



## EFFICIENT TREATMENT OF REAL ACID MINE DRAINAGE BY BIOELECTROCHEMICAL SYSTEM WITH ANODIC MICROBIAL SULFATE REDUCTION

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### ABSTRACT

*The efficiency for the treatment of real acid mine drainage (AMD) from sulfates and heavy metals was investigated in a bioelectrochemical system (BES). In a batch mode, two operational options of the BES were investigated – as a microbial fuel cell (MFC) and as a microbial electrolysis cell (MEC). It was found that the rates of sulfate removal and metal reduction in the cathodic area were achieved to different extents in the two BES modes of operation.*

*In a MFC mode for a time of 96 hours, the rate of removal of sulfates from AMD reached 27.7 %, while in the MEC mode it was 47.8%. Regarding Cu<sup>2+</sup> - in both modes as MFC and MEC the rate of reduction was 69,0% and 95,7 % respectively.*

**Key words:** *bioelectrochemical systems, acid mine drainage, sulfates, microbial sulfate reduction, heavy metals.*

### Introduction

Worldwide there are many current active, closed, abandoned or recovered mining sites. These industries often lead to formation of acid and metalliferous waters. Acid-mine drainage (AMD) is a result of a combination of chemical and biochemical oxidation processes during weathering of metal sulfides in mine wastes - sulfide minerals present in mining wastes (e.g., open pits, mining waste rock, and tailings) are inevitably oxidized to form AMD when exposed to water, air, and chemolithotrophic acidophiles [1]. Acid-mine drainage (AMD) is a serious environmental problem as it is toxic to aquatic life and lead to long-term water and soil pollution [2]. The AMD is a strong acidic wastewater characterized by low pH (between 1 and 4) and high concentrations of dissolved ferrous and nonferrous metal sulfates and other salts, presenting variable concentrations of dissolved metals such as aluminium, lead, cadmium, copper, zinc, manganese, nickel, etc. Such effluents represent a worldwide, challenging environmental problem if left untreated because they can contaminate ground and surface water courses, damaging the health of plants, humans and wildlife. Although, the sulfates by themselves are not extremely harmful pollutant [3], the discharge of excessive loads of sulfate also can seriously affect waters and ecosystems. Therefore, AMD require treatment before their discharge into the environment.

In practice, a way to partially solve this problem is remediation of surface water, groundwater and soil contaminated by AMD through different technologies, both abiotic and biotic methods [3; 4]. The huge variety of conventional technological solutions does not solve the two main problems - the high operating cost and significant amounts of sludge and waste formed.

In general, microbial electrochemical processes have potential in treating acid mine drainage (AMD) as they are relatively new proposed alternative to other conventional management/remediation methods for wastewaters which are highly acidic and rich in sulfate and heavy metals, without the need for extensive chemical dosing [5]. In recent years, bioelectrochemical systems (BESs) have arose as a promising technology for simultaneous wastewater treatment and energy consumption [6] (. They do not require further addition of external sources of alkali, acid or electron donor, but an external source of electricity [7].

In last decade the MFC technology has shown wide application prospects in the field of pollution control and their research grow exponentially each year [8; 9].

However, it was considered that bioelectrochemical systems using biocathodic electro-autotrophic sulfate reducing bacteria are limited by the achievable current density and are therefore restricted to relative low sulfate reduction rates of 0.015–1.87 g SO<sub>4</sub><sup>2-</sup> L<sup>-1</sup> d<sup>-1</sup>, while H<sub>2</sub> based sulfate reducing bioreactors have been shown to reach reduction rates up to 30 g SO<sub>4</sub><sup>2-</sup> L<sup>-1</sup> d<sup>-1</sup> [10].



The possibility of high strength sulfate wastewater treatment using bioelectrochemical systems with a recovery of elemental sulfur and without any external electron donor dosage has been shown [3]. The process was maintained by a mixture of SRB and SOB (mainly *Desulfovibrio* sp., respectively mainly *Sulfuricurvum* sp.) able to reduce sulfate to sulphide and partially oxidize sulfide to elemental sulfur in an autotrophic biocathode. Thus high sulfate reduction rates (up to  $388 \text{ mg SO}_4^{2-} \text{ L}^{-1} \text{ d}^{-1}$ ) were observed linked to a low production of sulfide.

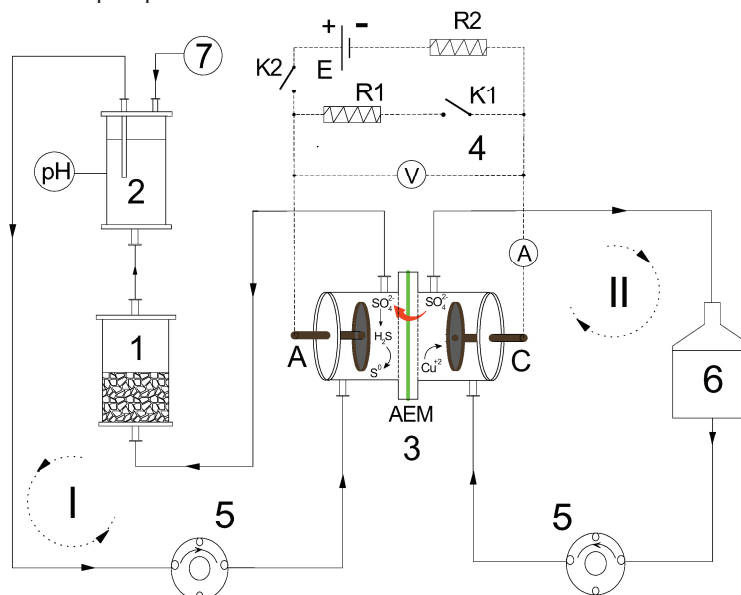
In previous researches it was found that there is an applicable possibility of simultaneous removal of sulfates and heavy metals from AMD through BES based on sulfate reduction in the anodic area with the use of waste ethanol stillage, cheap and affordable organic substrate for the microbial process [11].

The present study is the logical sequel as its general objective was to give more details in the simultaneous removal of sulfates and heavy metals from acid mine drainage in a two-section BES (MECs/MFCs).

### Materials and methods

The experiments were carried out in laboratory installation of a BES, which the possibility of working in two modes - both as MFC and MEC (Fig. 1). For this purpose, a two-chamber design of the BES was used, consisting of 2 cylindrical volumes (each one of  $200 \text{ dm}^3$ ) separated by an anion exchange membrane (AEM) type Fumasep FAM. Before the start of the experiments, the membrane was treated with a solution of  $0.5 \text{ M NaCl}$  at a temperature of  $25 \text{ }^\circ\text{C}$  for 24 h. Graphite cylinders (diameter - 45 mm, width - 6 mm) connected with graphite rods (diameter - 6 mm and length - 48 mm) were used for electrodes. The total geometrical area of each of the electrodes was approximately  $0.0038 \text{ m}^2$ , the distance between them - 15 mm, and respectively the area of the used anion exchange membrane was  $0.0028 \text{ m}^2$ .

The cultivation of sulfate-reducing bacteria (SRB) was carried out in a fixed biomass bioreactor (Fig. 1) with a volume of  $0.7 \text{ dm}^3$ . Approximately half of the bioreactor volume was filled with 0.3 kg of modified zeolite on which a biofilm of SRB and other metabolically related groups of microorganisms formed in time. The characterization and modification of the used natural zeolite and the microbial biofilm formation method have been described in a previous study [12]. The inoculum was a mixed culture of sulfate-reducing bacteria cultivated using lactate as a source of carbon and energy. This microbial consortium has been characterized in detail in a previous study [13]. The sulfidogenic bioreactor was connected to the BES anodic section and a buffer vessel ( $0.4 \text{ dm}^3$ ). The homogenization of the liquid phase with a total volume of  $0.9 \text{ dm}^3$  is ensured by recirculation with a peristaltic pump.



**Fig. 1.** A technological scheme of the laboratory-scaled installation; 1 - bioreactor with immobilized biomass for cultivation of SRB, 2 - Buffer volume for pH correction and electrical conductivity (EC) measurement, 3 - Bioelectrochemical system (MFC/MEC), 4 - load circuit of MEC/MFC, 5 - recirculation peristaltic pumps, 6 - Buffer volume for real AMD, 7 - 1N NaOH solution for pH correction.



As an electron donor for the microbial process of sulfate reduction a distillation residue was used. This waste residue is a complex substrate separated after distillation of alcohol from the fermentation medium with high COD values (35000 - 75000 mgO<sub>2</sub>.L<sup>-1</sup>), which has been found to be successfully utilized in the microbial sulfate reduction process [14]. The sulfate content of the used distillation residue ranged from 0.5 to 0.9 g.L<sup>-1</sup> I, which made it suitable for the process. Nevertheless, in order to reach an optimal COD/SO<sub>4</sub> ratio, during the initial inoculation of the bioreactor, additional sulfates (in the form of MgSO<sub>4</sub>.7H<sub>2</sub>O) were added to the mixture until a concentration of 1.5 g.L<sup>-1</sup> was reached in the medium. In order to adapt the SRB to the distillation residue, pre-cultivation was done in continuous mode with a contact time of 3 days for a period of 1 month in the volume of the bioreactor. After this initial preparation, the volumes in the anodic circuit of the BES (bioreactor, buffer volume and anodic chamber) were filled with fresh distillation waste medium without the addition of extra sulfates. At the same time, the cathodic volumes (cathode chamber - 0.2 dm<sup>3</sup> and buffer volume - 0.6 dm<sup>3</sup>) were filled with acidic mine waters from a real abandoned mine site in central Bulgaria, with a chemical composition indicated in Table 1. The table also shows the Individual emission limitations, which for some of the parameters significantly deviated from the permissible values.

Table 1. Physical and physicochemical parameters of the treated AMD.

Parameter	Acid mine drainage	IEL
pH	2.81±0.29	6-9
EC, µS/cm	4174±179	1800
Eh, mV	318±61	-
SO <sub>4</sub> <sup>2-</sup> , g.L <sup>-1</sup>	2.81±0.28	0.30
Al, mg.L <sup>-1</sup>	4.6±0.4	
As, mg.L <sup>-1</sup>	0.11±0.3	0.1
Ca, mg.L <sup>-1</sup>	478.5±32.2	-
Cu, mg.L <sup>-1</sup>	78.7±9.2	0.1
Fe, mg.L <sup>-1</sup>	10.8±1.1	3.5
Mg, mg.L <sup>-1</sup>	139.2±11.3	-
Mn, mg.L <sup>-1</sup>	4.6±0.6	-
Zn, mg.L <sup>-1</sup>	11.4±1.2	2.0

The experiments in the BES were carried out in a periodic mode of operation, for periods of 5 days each one, with the liquid phase in the BES being continuously recirculated by peristaltic pumps (5) with a flow rate of 5 L/h, both in the anode circuit (I - fig.1) and in the circuit of the cathode (II - fig.1). The pH value in the anolyte was adjusted daily to a pH value of 7.5 by dosing 1 N solution of NaOH into the buffer volume (position 7 in Fig.1.).

Electrical conductivity (EC) and pH were measured daily in the anolyte and catholyte. A WTW 3210 pH meter was used to measure pH. The electrical conductivity was measured using a device type WTW LF 197-S. The concentrations of sulfates were determined spectrophotometrically by BaCl<sub>2</sub> reagent at a wavelength of 420 nm and these of 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 [15]. The concentration of sulfates was measured every 24 hours, both in the cathodic and anodic zones.

The electrical parameters of the BES were measured with a Keithley 2110 digital multimeter, and a precision potentiometer with a maximum value of 11 kΩ was used for the load resistance. The maximum power value, P<sub>max</sub>, was measured by constructing polarization curves for each of the investigated variants. Current density and power were calculated based on the geometric area of the electrodes in the anodic/cathodic chambers and the electric voltage on the external resistance (R1 or R2).

The parameters pH, electrical conductivity, OCV and P<sub>max</sub> were monitored by means of a LabViewR controller. To provide an external voltage source, a stabilized adjustable rectifier type PS-3005D was used



when operating the BES in the microbial electrolysis cell (MEC) mode.

In BES mode of operation as a microbial fuel cell (MFC), a load resistance of  $R1=100 \Omega$  was applied. To investigate the performance of the BES as a microbial electrolysis cell (MEC), it was applied an external voltage (E) of 0.7 V and an external load resistance  $R2=10 \Omega$  was used, which is of a minimum value so as not to limit the current between the anode and the cathode in the BES.

### **Results and discussion**

The main objective of the study is to determine the effects on the AMD generated by a real mining site, when treated in BES under 2 modes of operation (such as MFC and MEC) and to compare them.

The obtained results relate to two parallel processes in the BES – heterotrophic microbial sulfate-reduction in the anodic area with an electron donor waste ethanol stillage and electrochemical reduction of the metals present in AMD in the cathodic zone. The occurrences of these processes is possible thanks to the generated electric voltage between the anode and the cathode in the case of the MFC mode and to the additionally supplied external voltage in the case of the MEC mode. In this way, the forced transfer of anions through the separator (anion exchange membrane - AEM) is also realized, as in the case of AMD, it turns out to be mainly sulfate ions. Once passed through the AEM from the cathodic to the anodic area, the sulfates enter into a zone of active microbial sulfate-reduction, where they are very quickly reduced to  $H_2S$  (at pH approximately 7.5) in the water-soluble form as  $HS^-$ . On the other hand, the produced  $H_2S$  acts as a mediator in the electron transfer to the surface of the anode, being oxidized, depending on various factors, to forms of sulfur with a higher valence, including elemental sulfur [16].

In this series of experiments, the performance of BES was investigated in two modes of operation – as a microbial fuel cell (MFC) and as a microbial electrolysis cell (MEC) in a batch mode, using a distillation residue (waste alcohol stillage) for the microbial sulfate reduction process for a period of 96 h.

Table 2. Dynamics of technological parameters in BES anolyte under MFC/ MEC operating mode for 96 hours.

	Parameter	0 h	24 h	48 h	72 h	96 h
MFC mode	pH	7.77	6.43	7.86	6.88	8.33
	EC, $\mu S/cm$	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
	$SO_4$ , $mg.L^{-1}$	974	543	218	398	140
MEC mode	$HS^-$ , $mg.L^{-1}$	10.4	87.2	132.7	215.3	178.4
	pH	6.89	7.21	7.43	7.56	7.57
	EC, $\mu S/cm$	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
	$SO_4$ , $mg.L^{-1}$	331	426	188	93	65
$HS^-$ , $mg.L^{-1}$	12.5	4.2	4.1	3.8	2.8	

The obtained results (table 2 and 3) can be commented regarding the ongoing processes in the anodic and cathodic zones of the BES in the two modes of operation such as MFC and MEC.

In MFC, in the anodic chamber (table 2) for a period of 96 hours, a transfer of sulfate ions from the cathode zone was established, which was evidenced by their reduction in the anodic area. The oxidation-reduction potential (ORP) at all modes was in the range of -384 to -409 mV, indicating strongly reducing conditions in the medium. The sulfate concentration decreased gradually from  $974 mg.L^{-1}$  (day 1) to  $140 mg.L^{-1}$  (day 5), indicating that the rate of microbial sulfate reduction was higher than the rate of sulfates migration through the AEM. The equalization of these two rates, under continuous operation mode, would lead to the optimization of the MSR process in the BES. At the same time, the concentration of  $HS^-$  in the medium also increased (from 10.4 to  $215 mg.L^{-1}$ ), which showed that the anodic current is not sufficient to oxidize the mediator ( $HS^-$ ) in electron transfer to a sufficient extent on the anodic surface. That means that for a higher



efficiency it would be necessary to take actions to reduce the total (internal and external) load resistance of the BES.

The behavior of BES under MEC mode in the anodic area was also interesting - the concentration of hydrogen sulfide and sulfates significantly decreased ( $\text{HS}^-$  from 12.5 to 2.8  $\text{mg.L}^{-1}$ ), so did the sulfates (from 331 to 65  $\text{mg.L}^{-1}$ ). In addition, the COD value in this mode also showed a greater decrease (up to 37.4  $\text{mg.L}^{-1}$ ) compared to the MFC variant (up to 40.1  $\text{mg.L}^{-1}$ ). These results showed an increase in MSR rate with the MEC variant compared to the MFC.

Table 3. Dynamics of technological parameters in BES catholyte under MFC/ MEC operating mode for 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, $\mu\text{S/cm}$	4284	3658	3136	2928	2605
	ORP, mV	250	210	225	204	229
	$\text{SO}_4$ , $\text{mg.L}^{-1}$	2752	2596	2363	2129	1989
	Cu, $\text{mg.L}^{-1}$	77.7	66.3	50.6	35.5	24.1
	Zn, $\text{mg.L}^{-1}$	10.2	-	9.19	-	9.03
	Fe, $\text{mg.L}^{-1}$	10.8	-	10.5	-	10.4
	Mn, $\text{mg.L}^{-1}$	4.6	-	4.5	-	4.5
MEC mode	Al, $\text{mg.L}^{-1}$	4.5	-	4.4	-	4.4
	pH	2.81	4.14	4.74	4.97	5.69
	EC, $\mu\text{S/cm}$	4272	3590	3055	2650	2118
	ORP, mV	229	240	206	203	178
	$\text{SO}_4$ , $\text{mg.L}^{-1}$	2797	2370	2205	1910	1459
	Cu, $\text{mg.L}^{-1}$	77.3	56.3	40.2	22.1	3.3
	Zn, $\text{mg.L}^{-1}$	10.4	-	7.4	-	4.3
	Fe, $\text{mg.L}^{-1}$	10.8	-	10.5	-	0.5
MEC mode	Mn, $\text{mg.L}^{-1}$	4.5	-	4.4	-	4.4
	Al, $\text{mg.L}^{-1}$	4.5	-	3	-	0.6

The dynamics of the technological parameters in the cathodic chamber of the BES in the two modes of operation also support the results presented above. As for the period of the experiment (96 hours) in MFC, the removal rate of sulfates from AMD reached 27.7 %, while in MEC mode it was 47.8 %. Regarding the heavy metals present in the composition of AMD - in a mode of MFC, the rate of reduction of  $\text{Cu}^{2+}$  is 69.0 %, while in relation to the other metals - no significant reduction was observed, respectively in a mode of operation such as MEC –  $\text{Cu}^{2+}$  - 95.7 %  $\text{Zn}^{2+}$  - 58.7 %,  $\text{Al}^{3+}$  - 86.7 % and  $\text{Fe}^{2/3+}$  - 95.4 %. For  $\text{Mn}^{2+}$ , no change was detected in both investigated variants.

These results can be explained by two mechanisms affecting the state of the metals in the treated mine waters. The first mechanism is related to the electrochemical potential of the metals, and the second for the change in the pH value of the medium. Heavy metals typical for AMD (such as Fe, Cu, Ni, Zn, Cr, Cd, etc.) have been found to be reduced and deposited on the cathodic surface in MECs/MFCs [17]. Metals with a positive red-ox potential such as  $\text{Cu}^{2+}$ ,  $\text{Cr}^{4+}$ , and  $\text{Co}^{2+}$  are successfully deposited on the MFC cathode. While for metals with lower reduction potentials, such as  $\text{Cd}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Zn}^{2+}$ , an additional potential is required to be applied, through an external voltage, which has been demonstrated in MEC [18].

In the present study (table 3), under the MFC mode of operation, only  $\text{Cu}^{2+}$  (69.0 %) was effectively removed, and in the MEC mode, in addition to  $\text{Cu}^{2+}$  (95.7 %), partly  $\text{Zn}^{2+}$  - 58.7 % and to a greater extent  $\text{Al}^{3+}$  - 86.7 % and  $\text{Fe}^{2/3+}$  - 95.4%. The removal of  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  was probably due to their electrochemical deposition on the cathode with (in the case of MEC) and without (in the case of MFC) an additional applied external voltage, while in the case of  $\text{Al}^{3+}$  and  $\text{Fe}^{2/3+}$  it was apparently due to their precipitation in the form of hydroxides with an increase of pH in the MEC variant. Regarding  $\text{Mn}^{2+}$ , no change was found in both investigated options. In the conducted experiments, both versions of the BES operation showed an increase in the pH value, with





the MFC reaching 3.68 and the MEC - 5.69. The effect of increasing pH in the treated AMD in BES has also been observed in other studies [7] and can also be considered as a positive impact in wastewater treating.

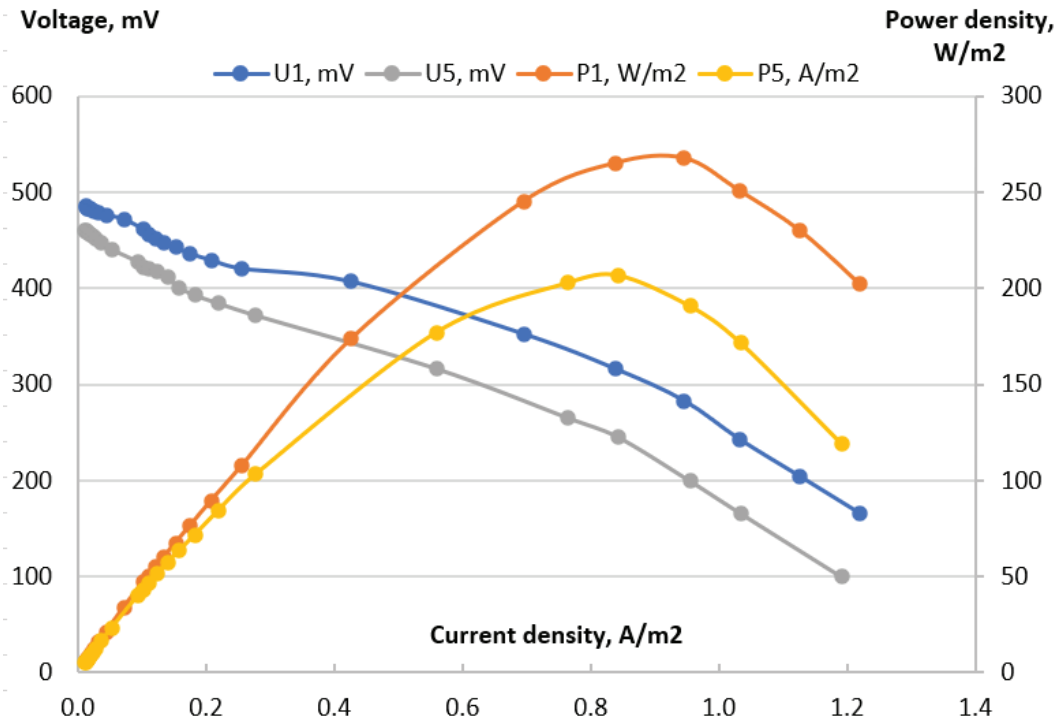


Fig.2. Polarization and power density curves in MFC mode, measured in 1st and 5th day of the experiment.

Regarding the variation of the electrochemical parameters in the BES, it is important to note the change of the maximum values of the power density and current at the beginning and at the end of the experiment. This is evident from the polarization and power curves (Fig. 2) measured on the first and fifth day of the experiment in the MFC mode of operation. Accordingly, the maximum power value decreased  $P_1=268 \text{ W.m}^{-2}$  to  $P_5=207 \text{ W.m}^{-2}$ , and these power values were recorded at correspondingly lower current density values -  $I_1=0.9 \text{ A.m}^{-2}$  and  $I_5=0.8 \text{ A.m}^{-2}$ . These changes were due to various factors – changes in pH and electrical conductivity in the anolyte and catholyte, changes in the concentration of  $\text{H}_2\text{S}$  in the anolyte, influence of the type and concentration of the electron acceptor in the cathode chamber (oxygen,  $\text{Cu}^{2+}$ , etc.) and others.

### Conclusions

The removal efficiency for sulfates and heavy metals when treating real acid mine drainage in a bioelectrochemical system under a batch mode was investigated. The studied BES was based on the process of heterotrophic microbial sulfate-reduction (MSR) in the anodic are, using distillation residue as an electron donor for the process. The treated acid mine waters were characterized by high acidity (pH of 2.8), significant concentrations of sulfates -  $2.8 \text{ g.L}^{-1}$  and heavy metal ions ( $\text{Cu}^{2+}$  -  $78.7 \text{ mg.L}^{-1}$ ,  $\text{Zn}^{2+}$  -  $11.2 \text{ g.L}^{-1}$ ,  $\text{Fe}^{2/3+}$  -  $10.8 \text{ g.L}^{-1}$ ,  $\text{Mn}^{2+}$  -  $4.6 \text{ g.L}^{-1}$ , etc.). Two operating modes of the BES were investigated – as a microbial fuel cell (MFC) and as a microbial electrolysis cell (MEC). It was found that for 96 hours, the sulfate removal rate was 27.7% for MFC mode, while in the MEC mode it was 47.8%. The reduction of  $\text{Cu}^{2+}$ , 69.0 % and 95.7 %, was found in MFC and MEC regimes respectively. Regarding the remaining heavy metals, their removal was achieved to a different rates, depending on the media conditions in the two modes of operation of the BES.

Based on the obtained results, it could be concluded that the use of BES for the treatment of AMD is an effective method by which such wastewater could be successfully treated, used for the selective extraction of various metals, and combined with traditional chemical and physicochemical methods. The use of BES also



has additional advantages because it is a reagent-free technology in which an additional amount of energy is also obtained.

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