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Microbial fuel cell based on electroactive sulfate-reducing biofilm

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ABSTRACT

A two chambered laboratory scale microbial fuel cell (MFC) has been developed, based on natural sulfate-reducing bacterium consortium in electroactive biofilm on zeolite. The MFC utilizes potassium ferricyanide in the cathode chamber as an electron acceptor that derives electrons from the obtained in anode chamber H_2S. The molecular oxygen is finally used as a terminal electron acceptor at cathode compartment. The generated power density was 0.68 W m^{-2} with current density of 3.2 A m^{-2} at 150 Ω electrode resistivity. The hydrogen sulfide itself is produced by microbial dissimilative sulfate reduction process by utilizing various organic substrates. Finally, elemental sulfur was identified as the predominant final oxidation product in the anode chamber. It was removed from MFC through medium circulation and gathering in an external tank. This report reveals dependence relationship between the progress of general electrochemical parameters and bacterial sulfate-reduction rate. The presented MFC design can be used for simultaneous sulfate purification of mining drainage wastewater and generation of renewable electricity.

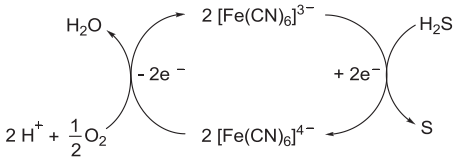
1. Introduction

Sulfate-rich waters are often found around the world as waste products from many mining, industrial processes or natural chemical reaction. Typically, they contain dissolved metals of high concentration and more than 3 g/L sulfate [11]. When wastewater happens uncontrolled, it oozes through to streams, rivers, subsoil water and the heavy metals are lethal to fish, other animals and plants. Under anaerobic conditions, dissimilatory sulfate reducing bacteria (SRB) use sulfate as a terminal electron acceptor for the degradation of organic compounds and produce hydrogen sulfide, which is considered as a broad-spectrum poison [16]. The disposal methods for treatment of sulfate-rich wastewater effluents are often one of the most expensive parts of the manufacturing process. Nevertheless, they do not examine the potential possibilities for conversion and storage of the energy obtained by SRB. The benefit of wastewater purification and electricity generation could be perceived as a sustainable modern way of treatment.

The microbial fuel cell (MFC) is a new form or renewable energy technology that can generate electricity from what would otherwise be considered waste [2], in particularly the dissolved sulfates. MFC based on the dissimilative microbial sulfate-reduction process enables simultaneously to remove the sulfates in wastewaters and to produce renewable energy. For that purpose, sulfate-reducing bacteria oxidaze organic compounds (for example lactate, acetate, butyrate or other fermentation products) and reduce sulfate anions. Hydrogen sulfide and bicarbonates are form, according to Eq. (1) below [17,7]:

$$(CH_2O)n + SO_4^{2-} \rightarrow H_2S + 2HCO_3^- \quad (1)$$

where CH_2O represents the organic substrate. The microbial produced hydrogen sulfide in Eq. (1) plays role of redox mediator and additional amount of other mediator is not necessary. H_2S is an electron donor, which means it gives off its electrons to the oxidant with the most positive redox potential, i.e. anode electrode. Then, the released protons in the anodic chamber migrate through a proton selective membrane into the cathode chamber. In one cathode configuration, the protons are taken up by ferricyanide; in another they are consumed by oxygen. Both ferricyanide and oxygen in the presence of electrons donated from the cathode surface react with protons and are reduced to ferrocyanide and water [9], as it shown in Eq. (2):



(2)

In this reaction scheme the oxygen in cathode chamber is defined as a terminal electrons acceptor. The reduction of molecular oxygen is the best choice for MFC, because the process occurs with high standard reduction potential, the end product is clean, non polluting H_2O [14,5]. However, elemental sulfur is formed in the anode chamber as by-product.

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The main aim of this study is to design and construct MFC that generate renewable energy from electroactive biofilm by use of sulfates and organic substrates. The biofilm is mobilized on zeolite particles, which are located in the anode chamber. It contains SRB species of *Desulfotomaculum*, *Desulfovibrium*, *Desulfomicrobium* and *Desulfobacterium* [3,12]. The production of H_2S is based on dissimilative microbial sulfate-reduction using SO_4^{-2}-rich wastewaters model solutions. The impact of several key parameters is investigated in order to achieve optimum rate of energy generation. It is proposed an engineering solution for removing of the obtained elemental sulfur in the anode chamber. The long-term performance of MFC is evaluated in respect for future application of the process.

2. Materials and methods

2.1. Design of laboratory scale microbial fuel cell

The microbial fuel cell is constructed with two different in volume chambers –cathode (0.06 dm^3) and anode (0.65 dm^3), which are separated with 0.0007 m^2 proton exchange membrane (CMI-7000S, Membrane International Inc.). The reactor is metal-free, i.e. it is made completely only from plastic material in order to prevent any corrosion from the dissolved hydrogen sulfide. Carbon rods with diameter of 8 mm and length of 9 cm are used as electrodes. Two electrodes are assembled in the anode chamber and one in the cathode chamber as it is shown on Fig. 1. The surface area of each electrode is 0.0024 m^2.

Almost half of the volume in the anode chamber is filled up with 0.4 kg modified zeolite with elemental composition as follow: 67.96% SiO_2, 11.23% Al_2O_3, 0.83% Fe_2O_3, 2.85% K_2O, 0.74% Na_2O, 3.01 CaO, 0.06% MgO, 0.90 TiO_2. The zeolite was saturated with NH_4Cl and KH_2PO_4, because these biogenic elements are important factor to achieve efficient sulfate-reduction rate. The particles size distribution is 2.5–5.0 mm and they are used as carrier of the electroactive sulfate-reducing biofilm. Cation exchange capacity and the exchanged ions in meq+/100 g are respectively: 112.75, K^+–33.88, Na^+ – 21.01, Ca^{2+} – 63.48, Mg^{2+} – 2.68 [10,15].

Thus, the reported MFC design is consists from two zones: (i) anode zone, where electroactive biofilm on zeolite derives electrons from organic substrates and produce H_2S and (ii) cathode zone, where the oxygen is the terminal electrons acceptor and react with the released protons (Fig. 2).

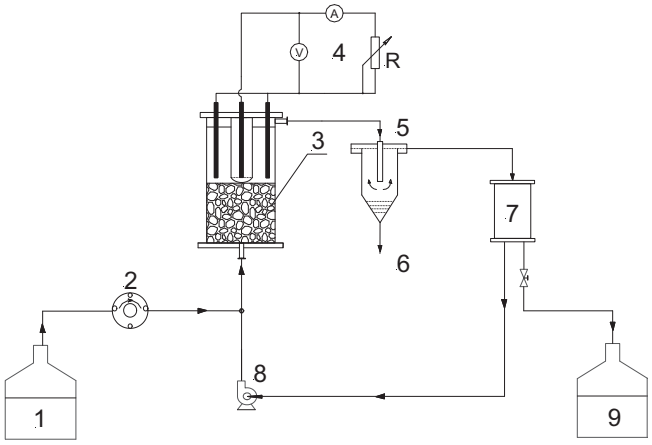

Fig. 1. Laboratory installation: (1) Stock solution with sulfate-rich wastewater, (2) peristaltic pump, (3) microbial fuel cell, (4) electricity chain with an external resistance, (5) precipitation tank, (6) elemental sulfur, (7) buffer stock solution, (8) recirculation pump and (9) collector tank.

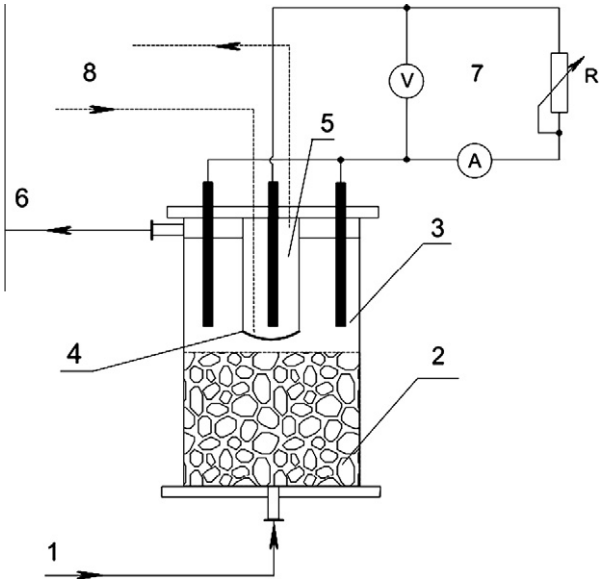

Fig. 2. Microbial fuel cell design: (1) entrance and (6) exit of the feeding solution, (2) zone filled with modified zeolite, (3) anode chamber, (4) cation exchange membrane, (5) cathode chamber, (7) electricity chain with a consumer and (8) air.

2.2. Cultivation of sulfate-reducing bacteria

The MFC volume is filled with 0.48 dm^3 modified culture medium of Postgate [6]. The culture medium contains 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 $MgSO_4 \cdot 7H_2O$, 6.0 g/l Na-lactate, 0.25 g/l yeast extract, pH 6.5 [1]. Sulfate concentration in the medium is 3 g/l, and thus the proportion between organic carbon and the terminal electron acceptor is 0.67.

2.3. Process operation

The cathode chamber is filled up with 100 mM $K_3[Fe(CN)_6]$ in 67 mM phosphate buffer with pH 7.0. The chamber is aerated with 0.15 dm^3/60 s air flow to ensure saturation of oxygen in the liquid. The anode chamber is inoculated with 40 ml mixed culture of sulfate-reducing bacteria. Then, MFC is fed continuously with culture medium after biofilm formation. The medium in stock solution (1) enters in the fuel cell with regulated debit by peristaltic pump (2). The adherence of active biofilm of SRB onto the natural occurred zeolite is carried out for a period of 3 months. The formation of active biofilm is carried out through repeat periodic replacement of 50% of the liquid phase of MFC with fresh medium. Replacement of the liquid phase is performed after sulfate concentration is reduced below 0.2 g/l. In the end of this cycle it is started continuous feeding of the anaerobic reactor with above mentioned culture medium of Postgate with various residence times. The homogenization process in MFC is realized by recirculation pump (8) at ascending flux run. The installation contains also a precipitation tank (5) with a volume of 2 dm^3 to gather the produced elemental sulfur. The outgoing from (5) solutions are collected in a reservoir (9) with a volume of 9 cm^3. The experiment is accomplished in the temperature range 21–22 °C.

2.4. Analytical methods

pH, Eh and mV are measured in key points of laboratory installation. At the same places are taken samples for spectrophotometric determination of sulfates by $BaCl_2$ (λ_{fixed} = 420 nm) and hydrogen sulfide (1-88/05.09 Nanocolor test, λ_{fixed} = 620 nm). Numerations of facultative aerobic heterotrophs, sugars-utilizing

Table 1
Nutrient media and cultivation conditions used for enumeration of main physiological groups microorganisms in the electroactive biofilm.

No.	Physiological groups	Nutrient media	Cultivation regime
1	Anaerobic heterotrophic bacteria	Nutrient broth + liquid paraffin	37 °C, 48 h
2	Fermenting sugars bacteria with gas production	Nutrient broth + 1% glucose + liquid parrafin	37 °C, 5 days
3	Sulfate-reducing bacteria	Postgate medium	30 °C, 5 days

Table 2
General technological parameters at various residence time of the feeding solution in the temperature range 21–22 °C.

Residence time (h)	72	60	48	36	24	16	9
pH	8.65	8.52	8.45	8.32	8.25	7.95	7.84
Eh (mV)	−276	−270	−260	−258	−253	−231	−217
TDS (g/l)	4.83	4.87	4.90	5.05	5.20	5.32	5.71
SO_4 (g/l)	0.745	0.85	0.94	1.32	1.49	1.6	2.02
H_2S (mg/l)	386	344	320	317	250	233	164
COD (mgO_2/l)	5781	5921	6136	6255	6485	6591	6945
OCV (mV)	720	704	685	680	675	668	644
COD removal efficiency (%)	29.8	28.1	25.5	24.1	21.3	20.0	15.7
V_{SO4}, $MgSO_4$/l h	31	38	43	55	63	88	109

fermentative bacteria and sulfate reducing bacteria in the effluent (number cells per milliliter) were determined by standard microbiological techniques – growing on nutrient agar in plate and most probable number of bacteria [13,18].

The nutrient media and cultivation regimes for this experiment are shown in Table 1.

MCF is measured with a portable digital multimeter Keithley Model 175. A precise potentiometer with maximum value of 13.5 k Ω is used for measuring of external resistances.

3. Results and discussion

The design of reported MFC precludes the possibility for oxygen ingress in the anodic chamber, because the both chambers are divided by cation-exchange membrane. It is permeable only for H^+ cations [8]. The production of elemental sulfur in the anodic chamber is due to electrochemical oxidation of H_2S on the anode surface. Approximately the half of anodic chamber chamber volume is filled with 0.4 kg saturated zeolite. It plays role as a biofilm bearer. Probably some small amount of biofilm is formed on the anode. However, it is consists from carbon rod, which possess very small surface. Therefore the immobilized on it biofilm is slightly small too. A critical technological problem at the management of dissimilative microbial sulfate-reduction is to achieve higher than 0.5 g/l produced hydrogen sulfide [4]. The reason is that these high concentration values cause toxicological effect on the microflora and repress the cultivation of SRB. The used bacterial suspension contained mesophilic SRB bacteria, which are most productive at 37 °C. Nevertheless, our experiments were performed at ambient temperature (20–22 °C) in order to reproduce the conditions in the industrial purification facilities. H_2S decrease concentration during its oxidation in the anode chamber. The pH is kept constantly (between 7 and 8.5) in the anode chamber, which is due to the generation of bicarbonate ions from the biofilm. All pointed fluctuations in the anolyte influence the technological parameters in MFC. Thus, series of attempts are accomplished to determine experimentally the optimal residence time by controlling the general technological parameters. The data are summarized in Table 2.

Table 3
Number of main physiological groups of microorganisms in the liquid phase of MFC.

Physiological group (cells/ml)	Number (cells/ml) Residence time (h)		
	72	24	9
Anaerobic heterotrophic bacteria	5.0×10^6	5.0×10^5	1.3×10^6
Fermenting sugars bacteria with gas production	2.5×10^6	6.0×10^4	2.5×10^3
Sulphate-reducing bacteria, using lactate	5.0×10^7	6.0×10^5	2.5×10^6

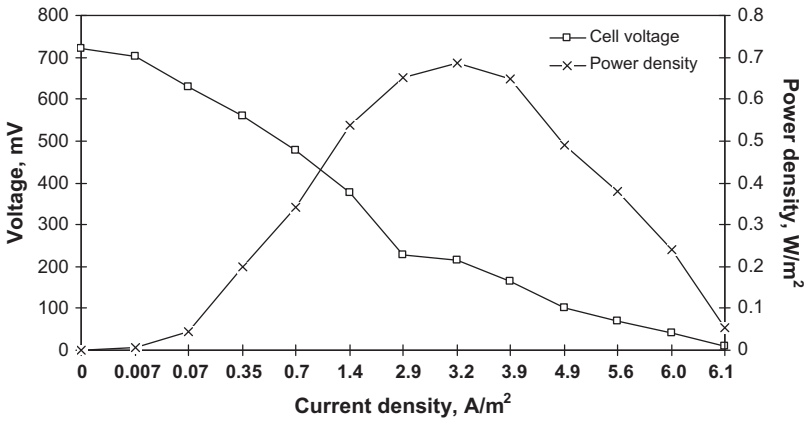

Fig. 3. Polarization curve of MFC at residence time of 72 h.

The measured COD of the feeding solution at pH 7.0 is 8240 mgO_2/l. It is ascertained that during the process the reduced residence time lead to pH decreasing. COD removal efficiency varies in the range 28.9–15.7% (Table 2) at reduced residence time. The measured microbial sulfate-reduction rate at various residence times is in the range 31–109 $MgSO_4$/l h. Another important parameter in MFC is the open circuit voltage (OCV). Its value is proportional to H_2S concentration in the anode chamber. From the data for H_2S and OCV in Table 2 it is ascertain linear dependence at various residence time. The maximum gained OCV value and H_2S concentration in the reported MFC installation is 720 mV and 386 mg/l respective. This higher H_2S concentration does not affect significantly the number of physiological groups microorganisms (Table 3).

The analytical microbiological data in Table 3 demonstrates the influence of residence time on the number of basic physiological groups microorganisms. The biggest number lactate-SRB (5.0×10^7 cells/ml) is registered at 72 h. With reducing the residence time from 72 h to 9 h the number of all investigated microorganisms in the liquid phase is decreased. Nevertheless, the highest rate of sulfate-reduction (109 mg/l) is achieved at 9 h due to the reason that the most SRB are immobilized in the electroactive biofilm on modified zeolite.

A polarization curve on Fig. 3 summarize the behavior of fuel element at various external resistances (0–13.5 k Ω).

The maximal values of power density is 0.68 W/m^2 and current density of 3.2 A/m^2 are obtained at 150 Ω external resistance. The presented polarization curve is measured at residence time of 72 h in respect of the fresh feeding nutrient medium in MFC. After 3 months exploitation of MFC with different residence times, cultivation regime and generated electricity we ascertained considerably amount of elemental sulfur in the precipitation tank (5). However, during this period we did not registered any worse efficiency of the MFC technological parameters.

4. Conclusion

The dissimilative microbial sulfate-reduction has a potential for electricity generation by treatment of sulfate-rich wastewaters. This process is depended of the amount produced H_2S and it can be managed successfully by variation of the residence time in the anode chamber of MFC. The reported installation propose engineering solution to remove the obtained as by-product elemental sulfur. During the exploitation period the technological and electrochemical parameters remain stable.

Acknowledgments

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