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Microbial fuel cell for metal sulfide oxidation and nitrate reduction. Part I. Preliminary investigation of electrogenic properties

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A fuel cell for wastewater treatment is developed. The aim of the study is the generation of electrical energy due to chemical oxidation of metal sulfide ions in the anode compartment and reduction of nitrate ions in the cathode one. Thus, energy will be obtained and sulfide and nitrate-containing wastewaters will be purified. In this part of the research a choice of the appropriate design of the cell, membrane, and electrodes is done. The electrogenic properties and the determination of the proper conditions for the generation of energy from fuel cells, with simultaneous removal of some severe environmental pollutants using the strain JCM 3863 *Acidithiobacillus ferrooxidans*, are studied through their ability to oxidize Fe^{2+} to Fe^{3+}. Their ability to oxidize metal sulfides will be an object of further investigations. The results for the obtained electrical power from the fuel cell in the course of the wastewater treatment process, as well as the rate of Fe^{2+}oxidation and nitrate depletion are shown.

Keywords: Fuel Cells, Metal Sulfides Oxidation, Nitrate Reduction

INTRODUCTION

In the face of the growing problem of fossil fuel depletion, there is a global interest in developing sustainable and environmentally friendly forms of energy. One form of alternative energy that may be viable in addressing this problem is bioenergy. In this context, Microbial fuel cells (MFCs) hold great potential as a green and carbon-neutral technology that directly converts biomass into electricity [1]. The advantages of MFCs over other biotechnologies for conversion of wastewater to energy are the higher energy conversion efficiency and the ability for treatment of waters that are not suitable for anaerobic digestion processes.

MFCs are bioelectrochemical systems (BESs) based on the transmission of electrons from living microorganisms to solid-phase electron acceptors located outside cells. In essence, they are electrochemical energy converters that generate electric current due to a certain oxidation-reduction reaction. Unlike conventional fuel elements that use chemical (mostly platinum) catalysts, the specificity of MFCs is the use of electrochemically active microorganisms that perform the function of biocatalysts. In the course of catabolic processes such as glycolysis, Krebs cycle, and fermentations, these microorganisms carry out the oxidation of the substrates present in the medium to produce electrons and protons. By extracellular electron transfer (EET), a portion of the generated electrons transferred to the anode and the outer circuit to the cathode are converted into electric current (Fig. 1).

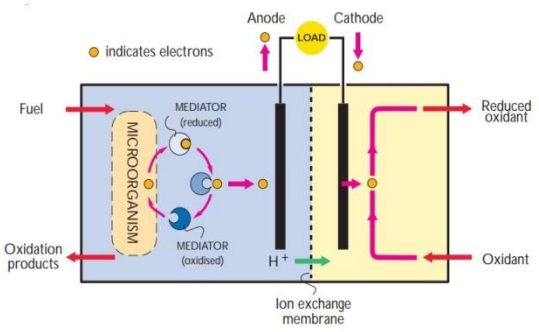

Figure 1. Schematic representation of the microbial fuel cell [2].

In their simplest form, MFCs consist of an anodic and a cathodic compartment that are generally separated by a membrane to avoid the migration of electrolytes from one chamber to the other [3].

Microbial fuel cells have been proven to be a promising technology to harvest energy and treat wastewater owing to their low cost and sustainability [1]. They can be used in generation of energy with simultaneous removal of some severe environmental pollutants.

Electrogenic bacteria are organisms that can transfer electrons to an anode, as a terminal electron acceptor and thus are classified as electrogenic bacteria. They are a heterogenic group that is united by the general property to transfer the electric charge to or from a solid electrode. First, Potter (1911) discovered the ability of microorganisms to produce electricity. He was using a suspension of *E. coli* and *Saccharomyces* sp. and a platinum electrode [4]. In 1980, Suzuki and colleagues used

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Clostridium butiricum for hydrogen production in a microbial fuel cell [5].

Acidophilic microorganisms focus the scientific interest in this area on both their practical application and the environmental risks associated with their metabolic activity. Acidophilic hemolithotrophic bacteria and archaea are the dominant microorganisms in naturally occurring acid habitats and those of anthropogenic nature. They generate energy from oxidation of ferro ions, elemental sulfur, metallic sulfides and other inorganic forms of sulfur to sulfates, transform a number of heavy metals into more mobile forms.

Ac. ferrooxidans are for the first time isolated from acid mine drainage in 1947 [6]. It is a major participant in consortia of microorganisms used for the industrial recovery of copper (bioleaching or biomining). It is a chemolithoautotrophic γ-proteobacterium using energy from the oxidation of iron- and sulfur-containing minerals for growth. *Ac. ferrooxidans* lives at extremely low pH (pH 1–2) and fixes both carbon and nitrogen from the atmosphere. It solubilizes copper and other metals from rocks and plays an important role in nutrient and metal biogeochemical cycling in acid environments [7].

There are a lot of investigations for the use of *Ac. ferrooxidans* for different purposes, mainly for leaching of metals [8-10], fuel desulfurization [11-13] and dewatering of sludge [14, 15]. Recently there are some publications that concern the use of the strain in fuel cells [16, 17].

The present study aims to explore the basic principles and to determine the appropriate conditions for obtaining energy from microbial fuel cells while simultaneously removing some environmental contaminants. The possibility of combining two streams with different types of pollutants (a stream subject to reduction and one to be oxidized) is considered. In this case, it is a stream containing nitrates and converting Fe^{2+} to Fe^{3+} ions by *Ac. ferroxidans*. This is a preliminary investigation that would afford in future the conversion of metal sulfides in the anodic compartment and reducing nitrates in the catodic one.

MATERIALS AND METHODS

In the present work, the strain *Ac. ferrooxidans* JCM 3863 from the Japanese Microorganism Collection was used. The lyophilized culture was rehydrated and cultured in liquid culture medium 9K [18] (JCM Medium No. 92). After a 3-day pre-cultivation by shaking at 30 °C, some of the microorganisms were inoculated into a fresh broth № 92 and shaken at 30 °C and the other part transferred to 2436 medium recommended by ATCC and cultured at 30 °C, again with shaking. It was found that the growth in medium 2436 was faster, there was no oxidation of Fe^{2+} as a result of autoclaving [18], as well as a reduced amount of the formed precipitate (jarosite). This required the use of 2436 medium in further experiments.

Cell density determination

The concentration of *Ac. ferrooxidans* cells was counted using a haemocytometer and a light microscope with 1000×magnification.

Measurement of ferrous and ferric iron concentrations

All components, used in this study, were of analytical grade. 5-Sulfosalicylic acid (SSA) forms a red-colored complex with ferric ions in aqueous solution. The complex of iron with SSA forms a yellow complex with ammonia.

Centrifuged and washed culture was added to broth medium containing 9.0 g/l of ferric ions. The final cell concentration was 4×10^8 cells/ml. After that, the concentration of ferric ions was determined spectrophotometrically: 0.1 ml of broth medium was mixed with 3 ml of 10% SSA followed by addition of 97 ml of deionized water. The light absorbance was measured at 500 nm. For the measurement of total iron 3 ml of 25% ammonium hydroxide solution was added to the above solution. The spectrophotometric measurement was performed at 425 nm. The concentration of Fe^{2+} ions was determined as a difference from the total iron concentration and the concentration of Fe^{3+} ions [19]. The measurements were performed at every 24 h – 0 h, 24 h, 48 h, 72 h and 96 h.

Measurement of nitrates and nitrites

The nitrates were determined spectrophotometrically by the method of Goldman & Jacobs [20].

Microbial fuel cell design

The scheme of the fuel cell is given in Fig. 2. It consists of two concentrically placed cylinders (each of 350 ml volume) separated by a membrane (0.02 m^2). The characteristics of the used membranes are given in Table 1.

The used anode electrodes are 5 graphite rods with overall surface area of 0.015 (5×0.003) m^2. In order to increase the electrode surface 300 ml of activated carbon (Fujikasui®, Japan, with an area of 680 $m^2.g^{-1}$) was added and a graphite rod as electricity collector was used as a cathode electrode.

Table 1. Characteristics of the studied membranes

Membrane	Type	Material	Thickness (μm)	Electrical resistance (Ω.cm^2)	Application
CelCard 3501	Anion	Polypropylene	25	2.55	Alkaline batteries separator
Fumapem® FAA-3-PK-75 OH form	Anion	Fumion® F polymer	55	1.26	Alkaline batteries separator
Neosepta® AFN	Anion	Polypropylene	160	0.5	Regeneration of acids
Nafion® 117	Cation	PTFE	180	-	Regeneration of acids, Electrolysis
Porous PVC	-	PVC	-	-	Batteries separator

The desired reactions are as follows:
Anode:
$4Fe^{2+} + H^+ + O_2 = 2Fe^{3+} + H_2O + 4e^-$

Cathode:
$NO_{3-} + H_2O + 2e^- = NO_{2-} + OH^-$;
$NO_{2-} + 2H_2O + 3e^- = 1/2N_2 + 4OH^-$

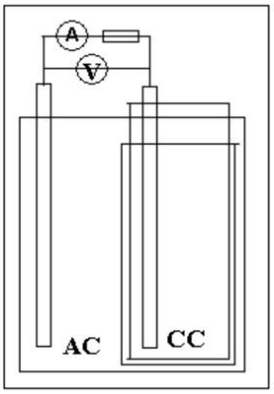

Figure 2. Scheme of the investigated fuel cell.

RESULTS AND DISCUSSION

Optimal conditions for cultivation of Ac. ferrooxidans

The pH, temperature and initial concentration of Fe^{2+} were studied. The results are shown in Figs. 3, 4, and 5.

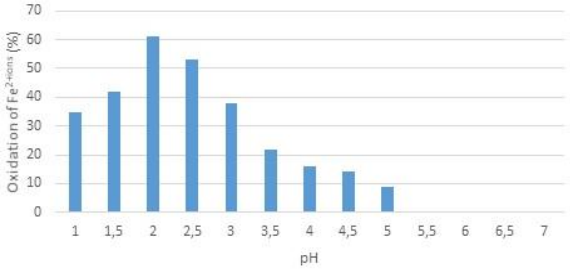

Figure 3. Influence of pH on the oxidation rate of *Ac. ferrooxidans* for a 96-h process.

Figure 3 shows that the maximum rate of oxidation is at pH 2. The oxidation of Fe^{2+} to Fe^{3+} is more than 60% and it rapidly decreases after pH 3.5. The maximum oxidation rate at pH 2 is achieved in the temperature range of 25-30 °C (fig. 4). That is about 50-60% of the initial Fe^{2+} concentration. For the other investigated temperatures it was determined that the oxidation rate is less than 30%.

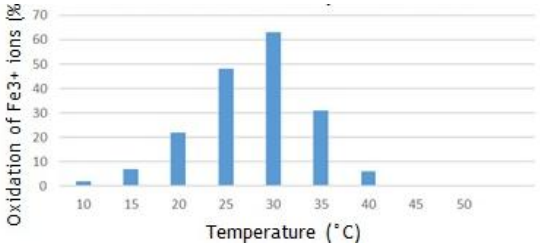

Figure 4. Influence of temperature on the oxidation rate of *Ac. ferrooxidans* for a 96-h process.

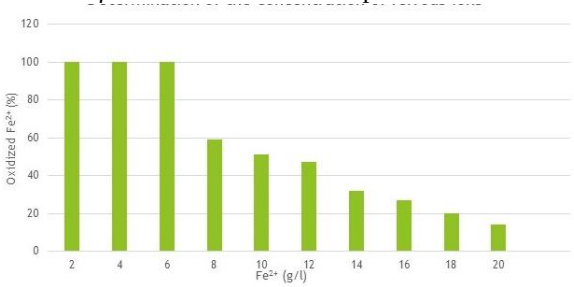

Figure 5. Influence of the initial concentration on the rate of oxidation of ferrous ions at pH 2 and 30 °C, 96 h.

From figure 5 it can be seen that in these conditions initial concentrations up to 6 g/l are totally oxidized before 72 h. Above a concentration of 10 g/l Fe^{2+}, the oxidation rate is less than 50% after 96 h. Probably, above a concentration of 10 g/l there is substrate inhibition of the process. For this reason, the concentration of 8 g/l was chosen as an operating concentration for the fuel cell. The other operating conditions were: pH = 2 and temperature 30°C.

Choice of membrane

The choice of membrane was conducted by varying the external resistance of the cell (1-1000 Ω) and measuring the resultant current and voltage

at two different pH values. The content of Fe^{2+} was 8.88 g/l. In this experiment no culture was used. The obtained results are shown in Figs. 6 and 7.

Fuel cell investigation

The fuel cell power was determined with a bacterial suspension at a density of 4.1×10^8, a Fe^{2+} concentration of 8.068 mg/ml and pH of 2.08, concentration of nitrates in the cathode compartment 500 mg/l. The obtained voltage as a function of the electrical current is plotted in figure 8. The slope of the curve shows that the internal resistance of the cell is relatively low – about 40 Ω.

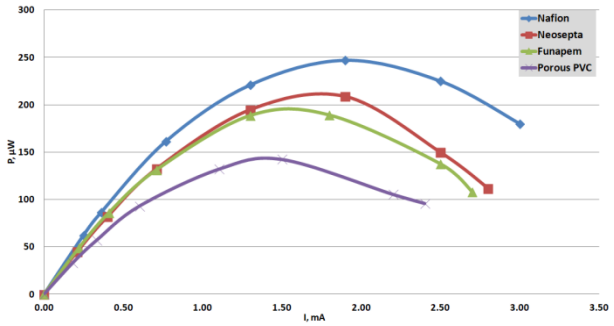

Figure 6. V-A characteristics of investigated membranes at pH = 2

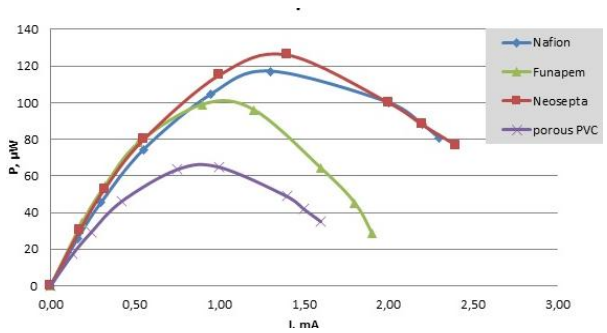

Figure 7. V-A characteristics of investigated membranes at pH = 3

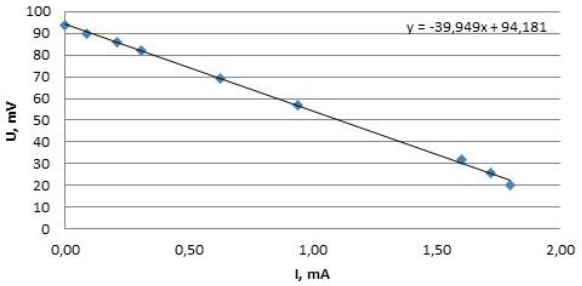

Figure 8. Voltage as a function of electric current

The results for the membrane CelCard 3501 are not given in the figures due to the very low values of the electrical power. At pH=2 the electrical indicators are nearly twice as high as these at pH=3. The membrane Nafion® shows the most appropriate characteristics for the process, followed by the membrane Neosepta®. That is why the further investigations were carried out with initial pH = 2, concentration of Fe^{2+} = 9 g/l using Nafion® as a membrane. The power of the fuel cell was measured for 4 days at 100 Ω external resistance.

The results are given in Fig. 9. The depletion of nitrates and Fe $^{2+}$ is shown in Fig 10. From the figures can be seen that although the electrical output of the cell is relatively low it is stable with time and the depletion for both reagents reaches up to about 80%.

Further possibilities to improve the operation of the cell are to immobilize the cells over an appropriate carrier and to conduct the process in continuous mode.

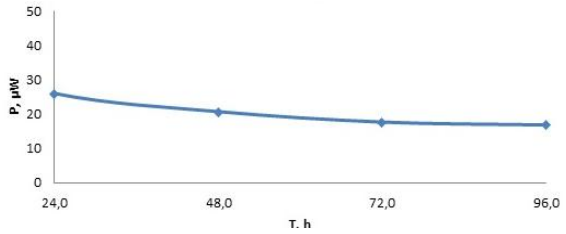

Figure 9. Power of the fuel cell for 4 days at 100 Ω external resistance

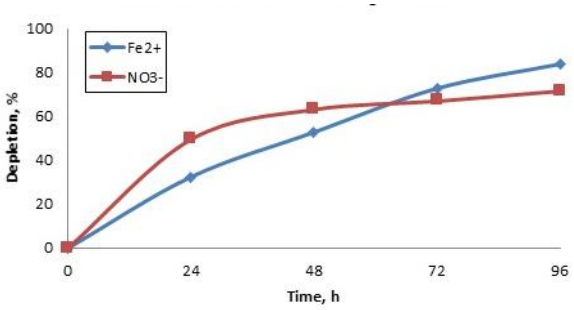

Figure 10. Depletion of Fe^{2+} / NO_3^- by the FC

CONCLUSIONS

The optimum conditions for the growth of *Ac. ferrooxidans* strain JCM 3863 were determined - 9K medium ATCC 2436, pH 2, temperature 30°C and ferrous ions concentration of 8 g/l. As most suitable for working in acidic environment the membrane Nafion was determined. We found that despite the low electrical power *Ac. ferrooxidans* shows electrogenic properties and chemical denitrification in the cathode compartment was successfully achieved. The selected microbial strain JCM 3863 *Ac. ferrooxidans* demonstrates promising properties as an electrogenic bacterium and could be used not only for the systems considered in this research. The experience gained in its cultivation can be applied to other fuel elements and pollutions. The designed FC is efficient, reliable and cheap as it doesn‘t incorporate noble metals.

Further investigations are going to be focused on the immobilization of the cells on the electrodes, investigation of the oxidation of different metal sulfides to sulfates, increase of the surface area of the electrodes, use of metal scrap as electrodes, exploitation of the cell at continuous work and use of denitrification bacteria.

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