FACTORS AFFECTING OF PERFORMANCE OF MICROBIAL FUEL CELL FOR TREATMENT OF SULPHATE POLLUTANTS

Anatoliy T. ANGELOV, Alexandre R. LOUKANOV*, Svetlana G. BRATKOVA*, Evgeni S. KRAICHEV**

ABSTRACT: *Microbial Fuel Cell (MFC) is considered as one of the alternative energy resources from renewable materials. By employing the natural sulfate-reducing bacterium consortium we demonstrate the possibility of electricity generation in a microbial fuel cell with concomitant sulfate removal. MFC constructed employing with cation-exchange membrane type CMI-7000S, graphite electrodes without addition of any toxic mediators electron acceptor ($K_3[Fe(CN)_6]$) in the cathode chamber without pH adjustment was assessed in the present study. In different resistant times were established the main technological parameters in microbial fuel cell. A maximum power density of 0,68 mW/cm^2 (normalized to geometric electrode area) was obtained with a two-chamber, continuous flow MFC operated in batch mode at 21 °C.*

Keywords: *Sulfide, Wastewater, Microbial Fuel cell, Electricity, Sulfate removal.*

1. Introduction

Sulfate-rich wastewaters are generated by many processes: that use sulphuric acid or sulphate rich feed stocks (e.g. fermentation or sea food processing industry). Also the use of reduced sulphur compounds in industrial processes, i.e. sulphide (tanneries, Kraft pulping), sulphite (sulphite pulping), thiosulphate (fixing of photographs) or dithionite (pulp bleaching) contaminates wastewater with sulphate. Sulphate rich wastewater also is a common problem for the mining and smelting industries throughout the world. This drainage waters typically contains dissolved metals of high concentration and more than 3 g/l sulphate (Kosinska & Miskiewics, 2009). Utilizing microbial metabolism to produce an electrical current from the degradation of organic/inorganic matter provides an elegant solution for simultaneous wastewater treatment and electricity generation; these systems are termed microbial fuel cells (MFCs) and represent a clean and renewable energy resource.

Sulphate-reducing bacteria oxidize simple organic compounds (such as lactate, acetate, butirate and other products of fermentations) with sulphate under anaerobic conditions, and thereby generate hydrogen sulphide and bicarbonate ions (Widdel et al., 1991, Guerrero-Rangel et al., 2010):

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

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 electroactive, 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 (Ieropoulos et al., 2005), as it shown in equation 2:

$$\begin{aligned} &4[Fe(CN)_6]^{3-} + 4e^- \rightarrow 4[Fe(CN)_6]^{4-}, \\ &4[Fe(CN)_6]^{4-} + 4H^- \rightarrow 4[Fe(CN)_6]^{3-} + H_2O \end{aligned} \qquad (2)$$

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 reduction product is clean, none polluting water (Rabaey & Verstraete, 2005). However, elemental sulfur is formed in the anode chamber as by-product.

Various parameters such as temperature, pH and sulphide in the solution will affect the growth and activity of sulphate-reducing bacteria.

The other important parameter is the total organic carbon (TOC) /SO_4^{2-} ratio (Vossoughi et al. 2003). Velasco et al. 2008 reported that the feed COD/SO_4^{2-} ratio can be an useful parameter to control hydrogen sulfide production in the metal precipitation process when ethanol is used as sole electron donor and carbon source. Kaksonen et al. 2004 showed that the stoichiometric COD/SO_4^{2-} ratio of 0.67 was adequate to attain around 60% of sulfate reduction with an initial sulfate

** University of Mining and Geology “St. Ivan Rilski”, Sofia, Bulgaria*

concentration of 2000 mg/L in a fluidized-bed reactor inoculated with SRB capable to completely oxidize ethanol to CO_2.

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*. The production of H_2S is based on dissimilative microbial sulfat-reduction using SO_4 -rich wastewaters model solutions. The impact of two key parameters – temperature and COD/SO_4 is investigated in order to achieve optimum rate of energy generation.

2. Material 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.). 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 % A_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 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 (Kononova, 1966).

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).

2.2. Cultivation of sulfate-reducing bacteria

The MFC volume is filled with 0.48 dm^3 modified culture medium of Postgate (Ghazy et al., 2011). 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 $MgSO_4.7H_2O$, 6.0 g/l Na-lactate, 0.25 g/l yeast extract, pH 6.5 (Ausubel et al., 1995). Sulfate concentration in the medium is 3 g/l, and thus the proportion between organic carbon and the terminal electron acceptor is 0.67.

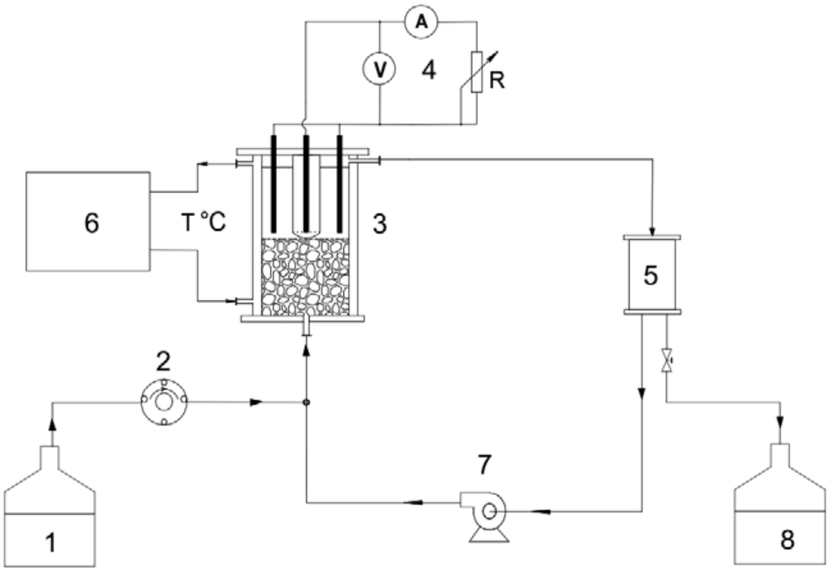

Fig. 1 *Laboratory installation: (1) Stock solution with sulfate-rich wastewater, (2) peristaltic pump, (3) microbial fuel cell, (4) electricity chain with an external resistance, (5) buffer volume, (6) water bath, (7)recirculation pump, (8) collector tank.*

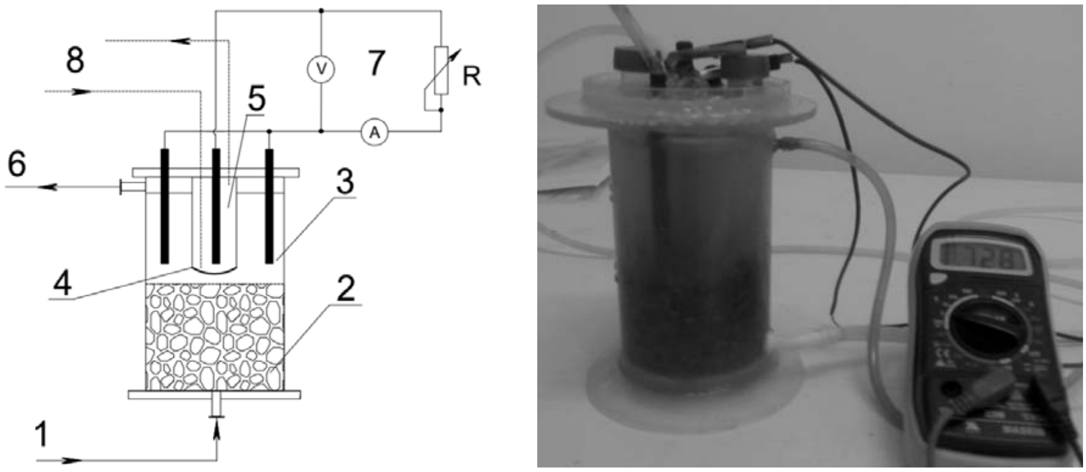

Fig. 2 *Microbial fuel cell design: (1) entrance and (6) exit of the feeding solution, (2) zone filled with modified zeolite, (3) anode chamber, (4) cation-exchange membrane, (5) cathode chamber, (7) electricity chain with external consumer, (8) air.*

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 three 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 buffer volume for pH correction (5) with a volume of 0.3 dm^3. The outgoing from (5) solutions are collected in a reservoir (9) with a volume of 9 dm^3. The experiment is accomplished in the three different temperature ranges (21-22°C, 30-31°C and 35-36°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). Number of sulfate reducing, aerobic heterotrophic and fermenting sugars bacteria in anode chamber is counted through standard microbiological method (Zhao et al., 2008).

The electrical parameters of MFC are measured with a portable digital multimeter Keithley Model 175. A precise potentiometer with maximum value of 13.5 kΩ is used for measuring of external resistances.

3. Results and discussion

A critical technological problem at the management of dissimilative microbial sulfate-reduction is to achieve higher than 0.5 g/l produced hydrogen sulfide (Cooney et al., 1996).

The reason is that these high concentration values cause toxicological effect on the microflora and repress the cultivation of SRB. However, H_2S decrease concentration during its oxidation in the anode chamber. 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 1.

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 1) 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. 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 group microorganisms (Table 2).

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 hours to 9 hours 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 hours due to the reason that the most SRB are immobilized in the electroactive biofilm on modified zeolite.

Polarization curves on Fig. 3 summarize the behavior of fuel element at various external resistances (0 - 13.5 kΩ). 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 hours in respect of the fresh feeding nutrient medium in MFC.

Table 1. *General technological parameters at various residence time of the feeding solution in the temperature range 21-22°C .*

Residence Time, h	72 h	48 h	24 h	16 h	9h
pH	8.65	8.45	8.25	7.95	7.84
Eh , mV	-276	-260	-253	-231	-217
TDS, g/l	4.83	4.90	5.20	5.32	5.71
SO_4, g/l	0.745	0.94	1.49	1.6	2.02
H_2S, mg/l	386	320	250	233	164
COD, mgO_2/l	5781	6136	6485	6591	6945
OCV, mV	720	685	675	668	644
COD removal efficiency, %	29.8	25.5	21.3	20.0	15.7
V_{SO4} , mgSO_4/l.h	31	43	63	88	109

Table 2. *Number of main physiological groups of microorganisms in the liquid phase of the microbial fuel cell.*

Physiological group	Number, cells/ml		
	Residence time,h		
	72 h	24 h	9 h
Anaerobic heterotrophic bacteria, cells/ml	$5,0.10^6$	$5,0.10^5$	$1,3.10^6$
Fermenting sugars bacteria with gas production, cells/ml	$2,5.10^6$	$6,0.10^4$	$2,5.10^3$
Sulphate-reducing bacteria, using lactate, cells/ml	$5,0.10^7$	$6,0.10^5$	$2,5.10^6$

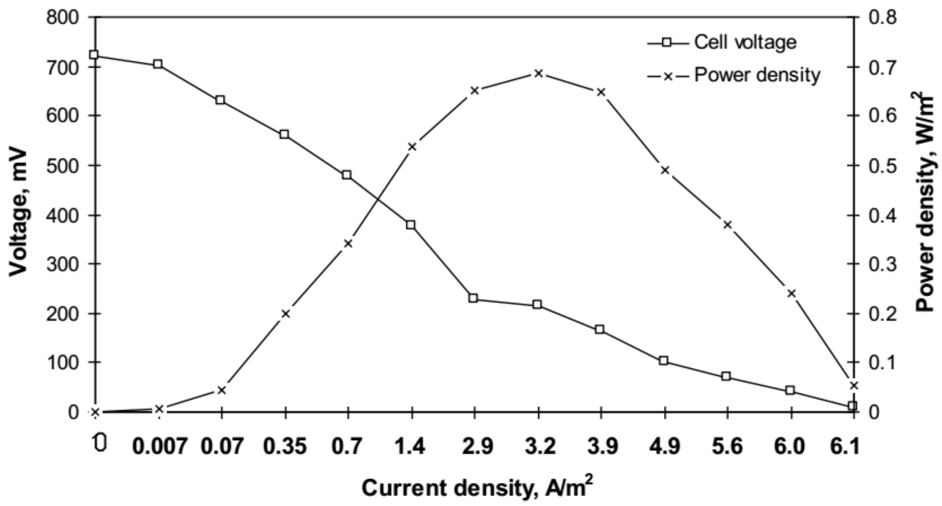

Fig. 3. *Polarization curve of MFC at residence time of 72 hours*

However, during this period we did not registered any worse efficiency of the MFC technological parameters. Influence of temperature at residence time of the feeding of 72 hours was demonstrated in table 4. The effect of temperature on the rate of sulphate-reduction was studied at COD/SO_4^{2-} ratio of 2.7 and residence time 72 h. It was found that in the range 21 – 36 ^oC process rate increases linearly. It was determined maximal rate of sulphate-reduction - 37 mg/l.h at 37 ^oC.

Nevatalo L. (2010) reported that the operation at these temperatures showed that mesophilic SRB processes can be operated at sub-optimal temperatures, but the activity is decreased by 10-40 % at 15°C when compared to optimal temperature.

The other important parameter is the total organic carbon (TOC) /SO_4^{2-} ratio – in table 5 are show the influence these parameter on rate of sulfate-reduction with residence time of the feeding of 72 hours**.** Velasco et al. (2008) reported that at a feed COD/SO_4^{2-} ratio of 2.5 with ethanol as sole donor of electrons most of the sulfate in the influent was converted to hydrogen sulfide. Wang et al., 2008 were studied sulfate reduction in a continuous flow, acidogenic reactor using molasses wastewater as the carbon source at varying chemical oxygen demand/sulfate (COD/SO_4^{2-}) ratios. The same authors were found that at COD/SO_4^{2-} ratio of 3.6, the removal rate of sulfate was higher than 97% and the ethanol content was largely reduced. In our case the final results are similar (table 5).

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 impact of two key parameters – temperature and COD/SO_4 is investigated in order to achieve optimum rate of sulfate removal rate. During the exploitation period the technological and electrochemical parameters remain stable.

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Table 4. *Influence of temperature at residence time of the feeding of 72 hours*

T, °C	21-22°C	30-31°C	35-36°C
pH	8.5	8.5	8.6
Eh , mV	-276	-295	-321
TDS, g/l	4.83	4.39	4.15
SO_4, g/l	0.745	0.550	0.334
H_2S, mg/l	386	452	511
COD, mgO_2/l	5781	5374	5147
COD removal efficiency, %	29.8	34.8	37.5
V_{SO4} , $mgSO_4/l.h$	31	34	37

Table 5. *Influence* of COD/SO_4^{2-} ratio *with residence time of the feeding of 72 hours*

COD/SO_4^{2-}	1.8	2.2	2.7
T, °C	21-22°C		
pH	8,48	8,56	8,65
Eh , mV	-262	-264	-271
TDS, g/l	5,02	4,94	4,81
SO_4, g/l	0,963	0,865	0,739
H_2S, mg/l	317	344	382
COD, mgO_2/l	5196	5558	5781
COD removal efficiency, %	36,9	32,5	29.8
V_{SO4} , $mgSO_4/l.h$	28,3	39,6	31,4

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