21	0.138	125.4	90.72	1.194	35.22
	31	0.094	85.2	91.04	0.809	40.20
	60	0.048	44.8	92.68	0.418	40.86
	72	0.040	38.2	94.74	0.348	44.85
	86	0.034	32.8	97.21	0.292	45.85
	108	0.027	26.4	98.25	0.232	48.17
	144	0.020	20.0	99.28	0.174	53.49

Table 22. Microbial metabolism products identified by HPLC analysis.

	Initial substrate			Substrate after MSR (60h HRT)		
	Vinasse 1:1	Stillage 1:1	Lactate	Vinasse 1:1	Stillage 1:1	Lactate
D(+)-cellobiose, g/l	0.255	0.26	-	-	0.02	-
D(+)-glucose, g/l	0.3	0.14	-	0.07	0.04	0.02
D(+)-xylose, g/l	1.115	0.065	-	0.02	0.01	-
D(+)-mannose, g/l	0.2	0.265	-	-	-	-
raffinose, g/l	2.28	4.575	-	2.9	1.42	2.3
D(+)-galactose, g/l	-	0.015	-	-	-	-
L(+)-arabinose, g/l	-	0.035	-	-	-	-
lactic acid, g/l	1.2	9.6	3.01	-	-	-
acetic acid, g/l	-	0.02	-	8.85	7.16	1.8
propionic acid, g/l	-	1.56	-	1.25	1.98	0.16
glycerol, g/l	2.19	-	-	-	-	-
ethanol, g/l	2.82	0.66	-	0.77	0.23	-
COD, g/l	30.1	27.2	6.45	17.8	18.5	4.6

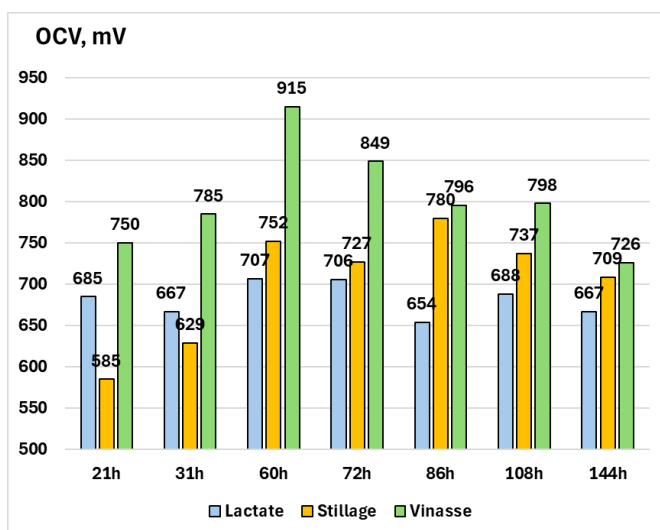


Figure 44. Open circuit voltage (OCV) values for the three investigated variants at seven different hydraulic retention times (HRTs).

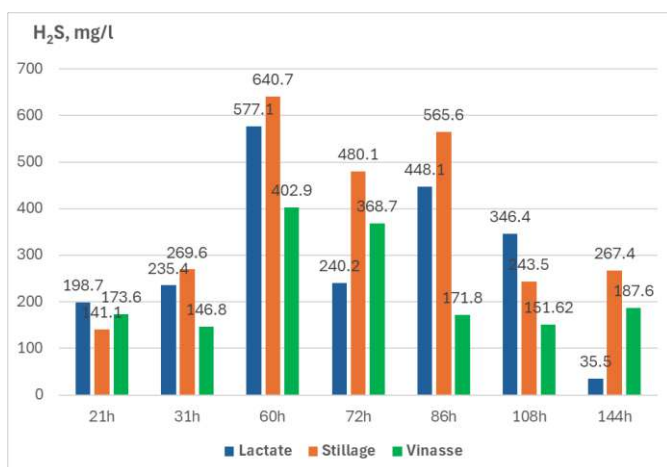


Figure 45. Hydrogen sulfide (H₂S) concentration values for the three investigated variants at seven different hydraulic retention times (HRTs).

The open circuit voltage (OCV) values in MFCs fed with vinasse solution are consistently higher compared to those operating with lactate or distillery stillage (Figure 44). The highest OCV values for all three fuel cells were observed at a hydraulic retention time of 60 hours. In the MFC with lactate, the OCV reached 707 mV, in the MFC with distillery stillage solution it reached 752 mV, while the highest value was recorded for the MFC with vinasse, reaching 915 mV.

A known relationship exists between the anode potential (and consequently the overall cell potential, OCV) and hydrogen sulfide (H_2S) concentration. Higher H_2S concentrations lead to an increase in anode potential during microbial sulfate reduction (MSR). In the present experiments, this relationship was confirmed, as at the 60-hour contact time the highest H_2S concentrations corresponded to the highest OCV values.

On the other hand, the potential negative impact of high H_2S concentrations on the activity of sulfate-reducing bacteria must be considered, as it may affect the long-term stability and performance of the MFC.

4.1.8. Analysis of the efficiency of the BES in the utilization of waste organic substrates from the alcohol production industry.

To evaluate the performance of microbial fuel cells, coulombic efficiency (CE) and the degree of organic matter utilization, expressed as chemical oxygen demand (COD), are most used. Coulombic efficiency describes the fraction of electrons released during the oxidation of the organic substrate that is captured in the form of electrical current. Theoretically, the number of electrons released is directly related to the extent of organic matter degradation, expressed as chemical oxygen demand (COD). A decrease in COD corresponds to a specific number of electrons that can potentially be converted into electrical current.

To assess the influence of different electron donors on MFC performance, three substrates were investigated: lactate (control substrate), distillery stillage, and vinasse.

The obtained results for the main parameters characterizing the energy efficiency of the microbial fuel cell (Table 23) show that the use of the three different electron donors for microbial sulfate reduction (MSR) in the anode chamber leads to significant variations in COD removal (ΔCOD), generated current (I), and coulombic efficiency (CE). Comparison of the three investigated substrates allows evaluation of the influence of substrate composition and biodegradability on the distribution of electron flow between electrogenic and metabolic processes within the anodic biofilm, as well as on the electrochemical performance of the microbial fuel cell.

Table 23. Variation of the main energy performance parameters* of the microbial fuel cell for the three investigated organic substrates at different hydraulic retention times.

	HRT	21 h	31 h	60 h	72 h	86 h	108 h	144 h
Lactate	OCV, mV	697	706	707	688	654	667	664
	I, mA	1.1	1,2	1,3	1,1	0,9	0,7	0,5
	DCOD, g/l	0.85	1.55	1.85	2.25	2.95	4.35	4.55
	COD, %	13.2	24.0	28.7	34.9	45.7	67.4	70.5
	CE, %	6.5	4	6.1	4.2	2.8	1.4	1.5
	VE, %	63.4	64.2	64.3	62.5	59.5	60.6	60.4
	EE, %	4.1	2.6	3.9	2.6	1.7	0.8	0.9

	HRT	21 h	31 h	60 h	72 h	86 h	108 h	144 h
Distillery stillage	OCV, mV	701	727	780	752	737	709	687
	I, mA	2,4	2,7	3,2	3,0	2,8	2,5	2,1
	DCOD, g/l	7.7	8.1	8.7	9.1	9.6	11.9	11.9
	COD, %	28.3	29.8	32.0	33.5	35.3	43.8	43.8
	CE, %	3.9	5.2	8.6	7.8	7.1	4	4.4
	VE, %	63.7	66.1	70.9	68.4	67	64.5	62.5
	EE, %	2.5	3.4	6.1	5.3	4.8	2.6	2.8
Vinasse	OCV, mV	715	749	798	756	726	695	679
	I, mA	2,1	2,3	2,5	2,4	2,2	2,0	1,9
	DCOD, g/l	10.6	12.1	12.3	13.5	13.9	14.5	16.1
	COD, %	35.2	40.2	40.9	44.9	46.2	48.2	53.5
	CE, %	2.6	2.9	4.8	4.1	3.6	3.1	3.1
	VE, %	65	68.1	72.5	68.7	66	63.2	61.7
	EE, %	1.7	2	3.5	2.8	2.4	2	1.9

*OCV, mV - open circuit voltage; I, mA - average value of the anode current at an external resistance of 100 Ω ; Δ COD, g/L - consumed chemical oxygen demand (COD); COD, % - COD removal efficiency; CE, % - coulombic efficiency; VE, % - voltage efficiency; EE, % - overall energy efficiency.

The comparative analysis shows that the highest current generation values were recorded when using distillery stillage, reaching 3.2 mA at a contact time of 60 h. The same substrate also provided the highest values of coulombic efficiency (up to 8.6%) and energy efficiency (up to 6.1%), indicating efficient utilization of organic matter and effective electron transfer to the anode.

Regardless of the type of electron donor, I, CE, and EE increased up to 60 - 72 h, after which they decreased due to depletion of readily biodegradable organic compounds, accumulation of hydrogen sulfide, and an increase in the internal resistance of the system. The obtained results demonstrate that a high degree of COD removal is not necessarily associated with high energy efficiency.

SCIENTIFIC AND APPLIED CONTRIBUTIONS

From the dissertation, the following contributions can be formulated, which are of both scientific-applied and applied character:

SCIENTIFIC AND APPLIED CONTRIBUTIONS

1. It has been demonstrated that the rate of dissimilatory microbial sulfate reduction in the anode chamber significantly exceeds the rate of sulfate migration through the anion-exchange membrane, which identifies membrane transport as the limiting factor of the process. The increase in electrode and membrane surface area leads to a significant improvement in sulfate and heavy metal removal efficiency, as well as enhanced electrochemical performance of the system.
2. MEC systems demonstrate higher efficiency compared to MFCs in terms of pollutant removal performance and metal ion reduction.
3. Competition between electrogenesis and sulfate reduction redistributes electron flow; therefore, COD removal does not directly correlate with energy efficiency in BES.

APPLIED CONTRIBUTIONS

1. Two- and three-chamber bioelectrochemical systems have been developed and validated for the efficient treatment of sulfate- and metal-containing waters (including acid mine drainage), enabling simultaneous wastewater purification and electricity generation.
2. The applicability of real waste substrates (vinasse and distillery stillage) as effective electron donors has been demonstrated, and optimal operational parameters have been determined to ensure a balance between efficient wastewater treatment and energy production.

LIST OF PUBLICATIONS RELATED TO THE DISSERTATION WORK

1. Maria Georgieva, Anatoliy Angelov (2016). "Application of microbial electrolysis cells in wastewater treatment and biological hydrogen production", National Scientific-Technical Conference with International Participation "Automation in Mining Industry and Metallurgy", BULKAMK'16, 10-11 November 2016, ISSN 1314-4537.
2. Georgieva, M., & Angelov, A. (2023). Treatment of acid mine drainage in a two-chamber microbial fuel cell. In Proceedings of the XX National Multidisciplinary Conference with International Participation (NMNPK 2023) (pp. 53-59), ISSN 1314-8931.
3. Angelov, A., & Georgieva, M. (2021). Microbial electrolysis cells - A new approach to wastewater treatment with high sulphate content. Proceedings of the XV International Conference on Open and Underwater Mining of Minerals (pp. 223-229). Varna, Bulgaria, ISSN 2535-0854, COBISS.BG-ID - 1280890596.
4. Georgieva, M. (2026). Application of microbial electrolysis cells in environmental protection systems. Proceedings of Scientific Conference "Knowledge, Science, Innovation, Technology", March 27, 2026, ISSN: 2815-3480 (CD).
5. Angelov, A., Georgieva, M., Genova, P. (2026). Analysis of the efficiency of a bioelectrochemical system for the treatment of organic- and sulfate-laden wastewater. National Scientific-Technical Conference "Environmental Aspects in the Mineral Resources Industry of Bulgaria", 16-17 June 2026, ISBN: 978-619-7777-00-0, COBISS-ID: 68556040.

SUMMARY

PhD Dissertation with title: *Treatment of Mining Wastewater Using Microbial Electrolysis Cells*, Author: Mariya Georgieva

The present dissertation investigates the application of bioelectrochemical systems for the treatment of mining wastewater containing sulfates and heavy metals. The study focuses on microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) utilizing the process of microbial sulfate reduction. The influence of key operational and design parameters, including sulfate concentration, membrane type, reactor configuration, and carbon source, on sulfate removal, metal recovery, and electrochemical performance was evaluated.

The obtained results demonstrate the potential of bioelectrochemical systems for simultaneous sulfate reduction, heavy metal removal, and resource recovery from mining wastewater. The research contributes to the development of sustainable and energy-efficient technologies for wastewater treatment and environmental protection.