

Treatment of Acid Mine Drainage in a Bioelectrochemical System, Based on an Anodic Microbial Sulfate Reduction

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ABSTRACT

The possibilities of simultaneous removal of sulfates and heavy metals (Cu, Ni, Zn) from acid mine drainage have been investigated in two-section bioelectrochemical system (BES). The used BES is based on the microbial sulfate reduction (MSR) process in the anode zone and abiotic reduction processes in the cathodic zone. In the present study, the model acid mine drainage with high sulfate (around 4.5 g/l) and heavy metals (Cu²⁺, Ni²⁺ and Zn²⁺) content was performed. As a separator in the laboratory, BES used an anionic exchange membrane (AEM), and for electron donor in the process of microbial sulfate reduction in the bioanode zone – waste ethanol stillage from the distillery industry was employed. In this study, the possibility of sulfates removal from the cathodic zone was established by their forced migration through AEM to the anode zone. Simultaneously, as a result of the MSR process, the sulfate ions passed through AEM are reduced to H₂S in the anode zone. The produced H₂S, having its role as a mediator in electron transfer, is oxidized on the anode surface to S⁰ and other forms of sulfur. The applicability of waste ethanol stillage as a cheap and affordable organic substrate for the MSR process has also been established. Heavy metals (Cu²⁺, Ni²⁺ and Zn²⁺) occur in the cathode chamber of BES in different degrees of the removal. As a microbial fuel cell (MFC) operating for 120 hours, the reduction rate of Cu²⁺ reaches 94.6% (in waste ethanol stillage) and 98.6% (in the case of Postgate culture medium). On the other hand, in terms of Ni²⁺ and Zn²⁺, no significant decrease in their concentrations in the liquid phase is found. In the case of microbial electrolysis cell (MEC) mode reduction of Cu²⁺– 99.9%, Ni²⁺– 65.9% and Zn²⁺– 64.0% was achieved. For 96 hours, the removal of sulfates in MEC mode reached 69.9% in comparison with MFC mode – 35.2%.

Keywords: BES, MFC, MEC, ethanol stillage, microbial sulfate reduction, acid mine drainage, heavy metals, sulphate.

INTRODUCTION

Acid mine drainage (AMD) are a serious environmental problem in both areas with active and completed mining activities. The reason for their formation is the oxidation of the sulfide minerals present in the mining waste (from open and underground mines, waste mining mass, tailings, etc.), which are oxidized on contact with air, water and chemolithotrophic acidophilic bacteria. AMD is characterized by high values of acidity, the concentration of sulfates, toxic heavy metals and arsenic (Favas et al., 2018).

Traditional technologies for the treatment of these waters are associated with the use of

neutralizing reagents to precipitate heavy metals and metalloids, such as hydroxides, carbonates and/or sulfides. Other technologies are applied, such as bioremediation, phytoremediation, electro dialysis, reverse osmosis and ultrafiltration, adsorption and passive systems (Rambabu et al., 2020). Despite the huge variety of technological solutions, a significant part of them is at a high cost, while others produce significant amounts of sludge and waste streams that require additional treatment.

Microbial electrolysis and fuel cells (MECs/ MFCs) have been found to be successfully applied to remove both organic and inorganic contaminants from wastewater (Venkata et al.,

2014). The basic process takes place when the electrons detach from the corresponding organic donor. During this process, they are transferred, to an insoluble anode of the bioelectrochemical system (BES), instead of the corresponding natural acceptors (oxygen, sulfates, ferriions, nitrates, etc.). Various electroactive microorganisms are used to carry out the process, including mixed cultures isolated from natural habitats (Kumar et al., 2015).

The use of the microbial sulfate reduction process in MECs/MFCs has been the subject of investigation in several publications (Rodrigues & Leão, 2020; Blázquez et al., 2019a; Blázquez et al., 2019b). An important effect in the process is the reduction of sulfates to biogenic H_2S , which is a mediator in electron transfer, being oxidized in the anode chamber on the anode surface to elemental sulfur (S^0) and its other forms (Rodrigues & Leão, 2020). Both some simple organic substrates such as – lactate, glucose, ethanol and acetate (Liamleam & Annachhatre, 2007) and complex substrates such as – wastewater from drainage streams of municipal waste landfills (Jin et al., 2020), from livestock (Zhang. M., & Wang. H., 2014) from the distillation industry (Gonçalves et al., 2007), etc. are used as electron donors for the MSR process in the anode zone. For the reduction of sulfates, microbial sulfate reduction is applied, both in the cathodic (as autotrophic MSRs) (Agostino & Rosenbaum, 2018) and in the anodic zones (as heterotrophic MSRs) of MECs/MFCs (Angelov et al., 2013).

Ethanol stillage is a liquid waste separated after the distillation of alcohol from the fermentation medium in the preparation of ethanol from starch. This organic waste is characterized by high COD values of 35000 to 65000 mg/l, has a variable chemical composition and very often, in the pre-treatment processes of the primary raw material, hydrolyzed the organic matter (in acid hydrolysis with H_2SO_4), leading to relatively high sulfate concentrations (Mikucka & Zielińska, 2020). This chemical composition of the ethanol stillage makes it a suitable organic substrate for the MSR process, which has been found in other studies (Gonçalves et al., 2007).

In addition to the high concentrations of sulfates, AMD are characterized by low pH values and significant heavy metal contents. Treatment of such waters in the process of microbial sulfate reduction is difficult and significantly reduced in intensity due to the typically lower pH values,

which are not sufficient for SRB (Sheoran et al., 2010; Liang et al., 2013).

One of the interesting approaches for the treatment of these waters is the use of microbial desalination cells (MDCs) in which a separation of MSR processes and AMD flow into separate volumes can be realized (Hemalatha et al., 2020; Vélez-Pérez et al., 2020). In this case, the separation of flows can be achieved by appropriate management of liquid phases, into two- and three-chamber configurations of anode and cathode zones, separated by the respective separators (CEM and AEM) (Blázquez et al., 2019a; Brewster et al. 2018; Pozo et al., 2017).

The removal of heavy metals in BES (MECs/MFCs) has been investigated in a significant number of publications (Kumbhar et al., 2021). The heavy metals (Fe, Cu, Ni, Zn, Cr, Cd, etc.) typically present in the AMD, were found to be reducible and deposited on the cathode surface in MECs/MFCs. A significant limitation in the reduction of metals in BES is their electrochemical potential. Metals with positive redox potential, such as Cu^{2+} , Cr^{4+} and Co^{2+} , have been successfully deposited at the MFC cathode (Kumbhar et al., 2021; Luo et al., 2014b). In contrast, in the case of metals with lower reduction potentials, such as Cd^{2+} , Ni^{2+} and Zn^{2+} , an additional potential needs to be supplied, via an external voltage, which is demonstrated in the MEC (Luo et al., 2014b; Tao et al., 2014). The efficiency of electrochemical reduction of metals on the cathode in BES depends on several factors – pH of the solution, electrical conductivity, the potential difference between the anode and cathode, electrochemical potential of metals, cathodic current density, presence of catalysts, dissolved oxygen content in the catholyte and others (Kumbhar et al., 2021).

Oxygen in the cathode chamber of BES is the preferred electron acceptor due to its high positive reduction potential – +1.23V. The ions of some metals with a positive reduction potential can also act as an electron acceptor, such as Cu^{2+} , Cr^{3+} , Mn^{2+} (Table 1) (Mathuriya & Yakhmi, 2014). The electrochemical reduction of dissolved heavy metals (which are present in the composition of AMD) can be carried out on a cathode surf, as in the BES mode of operation – such as MFC (for Cu, Cr, and Co, etc.), and when an additional external voltage is supplied in a mode, such as MEC (for Ni, Zn, Cd, etc.) (Kumbhar et al., 2021).

Table 1. The electrode potential of reduction, compared to the Standard Hydrogen Electrode (SHE) (Mathuriya & Yakhmi, 2014)

No.	Equation	The electrode potential of reduction
1	$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$	+ 1.23 V
2	$Cr_2O_7^{2-} + 14H^+ + 6e^- \rightarrow 2Cr^{3+} + 7H_2O$	+ 1.33 V
3	$Cu^{2+} + 2e^- \rightarrow Cu^0$	+ 0.286 V
4	$Cd^{2+} + 2e^- \rightarrow 2Cd^0$	- 0.40 V
5	$Ni^{2+} + 2e^- \rightarrow 2Ni^0$	- 0.25 V
6	$Zn^{2+} + 2e^- \rightarrow 2Zn^0$	- 0.764 V

A major objective of the study was to establish the possibility of simultaneous removal of sulfates and heavy metals (Cu, Ni and Zn) from acid mine drainage in a two-section BES (MECs/MFCs) where AEM is used as a separator between the cathode and the AMD flow separation bioanode from the heterotrophic sulfate reduction process with an electroactive biofilm formed. The possibility of an efficient heterotrophic MSR in the anode chamber was also investigated, using a waste ethanol stillage for electron donor and sulfate ions migrated through AEM, at two BES modes, such as MFC and MEC. The influence of oxygen on the electrochemical reduction of the copper ions (Cu^{2+}) in the cathode was also performed.

MATERIALS AND METHODS

The experiments were carried out in a laboratory installation of BES in which provision was made for operation in two modes – such as MFC and MEC (Fig. 1). For this purpose, a two-chamber construction of a sandwich type BES with a separator was used, separating the cathodic from the anodic zone by a – Fumasep FAM anion exchange membrane. Before the start of the experiments, the membrane was treated with a solution of 0.5 M NaCl at $T = 25^\circ C$ for 72 hours. For electrodes in BES, graphite plates with dimensions of $100 \times 100 \times 6$ mm and geometric area of $0.021 m^2$ were used; the area of the used anion exchange

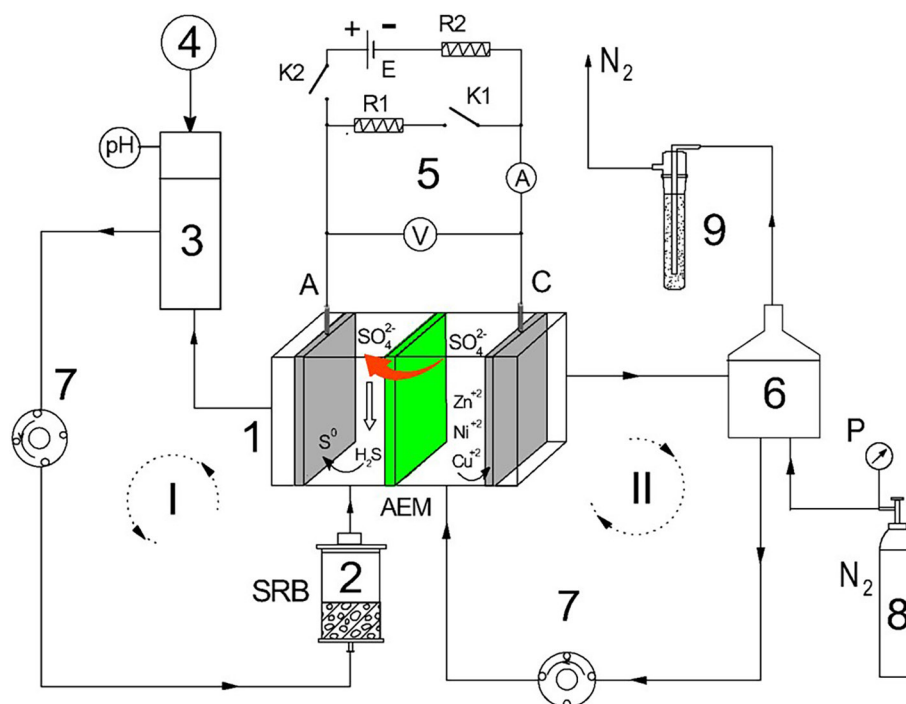


Figure 1. Flow diagram of the laboratory installation; 1 – MFC/MEC, 2 – bioreactor with immobilized biomass for cultivation of SRB, 3 – buffer volume for pH correction and electroconductivity measurement (EC), 4 – solution of 1N NaOH, 5 – MEC/MFC load circuit, 6 – buffer volume for the model AMD, 7 – recirculating peristaltic pumps, 8 – bottle with N_2 , 9 – hydro lock

membrane is 0.01 m². The volume of the cathode and anode sections are the same, i.e. 0.1 dm³. Before each series of experiments, the membrane and electrodes were replaced with new ones.

The cultivation of SRB is carried out in a fixed-bed bioreactor with a volume of 0.7 dm³. Approximately half the volume of the bioreactor was filled with 0.3 kg of modified zeolite, on which a biofilm of SRB and other metabolically related groups of microorganisms was formed. The characterization, modification of the natural zeolite used and the method of microbial biofilm formation have been described in a previous study (Angelov et al., 2013). The inoculum used was a mixed culture of sulfate-reducing bacteria cultivated using lactate as a carbon and energy source. The most abundant phylum in the bacterial community was *Proteobacteria* – 67%, of which the class *Deltaproteobacteria* accounted for 5.15%. In the microbial community, sulfate-reducing bacteria belong to the genera *Desulfomicrobium* (3.21%), *Desulfobulbus* (0.80%), *Desulfovibrio* (0.71%), *Desulfococcus* (0.11%) and *Desulfosphaera* (0.10%). This microbial consortium has been characterized in detail in previous studies (Bratkova et al., 2019).

The sulfidogenic bioreactor is connected to the anode section of the BES and a buffer vessel (0.3 dm³), and homogenization of the liquid phase with a total volume of 0.8 dm³ is ensured by recirculation with a peristaltic pump.

After the formation of the microbial biofilm in the fixed-bed bioreactor, the influence of two types of electron donors on the rate of the microbial sulfate-reduction process was studied – lactate and *distillery stillage*. In the first variant – electron donor lactate, a modified Postgate nutrient medium containing 3.0 g/l Na-lactate and 0.8 g/l sulfate, added in the form of MgSO₄·7H₂O was used. In the second variant, *distillery stillage*, obtained from bioethanol production, containing sulfate in the range of 0.7–0.9 g/l, was used as an electron donor.

To study the influence of the electron donor in the MSR process, after forming a biofilm of SRB on the zeolite surface, the volumes of the anaerobic reactor with immobilized biomass (0.4 dm³), the anode zone of BES (0.1 dm³) and the buffer vessel (0.3 dm³) were filled with 2 anolyte variants with a pre-made pH adjustment to 7.5: Option 1 – modified Postgate medium containing 3.0 g/l Na-lactate and 0.8 g/l SO₄²⁻, added in the form of MgSO₄·7H₂O and Option 2 – waste

ethanol stillage obtained from the production of bioethanol containing sulfate ions in the range 0.7–0.9 g/l.

All experiments were carried out in periodic mode, for one week each, and after forming the biofilm from the SRB, the bioreactor together with the anode zone of the BES and the buffer tank were filled with anolyte, and the liquid phase was recirculated by peristaltic pumps with a flow rate of 5 l/h in the loop of the anode (I – Fig. 1) and the cathode (II – Fig. 1). The pH value of the anolyte in both anolyte variants (modified medium of Postgate and waste ethanol stillage) was daily adjusted to the pH of 7.5 by 1N NaOH in the buffer volume (position 3 in Fig. 1.).

About the catholyte, a model solution of AMD was used, with chemical composition – Cu²⁺ – 150 mg/l in the form of CuSO₄, Ni²⁺ – 100 mg/l in the form of NiSO₄·7H₂O, Zn²⁺ – 100 mg/l in the form of ZnSO₄·7H₂O, SO₄²⁻ – 4.5 g/l in the form of NaSO₄, the pH of the catholyte was adjusted initially to pH = 2.0 with 1N H₂SO₄. The total volume of the catholyte in the cathode chamber (100 dm³) and the buffer volume (700 dm³) coincide with the total anolyte volume (800 dm³).

Electroconductivity (EC) and pH were measured daily in the anolyte and catholyte. A pH-meter HANNA HI-9021 was used to measure the pH. EC was measured by WTW LF 197-S. The concentrations of sulfates were determined spectrophotometrically – by BaCl₂ reagent at a wavelength of 420 nm and hydrogen sulfide – by using test 1-88/05.09 of “Nanocolor” at a wavelength of 620 nm. The organic substrate utilization was estimated by measuring the chemical oxygen demand (COD). The chemical oxygen demand of each batch was periodically determined and analyzed twice according to standard methods (APHA, 1989). The organic acids and alcohols were analyzed by high-performance liquid chromatography. An Aminex HPX-87H column from Bio-Rad coupled to a RI detector (LC-25RI) was used for HPLC; sulfuric acid (0.01 N) was used as an eluent.

The concentration of sulfates (SO₄²⁻) was measured every 24 hours, both in the cathodic and anodic zones. The removal rate of sulfates in the system was determined against the total concentration in the cathode and anode areas of the BES at the beginning of the experiment, with the approximate starting values in the catholyte and anolyte of sulfates being – 4 500 mg/l and 800 mg/l respectively:

$$C_{\text{TOTAL}}^{\text{SO}_4} = C_{\text{Anode}}^{\text{SO}_4} + C_{\text{Cathode}}^{\text{SO}_4}, \text{ mg/l} \quad (1)$$

$$K_{\text{SO}_4} = \frac{C_{\text{TOTAL}}^{\text{SO}_4}(0 \text{ h}) - C_{\text{TOTAL}}^{\text{SO}_4}(120 \text{ h})}{C_{\text{TOTAL}}^{\text{SO}_4}(0 \text{ h})} \cdot 100\% \quad (2)$$

The electrical parameters of the BES were measured with a Keithley 2110 digital multimeter using a precision potentiometer with a maximum value of 11 k Ω for load resistance. The maximum power value, P_{max} , was measured by constructing polarization curves for each of the studied BES modes. The current and power densities were also calculated based on the geometrical area of the electrodes in the anode/cathode chamber and the voltage on the external resistance (R1 or R2).

The parameters: pH, electrical conductivity, OCV and P_{max} were monitored using a controller type NI SensorDAQ^R and LabView^R. To provide an external voltage source, a stabilized adjustable rectifier type PS-3005D was used when operating BES in microbial electrolysis cell (MEC) mode.

In the mode of operation of BES (Fig. 1) a load resistance R1 = 100 Ω has been applied as a microbial fuel cell (MFC), in which it was previously established that stable fuel cell operation is reached with an optimal value of current density and power. To study the operation of the BES as a microbial electrolysis cell (MEC), an external load resistance R2 = 10 Ω was used, which has a minimum value in order not to limit the current between the anode and the cathode in the BES.

For SEM measurement, the samples were fixed with 2% glutaraldehyde solution in 0.1M phosphate buffer, dehydrated with ethanol, dried with carbon dioxide, and sprayed with carbon dust. Samples of the graphite electrodes were

analyzed by scanning electron microscope (SEM) JEOL/EO version 1.0.

RESULTS AND DISCUSSION

Treatment of AMD in BES operation mode as MFC

In these series of experiments, the operation of the BES as MFC with two different organic substrates for the heterotrophic MSR process, in the bioanode zone over a period of 120 hours was investigated. In the cathode zone electrochemical reduction, in the presence of heavy metal ions (Cu^{2+} , Ni^{2+} , Zn^{2+}) in the catholyte (the model AMD) with free access of the air was researched. According to the obtained results presented in (Fig. 2, Fig. 3 and Table 2), both organic substrates establish an effective course of the MSR process, with the total reduction of sulfates in both chambers of MFCs, when using Postgate medium is 47.53%, and when using ethanol stillage reaches 53.76% in 120 hours (Table 2). The rate of microbial sulfate reduction was commensurate in both studied variants – in the Postgate medium it was in the range 13.96 – 36.46 (mg/l).h, and when using ethanol stillage, it was 21.11–47.81 (mg/l).

In the studies carried out, the COD/ SO_4 ratio was changed in a fairly wide range – from 3.24 to 33.16 – in the Postgate culture medium and from 48.15 to 92.86 – for ethanol stillage. The COD/ SO_4 ratio in ethanol stillage is significantly higher than that in the Postgate medium, which is due to typically high COD values in this substrate (Fig. 2). The obtained values of the power density are in the range of 21.4 – 110.7 mW/m², with the

Table 2. Sulfate removal, sulfate reduction rate, COD/ SO_4 ratio and MFC power density at the used organic substrates for MSR- waste ethanol stillage and modified Postgate culture medium

Hours	SO_4 removal, %		Sulfate reduction rate, mg SO_4 /l.h		COD/ SO_4 *		Power density, mW/m ²	
	Ethanol stillage	Postgate B media	Ethanol stillage	Postgate B media	Ethanol stillage	Postgate B media	Ethanol stillage	Postgate B media
24	16.67	4.95	47.81	13.70	48.15	33.16	21.4	32.3
48	25.87	17.66	26.38	35.21	78.18	9.25	91.6	96.1
72	33.22	22.70	21.11	13.96	92.82	12.99	98.1	103.4
96	45.27	34.36	34.55	32.29	49.74	4.00	100.6	110.7
120	53.76	47.53	24.35	36.46	68.66	3.24	89.2	95.3

Note: * in determining the COD/ SO_4 ratio, the sum of the current concentration in the anolyte and the amount of reduced sulfates from the last 24h in the catholyte (the migrated SO_4 from the catholyte to the anolyte, for the last 24h) was used as the sulfates value.

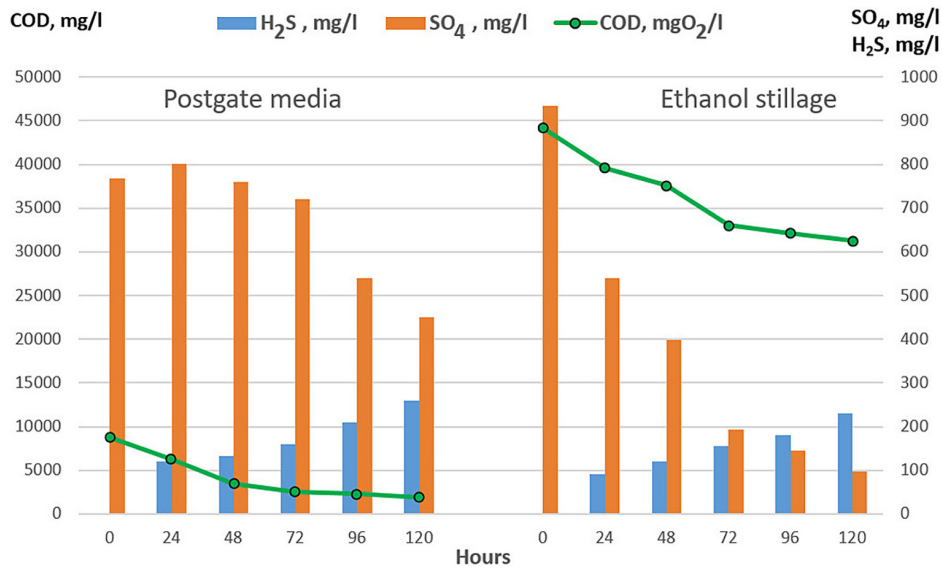


Figure 2. Change of COD, SO₄ and H₂S in analyte by ethanol stillage and Postgate B media using in the MFC mode for a period of 120 h

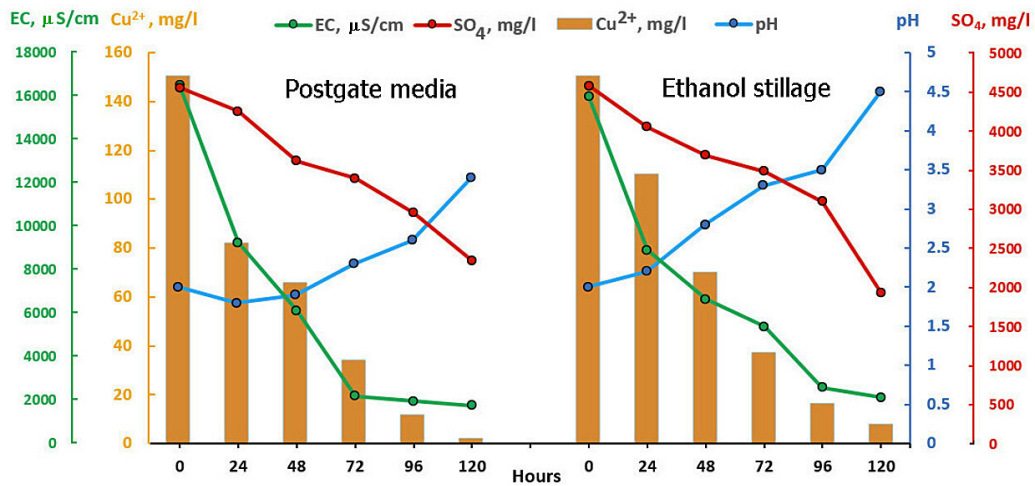


Figure 3. Variation of SO₄, Cu²⁺, pH and electrical conductivity in the catholyte when using ethanol stillage and Postgate B media in MFC mode for a period of 120 h

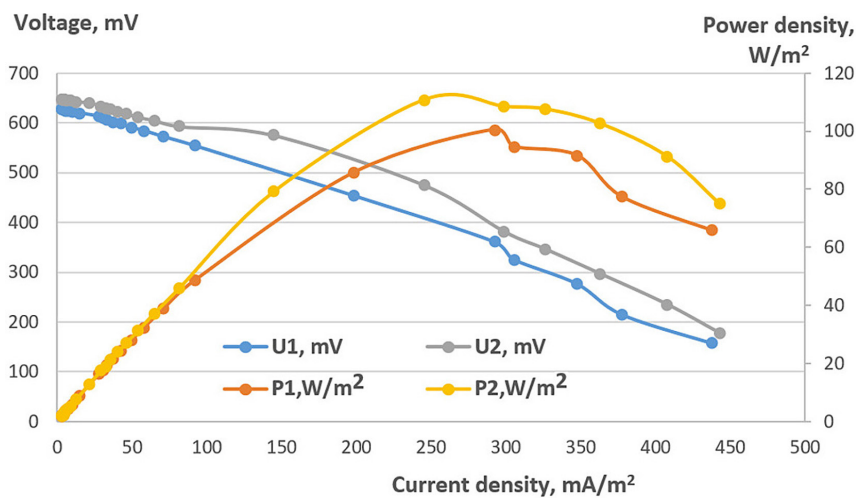


Figure 4. Polarization and power density curves in MFC measured by two electron donor variants for the MSR process – Waste ethanol stillage (U1, P1) and Postgate B media (U2, P2) at 96th hour

highest values being achieved at both organic substrates at 96 h (Table 2).

The theoretically calculated COD/SO₄ ratio showing the amount of COD consumed by SRB to reduce 1 g sulfate is 0.67 (Velasco et al., 2008). For comparison - Zhang et al., 2012, when studying ratios of COD/SO₄ in the range of 2.0 to 6.0, found the highest power density value of 997.1 mW/m² at COD/SO₄ = 2.3 in a combined UASB–MFC system.

Another significant result is related to the significant decrease in the electrical conductivity of the catholyte (in the model AMD waters) in both organic electron donor variants for the MSR process (Table 1), 86.7% for ethanol stillage and 89.4% in Postgate culture medium. This is undoubtedly mainly due to the migration of sulfate ions (Fig. 3) through AEM, and their concentration decreased significantly in the catholyte – 46.4% in ethanol stillage and 48.6% in Postgate medium.

In terms of MFC polarization and power density curves measured at the 96th hour, in both variants (Fig. 4), commensurate maximum current density values are also observed – 292.5 mA/m² (for ethanol stillage) and 298.8 mA/m² (in Postgate's culture medium) and maximum power density values – 100.6 W/m² (for ethanol stillage) and 110.7 W/m² (at Postgate B medium).

In the abiotic cathode zone, in this mode of operation (as MFC), the reduction rate of Cu²⁺ reaches 94.6% (for ethanol stillage) and 98.6% (for Postgate B media), as in terms of Ni²⁺ and Zn²⁺ for both of the studied variants, no decrease of their concentrations in the liquid phase was found, which can be explained by the negative electrochemical potentials of Ni²⁺ and Zn²⁺ (Table 1).

About the results of the HPLC analysis of the anolyte presented in Table 3, it can be concluded that waste ethanol stillage is characterized by a

complex organic composition and in the heterotrophic MSR process in the contour of the anodic zone of BES there is an accumulation of acetate in both investigated variants at the end of the experiment. These results can be explained by the incomplete oxidation of the organic substrate in MSR, where an increase in the concentration of acetate in the medium was found, which has also been commented on in other studies (Zhou et al., 2022; Timmers et al., 2018). The production of acetate in the microbial sulfate reduction process is a major disadvantage in sulfidogenic bioreactors, in which mainly SRB are present, oxidizing incompletely organic substances. The accumulated acetate as a final product in the treated wastewater contributes largely to the residual high COD values (Lens et al., 2002).

Effect of oxygen on the deposition of metals on the BES cathode

To establish the influence of the presence of oxygen in the abiotic cathodic section of the MFC, on the reduction efficiency of Cu²⁺, experiments were carried out in two variants – open-air mode and deaeration of the catholyte by blowing in the buffer volume (6) with N₂, with a flow rate of 1 dm³/h (Fig. 1), the chemical composition of the used catholyte was indicated above. In the anodic zone, waste ethanol stillage was used at COD and sulfate values indicated above.

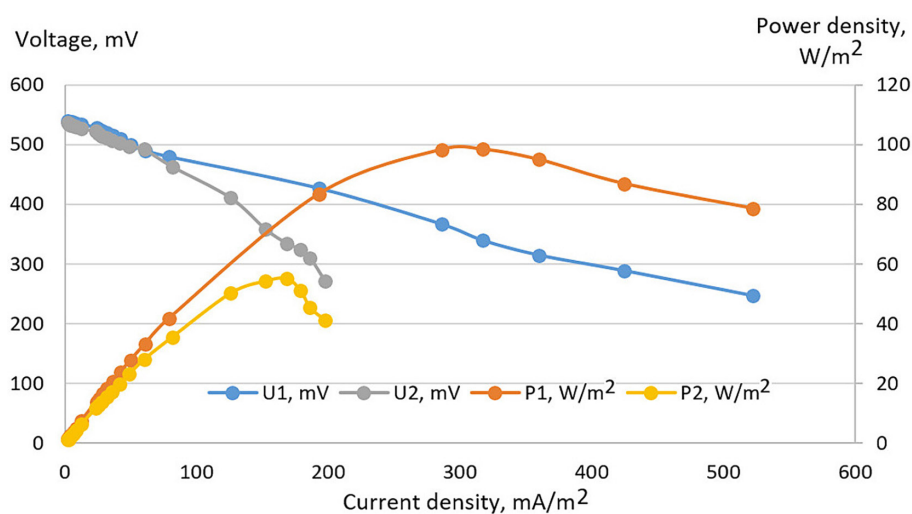
The obtained results (Table 4) showed a substantial influence of oxygen on the electrochemical reduction of Cu²⁺ on the cathode. A significant increase in the rate removal of Cu ions (98.2% at 72nd hour) was found in the deaeration of the catholyte compared to the open-air mode (75.3% at 72nd hour). Simultaneously, a decrease in the migration rate of sulfate ions through the AEM of the BES was also observed. This can be explained

Table 3. Results of HPLC analysis and COD of the anolyte at two studied variants of organic substrates for the MSR process at the beginning (0 h) and end (120 h) of the process

Time	Ethanol stillage		Postgate B media	
	0 h	120 h	0 h	120 h
Xylose (g/l)	10.62	1.43	-	-
Glucose (g/l)	0.52	0.33	-	-
Lactic acid (g/l)	1.13	-	2,97	-
Acetic acid (g/l)	8.65	9.43	-	1.58
Propionic acid (g/l)	3.9	5.17	-	0.90
Butyric acid (g/l)	0.10	0.15	-	0.01
COD (g O ₂ /l)	44.20	31.25	8.72	1.95

Table 4. The dynamics of Cu^{2+} , SO_4 and EC depend on the presence of oxygen in the catholyte of the BES

Hours	Open air mode			N_2 - deaeration		
	EC, $\mu\text{S}/\text{cm}$	SO_4 , mg/l	Cu^{2+} , mg/l (Removal, %)	EC, $\mu\text{S}/\text{cm}$	SO_4 , mg/l	Cu^{2+} , mg/l (Removal, %)
0	15980	4574	150.7	15986	4580	150.7
24	8910	4049	110.2(26.8%)	8994	4133	78.5(48%)
48	6650	3685	70.1 (53%)	6930	3965	23.1 (85%)
72	5390	3484	37.2 (75%)	5556	3650	2.7 (98.2%)
96	2580	2870	16 (89%)	2890	3180	0.3 (99.8%)
120	2130	2450	8.1 (94.6%)	2590	2910	0.2 (99.9%)
Removal, %	86.7	46.4	94.6	83.8	36.5	99.9

**Figure 5.** Polarization and power MFC curves (with ethanol stillage) measured at open-air mode (U1, P1) and aeration with N_2 (U2, P2) of the catholyte at 72 hours

by the lower values of the current density and power of the MFC in the absence of oxygen in the catholyte, which is the preferred electron acceptor. Figure 5 shows the polarization and power curves of the MFC, and it is clear from them the reduction of the maximum values of current density (from $317 \text{ mA}/\text{m}^2$ to $168.5 \text{ mA}/\text{m}^2$) and power (from $2.06 \text{ W}/\text{m}^2$ to $1.16 \text{ W}/\text{m}^2$) under anoxic and aerobic conditions in the catholyte. Analogous results have been reported by Heijne et al., 2010.

Treatment of AMD in BES operation mode as MEC

This series of experiments was carried out over a period of 96 hours, using a modified Postgate culture medium containing $3 \text{ g}/\text{l}$ Na-lactate and $0.8 \text{ g}/\text{l}$ sulfates for the MSR process in the anode zone. The choice of Postgate B media, over Waste Ethanol Stillage, was made to guarantee lower values of the ratio COD/SO_4 , at which higher speeds of MSR can be expected (Celis-García

et al., 2006). Another reason is the found constant decrease in the pH value in the anolyte using Waste Ethanol Stillage, through the period of the experiment, which required daily pH adjustment with 1 N NaOH to 7.5. In the Postgate B media variant, such a pH adjustment was not necessary after the first 24 hours. Changes in pH in the anodic and cathodic chambers of BES negatively affect their performance (Luo et al., 2014a). These pH changes in Waste Ethanol Stillage are probably due to the complex organic composition of this waste (Table 3) and the production of various organic acids, during the MSR process.

In the mode of operation of BES as MEC, 3 different external voltages were applied – 0.6 V , 0.9 V and 1.2 V . In all experiments, the conditions in the cathode space were identical as in the previous experiments, and free access to oxygen in the catholyte (open-air mode) was ensured. Although deaeration with N_2 was found to be a more effective removal of Cu^{2+} (Table 3 and Fig. 6), the open-air mode is preferred, due to the higher rate

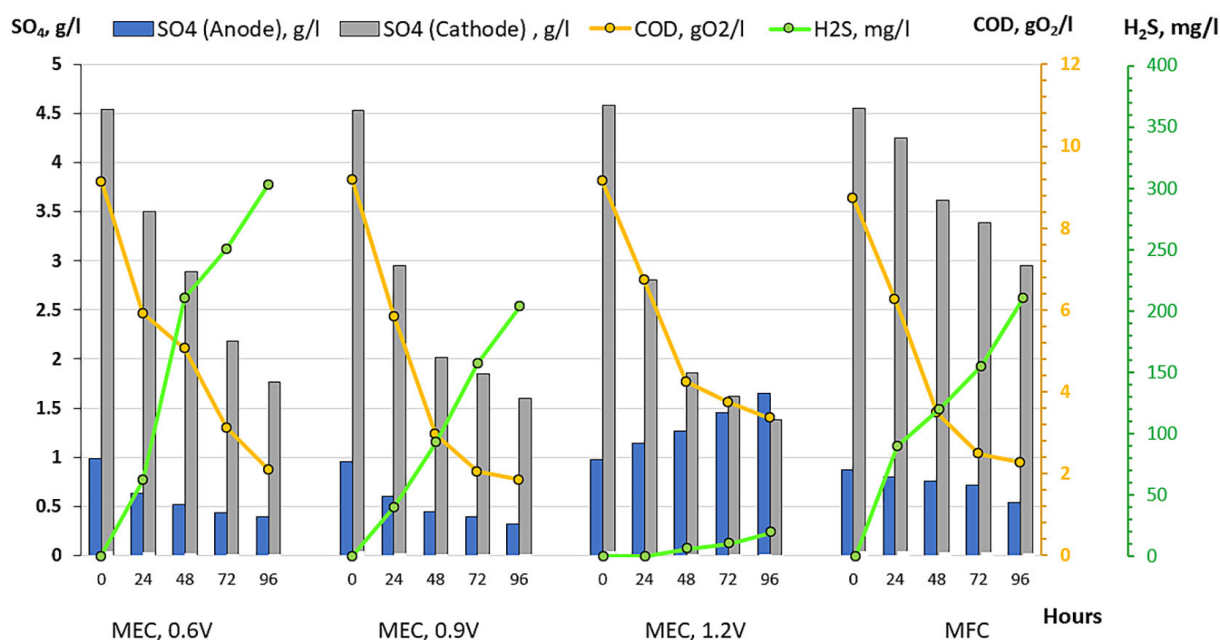


Figure 6. Removal of sulfates (in the anodic and cathodic zones), COD and H₂S values (in the anode zone), at different modes of operation of BES for a time of 96 hours

of sulfate removal (through the AEM migration and subsequent MSR in the anolyte), which appear to be the target contaminant in the AMD. Thus, it is aimed without further limiting the current density across the AEM in the BES (which is observed at deaeration with N₂ in Fig. 5), and conditions are provided for optimal migration of SO₄ in the anode zone. In almost all previous studies (Kumbhar et al., 2021; Tao et al., 2014; Mathuriya & Yakhmi, 2014), special measures for oxygen removal in the cathodic zone have not been taken, which would further increase the cost of technological implementation.

The obtained results (Fig. 6) showed a pronounced tendency of decrease the concentration of sulfate ions in the catholyte with increasing MEC voltage of 0.6 V, 0.9 V and 1.2 V for a time of 96 hours, which is due to their forced migration through the AEM when applying the external voltages in this mode. The rate of removal of sulfates from the catholyte reaches 69.9%, at an external voltage of 1.2V, for comparison at MFC, MEC_{0.6V} and MEC_{0.9V} reached 35.2%, 61.0% and 64.6% respectively. At the same time, the COD reduction in the anolyte reaches the highest degree of 79.9% at MEC_{0.9V}, 76.9% for MEC_{0.6V} and 73.9% for MFC and the lowest value of 63.3% for MEC_{1.2V} respectively. Similar are the results in terms of the reduction of sulfates in the anode zone, as they decrease from 66.6% (at MEC_{0.9V}), 60.6% (at MEC_{0.6V}) and 37.7% (at MFC). Interestingly,

the increase of sulfates in this zone at MEC_{1.2V} reached from 980 to 1650 mg/l. These results show a strong influence on the heterotrophic microbial sulfate reduction process in the bioanode zone by applying higher values of the external voltage. Analogous results were obtained by other authors (Venkata and Lenin, 2011), which found that the metabolism of bacteria was strongly negatively affected by the application of higher voltage values. In support of these results, significantly lower concentration of H₂S in the liquid phase in the anolyte is also observed (Fig. 6) in the MEC mode with an external voltage of 1.2V, in contrast to the other 3 modes (MFC, MEC_{0.6V}, MEC_{0.9V}) where the concentration is approximately one order of magnitude higher.

With regard to the removal efficiency of heavy metals in the catholyte, it could be underlined that the highest degree was achieved in MEC with an external voltage of 1.2V, where a reduction of Cu²⁺– 99.9%, Ni²⁺– 65.9% and Zn²⁺– 64.0% was reached. It is noted that in all variants of MEC with an applied voltage in the range 0.6–1.2 V, was found removal to varying degrees of Cu²⁺, Ni²⁺ and Zn²⁺, while when working BES as MFC, an effective reduction to Cu²⁺ was observed. In the study conducted by Luo et al., 2014, it is noted that in the cathodic zone, the Cu²⁺ are always removed first, and then of Ni²⁺ and Fe²⁺. From Fig. 7 it can be seen that firstly Cu²⁺ are removed and then ions of Ni²⁺ and Zn²⁺.

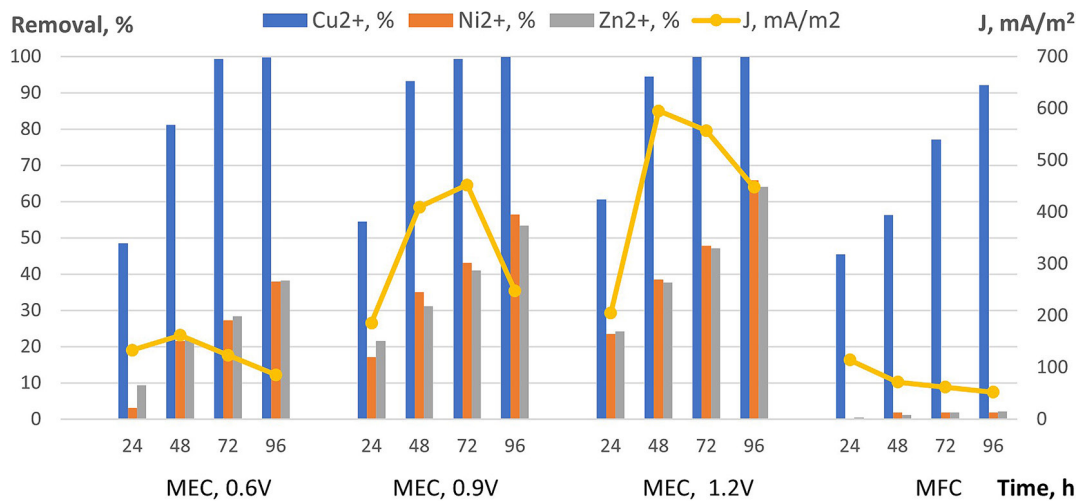


Figure 7. Comparison of the removal rate of Cu²⁺, Ni²⁺, Zn²⁺ and current density (J, mA/m²), in the cathodic zone of BES, under MEC (by 0.6V, 0.9V and 1.2V) and MFC modes

These results could be used to selectively remove Cu²⁺ in MFC mode and other metal ions with a positive reduction potential (present in the AMD) from the others, such as Ni²⁺ and Zn²⁺, in a mode such as MEC. This possibility has also been discussed by Luo et al., 2014a and Leon-Fernandez et al., 2021, and this can be achieved by a suitable combination of MFC and MEC. With regard to the results of the

SEM-analysis, on the surface of the cathode (in a MEC_{0.9V} mode), peaks of Cu are clearly reported, and to a lesser extent of Ni and Zn, deposited on the graphite surface (Fig. 8b). By variant of operation of the BES as MFC for a period of 96 hours (Fig. 8a), mostly observed peaks reporting the presence of Cu only, lacking such for Ni and Zn. These results also support the data presented in Figure 8.

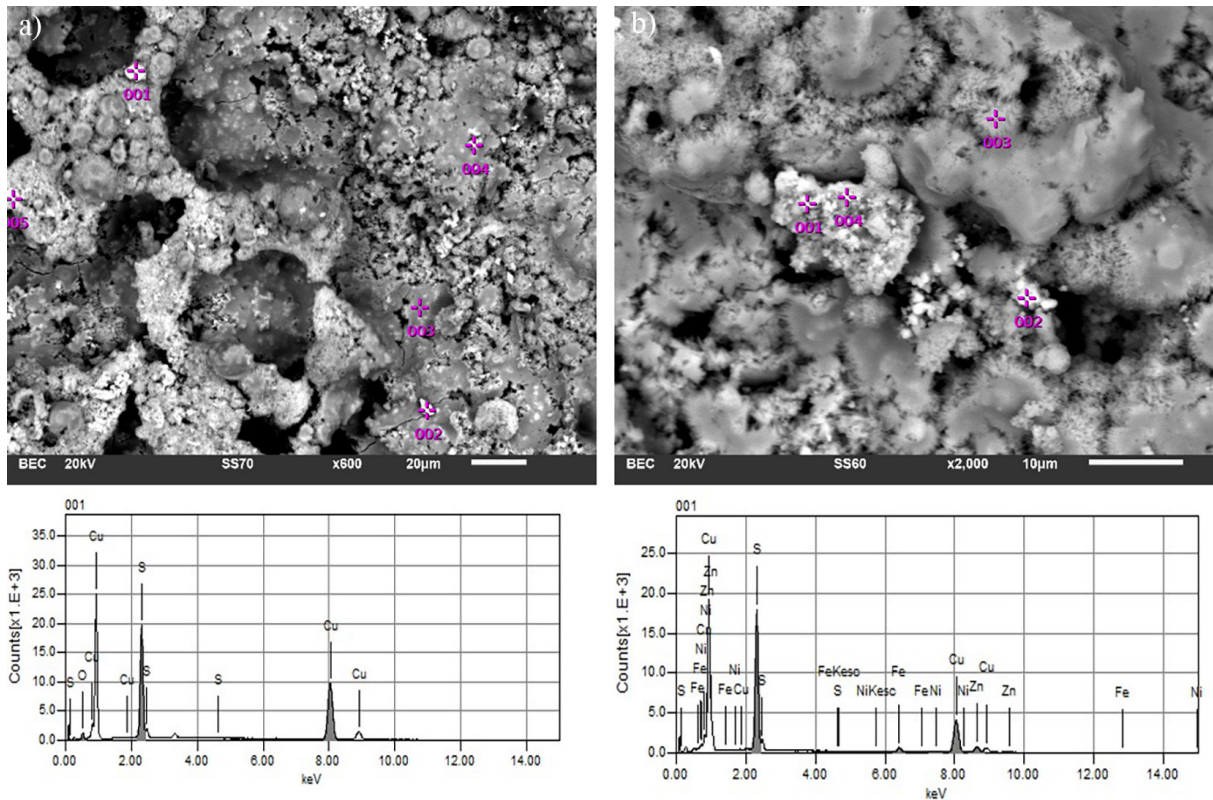


Figure 8. SEM images and EDX analysis of the cathode, after a mode of BES operation as MEC and MFC, a) SEM/EDX analysis of the BES cathode in mode as MFC at 0.9V over a period of 96 h, b) SEM/EDX analysis of the BES cathode in mode as MEC at 0.9V over a period of 96 h

Additional studies in the continuous operation of BES will be useful to establish the optimal migration rates of sulfates from catholyte through AEM compared to the recent MSR in the anolyte and the oxidation of the produced H_2S on the anode surface.

CONCLUSIONS

The present study demonstrated the possibility of simultaneous removal of sulfates and heavy metals from AMD by using BES based on the process of heterotrophic MSR in anode zone. Migration of sulfate ions through AEM of BES from AMD is a successful mechanism for their removal by AMD and their use as an electron acceptor for the heterotrophic MSR process in the bioanode zone. Simultaneously, the reduction of the metals on the cathode surface and oxidation of the produced biogenic H_2S of the anode is realized. In this scheme, simultaneously with the purification of AMD (in regard to sulfates and heavy metals), waste ethanol stillage is utilized, which is an inexpensive and affordable organic substrate for the MSR process, being a prerequisite for easier future practical implementation of the process.

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