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ANALYSIS OF THE EFFICIENCY OF MICROBIAL FUEL CELLS BASED ON SULFATE-REDUCTION PROCESS, INTEGRATED IN ANAEROBIC WETLANDS

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Abstract: Microbial fuel cells (MFCs) are systems in which microorganisms, using various energy sources (mainly organic compounds, but also inorganic substrates such as hydrogen sulfide, ferrous or ammonium ions) convert this energy directly into electricity. This process is accomplished as the electrons that are detached from the donor at its oxidation are transferred to an insoluble anode of the fuel cell instead to the relevant natural acceptor (oxygen, sulfates, ferric ions, nitrates). In MFCs based on microbial sulfate-reduction process, the electricity generation is related to the reduction of sulfates to hydrogen sulfide, which plays the role of a mediator and on the anodic surface it is oxidized to elemental sulfur. In most cases, at the continuous operational mode of the microbial fuel cell, the elemental sulfur is accumulated on the surface of the electrode or in the area around it and this leads to deterioration of the electrochemical characteristics of the cell. In this work three MFCs based on the process of microbial sulfate-reduction were tested for a period of over 18 months, as they were integrated in anaerobic wetlands for mining wastewater treatment. The influence of the formed microbial biofilm consisting of diverse microflora and products of the microbial metabolism and of chemical and electrochemical reactions (insoluble sulfides and elemental sulfur) on the efficiency of various components of MFCs was established.

INTRODUCTION

Wetlands, both artificial and natural, propose a cheaper alternative technology for mining wastewater treatment. The artificial wetlands are designed to optimize the occurrence of natural biogeochemical processes related to the removal of typical for mining wastewater contaminants as heavy metals, metalloids and sulfates. The anaerobic wetlands are hydro isolated facilities with a depth of more than 1 m. At the bottom of the anaerobic wetland a layer of limestone is lead, and upon it - organic matter (a mixture of mushroom compost, cow manure, straw, sawdust and soil). (Liu et al. 2015) The flow of treated water is passing through the substrate and it may be horizontal or vertical. In the substrate rich of organic matter anaerobic conditions that facilitate the occurring of the process of microbial sulfate-reduction are established (Vymazal et al., 2008). The generated H_2S, one of the final products of the process of sulfate-reduction, is highly reactive and precipitates heavy metals in the form of insoluble sulfides. Pollutants such as iron, copper, zinc, cadmium, nickel, arsenic, uranium and others are precipitated in anaerobic wetlands. An alkalinity is generated as a result of the bacterial activity and the limestone dissolution in anaerobic conditions. Due to the combination of various chemical, physical and biological processes, mining waters with pH in a wide range (acidic, neutral and alkaline water) can be treated through anaerobic wetlands (Johnson et al., 2005).

Energy shortage and environmental pollution are the two severe challenges that humanity is facing today. The current energy resource structure is unsustainable and the pollution control methods are mostly high energy consuming. Microbial fuel cells (MFCs), which can recover renewable energy from waste organic sources and convert chemical energy into electrical energy during wastewater treatment have drawn great attention of scientists and researchers (Zhou et al. 2013). Numerous studies on the efficiency of the microbial fuel cell have been conducted, based on the process of microbial sulfate-reduction (Rabaey et al., 2005). This type of fuel elements allows simultaneous removal of sulfates from wastewater with electricity production. In the anodic chamber the sulfate-reducing bacteria oxidize various organic compounds (lactate, acetate, butyrate or other fermentation products) by reducing the sulfate anions to hydrogen sulfide. It is known for this type of biological fuel cell that additional mediators are not necessary, as the microbially produced hydrogen sulfide plays such a role. The latter is oxidized on the surface of the anode to elemental sulfur. When closing the electrical circuit, the path of electrons is directed toward the cathode (Lee et al., 2012). Released protons pass through the proton-selective membrane and also reach the cathodec area. $KMnO_4$, $K_3[Fe(CN)_6]$ or $K_2Cr_2O_7$As can be used as an electron acceptor in this zone. The final electron acceptor is oxygen in the air, as it is reduced in the cathode area, and together with the hydrogen cations forms water. At a continuous operational mode of MFC, the elemental sulfur is accumulated on the surface of the electrode or in the area around it, as that leads to a deterioration of the electrochemical characteristics of the cell (Angelov et al., 2013).

For the purpose of this work an analysis of the operation of three MFCs based on the process of microbial sulphate-reduction is performed, integrated in anaerobic wetland, as it is estimated the impact of the changes due to the exploitation on their efficiency.

MATERIALS AND METHODS

Design of microbial fuel cells, incorporated into anaerobic wetland

The anaerobic wetland consists of three cascade connected anaerobic cells - cuboidal containers with length 190 mm, width 150 mm and height of 560 mm (Figure 1). The container was filled with mixture of 4.5 kg solid organic matter (cow manure, hay and sawdust in the ratio 4:1:1) and 2 kg limestone (particles size 5 – 10 mm). Water flows into the anaerobic wetland through a PVC tube (50 mm diameter) reaching the bottom of the container. Thus, an upstream of the treated water was provided and the volume of the substrate is optimally utilized. The effluent water passed through a perforated PVC pipe (50 mm diameter) placed in a depth of 10 cm in the substrate. This tube represents the MFC anodic chamber. Cathodic chamber is a cylinder with a volume of 0.06 dm^3, submerged in the volume of the anodic chamber. The two chambers are separated with 0.0007 m^2 proton exchange membrane (CMI-7000S, Membrane International Inc.). Carbon rods with diameter of 8 mm and length of 90 mm are used as electrodes. The surface area of each electrode is 0.0024 m^2. The cathode chamber is filled up with 100 mM $K_3[Fe(CN)_6]$ in 67 mM phosphate buffer with pH 7.0.

Each anaerobic cells was filled with 10 l solution, containing (in g/l): $MgSO_4.7H_2O$ – 3,85, and Na_2SO_4 – 0,74 and was inoculated with mixed cultures of sulfate-reducing bacteria obtained from laboratory sulfidogenic bioreactor for mine water treatment. The number of sulfate-reducing bacteria was $5{,}0.10^7$ cells/ml. The inoculum contains species, belonging to the genera *Desulfotomaculum*, *Desulfovibrium*, *Desulfomicrobium* and *Desulfobacterium*, identified on the basis of their morphological and biochemical characteristics according to Bergey's manual after isolation of pure cultures.

Polluted waters containing Cu – 60 mg/l ($CuSO_4.5H_2O$) and SO_4^{2-} – 2 g/l ($MgSO_4.7H_2O$ and Na_2SO_4), pH 4,5 were treated in the above-mentioned anaerobic wetlands under continuous-flow conditions.

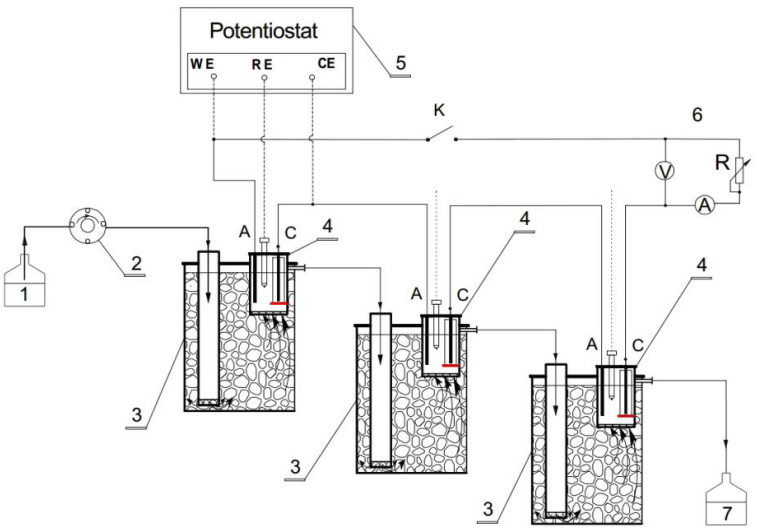


Fig. 1 *1 – Solution of wastewater; 2 – Peristaltic pump; 3 – Anaerobic cell; 4 – MFC; 5 – Potentiostat; 6 – Multimeter. 7 – Outflow container; 8 – PVC tube; A – Anode; C – Cathode; K – switch; R – Resistance;*

Analytical methods

The sulfate concentration was determined spectrophotometrically with $BaCl_2$ (Adams and Dean, 1990). The total sulfide concentration was measured spectrophotometrically at 620 nm immediately after sampling using Nanocolor test 1-88/05.09. The dissolved metals concentrations were determined by ICP (Inductively Coupled Plasma) spectrometry. The concentration of organic substrate was estimated by measuring the chemical oxygen demand (COD) as the method corresponds to DIN ISO 15715.

Quantification of different groups of microorganisms were determined by standard microbiological techniques. The samples were diluted tenfold according to the method of serial dilutions. Count of viable microbial cells was performed by the plate or liquid media count methods. Aerobic heterotrophic bacteria were counted by plating on agar, in three replicates for each dilution. A three-tube most-probable number technique was applied for estimation of the number of anaerobic heterotrophic bacteria, bacteria fermenting sugars with gas production, cellulose-degrading microorganisms, sulfate-reducing bacteria, denitrifying bacteria, Fe^{3+}-reducing bacteria, Fe^{2+} - oxidizing bacteria, pH 7,0, colorless sulfur bacteria, $S_2O_3^{2-}$oxidizing bacteria, pH 7,0 and nitrifying bacteria (Table 1).

Table 1. Media, used for quantification of different groups of microorganisms

Physiological groups of microorganisms	Medium
Aerobic heterotrophic bacteria	Nutrient Agar
Anaerobic heterotrophic bacteria	Nutrient broth + liquid paraffin
Bacteria fermenting sugars with gas production	Nutrient broth + glucose +liquid paraffin
Cellulose-degrading bacteria	Hutchinson and Clayton
Sulfate-reducing bacteria	Postgate B
Denitrifying bacteria	Giltay
Fe^{3+} - reducing bacteria	Bromfield
Fe^{2+} - oxidizing bacteria, pH 7,0	Ulf
Colorless sulfur bacteria	Borgard
$S_2O_3^{2-}$—oxidizing bacteria, pH 7,0	Starkey
Nitrifying bacteria	Saratchandra

MFC was monitored with a portable digital multimeter Keithley Model 175. A precise potentiometer with maximum value of 13.5 kΩ was used for measuring of external resistances. A potentiostat - ACM 3 connected to PC (Personal Computer) for reporting and analysis of the accumulated data was used for the establishment of the system electrochemical behavior.

RESULTS AND DISCUSSION

A stable microbial community was established in the cells within one month of cultivation at 20 – 22 ^{o}C under batch conditions. The data presented in Table 2 show the composition of microflora in the liquid phase of anaerobic cells.

Table 2. Composition of microflora in the liquid phase of anaerobic cells

Physiological groups of microorganisms	Anaerobic cell 1	Anaerobic cell 2	Anaerobic cell 3
Aerobic heterotrophic bacteria	10^4 - 10^5	10^4 - 10^6	10^5 - 10^6
Anaerobic heterotrophic bacteria	10^5 – 10^6	10^4 – 10^6	10^5 -10^7
Bacteria fermenting sugars with gas production	10^2 – 10^3	10^2 – 10^4	10^3 – 10^4
Cellulose-degrading bacteria	10^2 – 10^3	10^2 – 10^4	10^3 – 10^4
Sulfate-reducing bacteria	10^6 – 10^8	10^5 – 10^7	10^6 – 10^8
Denitrifying bacteria	10^5 – 10^6	10^4 – 10^5	10^5 – 10^7
Fe^{3+} - reducing bacteria	10^3 – 10^5	10^3 – 10^5	10^4 – 10^6
Fe^{2+} - oxidizing bacteria, pH 7,0	10^2 – 10^4	10^1 – 10^4	10^2 – 10^3
Colorless sulfur bacteria	10^4 – 10^5	10^4 – 10^5	10^4 – 10^6
$S_2O_3^{2-}$—oxidizing bacteria, pH 7,0	10^5 – 10^6	10^5 – 10^7	10^5 – 10^7
Nitrifying bacteria	10^1 – 10^3	10^2 – 10^4	10^2 – 10^3

The data presented in Table 3 shows that in the first anaerobic cell there was a complete removal of copper due to biogenic hydrogen sulfide. The average rate of microbial sulfate-reduction was 190.5 ± 12 mg SO_4^{2-}/l.d at the second month since the beginning of the experiment. Hydrogen sulfide was in excess and its effluent concentrations were in the range 129±23 mg/l. In this initial period of operation of the installation the measured OCV (Open Circuit Voltage) of MFC in the anaerobic cell 1 was 531 ±35 mV. The table also shows that as a result of bicarbonate ions, generated by sulphate-reducing bacteria, pH increased from 5.5 to 7.47.

The estimated rate of microbial sulfate reduction at the same period in anaerobic cell 2 was 85.7±9 mg SO_4^{2-}/l.d, and this in the anaerobic cell 3 was 43.8 ±8 mg SO_4^{2-}/l.d. The decrease of the process rate was due to the lower concentrations of sulfates, supplied to the input of each subsequent cascade cells. The concentration of hydrogen sulfide in the water was sufficient to provide a potential in the second and third MFC, respectively 641±40 and 647±30 mV.

Table 3. Basic parameters of the effluents from the subsequent cascade cells in the initial phase of the experiment

Facility	**pH**	**Copper, mg/l**	**Sulfates, mg/l**	**COD, mg/l**	**H_2S, mg/l**	**Voltage, mV**
Solution	5,5±0,03	60±2,98	2000±15	-	-	-
Anaerobic cell 1	7,47 ±0,05	<0,004	857±276	1150±20	167±24	634 ±50
Anaerobic cell 2	7,53 ±0,04	<0,004	283±152	1164±25	125±19	641±40
Anaerobic cell 3	7,49 ±0,06	<0,004	20±12	1172±20	45±21	647±30

At this initial moment of the installation operation (two months after the charge) in the liquid phase of the three anaerobic chambers high values of COD (above 1150 mg/l) were measured with a tendency to increase in each subsequent camera (Table 3). This result is related to the occurring of various reactions of hydrolysis of high molecular fast degradable organic polymers and to the fermentation of the resulting sugars and amino acids.

After 12 months of operation of the anaerobic passive system the main technological parameters of the three anaerobic cells were measured again (Table 4). The choice of the period was related to the fact that the fast degradable organic polymers had been depleted, the system had passed in dynamic equilibrium and the enriched liquid phase with low-molecular organic compounds was based on the transformation of slowly degradable under anaerobic conditions macromolecules.

Lower concentrations of dissolved organic compounds in water (COD in the range 413-462 mg/l) compared to the first months of operation of the plant were a prerequisite for a significant reduction in the rate of sulfate-reduction. The estimated rate of the process after one year of operation of the installation was in the range of 27.5 – 38.8 mg SO_4^{2-}/l.d.

Table 4. Basic parameters of the effluents from the subsequent cascade cells after 12 months of operation

Facility	pH	Copper, mg/l	Sulfates, mg/l	COD, mg/l	H_2S, mg/l	Voltage, mV
Solution	5,5±0,03	60±3,56	2000±15	-	-	-
Anaerobic cell 1	7,53 ±0,03	<0,004	1690±21	413±15	91±9	566±11
Anaerobic cell 2	7,61 ±0,04	<0,004	1390±18	441±25	83±13	466±26
Anaerobic cell 3	7,58 ±0,03	<0,004	1170±32	462±10	72±11	252±23

Figures 2 and 3 present the basic electrical parameters of the integrated fuel elements at the beginning of the experiment and after 12 months of work. It is obvious from the presented figures that in the first months of operation of the anaerobic passive system MFC 3 was characterized with the best performance, followed by MFC 1 and finally MFC 2. In MFC 3 a maximum power density of 101,1 mW/m^2 at a current density 267,1 mA/m^2, was established, obtained by applied resistance 90 Ω

The differences in the electrical parameters of fuel cells were mainly due to the specifics of the chemical composition of solutions flowing over the anode. The established lower residual concentrations of hydrogen sulfide in the effluent from chamber 3 were probably related to intensive processes of oxidation of hydrogen sulfide to elemental sulfur on the surface of the anode in MFC 3. Also, the highest values of COD were measured there (Table 3).

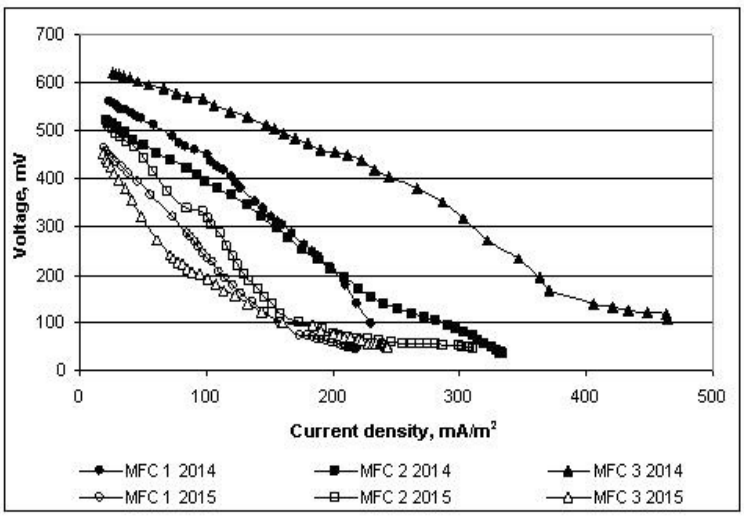


Fig. 2. *Comparison of OCVs generated in the three MFCs in the beginning of the experiment and after one year of operation*.

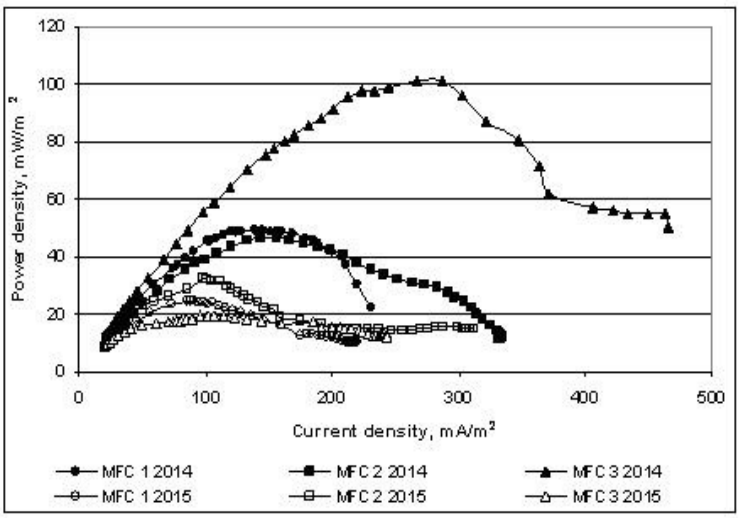
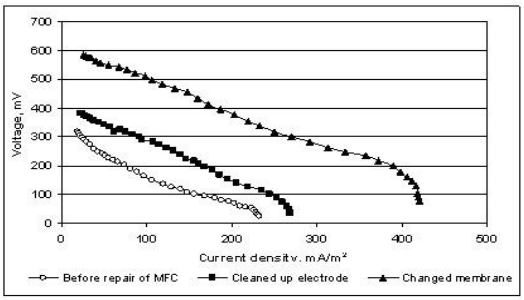


Fig. 3. *Comparison of power densities of the three MFCs in the beginning of the experiment and after one year of operation.*

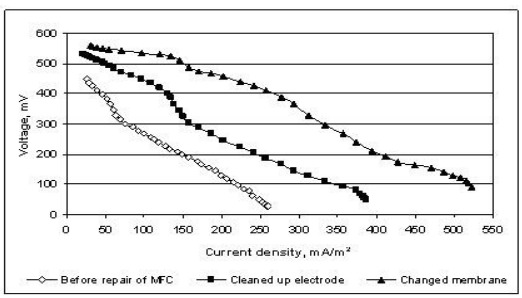
In all integrated MFCs a deterioration of operation was established - as a reduction of power densities and of generated voltages. In MFC 2 a maximum power density of 32,32 mW/m^2 at a current density 97.44 mA/m^2 was established, obtained by applied resistance 100 Ω.

This was due to various factors, affecting the efficiency of microbial fuel cells, such as reducing the concentration of dissolved organic matter in the anode area, retardation of the rates of the microbial sulfate-reduction and hence the amount of generated hydrogen sulfide, playing the role of a mediator, passivation of the anode from accumulated elemental sulfur, deterioration of membrane permeability from accumulated biofilm, aging of the solution in the cathodic area etc.

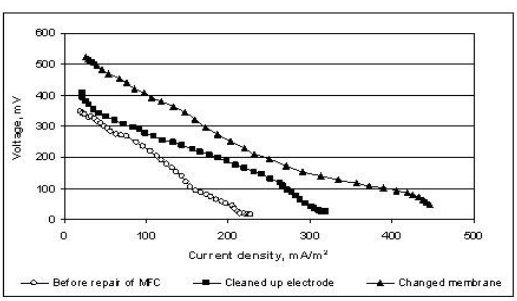
An experiment for the establishment of which exact changes in the MFC components had the strongest influence on electrochemical parameters of the system was performed. For this purpose parameters before MFCs repair were sequentially monitored, after the cleaning of the electrodes, and after replacement of the membrane. Figure 4 presents a comparison of the voltages in the three MFCs before and after the repairs.



MFC 1



MFC 2



MFC 3

Fig. 4. Comparison of voltages from polarization curves of MFCs

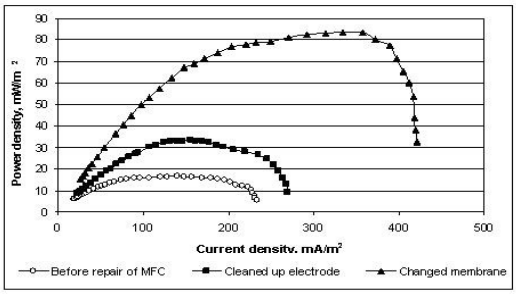
From Figure 4 it can be seen that the membrane replacement had the most significant effect on the voltages. The most presumptive reason for the reduction in efficiency of MFCs was that at a continuous operation a microbial biofilm was forming on the membrane, containing diverse microflora and products from microbial metabolism, and products of chemical and electrochemical reactions (insoluble sulfides and elemental sulfur). The formed biofilm significantly reduced the permeability of the cation-exchange membrane, as that negatively affected the parameters of the fuel elements. The replacement of the membranes of MFCs led to an increase of the voltages in all three fuel elements.

The best results were obtained for MFC, integrated in anaerobic cell 1. After the replacement of the membrane, the open circuit voltage increased with 265mV

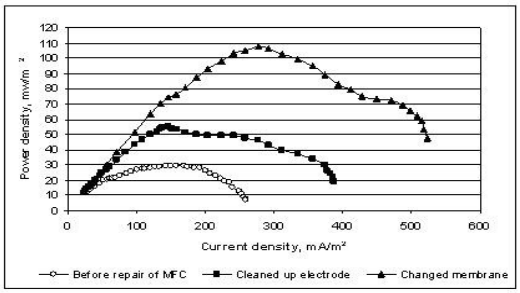
- from 318mV to 583mV. As the test pollutants, copper ions, were removed completely in the first anaerobic chamber, a deposition of significant amount of copper sulfide could be expected videlicet on the membrane of the integrated therein microbial fuel cell.

The cleaning of the electrodes also caused an increase in voltage values due to the removal of the passivating layer of sulfur and microbial biofilm, and the exposure of fresh contact surface. After cleaning the electrodes, the voltages in all three MFCs were increased with 50 to 80 mV.

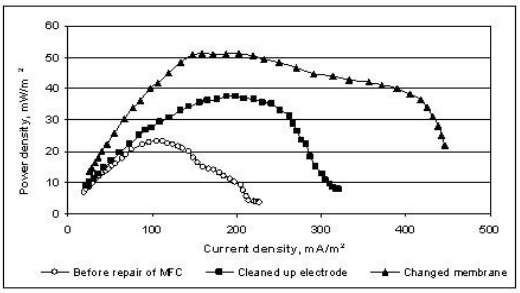
Figure 5 presents a comparison of the power densities in all three anaerobic cells before repair of MFCs, after cleaning the electrodes and after replacement of the membranes.



MFC 1



MFC 2



MFC 3

Fig. 5. Comparison of power densities of MFC

From these graphics it can be seen that after cleaning of the electrodes power densities of the fuel cells integrated in the anaerobic cells increased significantly. The effect was even better after the replacement of the membranes. In MFC 2 the highest increase of power density was established, that reached 107,58 mW/m^2 at a current density of 276,56 mA/m^2, obtained by applied resistance of 200 Ω after the shift of the membrane.

The results of cyclical VA (Volt Ampere) characteristics of MFCs further confirm the conclusion that the effectiveness of the used fuel elements significantly decreased over time. From figure 6 it can be established that the amplitudes of potentials significantly decreased, the same was set for the areas of received hystereses.

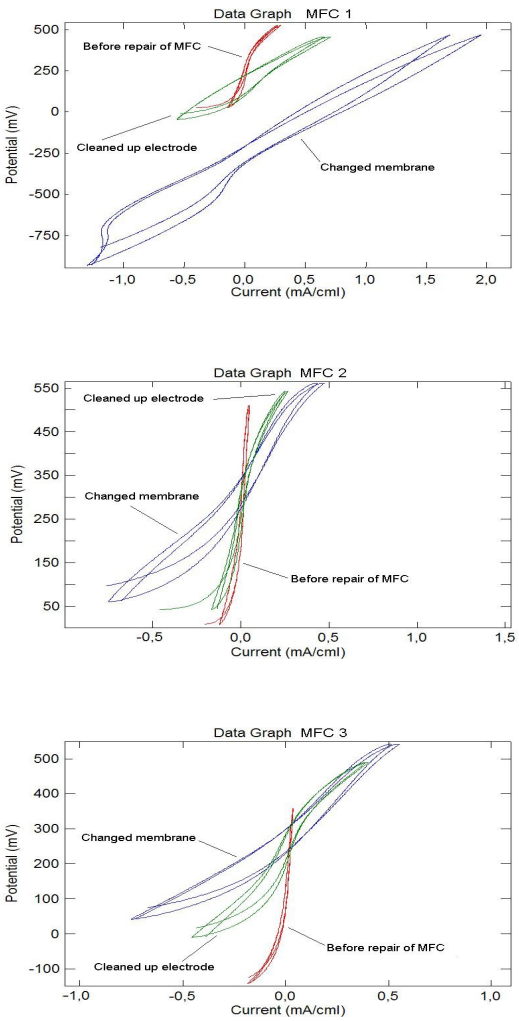


Fig. 6. Cyclical voltamperic diagram of MFCs

It can be concluded from the analysis of CVA (Cyclical Volt Ampere) diagrams that the most significant deterioration of electrochemical characteristics of MFC was due to „aging“ of the cation-exchange membranes. This process may be due to various factors - formation of a biofilm on the membrane, aging of the material, deposition of precipitates and other. The influence of anode and cathode „passivation“ as a result of various reasons had a smaller impact.

CONCLUSIONS

From the results it can be concluded that the efficiency of fuel cells is influenced by a complex of factors that depend largely on the period of operation of the anaerobic wetland. The main conclusions obtained from these experiments are as follows:

1. Higher values of open circuit voltages were measured in the beginning of the experiment, when the waters contained high concentrations of low molecular weight organic substances, produced in the media as a result of the hydrolysis of the fast degradable organic polymers;
2. The concentration of the biogenic hydrogen sulphide influenced the OCV, but in addition to the process of sulfate-reduction a large role in the generation of voltage had also other anaerobic microbial processes, occurring in the established conditions;
3. As a microbial biofilm was formed on the anode and there was deposited a passivating layer of elemental sulfur, the efficiency of the fuel cell decreased in time. A microbial biofilm was also formed on the surface of the cation exchange membrane, reducing its permeability;
4. It was found that the strongest influence on the effectiveness of the MFC had the membrane replacement. After that there was an average increase of the power densities of the three microbial fuel cells four times.
5. The passivation of the anode with elemental sulfur and formed biofilm also affected adversely the efficiency of microbial fuel cells. The cleaning of the electrodes led to a twofold increase in power densities of all three MFCs.
6. The analysis of the CVAs confirms the conclusion that the most significant deterioration of electrochemical characteristics of MFCs was due to the changes in the cation-exchange membranes. After replacement of the membranes in all three MFCs was observed an increase in the amplitudes of the potentials and areas of the resulting hystereses. The influence of anode and cathode „passivation“ had a smaller impact.

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