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# Wastewater treatment of sulfur and nitrate contaminated fluxes into fuel cells

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Fuel cells (FC) with microbial oxidation of sulfides and chemical denitrification and a microbial assisted process for both reactions were studied. A comparison between microbial and chemical fuel cells at the same conditions is also presented. A novel type of electrodes with pyrolyzed and activated carbon paddling is used for immobilization of the bacterial strain for sulfide oxidation. *Pseudomonas putida* 1046 is studied as a model strain for the anodic compartment and *Pseudomonas denitrificans* for the cathodic one.

**Keywords:** Fuel Cells, Sulfides Oxidation, Nitrate Reduction

## INTRODUCTION

Environmental protection, along with the search for new renewable energy sources, is one of the most essential topics nowadays.

Hydrogen sulfide is a pollutant the sources of which can be divided into natural and anthropogenic ones. Among the natural are volcanoes, thermal springs and closed deep water basins. Anthropogenic sources include different industries, such as petroleum, leather, textile, paper, etc. and it can be released both as gas or dissolved in wastewaters. Hydrogen sulfide is also formed in the sewage systems and wastewater treatment plants. It is highly corrosive, can take part in the sulfur cycle causing acid rains and is generally harmful to the environment even in small amounts [1, 2].

Other persistent pollutants are the nitrates. Their sources are animal farms, households, greenhouses, petroleum industry, fossil fuels burning, etc.

The classical methods for the decontamination of those two pollutants vary: for hydrogen sulfide processes such as adsorption, absorption, chemical oxidation, biochemical oxidation and Claus process among others are employed and for nitrates adsorption, electro-dialysis, reverse osmosis and bio-denitrification are more commonly used [3-9]. All those processes are expensive both as capital investments and equipment maintenance showing that the decontamination of fluxes containing sulfides and nitrates is an essential but expensive process. In light of this the concept for obtaining energy from their mutual decontamination in fuel cells seems promising [10, 11].

The aim of the present study is to investigate a simple and easy for maintenance construction of a FC for simultaneous sulfide oxidation and nitrate reduction, as well as comparing the biological and chemical processes at different operating conditions.

The expected reactions are shown below:

Anode:

$$\mathrm{S^{2-} + 6\,OH^- \rightarrow SO_3^{2-} + 3\,H_2O + 6e^-}$$

$$SO_3^{2-} + 2\,OH^- \rightarrow SO_4^{2-} + H_2O + 2e^-$$

Total:

$$S^{2-} + 8\,OH^- \rightarrow SO_4^{2-} + 4\,H_2O + 8e^-$$

Cathode:

$$\mathrm{2\,NO_3^- + 2\,H_2O + 4e^- \rightarrow 2\,NO_2^- + 4\,OH^-}$$

$$2\,NO_2^- + 4\,H_2O + 6e^- \rightarrow N_2 + 8\,OH^-$$

Total:

$$2\,NO_3^- + 6\,H_2O + 10e^- \rightarrow N_2 + 12\,OH^-$$

## *Materials and methods*

*Materials:* The solutions of sulfides and nitrates were prepared by dissolving appropriate amounts of analytical grade $Na_2S.9H_2O$ and $KNO_3$ (Sigma Aldrich), respectively. Concentrations are given as mg ions ($S^{2-}$ or $NO_3^-$) per liter ($mg.l^{-1}$). For improving the conductivity of the sulfide solutions, NaCl (analytical grade) was used in some cases. The neutral pH for the sulfide solutions was maintained by using phosphate buffer.

The concentration of the sulfide ions was determined photometrically by converting them to methylene blue by addition of N,N-p-phenylenediamine [11], and the concentration of nitrates – by UV photometry by the method of Goldman and Jacobs [12].

*Pseudomonas putida* 1046 was chosen as electrogenic strain capable of oxidizing sulfide ions [13, 14]. The medium for its cultivation was: 10 $g.l^{-1}$ meat extract, 10 $g.l^{-1}$ peptone and 5 $g.l^{-1}$ NaCl. After preparation it was shaken at 30°C in a mechanical shaker (50 rpm) for 24 h.

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The strain *Pseudomonas denitrificans* (NBIMCC 1625) was chosen to perform the microbial denitrification. This strain is facultative anaerobic, autotrophic and electrical stimulation enhances its metabolism [15]. In biological denitrification, the bacteria use nitrates as electron acceptors in their breathing process in the absence of oxygen. Denitrifying bacteria reduce inorganic nitrogen compounds, such as nitrates and nitrites, into harmless nitrogen gas. Nitrates are reduced to nitrogen, passing sequentially through nitrites and nitrogen oxides in accordance with the following reaction scheme:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow NO_2 \rightarrow N_2 \qquad (7)$$

The sequential reduction of nitrogen compounds takes place under the action of the catalytic enzymatic activity of *Pseudomonas denitrificans* under anaerobic conditions in the presence of a suitable electron donor [16, 17].

The strain was cultured in a medium containing: 10 g.l$^{-1}$ peptone, 1 g.l$^{-1}$ yeast extract, 10 g.l$^{-1}$ NaCl and was incubated at 30 °C in a rotary shaker at low agitation speed (50 rpm) for 24 h.

Activated granular carbon (Fujikasau®, Japan, 680 m$^2$.g$^{-1}$) was chosen to be used as a support for immobilization due to the fact that microbial cells are easily attached to its surface. It has the added benefit of adsorbing both substrate (contaminants) and product compounds, decreasing their concentrations to tolerable for the microorganisms levels so that substrate and product inhibition is avoided, allowing the fuel cell to operate at higher initial pollutant concentrations.

The membrane used was Fumapem® with performance characteristics given in Table 1. The electrodes used are shown in Fig. 1. They are graphite rods with dimensions (d = 6 mm, L = 200 mm, S = 3000 mm$^2$) with total working surface area of 0.015 (5×0.003) m$^2$ (Fig. 1a) and pyrolyzed and activated paddling (Fig. 1b) with the same geometrical surface area. Fig. 1c is a SEM image of the activated paddling. The technology of obtaining the paddling is pyrolyzation with simultaneous activation by a patented technology [18].

**Table 1.** Characteristics of the membrane.

| Membrane | Type | Material | Thickness (μm) | El. resistance (Ω.cm$^2$) | Usage |
|---|---|---|---|---|---|
| Fumapem® FFA-3-PK-75 (OH$^-$ form) | Anion | fumion® F Polymer | 55 | 1.26 | Alkaline battery separator |

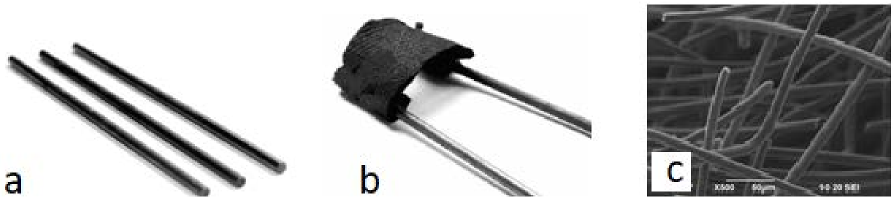


**Fig. 1.** Electrodes used in the fuel cell: a) Graphite rods, b) Paddling of activated carbon; c) SEM of a paddling of activated carbon.

The activated carbon described above was used in some sets of experiments in order to increase the geometrical surface of the electrodes.

Experiments for determination of the concentrations of sulfides that can be oxidized by *Pseudomonas putida* 1046 avoiding substrate or product inhibition were carried out in flasks at different sulfide concentrations. Three different darkened flasks with the same concentrations of sulfides were examined. Two of the flasks contained 300 ml of sulfide solution with 10 vol % of developed bioculture. In the first one – the *Pseudomonas putida* 1046 was cultivated without addition of sulfides in the nutrient medium and in the second one 3 mg.l$^{-1}$ sulfides were added. The third flask was a blank one containing only sulfide solution and was used to determine the effect of auto oxidation of the solution. The decrease of the concentrations was monitored as a function of time.

Experiments with increased conductivity of the solution by adding 8 g.l$^{-1}$ NaCl were carried out. The concentration of sodium chloride was determined as reasonable in our previous investigation [19].

The initial concentration of nitrates in all experiments was 500 mg.l$^{-1}$, also chosen from previous investigations [20].

The voltage obtained and electrical current were monitored as a function of time at 100 Ω external resistance.

The experiments were carried out at room temperature (20 °C). The processes were carried out in batch mode.

*Construction of the fuel cell*

A scheme of the construction of the fuel cell used is given (Fig. 2). It consists of two concentric compartments each with a volume of 300 ml, separated by a membrane (0.02 $m^2$). 300 ml of activated carbon is used in the cathode compartment in order to increase both conductivity and surface area of the electrode.

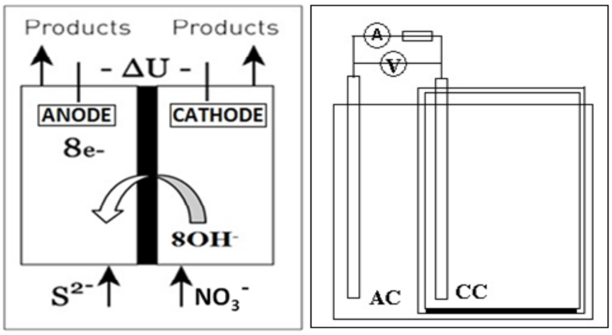


**Fig. 2.** Principle scheme of the fuel cell and the experimental installation

## RESULTS AND DISCUSSION

*Determination of the conditions for exploitation of Pseudomonas putida 1046*

The decrease of the sulfide concentrations with and without bioculture was monitored as a function of time and is presented in Fig. 3. There is a sharp decrease in concentration after the first hour. After this there is a certain plateau in the curves due to partial product inhibition due to the accumulation of various partially oxidized sulfur compounds, then the oxidation process continues again. At sulfide concentrations higher than 65 $mg.l^{-1}$, substrate inhibition of the process was observed. That is why a concentration of 65 $mg.l^{-1}$ was chosen for the subsequent experiments.

The electrodes used were 5 graphite rods in the anodic compartment and 300 ml activated carbon in the cathodic one.

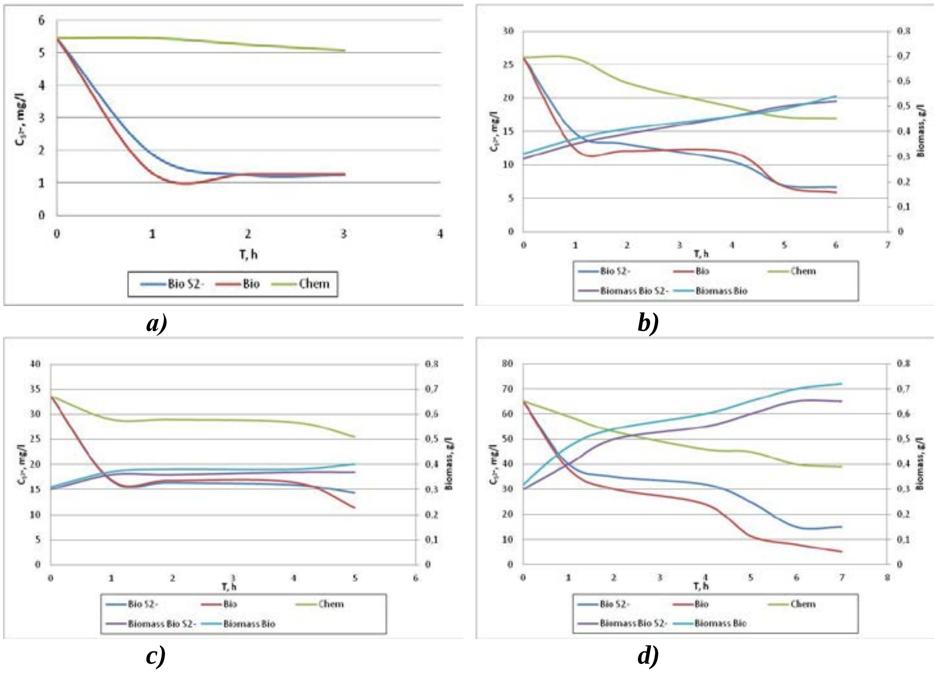


**Fig. 3.** Concentration of sulfides with suspended culture of *Pseudomonas putida 1046* as a function of time

*Fuel cell with suspended cells in the anode compartment*

The generated power of the cell was compared with that of an abiotic fuel cell. There was no significant difference in the generated power nor in the depletion of the pollutants. A little higher depletion rate of the sulfides and decrease in the depletion of the nitrates for the microbial fuel cell is observed. This can be explained with the degradation of the sulfides in the volume of the cell that doesn't correspond to electricity generation.

The addition of NaCl noticeably increases the power obtained (Fig. 4), as well as the depletion rates of both nitrates and sulfides (Fig. 5 and Fig. 6, respectively).

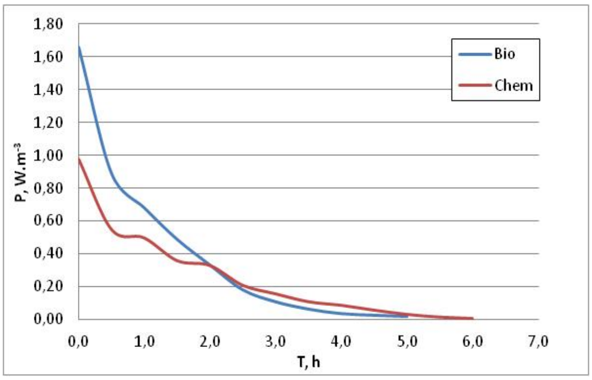


**Fig. 4**. Power generated by the fuel cell with suspended cells with increased conductivity of the solution (8 g.l$^{-1}$ NaCl). Comparison with chemical FC.

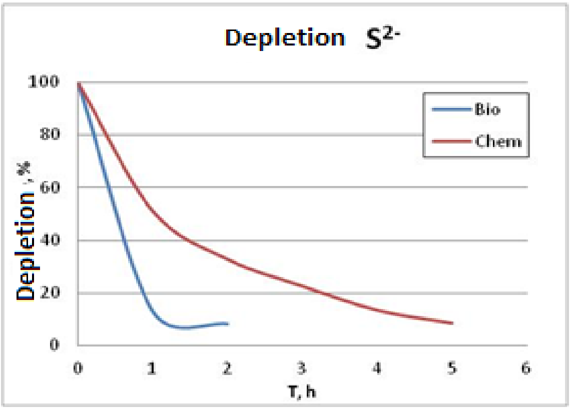


**Fig. 5.** Depletion of sulfides for microbial and chemical fuel cell with increased conductivity.

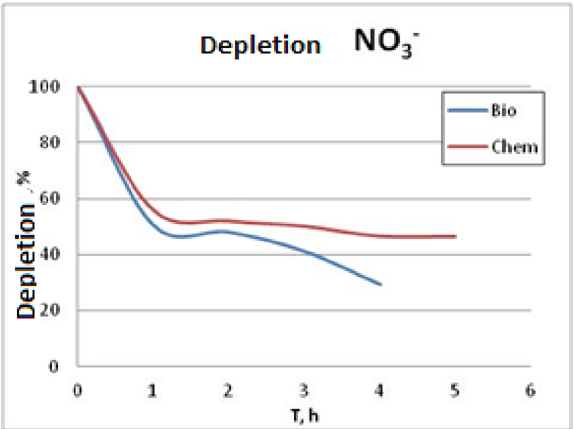


**Fig. 6.** Depletion of nitrates for microbial and chemical fuel cell with increased conductivity.

*Experiments with immobilized cells in the anode compartment*

*Pseudomonas putida* was immobilized on a pyrolyzed paddling. The comparison of the results with chemical and microbial fuel cell at the same operation conditions shows the superiority of the microbial cell (Fig. 7).

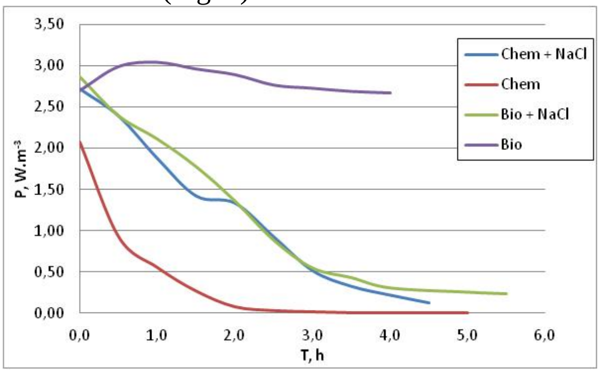


**Fig. 7.** Electrical power generation of the FC with immobilized cells. Comparison with chemical FC.

The depletions of the pollutants follow the same magnitude as the generated power.

*Fuel cell with microorganisms in the anode and cathode compartment*

*Pseudomonas denitrificans* was immobilized over 300 mg of activated carbon and pyrolyzed activated paddling was used for *Pseudomonas putida*. Unfortunately, the generated power is drastically lower (Fig. 8) compared to previous results at the same conditions.

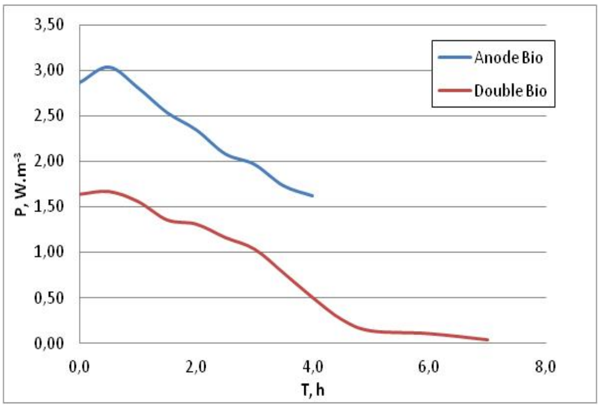


**Fig. 8.** Comparison between fuel cell with microorganisms on the anode electrode and a fuel cell with microorganisms on both electrodes.

However, at the expense of its reduced electrical performance, the fuel cell with immobilized *Pseudomonas denitrificans* in the cathodic compartment depletes the nitrate ions much faster (Fig. 9). Over 60 % of the pollutant is converted at the first hour mark, after which the process is slowed down, and product inhibition of microorganisms is most likely to be occurring. This is also the reason for the more rapid power drop in this configuration. The reason for the low electrical performance is the rapid reduction of the concentration of nitrate ions in the cathodic compartment. Another explanation of the results is that the contact between the individual beads of activated carbon is reduced due to the presence of immobilized microorganisms on it, resulting in lower conductivity of the electrode and hence decreased power.

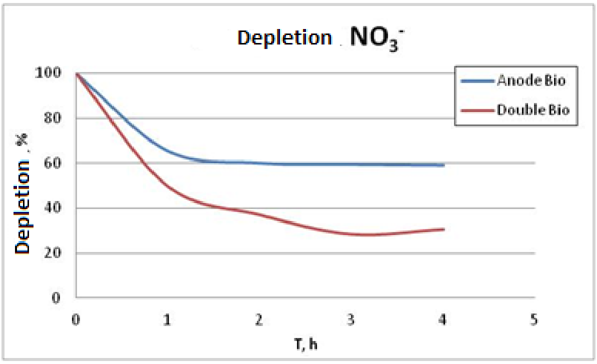


**Fig. 9.** Comparison between the nitrate depletion of a fuel cell with microorganisms on the anode electrode and a fuel cell with microorganisms on both electrodes.

The presence of sodium chloride in the sulfide solution does not significantly affect the performance of the microbial FC. This is illustrated in Fig. 10. Only during the first hours the fuel cell with the increased salinity has higher power output, after which the power drops more rapidly than the one without NaCl.

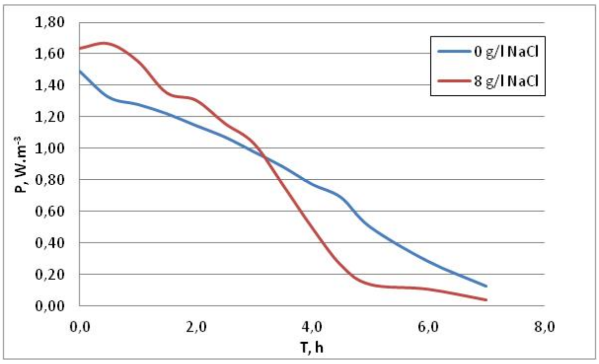


**Fig. 10**. Influence of the increased conductivity on the performance of fuel cell with microorganisms at the anode and the cathode.

A lot of factors affect the performance characteristics of a fuel cell for simultaneous sulfide oxidation and denitrification. The conductivity of the solutions used is an essential one. Obviously the support for the immobilization of the strain also plays a significant role.

In the current study a stable power density of over 3 $W.m^{-3}$, similar to the results in [10] was achieved by increased conductivity and microbial desulfurization.

Additional experiments are needed to explain the low performance characteristics of the process with microorganisms at both electrodes.

The depletion of sulfides in all cases reaches up to 96 % but depending on the presence of bioculture this is achieved for four hours (with microorganisms) and five hours (without microorganisms), which means an average depletion rate of 13 to 15 mg per hour.

The rate of denitrification was from 55 to 70% (equivalent to 55 to 87.5 mg per hour).

## CONCLUSIONS

Fuel cells can be successfully used for wastewater treatment with simultaneous energy generation. The presented configurations are simple and easy for construction and maintenance. The biological oxidation shows better characteristics for the studied fuel cells. Many different parameters affect their performance, requiring additional research for determining the optimal conditions (for energy generation, for neutralization of hazardous anthropogenic wastes, or both). The future challenges are many (to use pyrolyzed paddling for immobilization, to use paddling with implemented catalysts, to add organic substances, to create a stack of fuel cells, etc.), but nevertheless, the microbial fuel cells for treatment of wastewaters contaminated with sulfides and nitrates with simultaneous energy generation is a promising approach with undeniable benefits.

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